



Published in final edited form as:

Mol Psychiatry. 2021 February ; 26(2): 492–507. doi:10.1038/s41380-019-0376-6.

A *Drosophila* Model of Sleep Restriction Therapy for Insomnia

Samuel J. Belfer^{1,2}, Alexander G. Bashaw², Michael L. Perlis^{2,3,4}, Matthew S. Kayser^{1,2,4,5,*}

¹Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, United States

²Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, United States

³Behavioral Sleep Medicine Program, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, United States

⁴Center for Sleep and Circadian Neurobiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, United States

⁵Chronobiology Program, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, United States

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 towards novel treatment targets for insomnia.

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

*Lead Contact and Correspondence: Dr. Matthew S. Kayser, Clinical Research Building, Room 320, 415 Curie Boulevard, Philadelphia, PA 19143, kayser@penncmedicine.upenn.edu, Telephone: 215 898 8268, Fax: 215 898 0509.

Conflict of Interest

The authors declare no conflict of interest.

Introduction

Insomnia is the most common sleep disorder among adults, with significant public health and economic consequences¹⁻⁴. Cognitive Behavioral Therapy for Insomnia (CBT-I) is the first-line intervention for treatment of insomnia⁵. 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-I⁶. 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 sleep⁷. 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 treatment⁸⁻¹⁰, limited accessibility of practitioners and long duration of therapy are obstacles to broad implementation¹¹⁻¹³. If 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 impairment¹⁴. 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 systems¹⁵, perhaps informative about acute insomnia (stress-precipitated sleep loss), but less representative of chronic insomnia (conditioned sleeplessness). Neuroimaging, 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 sleep¹⁶⁻²⁰. 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 traits²³ 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 humans²⁴⁻²⁶. 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 (A β) worsens sleep while poor sleep accelerates A β accumulation²⁷⁻²⁹. Indeed, in *Drosophila*, A β accumulation in the brain leads to reduced and fragmented sleep³⁰ 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 AD³³, behavioral therapies show promise for improving sleep³⁴⁻³⁷. 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 A β 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*, *period01*, *fumin*, and *cry02* flies were obtained from A. Sehgal. *UAS-A β Arctic*³⁸ and *wide awake* were obtained from M. Wu. These lines were outcrossed at least 5x 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 degrees on a 12hr:12hr 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 minutes of consolidated inactivity³⁹. Data was processed using PySolo software⁴⁰. 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 hour 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 (9AM-9PM) 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 4 additional days. Under 10:14 LD, the dark period was from 8PM-10AM, while under 8:16 LD, the dark period was from 7PM-11AM. Day 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 (9AM-9PM) cycles to compare populations at baseline. On day 3, light schedules changed to the following (dark hours in parentheses): 20:4 LD (1AM-5AM) for days 3–4, 18:6 LD (12AM-6AM) for days 5–6, 16:8 LD (11PM-7AM) for days 7–8, and 14:10 LD (10PM to 8AM) for days 9–10 (Figure 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 described⁴¹. Briefly, a 685g rubber weight was dropped onto a rack supporting small DAMs monitors (Trikinetics, *wide awake*) or MultiBeam Activity Monitors (Trikinetics, β Arctic overexpression) at 12AM, 3AM, and 6AM. 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 12:12LD 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): 1AM-5AM for days 3–4, 12AM-6AM for days 5–6, 11PM-7AM for days 7–8, and 10PM to 8AM 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 12PM-6PM 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% corn meal, 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 hours, and 3 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 Figure 1D–J; Figure 2F–L; Figure 3B–E, G–J; Figure 4F–J; Figure 5B,D–I; Supplementary Figure 1A–L; Supplementary Figure 2A–C; Supplementary Figure 3A–I, L–M; Supplementary Figure 4A–D, F–I; Supplementary Figure 5A–G; Supplementary Figure 6D; and Supplementary Figure 7B–D. Student's t-test was used in Figure 3L–O; Figure 4B–D, N–O; Supplementary Figure 2D–H; Supplementary Figure 3J–K; Supplementary Figure 5E; Supplementary Figure 5H–I; and Supplementary Figure 6B–C, E–H; . Fisher's Exact test was used in Figure 3P and Figure 5J. Kolmogorov–Smirnov test was used in Figure 5A. Log-rank test was used in Figure 5K and Supplementary Figure 7F. For significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Each experiment was generated from a minimum of 3 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 \pm 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.^{41,42}

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 in humans. Darkness is a powerful sleep-promoting cue in humans and *Drosophila*, and wild type flies raised on a 12hr:12hr light:dark (LD) cycle exhibit high sleep efficiency (sleep time divided by total sleep opportunity) over the dark period^{43,44}. To control sleep timing and experimentally expand sleep opportunity, we examined sleep in wild type flies (*iso³¹*) following extension of the dark period from a baseline of 12 hours to 14 or 16 hours (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 deficits⁴⁵. 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 hours 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 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*¹.⁴⁶ 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 minutes 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 efficient⁴⁷. Applying this approach to *fmn* mutants, sleep time was compressed by initially contracting dark time to 4 hours, followed by titration of sleep opportunity by expanding the dark period by 2 hours 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 hours 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 hours 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 hours 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). With only 6 hours 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 hours of

