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ARTICLE

A *Drosophila* model of sleep restriction therapy for insomnia

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Abstract

Insomnia is the most common sleep disorder among adults, especially affecting individuals of advanced age or with neurodegenerative disease. Insomnia is also a common comorbidity across psychiatric disorders. Cognitive behavioral therapy for insomnia (CBT-I) is the first-line treatment for insomnia; a key component of this intervention is restriction of sleep opportunity, which optimizes matching of sleep ability and opportunity, leading to enhanced sleep drive. Despite the well-documented efficacy of CBT-I, little is known regarding how CBT-I works at a cellular and molecular level to improve sleep, due in large part to an absence of experimentally-tractable animals models of this intervention. Here, guided by human behavioral sleep therapies, we developed a *Drosophila* model for sleep restriction therapy (SRT) of insomnia. We demonstrate that restriction of sleep opportunity through manipulation of environmental cues improves sleep efficiency in multiple short-sleeping *Drosophila* mutants. The response to sleep opportunity restriction requires ongoing environmental inputs, but is independent of the molecular circadian clock. We apply this sleep opportunity restriction paradigm to aging and Alzheimer's disease fly models, and find that sleep impairments in these models are reversible with sleep restriction, with associated improvement in reproductive fitness and extended lifespan. This work establishes a model to investigate the neurobiological basis of CBT-I, and provides a platform that can be exploited toward novel treatment targets for insomnia.

Introduction

Insomnia is the most common sleep disorder among adults, with significant public health and economic consequences [1–4]. Cognitive behavioral therapy for insomnia (CBT-I) is the first-line intervention for treatment of insomnia [5]. CBT-I includes a combination of modalities: behavioral therapy (restriction of sleep opportunity and stimulus control), cognitive therapy (cognitive restructuring of dysfunctional beliefs about sleep and sleep disturbances), and sleep hygiene (education pertaining to behaviors that facilitate sleep continuity). Recent work suggests that restriction of sleep opportunity alone (sleep restriction therapy [SRT]) is sufficient to gain most of the benefits of CBT-I [6]. SRT addresses a prominent clinical feature of insomnia: the mismatch between sleep opportunity and sleep ability. Patients with insomnia often expand time in bed (sleep opportunity extension) with the goal of recovering lost sleep [7]. This adaptation is thought to perpetuate insomnia in the long term by promoting the mismatch between sleep ability (low) and opportunity (high), leading to less efficient, less consolidated sleep. By restricting time in bed, SRT optimizes matching of sleep ability and opportunity, leading to enhanced sleep drive (increased homeostatic pressure for sleep) and more consolidated sleep. Sleep opportunity is titrated as sleep ability stabilizes and increases. Although CBT-I has shown reliable and durable efficacy for insomnia treatment [8–10], limited accessibility of practitioners and long duration of therapy are obstacles to broad implementation [11–13]. If

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behavioral sleep interventions could be studied at a molecular/cellular level, this might guide new avenues for treatment.

Insomnia is characterized by persistent difficulty initiating or maintaining sleep despite adequate sleep opportunity, along with associated daytime impairment [14]. An animal model of insomnia should recapitulate these characteristics and, in particular, display decreased ability to sleep despite environmental circumstances that normally promote sleep. Rodent models of insomnia generally involve perturbations such as stress or fear conditioning to activate arousal systems [15], perhaps informative about acute insomnia (stress-precipitated sleep loss), but less representative of chronic insomnia (conditioned sleeplessness). Neuro-imaging, EEG, and genetic work in humans have not yielded molecular mechanisms involved in onset and treatment of insomnia at a causal level. In contrast, short-sleeping *Drosophila* mutants are compelling models of chronic insomnia: reductions in sleep seen in numerous single gene mutants are primarily due to severely decreased sleep bout length, indicating that flies can initiate but not maintain sleep [16–20]. It is unlikely that these short-sleepers simply do not need sleep, as mutants exhibit shortened lifespan and/or memory deficits [16–19, 21, 22]. In addition, a fly line generated by laboratory selection for insomnia-like traits [23] shares many features of human insomnia, including reduced sleep time and consolidation, along with shortened lifespan and learning deficits. These fly models might therefore serve an important role in studying insomnia etiology and treatment.

Sleep quantity and quality also decrease with aging across species, including humans [24–26]. Moreover, recent work suggests a bidirectional relationship between sleep and Alzheimer's disease (AD) pathology in flies, mice, and humans, where accumulation of the protein β-amyloid (Aβ) worsens sleep while poor sleep accelerates Aβ accumulation [27–29]. Indeed, in *Drosophila*, Aβ accumulation in the brain leads to reduced and fragmented sleep [30] and shortened lifespan [30, 31]. Related lines of work also suggest that sleep might serve as a modifiable risk factor in AD progression [27–30, 32]. While hypnotic use is associated with increased morbidity/mortality in individuals with AD [33], behavioral therapies show promise for improving sleep [34–36]. Here, using principles of human behavioral sleep therapies in *Drosophila*, we developed a behavioral paradigm that markedly improves sleep in fly models of insomnia. We applied this approach to an AD model and found that sleep impairments due to Aβ are reversible with behavioral sleep modification; animals with improved sleep also show lifespan extension. Our findings demonstrate efficacy of behavioral sleep therapy in an experimentally-tractable system, establishing a new model to investigate the neurobiological basis of CBT-I.

Methods

Fly strains

Iso^{31}, $sleepless^{P1}$, *redeye*, $period^{01}$, *fumin*, and cry^{02} flies were obtained from A. Sehgal. *UAS-AβArctic* [37] and *wide awake* were obtained from M. Wu. These lines were outcrossed at least 5× into the iso^{31} background. Canton S were obtained from E. Kravitz. Elav-Gal4 (#458) and $glass^{3}$ (#508) were obtained from the Bloomington Drosophila Stock Center. Flies were maintained on standard yeast/cornmeal-based medium (2% yeast, 5.4% cornmeal, 0.05% agar, 9.5% molasses, 0.12 of 5% Tegosept solution, 0.04% propionic acid) at 25 °C on a 12 h:12 h LD cycle.

Sleep analysis

Male and female flies were collected at 1–3 days old and aged in group housing, and flipped onto new food every 3–4 days. Flies aged 5–8 days were loaded into 5 × 65 mm Pyrex glass monitor tubes (Trikinetics) containing 5% sucrose and 2% agar. Locomotor activity was monitored using Drosophila activity monitoring (DAM) system (DAM2 monitors, Trikinetics, Waltham, MA). Activity was measured in 1-min bins and sleep was defined as 5 min of consolidated inactivity [38]. Data was processed using PySolo software [39]. All sleep measurements were quantified during the period of sleep opportunity (e.g., the dark period) or designated time period in non-sleep restricted conditions, not over the entire 24 h day, unless otherwise specified. Sleep latency (SL) was determined by time (minutes) until first sleep episode following start of the sleep period (e.g., lights off). Wake after sleep onset (WASO) was calculated as the minutes of wake after initiation of the first sleep episode until end of the sleep period. Activity index was calculated as the average number of beam breaks per minute of wake time. For all experiments, the first day of data following loading was discarded. Male flies were used for all experiments unless otherwise specified.

Dark time extension

Five- to eight-day-old flies were loaded into incubators and 2 days of data were collected under 12:12 LD (9 a.m.–9 p.m.) cycles to compare populations at baseline. On day 3, light schedules either remained at 12:12 LD or shifted to a 10:14 LD or 8:16 LD cycle. Sleep data was collected for four additional days. Under 10:14 LD, the dark period was from 8 p.m. to 10 a.m., while under 8:16 LD, the dark period was from 7 p.m. to 11 a.m. Days 4–5 of data collection was used for analysis.

Dark time restriction

Five- to eight-day-old flies were loaded into incubators and 2 days of data were collected at 12:12 LD (9 a.m.–9 p.m.) cycles to compare populations at baseline. On day 3, light schedules changed to the following (dark hours in parentheses): 20:4 LD (1 a.m.–5 a.m.) for days 3–4, 18:6 LD (12 a.m.–6 a.m.) for days 5–6, 16:8 LD (11 p.m.–7 a.m.) for days 7–8, and 14:10 LD (10 p.m.–8 a.m.) for days 9–10 (Fig. 2a). The 2nd day of each new LD cycle was used for analysis.