dark under 12:12 LD conditions (Fig. 2H); indeed, comparison of the same 6 hour 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 hours of sleep opportunity (16:8 LD), *fmn* flies initiated even more sleep bouts than within the entire 12 hour 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 towards 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 hours 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, 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 hours 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 observed no significant difference in sleep efficiency or SL between these conditions (Fig. 2L, 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^{43,44}. 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 hours of nocturnal sleep opportunity actually show increased day sleep efficiency compared to *fmn* flies on a 12:12 LD cycle during the equivalent 12 hour light period (Supplemental Fig. 4A). TST during the equivalent 12 hour 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^{43,44,48}. As such, sleep efficiency of *fmn* flies on a 12:12 LD schedule is elevated during this middle 6 hour period of the light phase in comparison to the entire 12 hour day (24.05±0.74% for ZT 3–9 vs. 16.18±0.52% for ZT 0–12; $p < 0.001$; Supplementary Fig. 4E). However, nocturnal sleep restriction of *fmn* mutants with 6 or 8 hours of dark potentiated daytime sleep efficiency even more during the 6 hour daytime siesta period (32.65±1.51% for 18:6 LD, 32.67±1.45% for 16:8 LD; 24.05±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 hours (Supplemental Fig. 4G-I). Although SRT in *fmn* mutants with a 14:10 LD schedule resulted in increased nocturnal sleep efficiency (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.

Sleep opportunity restriction is effective in multiple short-sleep 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*)^{17,49,50}. 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 humans⁵¹⁻⁵³. 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 clock⁵⁴. To determine whether enhanced sleep following sleep opportunity restriction requires molecular circadian rhythms,

we generated *per¹;fmm* 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 flies⁵⁵⁻⁵⁷, and under constant dark conditions, flies exhibit consolidated sleep at subjective night with lower temperature⁵⁸. Using temperature changes (TC) from warm (26°C) to cool (18°C) under constant darkness (DD), we assessed sleep in *fmm* 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 activity⁵⁹. Under 12:12 LD conditions, *fmm* mutants exposed to a 6 hour 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 *fmm* 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 dark period from 4 to 10 hours in comparison to direct initiation of sleep opportunity restriction at either 6, 8, or 10 hours in *fmm* mutants. Comparisons were made between groups of *fmm* 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 hours or restricted directly to 6, 8, or 10 hours 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 *fmm* mutants that the first day of sleep opportunity restriction (whether 4 or 6 hours) 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 outcome⁶⁰. We asked whether enhanced sleep with dark period compression persists with termination of sleep restriction. We restricted sleep opportunity in *fmm* mutants to 6 hours (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)⁶¹; genetic disruption of both of these pathways renders *Drosophila* insensitive to LD cycling and behavioral arrhythmicity^{61,62}. 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 system⁶³⁻⁶⁵. To test whether CRY plays a role in the response to sleep opportunity restriction, we generated *cry²;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*⁶⁶⁻⁶⁸ and humans⁶⁹. 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 flies⁵⁷, 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 cycles⁵⁷. 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 hours dark and coincident low temperature. We chose 10 hours 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 output⁷⁰, and reproductive output is also impaired with sleep deprivation⁷¹. We tested whether such decrements are modifiable with improved sleep. We assessed egg laying behavior after 5 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-hour 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 Alzheimer's disease (AD)²⁹. Several models of AD have been described in *Drosophila*, including those based on expression of aggregating β -amyloid (A β) peptides^{31,72}; A β accumulation results in decreased and fragmented sleep, while sleep deprivation increases A β burden³⁰. We examined sleep following pan-neuronal expression of A β Arctic, which carries a mutation to induce enhanced aggregation^{38,73}. Consistent with previous work³⁰, 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 A β Arctic 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 A β Arctic expression, though SL was unaffected (Fig. 5G,H).

We next examined whether sleep degradation related to A β 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 A β Arctic-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 A β Arctic-overexpressing animals whether given a 12 or 10 hour 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 A β Arctic 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 A β Arctic-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 A β aggregation.

Does enhancement of sleep in this model of AD have other beneficial effects? A β Arctic flies exhibit severely curtailed lifespan³⁰, so we tested whether correcting sleep can affect longevity. Comparing flies expressing A β Arctic 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 A β -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 response³⁵. However, CBT-I is

limited by obstacles to broad implementation¹¹⁻¹³. 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.

Towards 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^{23,74}. Single gene mutants such as those tested here^{17,20,49,50}, as well as a line generated by laboratory selection over many generations²³, 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^{75,76}, and while this disease is likely multigenic in nature⁷⁷, 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 brain⁷⁸, 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, β -overexpressing flies on an LD 12:12 schedule exhibit fragmented sleep (increased, short 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 towards 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 duration⁷⁹.

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 TST⁸⁰. 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 onset 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^{81,82}. 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^{83,84}. 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 progression²⁷⁻²⁹. In flies, pharmacologic and genetic approaches to improve sleep have been shown to ameliorate memory deficits in an Alzheimer’s Disease model³²; similarly, altering the sleep-A β interaction by modulating neuronal excitability with a pharmacotherapy prolongs lifespan in A β -expressing flies³⁰. We find that increased sleep efficiency through compression of sleep opportunity is alone sufficient to extend lifespan in A β -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 function⁸⁵.

Most pharmacological treatments in psychiatry are based on drugs discovered serendipitously over a half century ago⁸⁶. In recent years, significant advances in treating mental illness have been behavioral interventions⁸⁷, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Kyunghee Koh, Philip Gehrman, and members of the Kayser Lab for helpful discussions. This work was supported by NIH grants F30AG058409 (SJB), T32HL07953 (SJB), R21HD083628 (MLP), K24AG055602 (MLP), K08NS090461 (MSK), and a pilot grant (to MSK) from the University of Pennsylvania Alzheimer's Disease Core Center (P30-AG010124). Other support includes a Hearst Foundation Fellowship Award (SJB), and Burroughs Wellcome Career Award for Medical Scientists, March of Dimes Basil O'Connor Scholar Award, and Sloan Research Fellowship (MSK).

References

1. Dement W, Pelayo R. Public health impact and treatment of insomnia. *Eur Psychiatry* 1997; 12: 31s–39s.
2. Ozminkowski RJ, Wang S, Walsh JK. The direct and indirect costs of untreated insomnia in adults in the United States. *Sleep* 2007; 30: 263–273. [PubMed: 17425222]
3. Daley M, Morin CM, LeBlanc M, Grégoire JP, Savard J, Baillargeon L. Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents. *Sleep Med* 2009; 10: 427–438. [PubMed: 18753000]
4. Sarsour K, Morin CM, Foley K, Kalsekar A, Walsh JK. Association of insomnia severity and comorbid medical and psychiatric disorders in a health plan-based sample: Insomnia severity and comorbidities. *Sleep Med* 2010; 11: 69–74. [PubMed: 19410512]
5. Qaseem A, Kansagara D, Forcica MA, Cooke M, Denberg TD, Barry MJ et al. Management of chronic insomnia disorder in adults: A clinical practice guideline from the American college of physicians. *Ann Intern Med* 2016; 165: 125–133. [PubMed: 27136449]
6. Miller CB, Espie CA, Epstein DR, Friedman L, Morin CM, Pigeon WR et al. The evidence base of sleep restriction therapy for treating insomnia disorder. *Sleep Med. Rev* 2014; 18: 415–424. [PubMed: 24629826]
7. Spielman AJ. Assessment of insomnia. *Clin Psychol Rev* 1986; 6: 11–25.
8. Morin CM, LeBlanc M, Daley M, Gregoire JP, Mérette C. Epidemiology of insomnia: Prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors. *Sleep Med* 2006; 7: 123–130. [PubMed: 16459140]

9. Trauer JM, Qian MY, Doyle JS, Rajaratnam SMW, Cunnington D. Cognitive behavioral therapy for chronic insomnia: A systematic review and meta-analysis. *Ann Intern Med* 2015; 163: 191–204. [PubMed: 26054060]
10. Wu JQ, Appleman ER, Salazar RD, Ong JC. Cognitive behavioral therapy for insomnia comorbid with psychiatric and medical conditions a meta-analysis. *JAMA Intern Med* 2015; 175: 1461–1472. [PubMed: 26147487]
11. Sivertsen B, Vedaa Ø, Nordgreen T. The Future of Insomnia Treatment—the Challenge of Implementation. *Sleep* 2013; 36: 303–304. [PubMed: 23450899]
12. Kathol RG, Arnedt JT. Cognitive behavioral therapy for chronic insomnia: Confronting the challenges to implementation. *Ann Intern Med* 2016; 165: 149–150. [PubMed: 27136604]
13. Mitchell MD, Gehrman P, Perlis M, Umscheid CA. Comparative effectiveness of cognitive behavioral therapy for insomnia: A systematic review. *BMC Fam. Pract* 2012; 13. doi:10.1186/1471-2296-13-40.
14. Roth T. Insomnia: Definition, prevalence, etiology, and consequences. *J. Clin. Sleep Med* 2007; 3. doi:10.1378/chest.14-0970.
15. Toth LA, Bhargava P. Animal models of sleep disorders. *Comp Med* 2013; 63: 91–104. [PubMed: 23582416]
16. Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B et al. Reduced sleep in *Drosophila* Shaker mutants. *Nature* 2005; 434: 1087–1092. [PubMed: 15858564]
17. Koh K, Joiner WJ, Wu MN, Yue Z, Smith CJ, Sehgal A. Identification of SLEEPLESS, a Sleep-Promoting Factor. *Science* (80-) 2008; 321: 372–376.
18. Bushey D, Huber R, Tonomi G, Cirelli C. *Drosophila* Hyperkinetic mutants have reduced sleep and impaired memory. *J Neurosci* 2007; 27: 5384–5393. [PubMed: 17507560]
19. Stavropoulos N, Young MW. Article insomnia and Cullin-3 Regulate Sleep and Wakefulness in *Drosophila*. *Neuron* 2011; 72: 964–976. [PubMed: 22196332]
20. Kume K, Kume S, Park SK, Hirsh J, Jackson FR. Dopamine Is a Regulator of Arousal in the Fruit Fly. 2005; 25: 7377–7384.
21. Bushey D, Hughes K a, Tonomi G, Cirelli C. Sleep, aging, and lifespan in *Drosophila*. *BMC Neurosci* 2010; 11: 56. [PubMed: 20429945]
22. Zhang S, Yin Y, Lu H, Guo A. Increased dopaminergic signaling impairs aversive olfactory memory retention in *Drosophila*. *Biochem Biophys Res Commun* 2008; 370: 82–86. [PubMed: 18342622]
23. Seugnet L, Suzuki Y, Thimman M, Donlea J, Gimbel SI, Gottschalk L et al. Identifying Sleep Regulatory Genes Using a *Drosophila* Model of Insomnia. *J Neurosci* 2009; 29: 7148–7157. [PubMed: 19494137]
24. Robertson M, Keene AC. Molecular Mechanisms of Age-Related Sleep Loss in the Fruit Fly - A Mini-Review. *Gerontology* 2013; 59: 334–339. [PubMed: 23594925]
25. Hasan S, Dauvilliers Y, Mongrain V, Franken P, Tafti M. Age-related changes in sleep in inbred mice are genotype dependent. *Neurobiol Aging* 2012; 33. doi:10.1016/j.neurobiolaging.2010.05.010.
26. Mander BA, Winer JR, Walker MP. Sleep and Human Aging. *Neuron*. 2017; 94: 19–36. [PubMed: 28384471]
27. Kang J-EE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR et al. Amyloid-B Dynamics Are Regulated by Orexin and the Sleep-Wake Cycle. *Science* (80-) 2009; 326: 1005–1007.
28. Roh JH, Huang Y, Bero AW, Kasten T, Stewart FR, Bateman RJ et al. Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. *Sci Transl Med* 2012; 4: 150ra122.
29. Ju Y-ES, Lucey BP, Holtzman DM. Sleep and Alzheimer disease pathology—a bidirectional relationship. *Nat Rev Neurol* 2014; 10: 115–119. [PubMed: 24366271]
30. Tabuchi M, Lone SR, Liu S, Liu Q, Zhang J, Spira AP et al. Sleep interacts with $\alpha\beta$ to modulate intrinsic neuronal excitability. *Curr Biol* 2015; 25: 702–12. [PubMed: 25754641]