To evaluate the effects of tapering dark time, light schedules were changed directly to 18:6, 16:8, or 14:10 LD conditions, or the tapered restriction schedule above. 18:6 LD was compared to the tapered condition on day 6, 16:8 LD was compared on day 8, and 14:10 LD was compared on day 10.

Arousal threshold

Mechanical stimulation was performed as previously described [40]. Briefly, a 685 g rubber weight was dropped onto a rack supporting small DAMs monitors (Trikinetics, *wide awake*) or MultiBeam Activity Monitors (Trikinetics, AβArctic overexpression) at 12 a.m., 3 a.m., and 6 a.m. The absence of activity 5 min before a stimulus was counted as a sleep episode, and flies exhibiting beam crossings within 2 min after the stimulation were recorded as "aroused". We detected no differences in arousal within an experimental condition across the time points.

Temperature change

Five- to eight- day-old flies previously entrained to 12:12 LD conditions were loaded into incubators. For low temperature experiments, two days of data were collected under DD (constant dark) conditions at 26 °C to compare populations at baseline. On day 3, temperatures were reduced to 18 °C during the following periods (otherwise at 26 °C): 1 a.m.– 5 a.m. for days 3–4, 12 a.m.–6 a.m. for days 5–6, 11 p.m.–7 a.m. for days 7–8, and 10 p.m.–8 a.m. for days 9–10. The 2nd day of each new temperature cycle was used for analysis. High temperature experiments were performed in the same manner but under 12:12 LD conditions with a restricted period of 28 °C from 12 p.m. to 6 p.m. and temperature otherwise at 22 °C. The 4th day of restriction was used for analysis.

Aging

Male and female flies were collected at 1–3 days old and group housed at a density of approximately 10 male and 10 female flies per vial. Flies were maintained on a dextrose-based food mixture, containing 11.7% (wt/vol) dextrose, 0.6% cornmeal, and 0.3% yeast, and transferred to fresh food every 3–4 days. If fly density in vials became <10 flies, vials were combined to maintain original density. Flies were assayed for sleep and egg laying behaviors at 53 days post-eclosion.

Egg laying assay

Egg laying assays were performed in 60 mm Petri dishes. Dishes were first filled with 8 mL molten dextrose-based food which was allowed to cool and solidify. Dishes were visually examined to ensure that the surface was smooth. Twenty aged female flies were placed upon a dextrose dish in an embryo collection cage (Genesee Scientific, cat#: 59-100). Dishes were replaced after 24 h, and three consecutive days were averaged for each replicate experiment.

Longevity assay

Ten replicate vials, each containing 10 male and 10 female flies, were established for each condition. Flies were transferred to fresh dextrose-based food vials every 2–3 days, at which time dead flies were removed and recorded. Assays were conducted blind to genotype with a minimum of two replicates.

Statistical analysis and data reproducibility

Analysis was done using Prism (GraphPad Software). ANOVA with Tukey's test was used in Fig. 1d–j; Fig. 2f–l; Fig. 3b–e, g–j; Fig. 4f–j; Fig. 5b, d–i; Supplementary Fig. 1a–l; Supplementary Fig. 2a–c; Supplementary Fig. 3a–i, l, m; Supplementary Fig. 4a–d, f–i; Supplementary Fig. 5a–g; Supplementary Fig. 6d; and Supplementary Fig. 7b–d. Student's *t*-test was used in Fig. 3i–o; Fig. 4b–d, n–o; Supplementary Fig. 2d–h; Supplementary Fig. 3j–k; Supplementary Fig. 4e; Supplementary Fig. 5h–i; and Supplementary Fig. 6b, c, e–h. Fisher's exact test was used in Fig. 3p and Fig. 5j. Kolmogorov–Smirnov test was used in Fig. 5a. Log-rank test was used in Fig. 5k and Supplementary Fig. 7f. For significance: $*p \le 0.05$; $**p < 0.01$; $***p < 0.001$. Each experiment was generated from a minimum of three independent replicates. Samples were allocated based on genotype or experimental manipulation and statistics performed on aggregated data. Data generated from flies that died during sleep experiments were excluded. Bar graphs depict the mean ± SEM. Variance was similar between groups that were statistically compared. Preliminary experiments and previous work were used to assess variance and determine adequate sample sizes in advance of conducting experiments [40, 41].