31. Iijima K, Liu H-P, Chiang A-S, Hearn S a., Konsolaki M, Zhong Y. Dissecting the pathological effects of human A β 40 and A β 42 in *Drosophila*: A potential model for Alzheimer's disease. *Proc Natl Acad Sci U S A* 2004; 101: 6623–6628. [PubMed: 15069204]
32. Dissel S, Klose M, Donlea J, Cao L, English D, Winsky-Sommerer R et al. Enhanced sleep reverses memory deficits and underlying pathology in *drosophila* models of Alzheimer's disease. *Neurobiol Sleep Circadian Rhythm* 2017; 2: 15–26.
33. Saarelainen L, Tolppanen A, Koponen M, Tanskanen A, Tiihonen J, Hartikainen S et al. Risk of death associated with new benzodiazepine use among persons with Alzheimer disease: A matched cohort study. *Int J Geriatr Psychiatry* 2018; 33: 583–590.
34. Krystal AD, Edinger JD. Sleep EEG predictors and correlates of the response to cognitive behavioral therapy for insomnia. *Sleep* 2010; 33: 669–77. [PubMed: 20469809]
35. Riemann D, Perlis ML. The treatments of chronic insomnia: A review of benzodiazepine receptor agonists and psychological and behavioral therapies. *Sleep Med. Rev* 2009; 13: 205–214. [PubMed: 19201632]
36. Ngandu T, Lehtisalo J, Solomon A, Levälähti E, Ahtiluoto S, Antikainen R et al. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): A randomised controlled trial. *Lancet* 2015; 385: 2255–2263. [PubMed: 25771249]
37. Kivipelto M, Solomon A, Ahtiluoto S, Ngandu T, Lehtisalo J, Antikainen R et al. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): Study design and progress. *Alzheimer's Dement* 2013; 9: 657–665. [PubMed: 23332672]
38. Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FAI et al. Intraneuronal A β , non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience* 2005; 132: 123–135. [PubMed: 15780472]
39. Gilestro GF. Video tracking and analysis of sleep in *Drosophila melanogaster*. *Nat Protoc* 2012; 7: 995–1007. [PubMed: 22538850]
40. Gilestro GF, Cirelli C. PySolo: A complete suite for sleep analysis in *Drosophila*. *Bioinformatics* 2009; 25: 1466–1467. [PubMed: 19369499]
41. Kayser MS, Yue Z, Sehgal A. A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*. *Science* (80-) 2014; 344: 269–274.
42. Dilley LC, Vigderman A, Williams CE, Kayser MS. Behavioral and genetic features of sleep ontogeny in *Drosophila*. *Sleep* 2018. doi:10.1093/sleep/zsy086.
43. Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A et al. Rest in *Drosophila* Is a Sleep-like State. *Neuron* 2000; 25: 129–138. [PubMed: 10707978]
44. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of Sleep and Waking in *Drosophila melanogaster*. 2000; 287.
45. Sateia MJ, Doghramji K, Hauri PJ, Morin CM. Evaluation of chronic insomnia. *An American Academy of Sleep Medicine review. Sleep* 2000; 23: 243–308. [PubMed: 10737342]
46. Konopka RJ, Benzer S. Clock mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci U S A* 1971; 68: 2112–6. [PubMed: 5002428]
47. Kyle SD, Aquino MRJ, Miller CB, Henry AL, Crawford MR, Espie CA et al. Towards standardisation and improved understanding of sleep restriction therapy for insomnia disorder: A systematic examination of CBT-I trial content. *Sleep Med. Rev* 2015; 23: 83–88. [PubMed: 25771293]
48. Majercak J, Sidote D, Hardin PE, Edery I. How a circadian clock adapts to seasonal decreases in temperature and day length. *Neuron* 1999; 24: 219–230. [PubMed: 10677039]
49. Shi M, Yue Z, Kuryatov A, Lindstrom JM, Sehgal A. Identification of Redeye, a new sleep-regulating protein whose expression is modulated by sleep amount. *Elife*. 2014; 3:e01473. [PubMed: 24497543]
50. Liu S, Lamaze A, Liu Q, Tabuchi M, Yang Y, Fowler M et al. WIDE AWAKE mediates the circadian timing of sleep onset. *Neuron* 2014; 82: 151–166. [PubMed: 24631345]
51. Edinger JD, Wohlgemuth WK, Radtke R a, Marsh GR, Quillian RE. Cognitive behavioral therapy for treatment of chronic primary insomnia: a randomized controlled trial. *JAMA* 2001; 285: 1856–64. [PubMed: 11308399]

52. Smith MT, Perlis ML, Park A, Smith MS, Pennington J, Giles DE et al. Comparative meta-analysis of pharmacotherapy and behavior therapy for persistent insomnia. *Am. J. Psychiatry* 2002; 159: 5–11. [PubMed: 11772681]
53. Edinger JD, Wohlgemuth WK, Radtke RA, Coffman CJ, Carney CE. Dose-response effects of cognitive-behavioral insomnia therapy: A randomized clinical trial. *Sleep* 2007; 30: 203–212. [PubMed: 17326546]
54. Qiu J, Hardin PE. per mRNA cycling is locked to lights-off under photoperiodic conditions that support circadian feedback loop function. *Mol Cell Biol* 1996; 16: 4182–8. [PubMed: 8754817]
55. Okamoto-Mizuno K, Mizuno K. Effects of thermal environment on sleep and circadian rhythm. *J. Physiol. Anthropol* 2012; 31: 1–9. [PubMed: 22738301]
56. Kaneko H, Head LM, Ling J, Tang X, Liu Y, Hardin PE et al. Circadian rhythm of temperature preference and its neural control in *Drosophila*. *Curr Biol* 2012; 22: 1851–1857. [PubMed: 22981774]
57. Luo W, Chen WF, Yue Z, Chen D, Sowcik M, Sehgal A et al. Old flies have a robust central oscillator but weaker behavioral rhythms that can be improved by genetic and environmental manipulations. *Aging Cell* 2012; 11: 428–438. [PubMed: 22268765]
58. Ishimoto H, Lark A, Kitamoto T. Factors that differentially affect daytime and nighttime sleep in *Drosophila melanogaster*. *Front Neurol* 2012; FEB: 1–5.
59. Parisky KM, Agosto Rivera JL, Donelson NC, Kotecha S, Griffith LC. Reorganization of Sleep by Temperature in *Drosophila* Requires Light, the Homeostat, and the Circadian Clock. *Curr Biol* 2016; 26: 882–892. [PubMed: 26972320]
60. Matthews EE, Arnedt JT, McCarthy MS, Cuddihy LJ, Aloia MS. Adherence to cognitive behavioral therapy for insomnia: A systematic review. *Sleep Med. Rev* 2013; 17: 453–464. [PubMed: 23602124]
61. Yoshii T, Hermann-Luibl C, Helfrich-Forster C. Circadian light-input pathways in *Drosophila*. *Commun. Integr. Biol* 2016; 9. doi:10.1080/19420889.2015.1102805.
62. Helfrich-Förster C, Winter C, Hofbauer A, Hall JC, Stanewsky R. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 2001; 30: 249–261. [PubMed: 11343659]
63. Emery P, So WV, Kaneko M, Hall JC, Rosbash M. Cry, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 1998; 95: 669–679. [PubMed: 9845369]
64. Emery P, Stanewsky R, Helfrich-Förster C, Emery-Le M, Hall JC, Rosbash M. *Drosophila* CRY is a deep brain circadian photoreceptor. *Neuron* 2000; 26: 493–504. [PubMed: 10839367]
65. Agrawal P, Houl JH, Gunawardhana KL, Liu T, Zhou J, Zoran MJ et al. *Drosophila* CRY Entrains Clocks in Body Tissues to Light and Maintains Passive Membrane Properties in a Non-clock Body Tissue Independent of Light. *Curr Biol* 2017; 27: 2431–2441.e3. [PubMed: 28781048]
66. Koh K, Evans JM, Hendricks JC, Sehgal A. A *Drosophila* model for age-associated changes in sleep:wake cycles. *Proc Natl Acad Sci U S A* 2006; 103: 13843–13847. [PubMed: 16938867]
67. Brown MK, Chan MT, Zimmerman JE, Pack AI, Jackson NE, Naidoo N. Aging induced endoplasmic reticulum stress alters sleep and sleep homeostasis. *Neurobiol Aging* 2014; 35: 1431–1441. [PubMed: 24444805]
68. Vienne J, Spann R, Guo F, Rosbash M. Age-Related Reduction of Recovery Sleep and Arousal Threshold in *Drosophila*. *Sleep* 2016; 39: 1613–1624. [PubMed: 27306274]
69. Pandi-Perumal SR, Seils LK, Kayumov L, Ralph MR, Lowe A, Moller H et al. Senescence, sleep, and circadian rhythms. *Ageing Res. Rev* 2002; 1: 559–604. [PubMed: 12067601]
70. Curtsinger JW. Late-life fecundity plateaus in *Drosophila melanogaster* can be explained by variation in reproductive life spans. *Exp Gerontol* 2013; 48: 1338–1342. [PubMed: 24012993]
71. Potdar S, Daniel DK, Thomas FA, Lall S, Sheeba V. Sleep deprivation negatively impacts reproductive output in *Drosophila melanogaster*. *J Exp Biol* 2018; 221: jeb174771. [PubMed: 29361608]
72. Finelli A, Kelkar A, Song HJ, Yang H, Konsolaki M. A model for studying Alzheimer's Aβ₄₂-induced toxicity in *Drosophila melanogaster*. *Mol Cell Neurosci* 2004; 26: 365–375. [PubMed: 15234342]