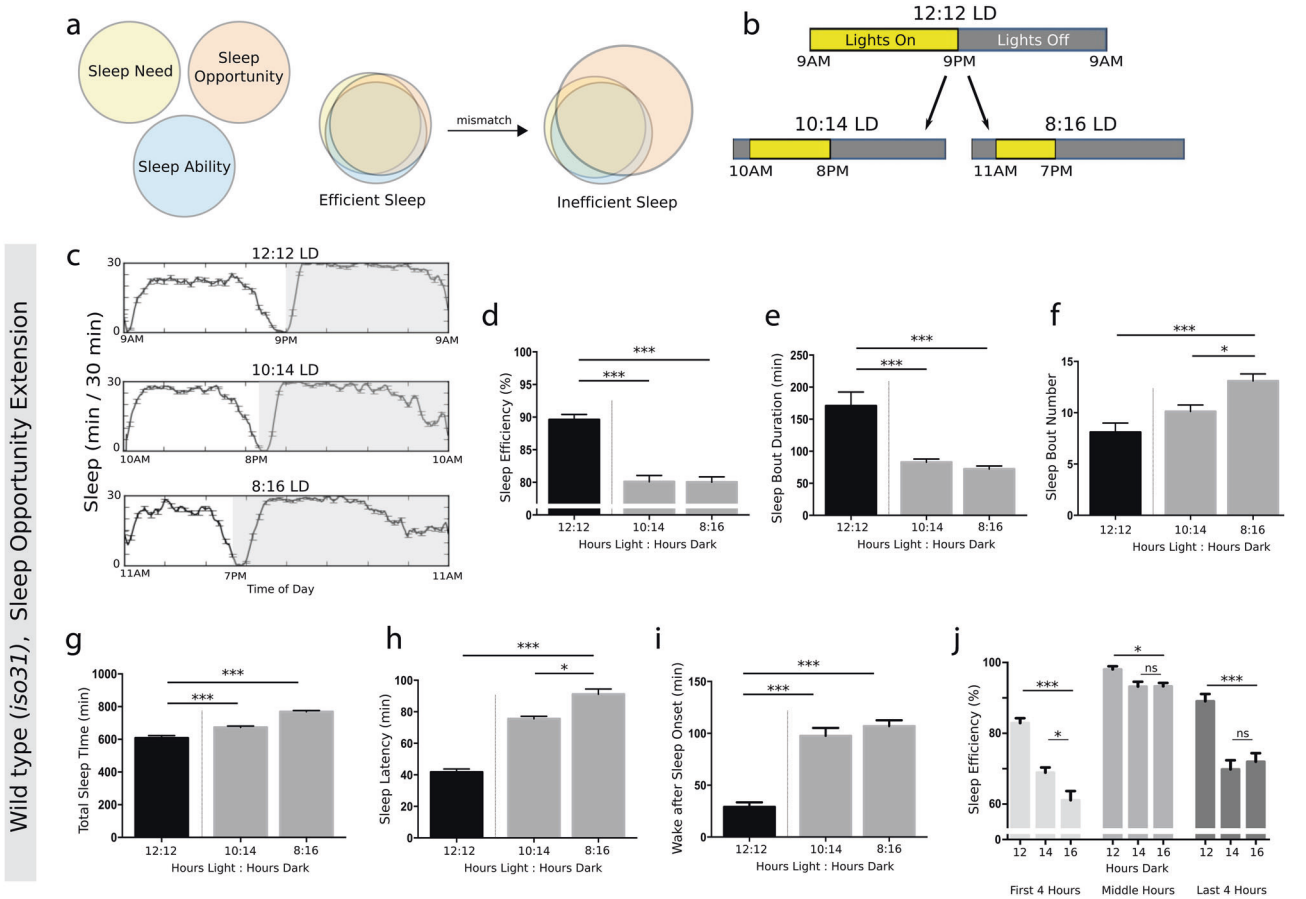


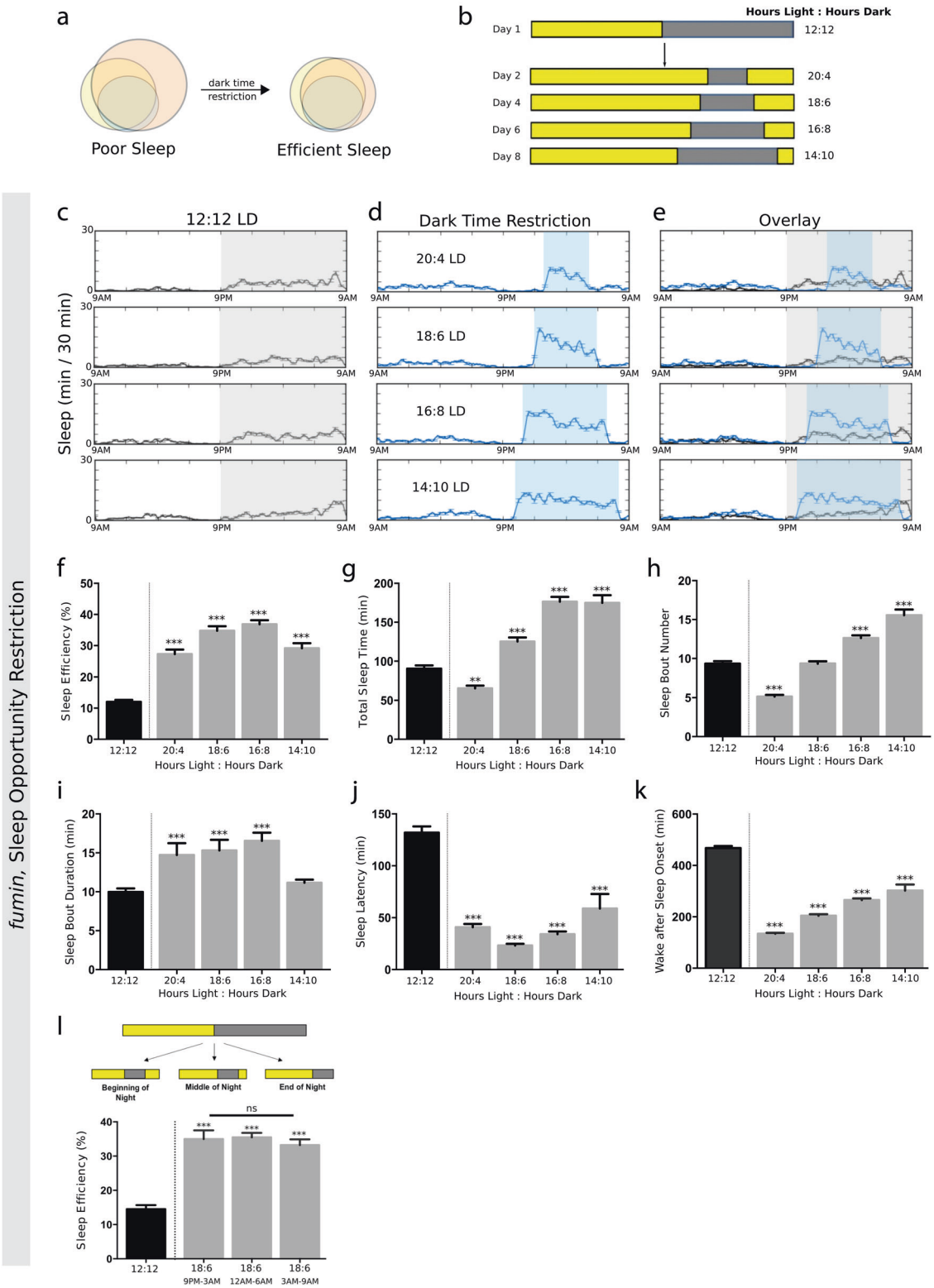
Fig. 1 Sleep opportunity extension impairs sleep in *Drosophila*. **a** Schematic of sleep degradation with mismatch of sleep opportunity and sleep ability. **b** Diagram of experimental extension of dark time from 12 h (12:12 LD) to 14 h (10:14 LD) or 16 h (8:16 LD). **c** Representative sleep traces of wild-type iso^{31} flies under 12:12 LD (top panel), 10:14 LD (middle panel) or 8:16 LD conditions (bottom panel). Gray shading indicates dark phase. Quantification of sleep efficiency (**d**), sleep bout duration (**e**), sleep bout number (**f**), total sleep time (**g**), sleep latency (**h**), and wake after sleep onset (**i**) following three nights of sleep opportunity extension in wild-type iso^{31} flies ($n = 48$ flies per condition). **j** Analysis of sleep efficiency based on time within the dark period. For all figures, error bars represent SEM; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Results

Sleep opportunity extension impairs sleep in *Drosophila*

In aiming to model human behavioral sleep interventions in *Drosophila*, we first asked whether mismatch of sleep opportunity and ability degrades sleep in fruit flies (Fig. 1a), as it does it humans. Darkness is a powerful sleep-promoting cue in humans and *Drosophila*, and wild-type flies raised on a 12 h:12 h light:dark (LD) cycle exhibit high sleep efficiency (sleep time divided by total sleep opportunity) over the dark period [42, 43]. To control sleep timing and experimentally expand sleep opportunity, we examined sleep in wild-type flies (iso^{31}) following extension of the dark period from a baseline of 12 h to 14 or 16 h (Fig. 1b, c). This manipulation significantly decreased sleep efficiency, and increased sleep fragmentation as evidenced by shorter, more frequent sleep bouts during the dark period (Fig. 1d–f). Total sleep time (TST) was only minimally increased despite an extended period of opportunity (Fig. 1g), and at the expense of all other sleep measures. A similar effect on sleep following extension of the dark period was observed across multiple wild-type strains (Supplementary Fig. 1a–d, 1g–j). In the clinical setting, measurement of sleep latency (SL) and time of wake after sleep onset (cumulative wake duration during the period of sleep opportunity after the first sleep episode; WASO) are used to measure severity of sleep deficits [44]. We also observed prolonged SL and increased WASO with sleep opportunity extension in flies (Fig. 1h, i, Supplementary Fig. 1e, f, 1k, l).

We next examined whether certain portions of the night were particularly affected by sleep opportunity extension. Flies specifically showed a large reduction in sleep efficiency during the first and last 4 h of the night with extension of the dark period; a small decrease was observed in the middle hours of the night, but sleep efficiency



remained over 90% (Fig. 1j). Reduced sleep efficiency at the beginning of the dark period was driven by prolonged SL and shorter sleep bouts (Fig. 1h; Supplementary Fig. 2a); reduced efficiency at end of the night reflected

◀ **Fig. 2** Sleep opportunity restriction enhances sleep in *fumin* mutants. **a** Schematic of hypothesis that sleep opportunity restriction aligns sleep opportunity and sleep ability, leading to efficient sleep. **b** Diagram of experimental protocol for restriction of sleep opportunity by manipulating the dark period. **c–e** Representative sleep traces of *fumin* mutants under 12:12 LD conditions (**c**, gray shading indicates dark phase), sleep restriction protocol (**d**, blue shading indicates dark phase), and both plots overlaid (**e**). **f–k** Quantification of sleep measures with restriction of sleep opportunity in *fumin* mutants ($n = 551$ flies for 12:12 LD; $n = 192$ for 20:4 LD; $n = 204$ for 18:6 LD; $n = 199$ for 16:8 LD; and $n = 55$ for 14:10 LD). **l** Sleep efficiency in *fumin* mutants with 18:6 LD dark period restriction occurring at different times of night ($n = 53$ for 9 p.m.–3 a.m., $n = 172$ for 12 a.m.–6 a.m., $n = 105$ for 3 a.m.–9 a.m.)

sleep fragmentation (Supplementary Fig. 2a, b). Together, these factors led to lower TST at the beginning and end of the dark period with sleep extension (Supplementary Fig. 2c). To test the role of the circadian clock in impaired sleep following sleep extension, we examined the *period* null mutant per^{01} [45]. While sleep efficiency was already low in these flies due to arrhythmicity (Supplementary Fig. 2d), sleep opportunity extension resulted in sleep fragmentation, prolonged SL, and increased WASO (Supplementary Fig. 2e–h), indicating that the response to sleep extension was not simply due to a mismatch in circadian timing. Together, these results suggest that, as in humans, flies cannot maintain efficient sleep when given an overabundance of sleep opportunity.