73. Nilsberth C, Westlind-danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C et al. The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced A β protofibril formation. *Nat Neurosci.* 2001; 4:18–23.
74. Perlis ML, Shaw PJ, Cano G, Espie CA. Models of insomnia In: Kryger M, Roth T, Dement W (ed). *Principles and Practice of Sleep Medicine*, 5th Edition. Saunders, 2011, pp 850–66.
75. Gehrman PR, Pfeiffenberger C, Byrne EM. The role of genes in the insomnia phenotype. *Sleep Med. Clin* 2013; 8: 323–331. [PubMed: 24072990]
76. Lind MJ, Gehrman PR. Genetic pathways to insomnia. *Brain Sci.* 2016; 6. doi:10.3390/brainsci6040064.
77. Veatch OJ, Keenan BT, Gehrman PR, Malow BA, Pack AI. Pleiotropic genetic effects influencing sleep and neurological disorders. *Lancet Neurol.* 2017; 16: 158–170. [PubMed: 28102151]
78. Dubowy C, Sehgal A. Circadian rhythms and sleep in *Drosophila melanogaster*. *Genetics* 2017; 205: 1373–1397. [PubMed: 28360128]
79. Harbison ST, McCoy LJ, Mackay TFC. Genome-wide association study of sleep in *Drosophila melanogaster*. *BMC Genomics* 2013; 14. doi:10.1186/1471-2164-14-281.
80. Bathgate CJ, Edinger JD, Krystal AD. Insomnia patients with objective short sleep duration have a blunted response to cognitive behavioral therapy for Insomnia. *Sleep* 2017; 40. doi:10.1093/sleep/zsw012.
81. Taub JM, Berger RJ. Performance and Mood Following Variations in the Length and Timing of Sleep. *Psychophysiology* 1973; 10: 559–570. [PubMed: 4750081]
82. Reynold A, Emily B, Saxena A, Rayad R, Youngstedt S. Negative Effects of Time in Bed Extension : A Pilot Study. *J sleep Med Disord* 2014; 1: 1–13.
83. Spielman A, Saskin P, Thorpy MJ. Treatment of Chronic Insomnia by Restriction of Time in Bed. *Sleep* 1987; 10: 45–56. [PubMed: 3563247]
84. Perlis ML, Corbitt CB, Kloss JD. Insomnia research: 3Ps and beyond. *Sleep Med Rev* 2014; 18: 191–193. [PubMed: 24685396]
85. Cassidy-Eagle E, Siebern A, Unti L, Glassman J, Hara OR, Veterans F et al. Cognitive Behavioral Treatment for Insomnia in Older Adults with Mild Cognitive Impairment in Independent Living Facilities : A Pilot Study. *J Sleep Disord Med Care* 2018; 1. doi:10.16966/jsdmc.105.
86. Insel TR. Next-generation treatments for mental disorders. *Sci Transl Med* 2012; 4: 155ps19.
87. Hofmann SG, Asnaani A, Vonk IJJ, Sawyer AT, Fang A. The efficacy of cognitive behavioral therapy: a review of meta-analyses. *Cogn Ther Res* 2012; 36: 427–440.

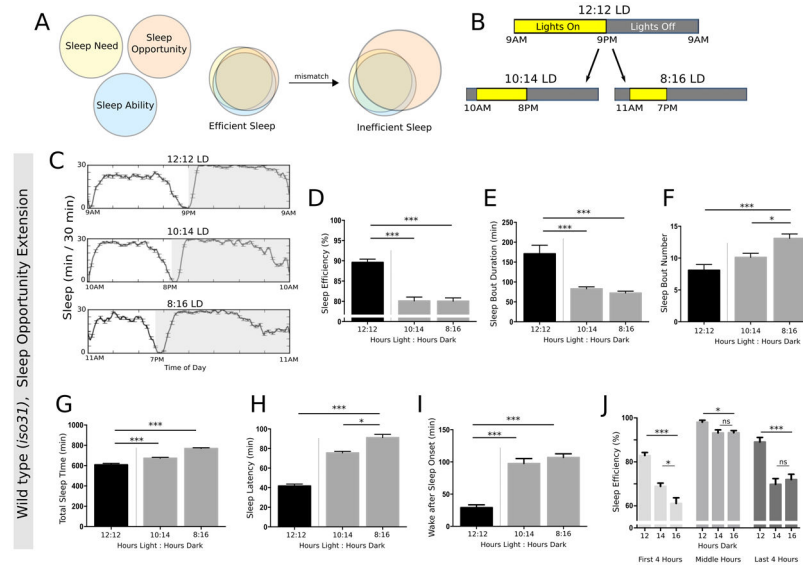


Figure 1. Sleep opportunity extension impairs sleep in *Drosophila*.

(A) Schematic of sleep degradation with mismatch of sleep opportunity and sleep ability. (B) Diagram of experimental extension of dark time from 12 hours (12:12 LD) to 14 hours (10:14 LD) or 16 hours (8:16 LD). (C) Representative sleep traces of wild type *iso³¹* 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 3 nights of sleep opportunity extension in wild type *iso³¹* 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.

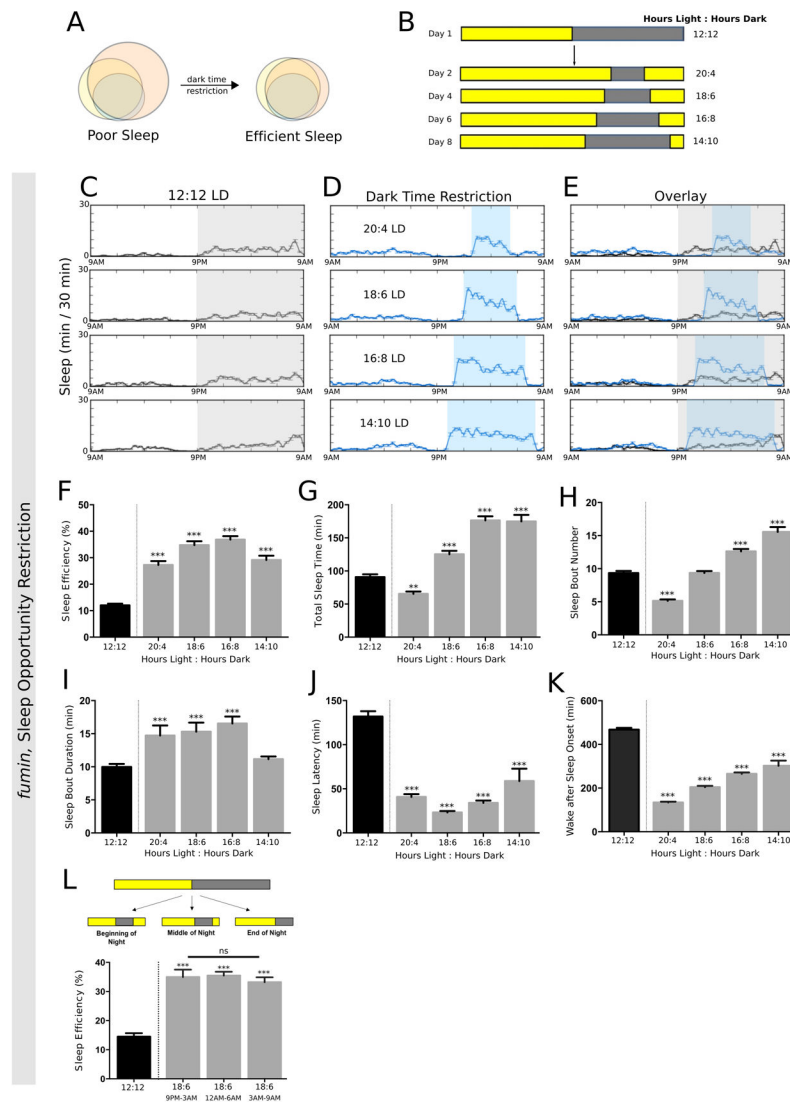


Figure 2. Sleep opportunity restriction enhances sleep in *fumin* mutants. (A) Schematic of hypothesis that sleep opportunity restriction aligns sleep opportunity and sleep ability, leading to efficient sleep. (B) Diagram of experimental protocol for restriction of sleep opportunity by manipulating the 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 9p-3a, n = 172 for 12a-6a, n = 105 for 3a-9a).

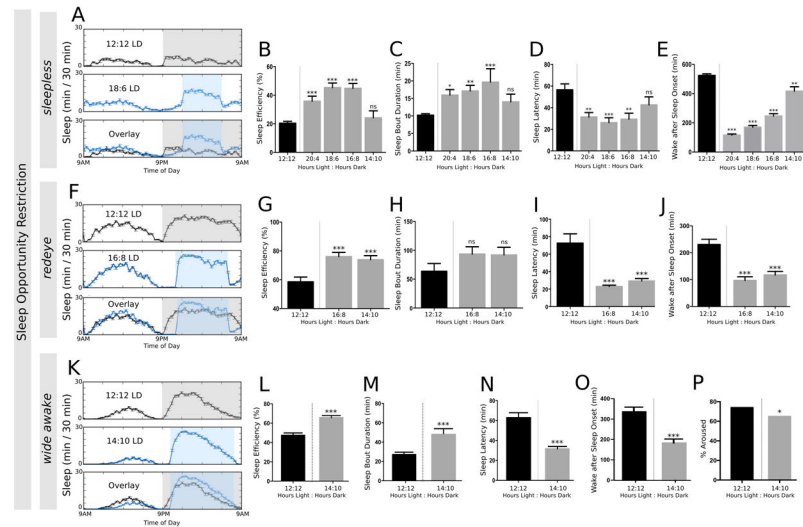


Figure 3. Sleep opportunity restriction improves sleep in multiple short-sleeping mutants. Representative sleep traces under 12:12 LD conditions (top panel, gray shading indicates dark phase), compressed sleep opportunity (middle panel, blue shading indicates dark phase) and overlaid plots (bottom panel) 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).