Sleep opportunity restriction enhances sleep in a short-sleeping mutant

If sleep extension results in analogous behavioral responses in humans and flies, can sleep opportunity restriction potentiate sleep efficiency in *Drosophila* short-sleeping mutants, as it does in humans with insomnia (Fig. 2a)? We first examined *fumin (fmn)* mutants, which lack a functional dopamine transporter and sleep ~200–300 min per day, representing a 70–80% reduction from wild-type levels (Fig. 2c) [20]. In humans with insomnia undergoing sleep restriction therapy (SRT), the initial amount of sleep restriction is determined based on an individual's TST; a titration procedure is then used to increase sleep opportunity as sleep is consolidated and becomes more efficient [46]. Applying this approach to *fmn* mutants, sleep time was compressed by initially contracting dark time to 4 h, followed by titration of sleep opportunity by expanding the dark period by 2 h every other day (Fig. 2b). Using this paradigm, we observed a threefold increase in sleep efficiency during the compressed dark period compared to *fmn* flies that remained under 12:12 LD conditions, with maximal improvement at 6–8 h sleep opportunity (Fig. 2c–f; Supplementary Table 1). Enhanced sleep efficiency was not simply a function of comparing sleep within a compressed dark period to the entire 12 h of dark: sleep efficiency in the restricted condition was also elevated in comparison to non-restricted flies (12:12 LD) during the same smaller time window or the equivalent number of hours following start of the dark period (Supplementary Fig. 3a–d). Interestingly, TST with compression of the dark period to 6–10 h was increased above 12:12 LD conditions (Fig. 2g), despite reduced opportunity. The enhancement in sleep efficiency and TST was driven by an increase in the frequency and duration of sleep bouts initiated during the dark period with sleep opportunity restriction (Fig. 2h, i). With only 6 h of sleep opportunity (18:6 LD), *fmn* flies initiated the same number of bouts during the dark period that normally occurred during the entire 12 h of dark under 12:12 LD conditions (Fig. 2h); indeed, comparison of the same 6 h dark period under 12:12 LD and 18:6 LD conditions revealed that restricted flies exhibit significantly more sleep bouts during this time (Supplementary Fig. 3e). Moreover, given 8 h of sleep opportunity (16:8 LD), *fmn* flies initiated even more sleep bouts than within the entire 12 h period under non-sleep restricted conditions (Fig. 2h). In addition, an increase in sleep bout duration was observed with compression of sleep opportunity (Fig. 2i), indicating that *fmn* flies initiate more bouts with matching of sleep opportunity and ability, along with improved sleep maintenance. Both SL and WASO during the dark period were significantly decreased under all dark time-restricted conditions (Fig. 2j, k), further indication of increased drive to sleep.

Importantly, similar restriction of sleep opportunity in wild-type flies did not increase sleep efficiency, perhaps because of a ceiling effect (baseline ~90%, Supplementary Fig. 3f). While there was a trend toward more consolidated nocturnal sleep in wild-type flies with a compressed dark period (Supplementary Fig. 3g, h), this occurred in the setting of daytime rebound sleep (Supplementary Fig. 3i). These results indicate that, as would be expected, restricting sleep opportunity in efficient-sleeping wild-type flies induces a state of sleep deprivation and associated homeostatic compensation. In contrast, restriction of sleep opportunity to as little as 6 h in *fmn* mutants did not induce subsequent daytime rebound sleep or a change to daytime activity (Supplementary Fig. 3j–l), suggesting that sleep opportunity and ability become better matched with sleep restriction.

Humans with insomnia who undergo behavioral sleep modification might restrict sleep from the beginning of the night, end of the night, or both depending on patient preference. We initially modeled *Drosophila* sleep restriction by limiting sleep opportunity from both start and end of the night (e.g., Zeitgeber time (ZT) 15–21 for 6 h of restriction, Fig. 2b). To test whether this behavioral paradigm depends on timing of sleep restriction or only total amount, we limited sleep opportunity to either the first 6 (ZT 12–18) or last 6 (ZT 18–24) hours of the subjective night. We

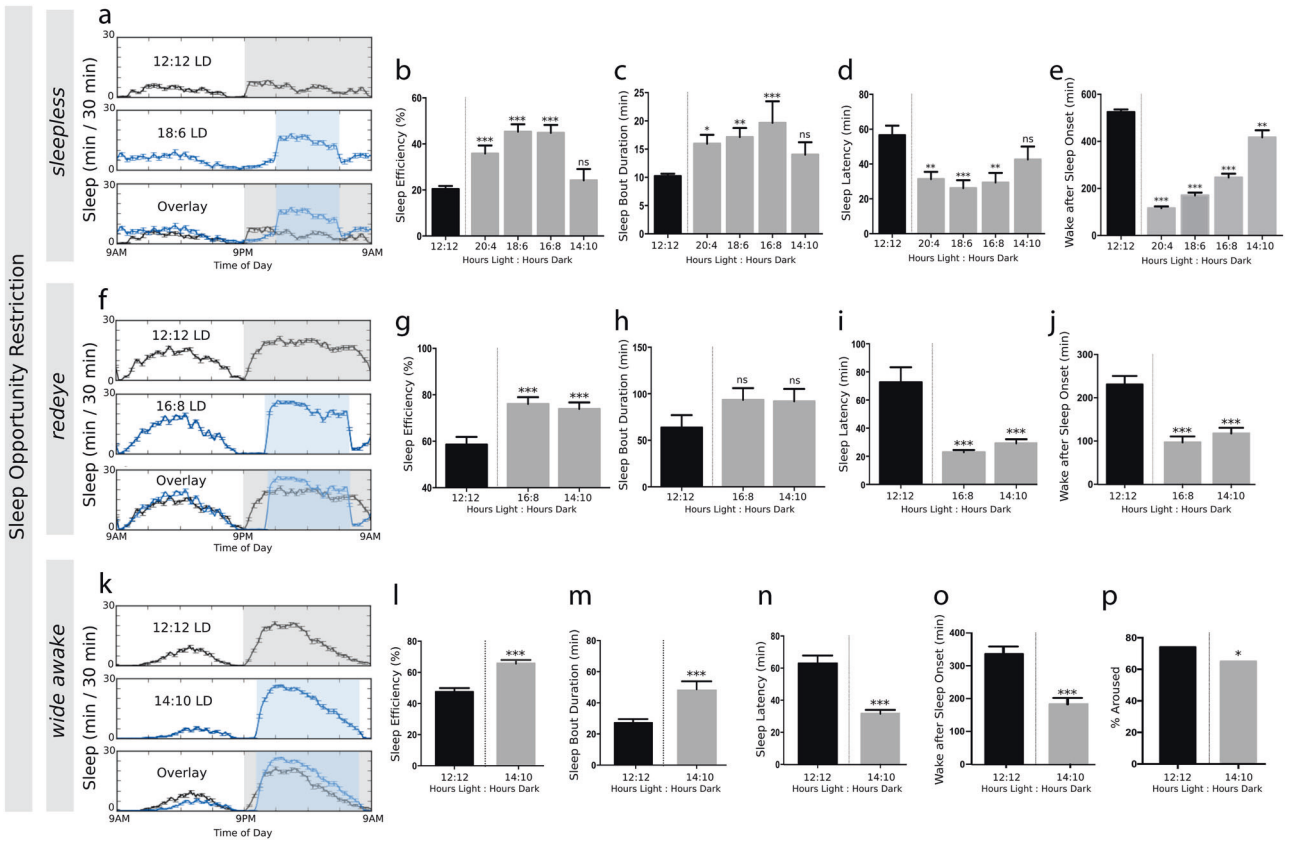


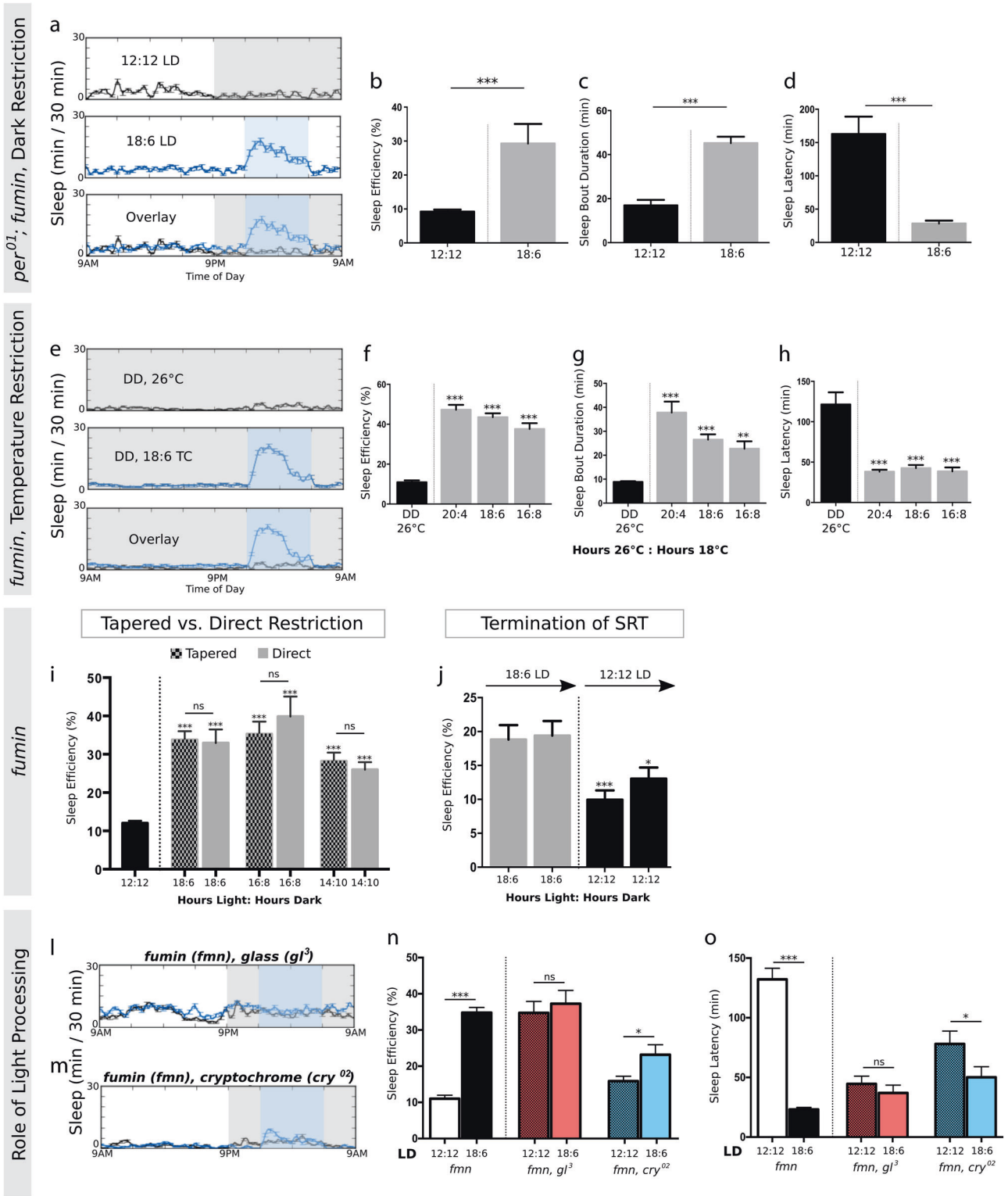
Fig. 3 Sleep opportunity restriction improves sleep in multiple short-sleeping mutants. Representative sleep traces under 12:12 LD conditions (top panel, gray shading indicates dark phase), compressed sleep opportunity (middle panel, blue shading indicates dark phase) and overlaid plots (bottom panel) for *sleepless* (**a**), *redeye* (**f**), and *wide awake* (**k**) mutants. Quantification of sleep efficiency (**b**, **g**, **l**), sleep bout duration (**c**, **h**, **m**), sleep latency (**d**, **i**, **n**), and wake after sleep onset (**e**, **j**, **o**) for each genotype (*sleepless*: $n = 210$ for 12:12 LD, $n = 64$ for 20:4 LD, $n = 69$ for 18:6 LD, $n = 68$ for 16:8 LD, and $n = 33$ for 14:10 LD; *redeye:* $n = 63$ for 12:12 LD, $n = 60$ for 16:8 LD, $n = 58$ for 14:10 LD; *wide awake:* $n = 62$ for 12:12 LD, $n = 62$ for 14:10 LD). **p** Arousal threshold of *wide awake* mutants following mechanical stimulation ($n = 246$ sleep episodes in 96 flies for 12:12 LD and $n = 250$ sleep episodes in 96 flies for 14:10 LD)

observed no significant difference in sleep efficiency or SL between these conditions (Fig. 2i, Supplementary Fig. 3m), indicating that the amount of sleep opportunity, not the timing, determines response.

Nocturnal sleep opportunity restriction improves daytime sleep

In contrast to humans, flies have a major sleep phase during the day [42, 43]. How does increased nocturnal sleep efficiency affect daytime sleep? We compared *fmn* mutants undergoing SRT at 18:6 LD, 16:8 LD, or 14:10 LD to those on a 12:12 LD schedule, focusing on sleep characteristics during the light period. *Fmn* flies on a 20:4 LD schedule were not included in the analysis because they exhibit sleep rebound during the light period following such stringent restriction of the dark period (Supplementary Fig. 3l). We found that *fmn* mutants restricted to 6 or 8 h of nocturnal sleep opportunity actually show increased day sleep efficiency compared to *fmn* flies on a 12:12 LD cycle during the equivalent 12 h light period (Supplemental Fig. 4a). TST during the equivalent 12 h light period was also increased (Supplemental Fig. 4b), driven by more frequent sleep bouts without a change in bout duration (Supplemental Fig. 4c, d).

The normal *Drosophila* light phase sleep period, or siesta, is consolidated into the middle portion of the day [42, 43, 47]. As such, sleep efficiency of *fmn* flies on a 12:12 LD schedule is elevated during this middle 6 h period of the light phase in comparison to the entire 12 h day ($24.05 \pm 0.74\%$ for ZT 3–9 vs. $16.18 \pm 0.52\%$ for ZT 0–12; $p < 0.001$; Supplementary Fig. 4e). However, nocturnal sleep restriction of *fmn* mutants with 6 or 8 h of dark potentiated daytime sleep efficiency even more during the 6 h daytime siesta period ($32.65 \pm 1.51\%$ for 18:6 LD, $32.67 \pm 1.45\%$ for 16:8 LD; $24.05 \pm 0.74\%$ for 12:12 LD; $p < 0.001$; Supplementary Fig. 4f). TST and number of sleep bouts were likewise elevated during this siesta period following compression of the dark phase to 6–8 h (Supplemental Fig. 4g–i). Although



SRT in *fmn* mutants with a 14:10 LD schedule resulted in increased nocturnal sleep effiency (Fig. 2f), daytime sleep was not improved compared to LD 12:12 *fmn* controls (Supplemental Fig. 4a, f). Together, these results indicate that optimal matching of nocturnal sleep opportunity and ability also improves sleep behaviors during the day.

◀ **Fig. 4** Response to sleep restriction requires ongoing environmental cues. **a** Representative sleep traces under 12:12 LD conditions (top panel, gray shading indicates dark phase), 18:6 LD dark time restriction (middle panel, blue shading indicates dark phase) and overlaid plots (bottom panel) for per^{01}*; fumin* mutants. Quantification of sleep efficiency (**b**), bout duration (**c**), and sleep latency (**d**) in per^{01}*; fumin* mutants ($n = 61$ for 12:12 LD, $n = 62$ for 18:6 LD). **e** Representative sleep traces in *fumin* mutants under constant dark (DD) conditions (top panel, gray indicates 26 °C) or with compressed sleep opportunity using temperature change (TC; middle panel, blue indicates 18 °C). Quantification of sleep efficiency (**f**), bout duration (**g**), and sleep latency (**h**) in *fumin* mutants under DD conditions with sleep opportunity restriction using temperature changes ($n = 144$ for DD, $n = 62$ for 20:4 TC, $n = 56$ for 18:6 TC, $n = 28$ for 16:8 TC). **i** Sleep efficiency in *fumin* mutants with sleep restriction via tapered protocol versus sleep restriction initiated with the indicated dark period ($n = 54$, 33 for 18:6 LD, $n = 25$, 24 for 16:8 LD, $n = 54$, 54 for 14:10 LD). **j** Sleep efficiency in *fumin* mutants under 18:6 LD conditions and after shift back to 12:12 LD ($n = 32$). **l–o** Sleep opportunity restriction in light-processing mutants. Overlaid sleep traces of $fumin;glass^{3}$ (**l**) and $fumin;cry^{02}$ (**m**). Black traces indicate 12:12 LD (gray shading indicates dark period); blue traces indicate sleep restriction (blue shading indicates dark period). Quantification of sleep efficiency (**n**) and sleep latency (**o**; $n = 48$ for $fumin;glass^{3}$, $n = 54$ for $fumin;cry^{02}$)

Sleep opportunity restriction is effective in multiple short-sleeping mutants

To test whether enhanced sleep with SRT is specific to *fmn* mutants, we next examined this paradigm in other mutants with distinct genetic lesions underlying a short-sleep phenotype: *sleepless (sss), redeye (rye)*, and *wide awake (wake)* [17, 48, 49]. The restricted dark period was calculated based on average TST for each mutant under 12:12 LD cycles. For a given genotype, we compared nocturnal sleep measures under control (12:12 LD) versus dark-restricted conditions. We found that restriction of sleep opportunity in each mutant increased nocturnal sleep efficiency, while reducing SL and WASO (Fig. 3; Supplementary Table 1; and Supplementary Fig. 5). The effect on sleep bout number and duration was more variable, with only some mutants (*sss* and *wake*) exhibiting longer sleep bouts (Fig. 3c, h, m; Supplementary Fig. 5). TST was largely unchanged with sleep compression (Supplementary Fig. 5), consistent with CBT-I findings in humans [50–52]. These results demonstrate that behavioral sleep modification can be applied across a variety of short-sleep etiologies, and indicate there is a ceiling beyond which sleep cannot be improved (i.e., sleep mutants cannot be fully restored to wild-type sleep levels).

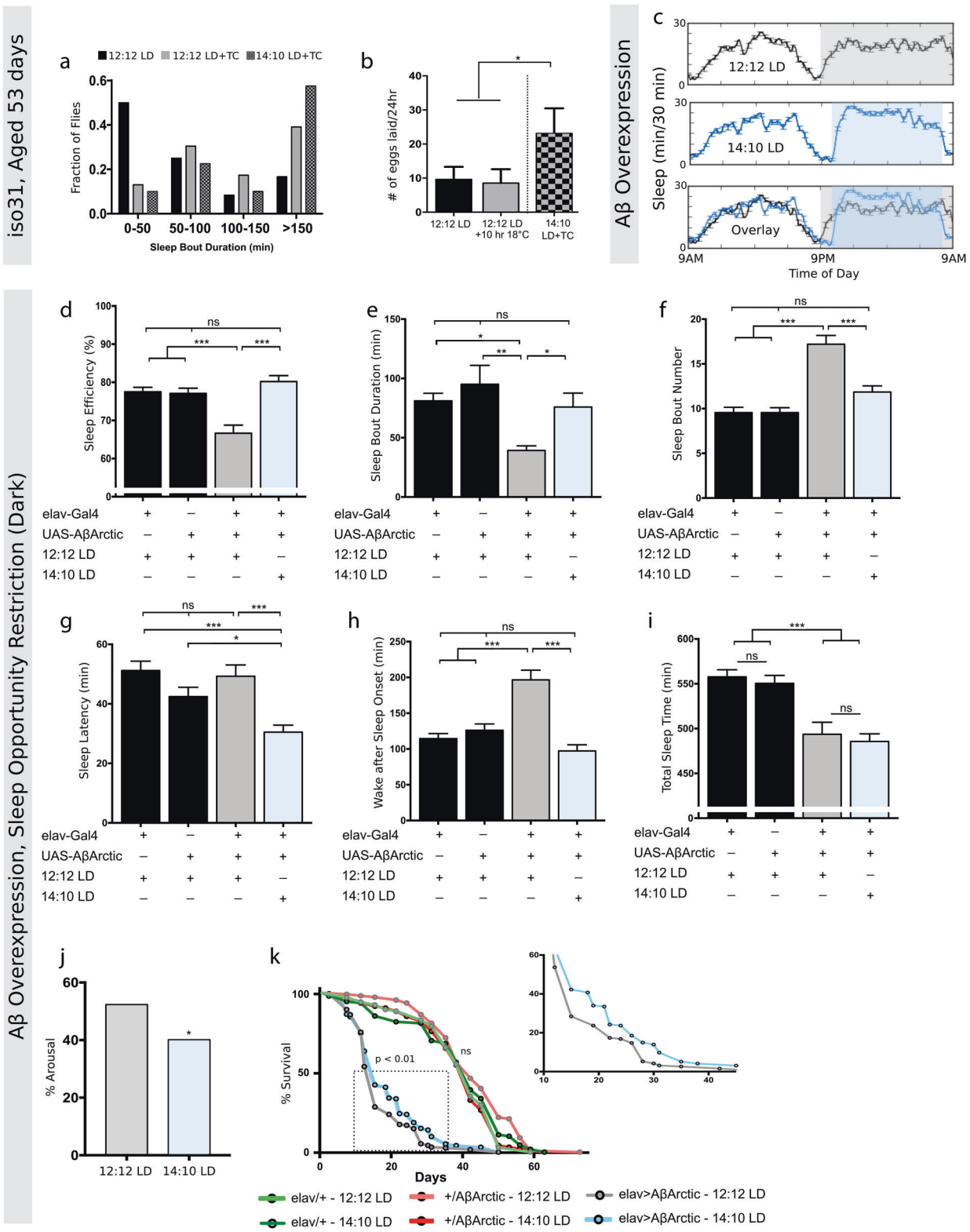
Do SRT-induced changes to sleep efficiency in flies coincide with deeper sleep? To begin answering this question, we examined whether restriction of sleep opportunity increases the arousal threshold during sleep compared to animals on a standard LD cycle. We focused on *wake* mutants because of the less severe sleep duration phenotype compared to other mutants, and thus higher probability of encountering a sleep episode. Delivery of a mechanical stimulus to *wake* mutants during the dark period aroused significantly fewer sleeping flies under SRT (14:10 LD) compared to control (12:12 LD) conditions (Fig. 3p). These findings provide evidence that restriction of sleep opportunity is associated with increased sleep depth. Together, our data establish a paradigm for SRT in flies, and suggest that sleep ability is plastic in *Drosophila* short-sleeping mutants.

Response to sleep restriction requires ongoing environmental cues

Aberrant light cycles affect function of the molecular clock [53]. To determine whether enhanced sleep following sleep opportunity restriction requires molecular circadian rhythms, we generated per^{01}*;fmn* double mutants that lack a functional molecular clock in addition to exhibiting a short-sleep phenotype. With SRT via dark period compression, we observed that increased sleep efficiency and decreased SL persist, indicating that sleep restriction is clock-independent (Fig. 4a–d).

We next asked if sleep restriction is specific to dark as a sleep-permissive cue. Cool temperatures are also sleep-permissive in both humans and flies [54–56], and under constant dark conditions, flies exhibit consolidated sleep at subjective night with lower temperature [57]. Using temperature changes (TC) from warm (26 °C) to cool (18 °C) under constant darkness (DD), we assessed sleep in *fmn* flies exposed to restricted periods of low temperature in comparison to those at a constant 26 °C. Restriction of sleep opportunity with low temperature, like darkness, resulted in increased sleep efficiency, increased bout length, and decreased SL (Fig. 4e–h). Low temperature can reduce overall locomotion in flies, raising the possibility that improved sleep measures observed with temperature-based SRT reflect non-specific activity changes. To address this issue, we took advantage of the fact that elevated temperatures are sleep-promoting during the day in flies, without altering activity [58]. Under 12:12 LD conditions, *fmn* mutants exposed to a 6 h daytime period of elevated temperature (28 °C) exhibited increased sleep efficiency and bout duration compared to flies at a constant temperature (Supplementary Fig. 6a–c). Together these results indicate that enhanced sleep with sleep restriction is not specific to light/dark inputs. Lastly, we assessed SRT using coincident darkness and low temperature. Combining these sleep-permissive cues yielded similar increases in sleep efficiency to darkness or low temperature alone (Supplementary Fig. 6d), suggesting that either cue is sufficient for the maximum sleep improvement in *fmn* mutants.

How do other features of behavioral sleep modification in humans function in our fly model? First, in humans, SRT



initiates with the greatest restriction of sleep opportunity and the goal of enhancing sleep drive/stabilizing sleep ability. This is followed by increased periods of sleep opportunity (titration) that would not have yielded efficient sleep at the outset. To test whether the titration paradigm is necessary in flies, we examined gradual extension of the

◀ **Fig. 5** Sleep opportunity restriction improves sleep degradation associated with aging and Aβ accumulation. **a** Histogram of sleep bout durations of aged flies (53 days old) under 12:12 LD (black bars, $n = 75$), 12:12 LD + TC (26 °C:18 °C, gray bars, $n = 78$), or 14:10 LD + TC conditions (blue bars, $n = 77$). **b** Number of eggs laid by aged female flies under 12:12 LD, 12:12 LD plus 10 h of low temperature during the light phase, or 14:10 LD + TC conditions ($n = 100$ flies per condition). **c** Representative sleep traces in flies with pan-neuronal overexpression of AβArctic under 12:12 LD conditions (top panel; gray shading indicates dark phase), sleep opportunity restriction (middle panel; blue shading indicates dark phase) and overlaid plots (bottom panel). **d–i** Quantification of sleep measures for *elav-Gal4/+* ($n = 60$), *UAS-AβArctic/+* ($n = 53$), and *elav-Gal4/UAS-AβArctic* flies under 12:12 LD ($n = 59$) or 14:10 LD conditions ($n = 60$). **j** Arousal threshold of *elav-Gal4/UAS-AβArctic* flies following mechanical stimulation ($n = 149$ sleep episodes in 32 flies for 12:12 LD and $n = 147$ sleep episodes in 32 flies for 14:10 LD). **k** Survival curves with pan-neuronal overexpression of AβArctic or genetic controls under 12:12 LD or 14:10 LD conditions ($n = 100$ males for each condition; *elav-Gal4/+*: 12:12 LD (light green) and 14:10 LD (dark green); *UAS-AβArctic/+*: 12:12 LD (light red) and 14:10 LD (dark red); *elav-Gal4 > UAS-AβArctic*: 12:12 LD (gray) and 14:10 LD restriction (blue). Inset shows enlarged survival curves of *elav-Gal4 > UAS-AβArctic* flies under each condition

dark period from 4 to 10 h in comparison to direct initiation of sleep opportunity restriction at either 6, 8, or 10 h in *fmn* mutants. Comparisons were made between groups of *fmn* mutants either tapered to or directly initiated on a given LD schedule. We found that enhanced sleep efficiency and other sleep measures were similar whether tapered from 4 h or restricted directly to 6, 8, or 10 h of dark (Fig. 4i, Supplementary Fig. 6e–f). Second, improved sleep with SRT in humans can take days to manifest, as sleep drive builds. We found in *fmn* mutants that the first day of sleep opportunity restriction (whether 4 or 6 h) did not induce a maximal improvement in nocturnal sleep efficiency or SL compared to 12:12 LD conditions; improvement reliably maximized by day 3 of restriction (Supplementary Fig. 6g–h), suggesting that homeostatic sleep drive has to build over time. Third, adherence to the components of CBT-I, including sleep restriction, is strongly related to treatment outcome [59]. We asked whether enhanced sleep with dark period compression persists with termination of sleep restriction. We restricted sleep opportunity in *fmn* mutants to 6 h (18:6 LD) for 5 days, and then shifted the flies back to a 12:12 LD cycle to test if increased sleep efficiency continues. With this manipulation, we found an immediate regression of nocturnal sleep efficiency back to baseline (Fig. 4j), suggesting that improvements in sleep with SRT require ongoing restriction of sleep opportunity.

Our results suggest that blocking sensory processing of LD cues should occlude the response to sleep restriction with dark period compression. Flies process light through canonical visual pathways as well as other light sensors such as CRYPTOCHROME (CRY) [60]; genetic disruption of both of these pathways renders *Drosophila* insensitive to LD cycling and behavioral arrhythmicity [60, 61]. We generated *glass;fumin* double mutants, which lack all functional eye components and are short-sleepers. These double mutants exhibited no change in sleep efficiency or SL with dark period restriction (Fig. 4l, n–o), indicating that a functional eye is necessary for induction of SRT using altered LD cycles. CRY is a UV- and blue light-sensitive protein that communicates light information to the circadian system [62–64]. To test whether CRY plays a role in the response to sleep opportunity restriction, we generated cry^{02}:*fumin* double mutants. These flies exhibited increased sleep efficiency and reduced SL with sleep restriction (Fig. 4m–o), though the responses were attenuated compared to *fmn* mutants alone, suggesting that maximal increases in sleep efficiency with restriction of the dark period utilize multiple light-processing systems. Together, these data demonstrate that sleep restriction has a direct reliance on environmental cues to produce its effect regardless of prior experience, and that sleep opportunity restriction in flies does not cause a long-lasting change in the absence of these cues.

Sleep restriction improves sleep in aging and Alzheimer's disease models

Aging is associated with increased sleep fragmentation in *Drosophila* [65–67] and humans [68]. We next investigated whether behavioral sleep modification through sleep restriction might improve sleep in aged flies. Behavioral response to light cues are weakened in aged flies [56], so a compressed dark period is not sufficient to restrict sleep opportunity (Supplementary Fig. 7a); however, sleep can be consolidated by adding coincident temperature cycles to 12:12 LD cycles [56]. To investigate whether aged flies further consolidate sleep with sleep opportunity restriction, we compared aged female flies (53 days post-eclosion) under 12:12 LD + TC (26 °C:18 °C) conditions to flies that were restricted to 10 h dark and coincident low temperature. We chose 10 h of sleep opportunity to match TST during the night at baseline. We observed an increase in sleep bout length in restricted flies, above that of 12:12 LD + TC alone, indicating a consolidation of nocturnal sleep with restriction (Fig. 5a). No significant increase in sleep efficiency was observed above 12:12 LD + TC, likely due to a ceiling effect in sleep efficiency in aged flies (Supplementary Fig. 7b). To assess behavioral consequences of consolidating sleep in aged flies, we examined reproductive fitness following sleep opportunity restriction. Aged female flies normally exhibit a dramatic reduction in reproductive output [69], and reproductive output is also impaired with sleep deprivation [70]. We tested whether such decrements are modifiable with improved sleep. We assessed egg laying behavior after five nights of sleep opportunity restriction in

53 day old mated female flies, and found that flies with improved sleep laid significantly more eggs in a 24-h period than controls (Fig. 5b). This increase was not simply due to exposure to cool temperatures, as addition of an equivalent low temperature period during the day under 12:12 LD conditions was indistinguishable from control flies (Fig. 5b). These results raise the possibility of potential behavioral benefits to improved sleep in aged flies following restriction of sleep opportunity.

Sleep quality degrades with normal aging, but disruptions to sleep are also increasingly appreciated in neurodegenerative processes like AD [29]. Several models of AD have been described in *Drosophila*, including those based on expression of aggregating β-amyloid (Aβ) peptides [31, 71]; Aβ accumulation results in decreased and fragmented sleep, while sleep deprivation increases Aβ burden [30]. We examined sleep following pan-neuronal expression of AβArctic, which carries a mutation to induce enhanced aggregation [37, 72]. Consistent with previous work [30], we observed a reduction in TST and increase in sleep fragmentation during the nocturnal period in 7–10-day-old male flies with pan-neuronal AβArctic expression under 12:12 LD cycles (Fig. 5c, e, f, i). Sleep during the night was less efficient, due to a reduction in sleep bout duration and increase in number of sleep bouts (Fig. 5d–f); WASO was likewise increased with pan-neuronal AβArctic expression, though SL was unaffected (Fig. 5g, h).

We next examined whether sleep degradation related to Aβ accumulation is reversible with sleep opportunity restriction using dark period compression. In contrast to aged wild-type flies, compression of the dark period alone was sufficient to alter sleep/wake patterns in AβArctic-overexpressing flies, eliminating the need for coincident temperature changes. We found SRT restored sleep efficiency, sleep bout length, and number of sleep bouts back to control levels during the dark period (Fig. 5d–f, Supplementary Fig. 7c); WASO was also normalized, and SL was shortened (Fig. 5g, h). TST during the dark period was equivalent in AβArctic-overexpressing animals whether given a 12 or 10 h night, meaning the flies were able to achieve the same amount of sleep in a compressed dark window (Fig. 5i). Pan-neuronal ectopic expression of AβArctic did not consistently impair daytime sleep efficiency compared to genetic controls, and nocturnal sleep restriction had no further effect on day sleep (Supplementary Fig. 7d). We also assessed nocturnal sleep depth in AβArctic-overexpressing flies under either 12:12 or 14:10 LD conditions, and found that SRT (14:10 LD) was associated with increased arousal threshold (Fig. 5j). Thus, manipulation of environmental cues is sufficient to improve sleep despite pan-neuronal Aβ aggregation.

Does enhancement of sleep in this model of AD have other beneficial effects? AβArctic flies exhibit severely curtailed lifespan [30], so we tested whether correcting sleep can affect longevity. Comparing flies expressing AβArctic pan-neuronally under either 12:12 LD or dark-restricted (14:10 LD) conditions, we found that sleep opportunity restriction was associated with a small but significant extension of lifespan in both males and females (Fig. 5k, Supplementary Fig. 7e). This longevity extension was not due to changes in the LD cycle, as genetic controls showed no alteration in longevity with sleep opportunity restriction. Taken together, these data suggest that SRT mitigates Aβ-related sleep disturbances and shortened lifespan.

Discussion

CBT-I is the first-line treatment for insomnia, offering advantages over existing pharmacotherapies with regard to safety and durability of response [35]. However, CBT-I is limited by obstacles to broad implementation [11–13]. Research in *Drosophila* has yielded numerous insights into basic sleep neurobiology, and here, we have leveraged this system to develop a tractable experimental model of sleep restriction therapy (SRT) for insomnia. We find that mismatch of sleep opportunity and ability degrades sleep continuity in flies, as in humans. Surprisingly, compression of sleep opportunity in short-sleeping genetic mutants improves sleep efficiency along with multiple other measures of sleep. We apply this paradigm to normal aging and neurodegeneration, both of which are associated with impaired sleep, and find that behavioral sleep modification restores sleep consolidation and extends lifespan. These data establish a new platform for deciphering mechanistic principles of a behavioral sleep therapy that improves sleep across species.

Toward a molecular and genetic basis of SRT using *Drosophila*

Previous work has argued that short-sleeping flies are a compelling model for studying human insomnia [23, 73]. Single gene mutants such as those tested here [17, 20, 48, 49], as well as a line generated by laboratory selection over many generations [23], recapitulate central features of human insomnia: reduced sleep time, increased sleep latency and sleep fragmentation, and daytime impairments. The conserved response in flies to both sleep opportunity extension and restriction provides further support for the idea that this organism can serve as a valid model for insomnia. Human evidence is consistent with a genetic component to insomnia [74, 75], and while this disease is likely multigenic in nature [76], highly penetrant single gene mutations are important for studying disorder

mechanisms and treatment approaches. The fact that genetically-distinct *Drosophila* sleep mutants all respond to the sleep compression paradigm suggests these lesions might converge on a shared physiological, and perhaps cellular, endpoint. Future work will use these models to understand how SRT alters function of well-characterized sleep circuits in the fly brain [77], with the ultimate goal of identifying molecular changes in these circuits induced by SRT.

While SRT increases sleep efficiency in multiple short-sleeping models, other sleep characteristics do not show a uniform response. For example, *fmn* mutants initiate more frequent sleep bouts during the compressed nocturnal period (LD 18:6) in comparison to the same genotype under LD 12:12 conditions, but the duration of sleep episodes is only modestly increased, suggesting a persistent deficit in sleep maintenance. In contrast, Aβ-overexpressing flies on an LD 12:12 schedule exhibit fragmented sleep (increased frequency of brief sleep bouts) compared to genetic controls on the same schedule; however, SRT restores sleep efficiency by promoting more consolidated sleep (fewer, longer sleep bouts), along with increased sleep depth. The distinct response patterns to SRT elicited in different short-sleep models might prove informative toward understanding how this paradigm acts at a genetic level. This line of work will be complemented by examination of SRT in outbred fly populations exhibiting natural variation in sleep need and duration [78].

Implications for human insomnia and SRT

The efficacy of sleep opportunity restriction in multiple mutants suggests that, in humans, SRT should be effective across insomnia subtypes, provided there is a mismatch between sleep opportunity and ability. A possible exception is evidence that insomnia patients with objective short sleep duration do not respond as well to CBT-I as those with relatively normal TST [79]. This stands in contrast to our model which explicitly focuses on genetic models of short sleep. The difference between fly and human might reflect limitations of the paradigm in modeling insomnia, but our findings in *Drosophila* also raise the possibility that more significant curtailment of sleep opportunity is necessary for clinically-improved insomnia in patients with short sleep duration. Interestingly, results in *fmn* mutants suggest that titration of sleep opportunity might not be necessary in flies, in contrast to humans. Future work will examine whether this result is generalizable to other short-sleep etiologies, and if modification of the titration protocol can in fact yield additional benefits to sleep.

As with sleep opportunity restriction, we find sleep extension yields a conserved response from flies to humans. Wild-type flies exhibit impaired sleep continuity when presented with an overabundance of sleep opportunity, along with increased sleep latency. TST does increase with sleep extension, but at the expense of other sleep measures. This response to sleep extension is consistent with findings in humans that suggest time in bed extension is associated with increased TST, but impairments in sleepiness, mood, and performance [80, 81]. We also find that degradation of sleep measures occurs primarily at the beginning and end of the extended nocturnal sleep period in flies. It will be of interest to examine whether similar temporally-specific disruptions to sleep continuity occur in humans.

Our data indicate that sleep ability is plastic: optimizing environmental conditions can enhance sleep efficiency (and even total sleep time in *fmn* flies; Fig. 2g) despite fixed genetic mutations, suggesting biological determinants of sleep are highly mutable. This idea is conceptually informative for humans with insomnia, and provides empirical evidence for focusing on mechanisms of sleep opportunity restriction as the core insomnia treatment modality. Indeed, the Spielman model for insomnia (also known as the 3P model) identifies predisposing (e.g., genetic) and precipitating factors (e.g., acute stressor) that lead to acute insomnia, with perpetuating factors (e.g., sleep extension) that shift acute insomnia to chronic [7, 82, 83]. This model has served as the basis for using sleep restriction in humans to target sleep extension (a perpetuating factor). Our results raise the possibility that sleep restriction also targets predisposing genetic factors, by better matching intrinsic sleep ability with opportunity. In other words, humans with a genetic predisposition to insomnia might be sleep "over-extended" even if sleep opportunity appears normal; restriction of sleep opportunity would therefore increase sleep efficiency and perhaps potentiate sleep ability.

Modeling SRT in neurodegenerative and psychiatric disorders

Poor sleep has long been appreciated as a comorbidity of aging and neurodegeneration [26, 29], but more recently identified as a potential modifiable risk factor for neurodegenerative disease progression [27–29]. In flies, pharmacologic and genetic approaches to improve sleep have been shown to ameliorate memory deficits in an Alzheimer's disease model [32]; similarly, altering the sleep-Aβ interaction by modulating neuronal excitability with a pharmacotherapy prolongs lifespan in Aβ-expressing flies [30]. We find that increased sleep efficiency through compression of sleep opportunity is alone sufficient to extend lifespan in Aβ-expressing flies. An intriguing future direction is that behavioral approaches to treating insomnia could slow progression of disease, consistent with evidence in humans demonstrating that CBT-I in older adults with mild cognitive impairment improves cognitive function [36].

Most pharmacological treatments in psychiatry are based on drugs discovered serendipitously over a half century ago [84]. In recent years, significant advances in treating mental illness have been behavioral interventions [85], yet little is known regarding the mechanistic basis of such interventions. How can behavioral therapies be studied at a molecular level? This fly model of behavioral sleep modification can be used to generate such granular insights. Our initial results demonstrate that therapeutic sleep restriction does not require a functional molecular clock, and that manipulating light:dark cycles to enhance sleep drive requires canonical light sensory pathways. Future work will use this model to define the neural circuits required for, and molecular changes occurring with, sleep restriction, with the goal of identifying new insomnia treatment targets that are conceptually based on the established efficacy of CBT-I.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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