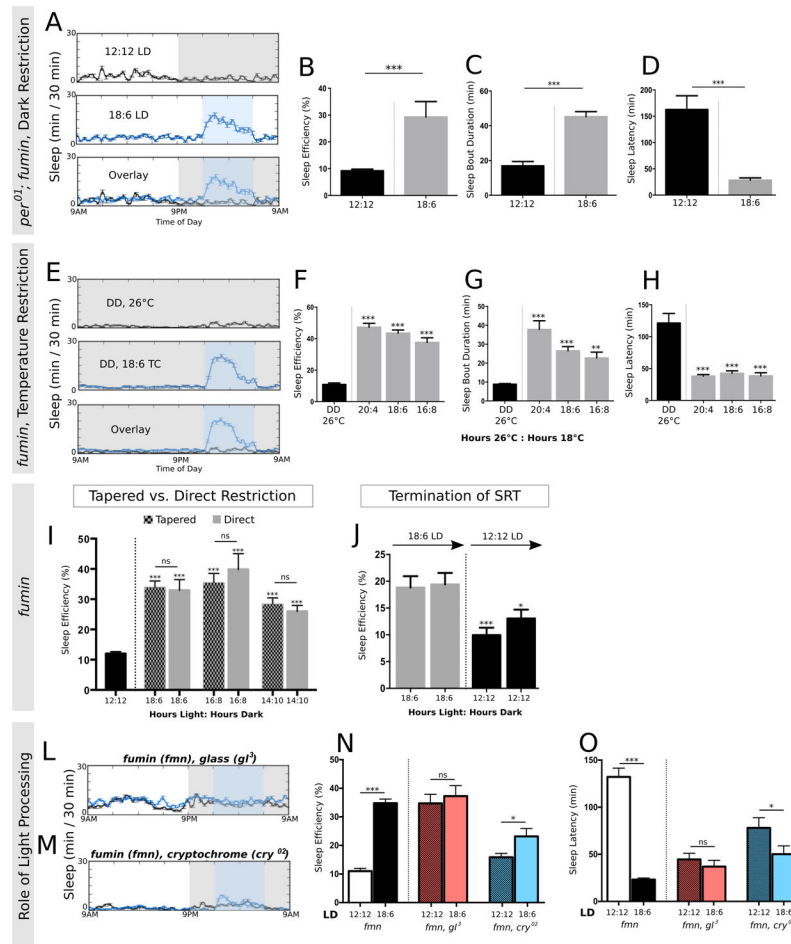


Figure 4. Response to sleep restriction requires ongoing environmental cues. (A) Representative sleep traces under 12:12 LD conditions (top panel, gray shading indicates dark phase), 18:6 LD dark time restriction (middle panel, blue shading indicates dark phase) and overlaid plots (bottom panel) for *per⁰¹; fumin* mutants. Quantification of sleep efficiency (B), bout duration (C), and sleep latency (D) in *per⁰¹; 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;glass³* (L) and *fumin;cry⁰²* (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;glass³*, n = 54 for *fumin;cry⁰²*).

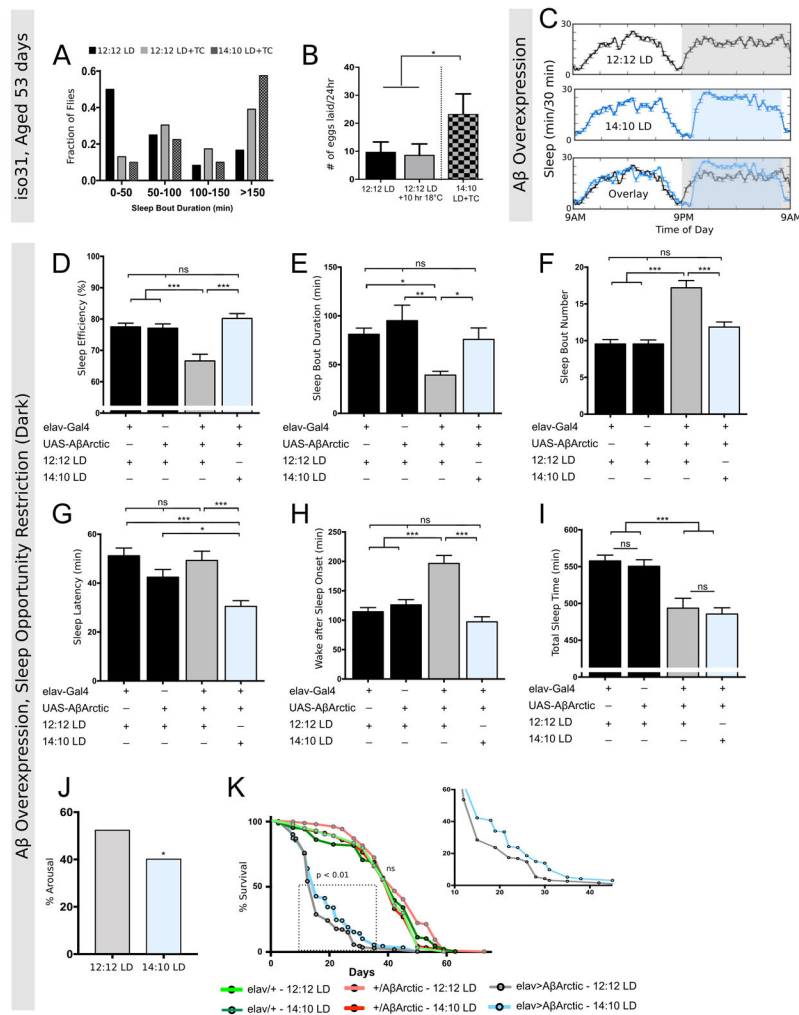


Figure 5. Sleep opportunity restriction improves sleep degradation associated with aging and A β accumulation

(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 hours 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 A β Arctic 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-A β Arctic*⁺ ($n = 53$), and *elav-Gal4/UAS-A β Arctic* flies under 12:12 LD ($n = 59$) or 14:10 LD conditions ($n = 60$). (J) Arousal threshold of *elav-Gal4/UAS-A β Arctic* 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 A β Arctic 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-A β Arctic*⁺: 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.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript