




Publicly Accessible Penn Dissertations

2019

A Drosophila Model Of Sleep Restriction Therapy For Insomnia And Neurodegenerative Disease

Samuel Belfer
University of Pennsylvania

Follow this and additional works at: <https://repository.upenn.edu/edissertations>

 Part of the [Neuroscience and Neurobiology Commons](#)

Recommended Citation

Belfer, Samuel, "A Drosophila Model Of Sleep Restriction Therapy For Insomnia And Neurodegenerative Disease" (2019). *Publicly Accessible Penn Dissertations*. 3583.
<https://repository.upenn.edu/edissertations/3583>

This paper is posted at ScholarlyCommons. <https://repository.upenn.edu/edissertations/3583>
For more information, please contact repository@pobox.upenn.edu.

A Drosophila Model Of Sleep Restriction Therapy For Insomnia And Neurodegenerative Disease

Abstract

Insomnia is the most common sleep disorder among adults, especially affecting individuals of advanced age or with neurodegenerative disease. Humans with insomnia often expand the amount of time they spend in bed in an attempt to compensate for inability to sleep. However, this mismatch of time in bed (high) with sleep ability (low) perpetuates insomnia symptoms. Cognitive Behavioral Therapy for Insomnia (CBT-I) is the first-line insomnia treatment. Sleep restriction – a key component of CBT-I – addresses mismatch between sleep opportunity and ability by restricting time in bed to an amount equal to average sleep ability, leading to enhanced sleep drive and consolidation. Though effective, limited accessibility of practitioners and long duration of therapy are barriers to broad implementation of CBT-I. Deciphering a molecular basis for this behavioral therapy has potential to open new treatment avenues. In Chapter 1, I discuss the utility of modeling insomnia, behavioral therapy, and neurodegenerative disease in *Drosophila*. In Chapter 2, we develop a *Drosophila* model for sleep restriction therapy (SRT). We find that restriction of sleep opportunity through manipulation of environmental cues improves sleep efficiency and continuity in multiple short-sleeping *Drosophila* mutants. We apply SRT to a *Drosophila* model of Alzheimer's disease, in which A β accumulation causes decreased and fragmented sleep, and demonstrated that sleep restriction reverses these sleep deficits, with associated extension in lifespan. In Chapter 3, we expand our search for fly models of human neurodegenerative disease associated with short-sleeping phenotypes. We find that overexpression of human TDP-43, the protein deposited in intracellular inclusions in ALS and FTD, cause profound sleep disturbances that can be rescued by SRT. TDP-43 flies also exhibit increased arousal threshold and extended longevity with SRT, suggesting deeper sleep with sleep opportunity restriction confers health benefits. In Chapter 4, I discuss ongoing work investigating the intracellular localization of TDP-43, and how improved sleep might mediate toxicity of this protein. Finally, I discuss use of this model to identify molecular signals mediating the response to sleep restriction therapy. These findings have important implications for our understanding of behavioral sleep therapy and its potential as a therapeutic intervention for neurodegenerative disease.

Degree Type

Dissertation

Degree Name

Doctor of Philosophy (PhD)

Graduate Group

Neuroscience

First Advisor

Matthew S. Kayser

Keywords

Cognitive Behavioral Therapy for Insomnia, *Drosophila*, Insomnia, Neurodegeneration, Sleep Restriction

Subject Categories

Neuroscience and Neurobiology

A *DROSOPHILA* MODEL OF SLEEP RESTRICTION THERAPY FOR INSOMNIA AND
NEURODEGENERATIVE DISEASE

Samuel J. Belfer

A DISSERTATION

in

Neuroscience

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2019

Supervisor of Dissertation

Matthew S. Kayser, MD, PhD
Assistant Professor of Psychiatry

Graduate Group Chairperson

Joshua Gold, PhD
Professor of Neuroscience

Dissertation Committee

David M. Raizen, MD, PhD – Associate Professor of Neurology

Philip Gehrman, PhD, CBSM – Assistant Professor of Psychiatry

F. Bradley Johnson, MD, PhD – Professor of Pathology and Laboratory Medicine

Kyunghee Koh, PhD – Associate Professor of Neuroscience, Jefferson University

Dedication

This dissertation is dedicated to my Zayde, Sheldon Belfer, who would have loved nothing more than reading it.

He would have done so with a pen in hand to make sure he could write notes in the margins, and clipped articles from the New York Post and Daily News for months about anything that reminded him of the topics discussed herein.

זיכרונו לברכה



Acknowledgments

Completion of this work would not have been possible without the endless dedication of my advisor, Dr. Matthew Kayser. You have an unbelievable way of fostering excitement when the science gets interesting, and motivating through difficulty when it seems like everything is going sideways. I am indebted to you for your passion and patience, and reminding me to laugh all the while. Your lab is truly an extension of your family, and we all feel that closeness and collective drive to succeed. Thank you for making me a better scientist and giving me an amazing role model of what great mentorship looks like.

To the members of the Kayser Lab – Dave, Milán, Emilia, Christine, Jackie, Amy, Leela, Natalie, Ben, Charlette, Zander, Austin, Anyara, Lucy, and Jackie – thanks for making these PhD years so much fun. I always look forward to coming to lab in the morning, not being able to imagine what we might talk about.

Collaborating and debating ideas has challenged me at every turn. Thank you.

I would like to thank the members of my thesis committee, David Raizen, Phil Gehrman, Brad Johnson, and Kyunghee Koh for their guidance and support throughout this journey. In particular, I thank David for acting as an invaluable mentor since I joined his lab as an undergraduate in 2010. Thank you for giving me my scientific start, and inspiring in me an undying love for sleep and model organisms.

To my parents, Mindy and Rich – the foundation of hard work and the value of education that you raised us with has somehow, 29 years later, gotten me to this day. Thank you for always fostering my love for science, encouraging me to experience science in camps and specialized high schools, and by always being willing to turn our house into a greenhouse for tomato plant grafting experiments if that's what was necessary. Thank you for always being there to listen, and for always reminding me of what is possible.

To Allie – as the first doctor in the family, I expect you know most of what's written here already.

To Jeremy – if you are still reading this far into the dissertation, consider me impressed. Thank you both for the laughs and the finger-letter N's to get me to this point.

To Rachel – even when you met me as a 23-year-old medical student, you (of course) were able to see this day clearly. Thank you for always being the patient one, the wise one, but not the numerate one, and keeping me grounded and focused on what was next. Your attention to every detail of our lives has enabled me to work the long days and long nights I needed to get this all done. You know just how to motivate me to be better, and everything I have accomplished in the last 5 years is a direct result of knowing you were always by my side. Now onto the next step of our journey together. I love you.

To Izzy – You are the best gift that these PhD years could have ever given me. I swear that I'll be around for you/ I'll do whatever it takes/ I'll make a million mistakes/ I'll make the world safe and sound for you. This is all for you, sweetheart.



Helping Abba finish his dissertation.

ABSTRACT

A *DROSOPHILA* MODEL OF SLEEP RESTRICTION THERAPY FOR INSOMNIA AND NEURODEGENERATIVE DISEASE

Samuel J. Belfer

Matthew S. Kayser

Insomnia is the most common sleep disorder among adults, especially affecting individuals of advanced age or with neurodegenerative disease. Humans with insomnia often expand the amount of time they spend in bed in an attempt to compensate for inability to sleep. However, this mismatch of time in bed (high) with sleep ability (low) perpetuates insomnia symptoms. Cognitive Behavioral Therapy for Insomnia (CBT-I) is the first-line insomnia treatment. Sleep restriction – a key component of CBT-I – addresses mismatch between sleep opportunity and ability by restricting time in bed to an amount equal to average sleep ability, leading to enhanced sleep drive and consolidation. Though effective, limited accessibility of practitioners and long duration of therapy are barriers to broad implementation of CBT-I. Deciphering a molecular basis for this behavioral therapy has potential to open new treatment avenues. In Chapter 1, I discuss the utility of modeling insomnia, behavioral therapy, and neurodegenerative disease in *Drosophila*. In Chapter 2, we develop a *Drosophila* model for sleep restriction therapy (SRT). We find that restriction of sleep opportunity through manipulation of environmental cues improves sleep efficiency and continuity in multiple short-sleeping *Drosophila* mutants. We apply SRT to a *Drosophila* model of Alzheimer's disease, in which A β accumulation causes decreased and fragmented sleep, and demonstrated that sleep restriction reverses these sleep deficits, with associated extension in lifespan. In Chapter 3, we expand our search for fly models of human neurodegenerative disease

associated with short-sleeping phenotypes. We find that overexpression of human TDP-43, the protein deposited in intracellular inclusions in ALS and FTD, cause profound sleep disturbances that can be rescued by SRT. TDP-43 flies also exhibit increased arousal threshold and extended longevity with SRT, suggesting deeper sleep with sleep opportunity restriction confers health benefits. In Chapter 4, I discuss ongoing work investigating the intracellular localization of TDP-43, and how improved sleep might mediate toxicity of this protein. Finally, I discuss use of this model to identify molecular signals mediating the response to sleep restriction therapy. These findings have important implications for our understanding of behavioral sleep therapy and its potential as a therapeutic intervention for neurodegenerative disease.

TABLE OF CONTENTS

Acknowledgments	iii
ABSTRACT	v
List of Tables	ix
List of Figures	x
Chapter 1: Insomnia and Behavioral Therapy in Animal Models	1
Introduction	2
Insomnia – Epidemiology, Causes, and Consequences	5
The Treatment of Insomnia	10
Animal Models of Insomnia	14
Sleep, Aging, and Neurodegenerative Disease	19
<i>Drosophila</i> Models of Aging and Neurodegenerative Disease	25
Concluding Remarks	29
References	30
Chapter 2:	
A <i>Drosophila</i> Model of Sleep Restriction Therapy for Insomnia	49
Abstract	50
Introduction	51
Methods	54
Results	58
Sleep opportunity extension impairs sleep in <i>Drosophila</i>	58
Sleep opportunity restriction enhances sleep in a short-sleeping mutant	60
Nocturnal sleep opportunity restriction improves daytime sleep	62
Sleep opportunity restriction is effective in multiple short-sleep mutants	63
Response to sleep restriction requires ongoing environmental cues	64
Sleep restriction improves sleep in aging and Alzheimer's Disease models	67
Discussion	71
Figures	76

Figure 1. Sleep opportunity extension impairs sleep in <i>Drosophila</i> .	76
Figure 2. Sleep opportunity restriction enhances sleep in <i>fumin</i> mutants.	78
Figure 3. Sleep opportunity restriction improves sleep in multiple short-sleeping mutants.	79
Figure 4. Response to sleep restriction requires ongoing environmental cues.	80
Figure 5. Sleep opportunity restriction improves sleep degradation associated with aging and A β accumulation.	83
Supplementary Figures.	84
References	92
Chapter 3:	
A <i>Drosophila</i> Model of Sleep Restriction Therapy for Neurodegenerative Disease	99
Abstract	100
Introduction	101
Methods	103
Results	107
TDP-43 overexpression impairs sleep in <i>Drosophila</i> .	107
Ataxin 2, a known modifier of TDP-43 toxicity, modifies sleep phenotype.	109
Sleep screen for genetic modifiers of TDP-43 toxicity reveals novel hits.	110
m6a nuclear reader YT5-21B is a novel suppressor of TDP-43 toxicity.	111
Sleep Restriction Therapy improves sleep in <i>Drosophila</i> model of ALS/FTD.	113
Discussion	115
Figures	119
Figure 1. TDP-43 overexpression causes sleep deterioration.	120
Figure 2. Ataxin 2 knockdown modifies sleep phenotype with TDP-43 overexpression.	121
Figure 3. TDP-43 modifier screen identifies m6a nuclear reader YT5-21B as a suppressor of toxicity.	122
Figure 4. Sleep Restriction Therapy improves sleep and longevity in TDP-43 overexpression model of ALS.	124
Supplementary Figures.	125
References	133
Chapter 4: Conclusions and Future Directions	137
Modeling Behavioral Sleep Therapy in <i>Drosophila</i>	139
The Purpose of Sleep	150
Behavioral Therapies in Animal Models	153
Sleep and Neurodegeneration	157
References	163

List of Tables

Chapter 3:

Supplementary Table 1: Table of TRiP RNAi Lines Screened for Modifiers of
TDP-43 Toxicity.....132

List of Figures

Chapter 1:

Figure 1.1: The 3P Behavioral Model of Insomnia	9
---	---

Chapter 2:

Figure 1: Sleep opportunity extension impairs sleep in <i>Drosophila</i>	78
Figure 2: Sleep opportunity restriction enhances sleep in <i>fumin</i> mutants.....	79
Figure 3: Sleep opportunity restriction improves sleep in multiple short sleeping mutants	81
Figure 4: Response to sleep restriction requires ongoing environmental cues.....	82
Figure 5: Sleep opportunity restriction improves sleep degradation associated with A β accumulation.....	84
Supplementary Figure 1: Sleep opportunity extension in wild type flies and <i>period</i> mutants.....	86
Supplementary Figure 2: Binned time-of-day analysis of sleep opportunity extension in wild type flies.....	87
Supplementary Figure 3: Response to sleep opportunity restriction in <i>fumin</i> and wild type flies	88
Supplementary Figure 4: Daytime changes to sleep with nocturnal sleep opportunity restriction.....	89
Supplementary Figure 5: Response to sleep opportunity restriction in short-sleeping mutants.....	90
Supplementary Figure 6: Sleep opportunity restriction with alternative sleep-	

promoting paradigms and temporal dynamics of sleep response.....	91
Supplementary Figure 7: Sleep opportunity restriction in aged flies and A β females	93
Chapter 3:	
Figure 1: TDP-43 overexpression causes sleep deterioration.....	121
Figure 2: Ataxin 2 knockdown modifies sleep phenotype with TDP-43 overexpression.....	123
Figure 3: TDP-43 modifier screen identifies m6a nuclear reader YT5-21B as a suppressor of toxicity.....	124
Figure 4: Sleep Restriction Therapy improves sleep and longevity in TDP-43 overexpression model of ALS/FTD.....	126
Supplementary Figure 1: TDP-43 overexpression models of ALS/FTD cause sleep deficits.....	127
Supplementary Figure 2: Ataxin 2 knockdown improves sleep only in TDP-43 overexpression.....	128
Supplementary Figure 3: Results of modifier screen.....	129
Supplementary Figure 4: SRT improves sleep in TDP-43 overexpression model of ALS/FTD.....	131
Chapter 4:	
Figure 4.1: Adapted <i>Drosophila</i> 3P Behavioral Model	146

Chapter 1: Insomnia and Behavioral Therapy in Animal Models

Samuel J. Belfer^{1,2} and Matthew S. Kayser^{1,2,3,4}

¹Department of Neuroscience,

²Department of Psychiatry,

³Center for Sleep and Circadian Neurobiology, and

⁴Chronobiology Program,

Perelman School of Medicine at the University of Pennsylvania

Introduction

יוצאין ... ובשן של שועל דעבדי לשינתא דחייא למאן דניים דמיתא למאן דלא ניים

“One may go out on the Sabbath ... with a fox tooth, as they use it as a talisman for sleep; the tooth of a live fox for one who sleeps too much to wake him up, and the tooth of a dead fox for one who does not sleep”

- Babylonian Talmud, Tractate Shabbat 67a

While modern medicine has not fully embraced fox teeth as a therapeutic strategy for sleep disorders, the search for effective approaches to treat disturbances in sleep is at least as old as the codification of the Mishnah in 200 CE. In modern times, the need for therapy is at an all-time high: more than 1 in 3 adults in the United States has insufficient sleep according to CDC estimates. This problem continues to grow in teenagers, as 68% of high school students report not getting adequate sleep (Wheaton *et al.*, 2016). With the strong association between poor sleep and chronic disease, it is becoming clear that treating disordered sleep should be a priority in all disciplines of medicine. Furthermore, changes in sleep represent an important characteristic of the normal aging process. Aging is associated with a degradation of sleep quality, which includes decreased sleep efficiency, sleep fragmentation, and early morning awakening. Sufficient sleep is necessary to support a large number of brain functions, and disruption of sleep negatively impacts vigilance, cognition and learning (Roth, 2007). Sleep is

hypothesized to affect these functions by serving as a regulator of neural clearance of cellular products and waste, while loss of sleep leads to increased production of these compounds (Kang *et al.*, 2009; Musiek and Holtzman, 2016). Protein accumulation can serve to worsen sleep, resulting in a vicious cycle of poor sleep and pathogenic aggregation. It is therefore no surprise that sleep quality is worsened by the onset of dementia. Although dementia is typically characterized by progressive memory loss and deterioration of other neurocognitive functions, sleep disturbance represents a significant toll for both patients and caregivers. In fact, up to 45% of Alzheimer's disease patients report sleep disturbances in epidemiological studies (Moran *et al.*, 2005), while sleep degradation represents a significant risk factor for institutionalization (Peter-Derex *et al.*, 2015). Interestingly, degraded sleep can occur up to 10 years before the clinical onset of cognitive decline (Sterniczuk *et al.*, 2013), consistent with the finding that brain regions involved in sleep are affected early in the pathogenesis of disease (Y. E. S. Ju, Lucey and Holtzman, 2014).

While many patients turn to hypnotics to treat sleep deficits, use of these drugs is associated with increased mortality in the elderly, a population especially at risk for cognitive and behavioral decline (Kripke, Langer and Kline, 2012). Fortunately, behavioral therapies to treat sleep disorders present safe and effective treatment options for patients. Yet, with the high demand for practitioners outpacing their availability, behavioral therapies are not available to all patients. Moreover, the long duration of treatment can disincentivize patients who *can* access behavioral therapy from pursuing it. Clearly, mechanistic insight into how behavioral therapies work is necessary to devise new strategies to treat sleep. Understanding behavioral therapy on a cellular and molecular level would create novel approaches that target specific disease pathways to revolutionize treatment strategies.

Here, I discuss the epidemiology and deleterious sequelae associated with untreated sleep deterioration, as well as the first-line treatment for insomnia in humans, Cognitive Behavioral Therapy for Insomnia (CBT-I). CBT-I includes a combination of modalities: Behavioral Therapy (sleep restriction and stimulus control), Cognitive Therapy (e.g., cognitive restructuring of dysfunctional beliefs about sleep), and sleep hygiene. I consider the components of this behavioral therapy, and examine the physiologic rationale for each part of CBT-I. The model organism *Drosophila melanogaster* has become one of the most powerful systems for studying how brain circuits give rise to complex behaviors (Bellen, Tong and Tsuda, 2010). The discovery that *Drosophila* sleep (Hendricks *et al.*, 2000; Shaw *et al.*, 2000) has opened the door to using this tractable genetic system to explore the regulation of sleep, leading to novel insights that scale to mammalian systems. I discuss the utility of model systems in the mechanistic study of sleep and insomnia, and how the study of sleep across phylogeny has sought to further understand the underlying function of sleep. In addition to short sleeping *Drosophila* mutants being among the best animal models of chronic insomnia, models of neurodegenerative disease have been established as well. Transgenic lines can incorporate pathogenic mutations found in humans afflicted with neurodegenerative disease, recapitulating cellular and molecular changes that lead to cognitive decline. Moreover, I discuss *Drosophila* as the ideal model system to lay the groundwork for this pursuit; well-validated insomnia, aging, and neurodegenerative disease models that can be studied in a reasonable time course to allow for mechanistic insight. I detail how tenets of CBT-I can be adapted for experimental feasibility in *Drosophila*, and how findings in this system can be extrapolated to human patients. In this way, behavioral therapy can be modeled in the fly to identify and elucidate novel therapeutic treatment strategies. A mechanistic understanding of therapies for sleep disturbances opens new

doors for treatment of aging-related sleep decline, neurodegenerative disease, or other maladies that feature significant sleep disturbances.

Insomnia – Epidemiology, Causes, and Consequences

Insomnia is characterized by difficulty initiating or maintaining sleep, despite adequate opportunity (Roth, 2007). Insomnia can take many clinical forms: patients who report inability to fall asleep, frequent night time awakenings, or persistent early awakenings all meet diagnostic criteria. There have been some efforts to distinguish the clinical features of sleep onset insomnia, sleep maintenance insomnia, and early morning awakening insomnia but studies have demonstrated the limited durability of these specific subtypes (Hohagen *et al.*, 1994). Moreover, chronic nonrestorative or poor quality sleep is also classified as insomnia, provided it is not as a response to poor conditions for sleep. Importantly, patients must also experience daytime impairments that include but are not limited to: fatigue, attention or memory impairment, mood instability, daytime sleepiness, negative feelings about sleep, or physiological symptoms related to poor sleep (Thorpy, 2017). Clinical insomnia specifies ample opportunity and conditions for sleep, as to distinguish from sleep deprivation due to inadequate time in bed. There is no specific criteria for sleep amount, as genetic and environmental factors cause variability in baseline sleep amount necessary for each individual. Therefore, differences in sleep amount and quality from each patient's baseline sleep characteristics (which often cannot be measured quantitatively until insomnia resolves) are used to track progression of the disorder. Other than total sleep amount, sleep efficiency (total sleep time divided by time in bed), time to fall asleep (sleep latency), and total wake time after sleep onset are also followed to determine severity of disease.

Insomnia has historically been classified as primary or secondary insomnia, based on whether another medical or psychiatric condition was identified as the cause of insomnia. However, insomnia is now conceptualized as an independent risk factor for numerous psychiatric and medical conditions, making it difficult to establish causality. DSM-V has therefore eliminated this distinction, uniting all forms of insomnia under the umbrella diagnostic category of “insomnia disorder” (American Psychiatric Association, 2013).

Insomnia symptoms are quite prevalent in the population, with greater than 50% of adults experiencing acute symptoms each year (Riemann *et al.*, 2019). It is estimated that 40-70% of individuals who experience acute insomnia symptoms progress to chronic insomnia, with the remaining cases self-resolving, likely with the removal of an acute stressor (Morin, Bélanger, *et al.*, 2009). Chronic insomnia indicates that symptoms of insomnia have recurred at least three times per week over a period of three months. Which cases of insomnia will progress to chronic insomnia and which will be self-delimited has been the focus of much scientific work to date. One study identified higher baseline anxiety and depressive symptoms, and negative overall health as independent risk factors for progression to chronic insomnia (LeBlanc *et al.*, 2009). Another study identified dysfunctional beliefs about sleep as a critical factor in identifying which patients will progress to a chronic insomnia phase (Yang, Lin and Cheng, 2013). However, a neurobiological understanding of who may be at higher risk for prolonged sleep degradation has been elusive.

One helpful model in framing the risk of progression to chronic disease is the 3P behavioral model (Spielman *et al.* 1987). This model supposes that three factors can describe how acute insomnia occurs and how actions taken in response to acute insomnia can cause prolonging of symptoms into a chronic phase. The first of the 3P's

described in this model is predisposing factors, and they include biological, environmental, and social variables that affect sleep. Genetic factors that contribute to insomnia have been well-studied in human cohorts (Lind and Gehrman, 2016). Chief among these have been polymorphisms in genes that encode components of neurotransmitter systems. A 'short' allele in a serotonin transporter gene-linked polymorphic region predicted increased sleep latency in a population of caregivers whose jobs included frequent night time awakenings (Brummett *et al.*, 2007). Another study implicated this same locus as predisposing to insomnia in shift workers (Pallesen *et al.*, 2019). However, other studies found no direct correlation between allelic status at the serotonin transporter locus and risk of insomnia, observing an interaction only between allele and stress level on subjective sleep quality (van Dalen and Markus, 2019). Genome-wide association studies have been essential in the discovery of novel genes that have never been previously associated with insomnia, as well as validation of loci discovered by other means. A recent analysis in 1.3 million individuals identified numerous novel genes, including one associated with type 2 diabetes and autism spectrum disorder (diseases with prominent sleep deficits) that has never before been associated with insomnia (Jansen *et al.*, 2019). Causal genetic interactions between loci associated with psychiatric disorders and insomnia have also been explored (Gao *et al.*, 2019). While biological explanations receive much focus to explain the prevalence of chronic insomnia, research into predisposing social determinants of health have lagged. Recent work has pointed to an association between perceived neighborhood safety and sleep duration (Ruff *et al.*, 2018), while another study has shown high rates of community unemployment predisposes to insomnia (Riedel *et al.* 2012).

The second of the 3P's describes precipitating factors, acute triggers that initially disturb sleep, often categorized as the causes of the initial insomnia symptoms. These

can include a new medical or psychiatric illness, stressful life event, the physiological changes of pregnancy, or other factors that directly affect sleep. While precipitating factors are often identified as the cause of acute bouts of insomnia symptoms, the stress and arousal associated with these events is reduced over time. In this manner, precipitating events are usually not the sole cause of chronic cases of insomnia. Finally, perpetuating factors refer to cognitive and behavioral adaptations to the poor sleep that the person suffering from insomnia undertakes to compensate for the lack of sleep caused by precipitating factors. These include incorporating non-sleep activities into sleep routines, frequent daytime napping, and spending longer durations in bed to compensate for poor sleep. While self-reinforcing in the short term, perpetuating factors can predict the progression to chronic insomnia

(Perlis et al. 2014). The 3P model posits that there is a threshold above which insomnia symptoms occur (Figure 1.1). When predisposing factors and precipitating stressors combine to overcome this threshold, acute insomnia occurs. As individuals with insomnia adapt to the acute stressor (precipitating factor), or it resolves, sub-threshold levels are reached and insomnia will not progress to a chronic phase. However, if

perpetuating behaviors are adopted, these can combine with predisposing and precipitating factors and enable insomnia symptoms to occur over a longer term. This combination of these three factors therefore models who is at the most risk for development of chronic insomnia, as well as identifies behaviors and ideation that can be targeted for therapy.

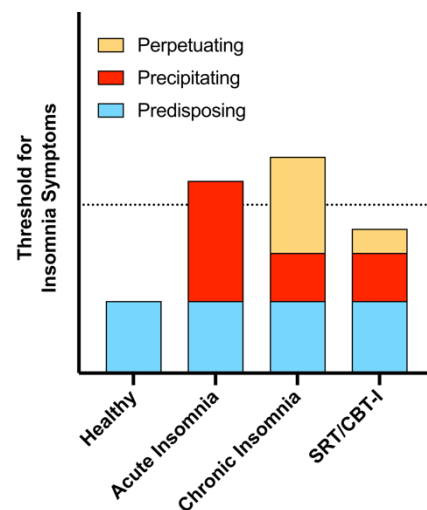


Figure 1.1 - The 3P Behavioral Model of Insomnia. Adapted from Perlis et al. 2014. Perpetuating factors remain fairly constant over time. Precipitating factors increase with onset of acute stressor, but are diminished over time. Perpetuating factors cause progression from acute insomnia to chronic disturbances. SRT and CBT-I affect perpetuating factors by diminishing maladaptive behaviors causing chronic insomnia.

One instance of the 3P model guiding therapeutic strategies is sleep restriction therapy (SRT). SRT seeks to treat chronic insomnia by addressing the perpetuating factor of sleep time extension, or spending too much time in bed. Patients with insomnia often expand time in bed in hopes of recovering lost sleep (Spielman, 1986). This adaptation perpetuates symptoms by encouraging fragmentation of sleep times, as well as encouraging non-sleep behaviors while in bed. By restricting time in bed, SRT leads to enhanced sleep drive and consolidation by EEG (Krystal and Edinger, 2010). Once efficient sleep is established (sleep efficiency > 90%), sleep opportunity is carefully titrated as sleep ability stabilizes and increases. The 3P model predicts that the elimination of perpetuating symptoms should resolve chronic insomnia, by bringing pro-insomnia factors to subthreshold levels. In the case of SRT, this model is supported by clinical evidence. A 3P model-based approach has been quite influential among sleep medicine practitioners, and evaluation approaches for chronic insomnia feature questions about time in bed as one of the first line of inquiry for patients (Sutton, 2014).

The 3P model fails to predict every factor that influences disease course, however. In chronic anxiety patients, circadian chronotypes that are incompatible with typical sleep schedules have significantly greater sleep anxiety than other chronotypes (Passos *et al.*, 2017). Delayed- or advanced sleep phase syndromes can cause mistimed therapies that prove ineffective simply because they were offered at inopportune times for sleep. Additionally, the 3P model fails to provide a physiological rationale for how precipitating factors cause sleep fragmentation (Perlis *et al.* 2014). Rumination about a stressful life event can partially explain sleep onset insomnia, but other subtypes are less clear, especially as sleep need remains unchanged. Despite its flaws, the 3P model gives a conceptual framework to understand the onset and

persistence of insomnia symptoms, and suggests efficacious behavioral adaptations for patients.

The Treatment of Insomnia

A low threshold for treatment of insomnia during the acute and early chronic stages is critical to prevent continued daytime impairment, as well as preventing perpetuating behaviors from becoming part of patients' routine (Sutton, 2014). Cognitive Behavioral Therapy for Insomnia (CBT-I) is the first-line intervention for treatment of insomnia. Meta-analysis has shown this behavioral modality to be equal to pharmacologic therapy at treating acute insomnia, and as more effective in the long term (Smith *et al.*, 2002). Furthermore, the effectiveness of CBT-I has made it an attractive alternative to treat insomnia that occurs in the context of psychiatric comorbidities, like bipolar disorder (Kaplan and Harvey, 2013), PTSD (Ho, Chan and Tang, 2016), and depression (Cunningham and Shapiro, 2018). While CBT-I was conceived as a therapy for chronic insomnia due to its long duration, one-visit adaptations to treat acute insomnia have shown some efficacy (Ellis, Cushing and Germain, 2015).

CBT-I includes a combination of modalities: Behavioral Therapy (sleep restriction and stimulus control), Cognitive Therapy (e.g., cognitive restructuring of dysfunctional beliefs about sleep), and sleep hygiene. Behavioral therapy addresses prominent clinical feature of insomnia: the mismatch between sleep opportunity and sleep ability. Patients with insomnia often expand time in bed in hopes of recovering lost sleep (Spielman, 1986). This adaptation worsens symptoms by mismatching sleep ability (low) and opportunity (high), leading to less efficient and more fragmented sleep. By restricting time in bed, sleep restriction allows for better matching. In practice, patients

are asked to provide an average of the total number of hours of sleep per night, by keeping a sleep diary. Parameters for time in bed are set to match patients' average sleep time; patients are instructed to remain out of bed at all other times. While patients often report feeling tired in the first few days of implementation (Sutton, 2014), this represents a necessary part of the therapeutic process as sleep drive builds, subsequently increasing the intensity of slow wave sleep (Krystal and Edinger, 2010). A sleep diary is kept throughout this process, and sleep efficiency is monitored such that patients are sleeping for >90% of time spent in bed. After days or weeks of obtaining efficient, but restricted sleep opportunity, time in bed is slowly titrated by 15-30 minutes per week until a duration is reached that is efficient and restorative by patient report. Sleep restriction therapy has been used as monotherapy; studies show that sleep restriction alone is sufficient to gain most of the benefits of CBT-I (Miller *et al.*, 2014).

Stimulus control has also been utilized effectively and coupled with counseling on sleep hygiene to compose an important part of CBT-I. At its core, stimulus control encourages pro-sleep behaviors while in bed, while eliminating extraneous activities not associated with sleeping. Avoidance of daytime napping, non-sleep activities in bed, and caffeine, while encouraging regular exercise during wake times are important tenets of stimulus control (Sutton, 2014). Patients are also advised to get out of bed if they are having trouble falling asleep for 20 minutes or more; they can return to bed if tired after pursuit of a quiet activity in a low-lit room (reading, meditation, mindfulness exercises). Cognitive therapy aims to combat negative emotions that have accumulated due to disordered sleep.

CBT-I has shown efficacy in treatment regardless of etiology of insomnia (Riemann and Perlis, 2009), but limited accessibility of practitioners and long duration of therapy are the biggest roadblocks to broad implementation. To this end, adaptations to

traditional CBT-I have sought to bridge this gap and provide access to additional patients. Abbreviated treatment courses have been studied to treat primary insomnia, as well as insomnia comorbid with cancer (Palesh *et al.*, 2018) and depression (Wagley *et al.*, 2013; Pigeon *et al.*, 2017). Further extending the reach of CBT-I, telephone visits with practitioners or teleconferences with other patients (Brenes *et al.*, 2016), CBT-I ‘coaches’ on a mobile device (Koffel *et al.*, 2018), and text message services (Filion *et al.*, 2015) represented the first wave of technological applications to CBT-I. Recent advances have allowed patients to engage with mobile apps (Horsch *et al.*, 2017), even incorporating data from actigraphy to achieve accurate accounts of sleep timing and treatment compliance.

Despite the efficacy and increasing access to CBT-I, drug treatments still represent an outsized portion of treatments for insomnia. Two classes of drugs represent a majority of prescriptions for sleep disturbances: benzodiazepine receptor agonists and benzodiazepines. Sharing a common mechanism of action, these drugs increase inhibitory tone by binding the GABA_A receptor, and counteract pro-arousal cues throughout the brain. For short term use in acute insomnia, benzodiazepines and benzodiazepine receptor agonists have been shown to be safe and effective (Smith *et al.*, 2002). Their primary danger is to overuse, overdose, and dependency on these drugs for sleep in the long term. For this reason, these drug classes are no longer approved to treat chronic insomnia in Europe (Riemann *et al.*, 2015).

New treatments are in various stages of development; perhaps the most exciting is suvorexant, a receptor agonist that affects orexin receptors. Compared to placebo, patients taking suvorexant reported improvements in many subjective sleep metrics, including sleep latency and total sleep time (Kuriyama and Tabata, 2017). Notably, these results were after one year of treatment, longer time periods than benzodiazepines

are recommended to be used (Riemann *et al.*, 2015). Suvorexant is FDA-approved for the treatment of primary insomnia, and its limited side effect profile makes it an attractive option for individuals with comorbid dementia (Kuriyama and Tabata, 2017). Dual therapy with suvorexant and benzodiazepines causes high rates of oversedation and these patients had a high discontinuation rate in a retrospective study (Hatano *et al.*, 2018). Suvorexant alone causes somnolence and drowsiness as side effects, and strange dreams have also been reported as a side effect that can affect drug compliance. Perhaps most critically, suvorexant is metabolized via the cytochrome P450 system and levels can be directly altered by other drugs that affect this system (macrolide antibiotics, warfarin), leading to dangerous drug-drug interactions (Kishi, Matsunaga and Iwata, 2015). A direct comparison of efficacy of suvorexant to drugs in the benzodiazepine class would clarify under what conditions each drug should be used for maximal improvement of insomnia symptoms (Kuriyama and Tabata, 2017), but this new drug class represents an exciting development for pharmacologic therapies for insomnia.

Despite the numerous treatment options available, 40% of patients do not respond to treatment, even when CBT-I is combined with pharmacologic therapy (Morin, Vallières, *et al.*, 2009). Are there biological predictors that could identify who is at increased risk to be resistant to therapy? Perhaps lessons in prediction of treatment resistance can be gleaned from major depressive disorder, in which a third of patients are resistant to therapy (De Carlo, Calati and Serretti, 2016). Recent studies have used machine learning to accurately identify patients who are unlikely to respond to treatment (Kautzky *et al.*, 2017, 2018). Additionally, several studies have attempted to identify transcriptomic biomarkers and neuroimaging trends that could predict non-responders (Thase, 2014). While a single biological variable remains elusive in predicting resistance

to therapy in major depression, such investigations are lagging in the study of insomnia. Further investigation into treatment resistance is necessary to better understand this population.

Animal Models of Insomnia

Sleep research in humans and animal models alike has sought to answer a fundamental question: what essential function does sleep behavior serve? While they are sleeping, animals must overcome several evolutionary disadvantages. During sleep an animal is not eating or drinking, not seeking a mate for reproduction, and not searching for safe locations for shelter. Meanwhile, they are vulnerable to predation due to decreased arousability in response to external stimuli. Therefore, for sleep to be evolutionarily maintained, it must serve an essential purpose. Lending support to this idea, every animal that has been carefully studied has revealed robust sleep states. Initial studies of sleep states in animal models were classified according to the widely-accepted two-process model of sleep regulation (Borbély, 1982). Briefly, this model describes sleep behavior as being regulated by a homeostatic process depending on sleep and wake (Process S) and by the circadian pacemaker (Process C). Process S represents the need for sleep. It therefore increases throughout the waking period and is subsequently reduced by sleep behavior. Process C varies directly with an organisms circadian timing, carefully entrained to light/dark cycles. Measurement of these processes in animals lends itself to behavioral observation; the two-process model predicts that deprivation of sleep should further increase process S, meaning that organism should compensate for lost sleep by sleeping deeply in the subsequent period.

Furthermore, the model predicts that sleep behavior should have periodicity as the circadian system delineates opportunistic times for rest.

Therefore, sleep in animals has been classified according to a series of behavioral criteria: (1) animals must display decreased responsiveness to external stimuli, (2) but sleep must be reversible with sufficiently strong stimulation. (3) Sleep also must be subject to homeostatic regulation, such that sustained arousal from sleep induces subsequent compensatory increases in sleep amount and sleep depth (Allada and Siegel, 2008). In addition to behavioral measures, EEG monitoring of animals during sleep has enabled electrical signatures of sleep states to be elucidated. Regional brain differences in EEG in mammals as well as inability to adapt whole-brain electrical modeling to simpler model organisms has led to a preference for behavioral characterization of sleep. Through these behavioral classification schemes, common molecular criteria and neurotransmitter systems have been implicated in control of sleep across phylogeny (Crocker and Sehgal, 2010; Anafi, Kayser and Raizen, 2019).

The mechanistic study of sleep in invertebrates began in earnest at the turn of the millennium when sleep was described in *Drosophila melanogaster*, the vinegar fly (Hendricks *et al.*, 2000; Shaw *et al.*, 2000). In addition to meeting the behavioral criteria detailed above, sleep timing in flies was found to be regulated, but separable, from the function of circadian clocks, such that clock mutants achieved the same overall amount of total sleep time, though it was dispersed evenly throughout the day (Hendricks *et al.*, 2000).

The finding that *Drosophila* sleep likewise set the stage for forward genetic sleep screens, heralding discovery of new sleep-regulating genes and mapping of sleep neural microcircuitry. Critically, the deep understanding of genetic contributors to sleep has established many short-sleeping mutant lines that are potential models for insomnia in

Drosophila. An animal model of insomnia should recapitulate the key pathology of disease (decreased ability to sleep despite environmental circumstances that normally promote sleep, with associated daytime impairment). Several *Drosophila* mutants meet this criteria; mutations and alterations in expression of Shaker potassium channels produce changes to sleep. *Shaker*, a mutant line which carries a point mutation in a domain conserved in mammals, sleep for only 3-4 hours per day, whereas wild type control flies sleep 9-13 hours (Cirelli *et al.*, 2005). Two other mutant lines, *hyperkinetic* and *sleepless*, feature mutations in proteins that bind directly to Shaker channels or modify its expression, altering Shaker current kinetics (Bushey *et al.*, 2007; Wu *et al.*, 2010). These mutant lines also have severely shortened sleep duration (Bushey *et al.*, 2007; Koh *et al.*, 2008). These lines are compelling models of insomnia: reductions in sleep seen in *shaker*, *hyperkinetic*, and *sleepless* mutants are primarily due to severely decreased sleep bout length, indicating that flies initiate but cannot maintain sleep (Cirelli *et al.*, 2005; Bushey *et al.*, 2007; Koh *et al.*, 2008). It is unlikely that these short sleepers simply do not need sleep, since all three mutations reduce lifespan and *shaker* and *hyperkinetic* mutants have memory deficits (Cirelli *et al.*, 2005; Bushey *et al.*, 2007, 2010; Koh *et al.*, 2008). Several additional mutant lines with etiologies distinct from Shaker currents also produce short sleeping phenotypes in *Drosophila*. *Insomniac* mutants, who have aberrant ubiquitination, also have decreased sleep bout length and show an increased number of sleep episodes, indicating that they repeatedly initiate sleep to meet need, but cannot stay asleep (Stavropoulos and Young, 2011). The gene *redeye*, which encodes an α subunit of a nicotinic acetylcholine receptor, causes severe sleep deficits when mutated in *Drosophila*, and levels of REDEYE increase with high homeostatic drive for sleep (Shi *et al.*, 2014). *Fumin* flies, with a mutation in the

dopamine transporter, have significant reductions in sleep time as well as decreased arousal threshold and memory problems, again suggesting mutants are not able to fulfill their sleep need, as with human insomnia (Kume *et al.*, 2005). Mutant *Drosophila* lines provide compelling models of insomnia from a diversity of etiologies, mimicking the heterogeneity of human disease.

In addition to mutants discovered through screens, advanced understanding of arousal networks in *Drosophila* provide another potential avenue to model insomnia. Despite anatomical differences, the neural logic underpinning how specific *Drosophila* brain regions interact to control sleep and wakefulness can prove immensely useful for understanding similar processes in humans, as well as their potential contribution to disease. Experimental activation of 2 dopaminergic neurons (Liu *et al.*, 2012; Ueno *et al.*, 2012), a small cluster of octopaminergic neurons (Crocker *et al.*, 2010), or well-described populations of neuropeptidergic cells (Parisky *et al.*, 2008), each induces sustained wakefulness. Constitutive activation or silencing of these specific neurons is possible with precise genetic control in *Drosophila* (Baines *et al.*, 2001; Luan *et al.*, 2006). Temporally-regulated manipulation can also be attained by expression of the heat-activated depolarizing channel TrpA1 (Hamada *et al.*, 2008), while the temperature-sensitive dominant negative allele of *shibire* blocks synaptic transmission (Kitamoto, 2001). Recent work has also harnessed optogenetic and chemogenetic tools to alter activation of specific groups of neurons, enabling rapid reversibility (Becnel *et al.*, 2013; Klapoetke *et al.*, 2014).

The ease of use and powerful genetic tools to study sleep in *Drosophila* spawned additional models in even simpler organisms. *Caenorhabditis elegans*, a nematode with only 302 adult neurons, exhibits sleep between transitions of larval stages, as well as in response to cellular stressors (Raizen *et al.*, 2008; Hill *et al.*, 2014). Recent work

indicates that Cnidarians, which lack a brain structure and instead have a simple nerve net, demonstrate behavioral quiescence that meets criteria for sleep (Nath *et al.*, 2017). That these tissues can control sleep in their limited behavioral repertoire speaks to the essential function of sleep behavior.

While rodent models of normal sleep/wake function have been remarkable in helping to understand neuronal networks and electrical signatures involved in sleep, only a few valid insomnia models are widely accepted (Revel *et al.*, 2009). While sleep fragmentation is a characteristic symptom of human insomnia, baseline sleep in rodents is already quite fragmented. REM sleep cycles, which occur every 60-90 minutes in humans, occur on the order of seconds to minutes in several wild type mice strains (McShane *et al.*, 2010). Therefore, hypnotics or behavioral therapeutics that extend sleep bout duration can be tested in these strains, but relevance of these outcomes is difficult to interpret. Beyond viewing wild type mice as models of insomnia, stress is often leveraged to disrupt sleep and cause arousal, thereby decreasing total sleep and sleep continuity. Paradoxically, mild and brief stressors tend to cause increases to REM sleep, while more intense and prolonged stress leads to phenotypes that more closely resemble human insomnia (Revel *et al.*, 2009). Stressors can involve perception of a dangerous or taxing situation (viewing water underneath a transparent cage, seeing an aggressive conspecific in an adjacent cage), direct noxious stimuli like a foot shock, or gentle handling for long periods of time. The effect of individual stressors on sleep has been well-studied (Pawlyk *et al.*, 2008), but it remains difficult to generalize these models to human insomnia. Furthermore, these models more closely mimic acute insomnia as conceptualized by the 3P framework, as they feature acute stress in the absence of adaptations that perpetuate short sleep. Other models of insomnia leverage activation of arousal systems by caffeine or high-dose nicotine, or suppression of

serotonin production by synthesis inhibitors (Borbély, Neuhaus and Tobler, 1981; Revel *et al.*, 2009). These manipulations, while successful in limiting slow wave and REM sleep, are likely to have broad effects on neuronal function. Recent work in rodents has identified discrete neuronal subtypes involved in activating or suppressing REM behavior. GABAergic neurons with cell bodies in the dorsomedial hypothalamus that project to the preoptic area suppress REM sleep, while neurons projecting to the raphe pallidus promote REM (Chen *et al.*, 2018). Similarly, periaqueductal gray neurons have a privileged role in gating REM sleep (Weber *et al.*, 2018). Dissecting neuronal networks to understand regulation of sleep phases is essential to the understanding of behavior, but these genetic tools have not yet been applied to modeling insomnia.

Sleep, Aging, and Neurodegenerative Disease

Sleep is a dynamic process that changes throughout lifespan. Infants and toddlers require long sleep times, and spend a large percentage of time in REM sleep (Roffwarg, Muzio and Dement, 1966). These ontogenetic changes are mirrored by young zebrafish, fruit flies, and roundworms (Kayser and Biron, 2016). As children mature into adults, sleep times decline overall, and the REM/NREM ratio reaches a steady state. In the 5th decade of life, sleep again begins changing as a result of normal aging. Many of the effects of sleep on aging have been investigated in animal models. *Drosophila* studies on aging will be discussed in a separate section below. Here, I will detail how studies in humans and rodent models have informed our understanding of the effects of aging on sleep.

The most salient effect of age on sleep in both rodents and humans is sleep fragmentation, defined as a weakened ability to stay asleep during normal sleep times, as well as an inability to sustain wakefulness when usually awake (Van Gool and Mirmiran, 1983; Bliwise, 1993). In humans, these changes are reported to physicians as shorter sleep duration, earlier bedtimes and rise times, increased latency to sleep onset, increased sleep fragmentation, and greater likelihood of being awoken in response to external stimuli (Mander et al. 2017). During waking hours, there is increased propensity to nap at a population level, but this finding may be obscured by sleep disruption due to comorbid chronic conditions or frequent nighttime awakenings (Foley *et al.*, 2007). In fact, there is evidence to suggest healthy aging may reduce the susceptibility to daytime naps (Dijk *et al.*, 2010). Objective changes to sleep with age can be quantified by polysomnography. When the sleep of aging adults is measured by polysomnography, a reduction in time spent in slow wave sleep (SWS, deep NREM stages) is observed, as is a decrease of the total number and duration of NREM-REM cycles. EEG results in rodents corroborate the human polysomnographic findings; aging male mice have shorter time spent in SWS, and a slowing of theta peak frequency, a correlate of arousal (Wimmer *et al.*, 2013). In sum, normal aging causes decreased sleep, sleep that is more fragile and fragmented, and impaired sleep depth.

Influences of circadian rhythm degradation

Some neurobiological insights have been gleaned as to how age-related changes to sleep occur. Circadian rhythm strength has long been proposed as a potential cause of age-related sleep changes. Amplitude of peripheral rhythms as measured by body temperature regulation are found to be attenuated in older people, and aging affects one's ability to adapt to changing or irregular rhythms (Dijk, Duffy and

Czeisler, 2000). These changes were noted in rodent models nearly 20 years earlier (Halberg *et al.*, 1981). Additional work has implicated changes in the suprachiasmatic nucleus (SCN), the master pacemaker in the hypothalamus, as a site of aging-related changes. The volume of the SCN has been reported to decrease with age in some studies in both humans and rats (Swaab, Fliers and Partiman, 1985; Tsukahara *et al.*, 2005), but not others (Madeira *et al.*, 1995). A more consistent change with age has been observed in the synchrony of firing in SCN neurons themselves. Aged mice have a larger number of neurons firing out of phase with other neurons (Farajnia *et al.*, 2012), which is likely a cause of dampened output function with increasing age. Others have found that altered production of neuropeptides that serve to align firing patterns is another consequence of aging (Kawakami *et al.*, 1997), and can be another potential cause of desynchrony.

Homeostatic drive to sleep and aging

After sleep deprivation, the homeostatic drive for sleep responds by increasing SWS amount to compensate for time missed. Older adults have decreased slow wave sleep; does this indicate age-related degradation of the homeostat? EEGs of older adults who underwent sleep deprivation exhibited increased slow wave activity following a missed night sleep, indicating that homeostatic rebound is still present (Dijk, Duffy and Czeisler, 2001; Schmidt, Peigneux and Cajochen, 2012). However, slow wave activity response is diminished with age in frontal brain regions (Münch *et al.*, 2004), raising the possibility that changes with aging occur differently in different brain regions. Taken together, these findings indicate that while homeostatic drive to sleep is present in older adults, it may be weakened.

Functional consequences

Are there functional consequences for these sleep changes in older adults? Individuals with higher latency to sleep onset and increased wake time after sleep onset were found to have worse working memory than age-matched individuals with better sleep (Cavuoto *et al.*, 2016). Furthermore, long sleep duration (which may predict sleep disruption or fragmentation) was associated with poorer memory and processing speed (Low, Wu and Spira, 2019). Intriguingly, recent work indicates that age-related cognitive decline associated with poor sleep may be reversible. Improving slow wave sleep via auditory stimulation or transcranial direct current stimulation has shown promise in improving declarative and visual memory (Mander, Winer and Walker, 2017a). Pharmacologic induction of increased slow wave sleep failed to show memory effects, however (Feld *et al.*, 2013). Therefore, more studies are needed to elucidate if improving sleep may combat the effects of aging-related cognitive deterioration, thereby improving cognition and memory in the aging population.

Sleep and Dementia

While sleep disturbances impact the quality of life in the healthy aging population, in patients with dementia, the impact of sleep degradation is magnified. The association between sleep deficits and the progression of dementia has been best characterized in Alzheimer's disease (AD) patients. Up to 45% of AD patients report sleep disturbances in epidemiological studies (Moran *et al.*, 2005), and sleep degradation represents a significant risk factor for institutionalization (Peter-Derex *et al.*, 2015). Pathologically, self-reported somnolence, sleep problems, and short sleep duration predicted amyloid β burden in brain tissue (Spira *et al.*, 2013; Sprecher *et al.*, 2015), while CSF levels of

amyloid β were associated with decreased sleep efficiency and increased sleep latency (Liguori *et al.*, 2014). Interestingly, degraded sleep can occur up to 10 years before the clinical onset of cognitive decline (Musiek and Holtzman, 2016), consistent with the finding that brain regions involved in sleep are affected early in the pathogenesis of disease (Ju *et al.* 2014).

The link between sleep and Alzheimer's disease is bidirectional: progression of disease is associated with worsened sleep, and poor sleep causes worsening cognitive function (Spira *et al.*, 2013; Iranzo, 2016). In rodent models of A β deposition, sleep deprivation accelerates accumulation, whereas promoting sleep inhibits plaque formation by decreasing A β production and increasing the rate of protein clearance (Kang *et al.*, 2009; Xie *et al.*, 2013). While initial studies focused on A β accumulation as the cause of neurodegeneration, recent work has implicated tau pathology as the primary driver of AD. In fact, tau pathology in postmortem brains more closely associates with the onset of cognitive symptoms than A β . Whatever the pathologic cause, facilitating clearance of accumulated proteins while sleeping may explain the association between sleep and Alzheimer's disease. Recent work indicates that flow through glymphatic fluid transport system robustly increases during sleep versus wake, and proposes sleep as a privileged time for increased flow and clearance of accumulated toxins (Acharyar *et al.*, 2017). Specifically, sleep deprivation increases tau spreading throughout brain tissues (Holth *et al.*, 2019). Furthermore, levels of tau in CSF while awake were nearly 100% higher than sleeping levels (Holth *et al.*, 2019). Sleep behavior is clearly critical for the progression of disease, and these findings propose promotion of healthy sleep should be included as an essential part of disease therapy.

Other forms of dementia have unique sleep degradation signatures that are helpful in diagnosis and stratifying risk of disease progression. Idiopathic REM Behavior Disorder, a parasomnia that features loss of atonia and dream enactment during REM sleep, is considered one of the strongest risk factors for later development of α -synucleinopathy, which includes Parkinson's disease (PD), dementia with Lewy Bodies, and multiple system atrophy (Schenck, Bundlie and Mahowald, 1996; Iranzo *et al.*, 2006; Postuma *et al.*, 2009; Schenck, Boeve and Mahowald, 2013). Diagnosis of REM Behavior Disorder can be used as a predictor of PD subtype and disease course, as REM Behavior Disorder can precede the onset of cognitive symptoms by a decade (Kumru *et al.*, 2007; Postuma *et al.*, 2008; Lin and Chen, 2018). Due to this relationship, serial cognitive testing is common in the workup of parasomnias, and is often responsible for early diagnosis of neurodegenerative diseases (Gagnon *et al.*, 2009, 2010).

While sleep disturbances have been well-characterized in AD and α -synucleinopathy, sleep disturbances are rarely mentioned in the disease course of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). Long considered a disease that solely affected motor neurons, recent work has implicated cognitive and autonomic dysfunction as being involved in the disease (Congiu *et al.*, 2017). Moreover, polysomnography studies indicate that sleep is significantly disrupted in ALS/FTD patients, despite a dearth of patient reports of sleep disturbances (Congiu *et al.*, 2019; Panda, Gourie-Devi and Sharma, 2019). This study implies that sleep degradation may be more widespread than has been previously reported in this population. Therefore, a comprehensive study of sleep in ALS/FTD, and across all related disorders remains

necessary to fully understand the associations between sleep and neurodegenerative disease.

***Drosophila* Models of Aging and Neurodegenerative Disease**

Flies can live a maximum of about 90 days at normal rearing temperature of 25°C (Ziehm, Piper and Thornton, 2013). This duration makes aging studies less tedious than mammalian models, which requires months to years of aging before age-related changes occur. It is therefore no surprise that *Drosophila* has been embraced as a model system for elucidating mechanisms of behavioral change with age. Flies demonstrate numerous behavioral changes with advanced age, including reduced feeding, egg laying behavior, and physical activity (Piper and Partridge, 2018). This is coupled with physiological changes to metabolic rate and decreased cardiac function. Mechanisms of longevity extension discovered in other model systems are evolutionarily conserved in *Drosophila*, indicating that lessons learned in flies can be broadly applicable (Altintas, Park and Lee, 2016). Complicating the study of aging is that it is unclear what exactly causes death in flies, although gut leakiness is the leading hypothesis (Rera, Azizi and Walker, 2013).

Critical for the research undertaken here has been the thorough characterization of the effects of aging on sleep in *Drosophila*. Koh and colleagues characterized that age-advanced flies demonstrate sleep fragmentation, characterized by a decrease in average sleep bout length, increased number of sleep bouts, and an increased number of brief awakenings when compared to young adults, recapitulating the most striking sleep changes in humans. Furthermore, a redistribution of sleep was noted; aged flies show increased sleep during the day and decreased sleep amount at night (Koh *et al.*, 2006). These changes to sleep were modulated by altering rearing temperature,

indicating they reflect the physiological age of the fly (Koh *et al.*, 2006). Building upon this foundation, recent work has revealed that aging causes decreased arousal threshold during sleep and reduced rebound sleep following mechanical sleep deprivation (Vienne *et al.*, 2016), again mirroring aging effects in humans.

In addition to robust models of aging, *Drosophila* has been leveraged to study mechanisms of neurodegenerative diseases *in vivo*. Since the first neurodegenerative mutant was discovered in a screen for aberrant phototaxis in flies (Hotta and Benzer, 1972), *Drosophila* has been viewed as a dynamic system for studying neurodegeneration (Zhang, Coyne and Lloyd, 2018). Increasing understanding of human disease has enabled compelling models to be developed in *Drosophila*.

Alzheimer's disease

Alzheimer's disease is characterized in human postmortem brains by two characteristic pathologic findings: extracellular plaques composed mainly of amyloid β (A β) peptides, and intracellular microtubule-associated tangles that are composed primarily of tau. Since A β and tau deposits are pathognomonic for Alzheimer's disease, exploring these peptides' roles in *Drosophila* represented the first models in the fly field. Flies have a homolog of amyloid precursor protein (APP), the gene from which A β is made. Knockdown of endogenous APP produces aberrant phototaxis phenotypes, and remarkably these deficits can be rescued by ectopic expression of human APP protein, indicating their close homology (Luo, Tully and White, 1992). However, APP overexpression does not on its own produce visible plaques in *Drosophila* brains. It requires combination with overexpression of A β cleavage enzymes to produce meaningful memory phenotypes and neurodegeneration (Mhatre *et al.*, 2014; Dissel *et al.*, 2017). While this combination may provide interesting clues into the mechanisms of

accumulation of wild type A β , to expedite the phenotypes of interest, expression of pre-processed A β peptides has been embraced in *Drosophila*. Two A β peptides accumulate in extracellular plaques, A β 40 and A β 42, with the numbers indicating the amino acid lengths of the peptides. A β 40 on its own does not induce pathology with overexpression in *Drosophila*. A β 42 however, forms deposits during aging in the fly brain (Koichi Iijima *et al.*, 2004). Furthermore, known mutations in APP that cause early-onset familial Alzheimer's disease have been leveraged in *Drosophila* to expedite the onset of observable phenotypes. A β Arctic, a fast-aggregating peptide derived from a familial mutation, has been demonstrated to have more severe neurodegenerative phenotypes and deposition of plaques than overexpression of A β 42 (Crowther *et al.*, 2005; Iijima *et al.*, 2008). These flies also have locomotor defects as well as truncated lifespan (Nilsberth *et al.*, 2001). Several models of A β 42 overexpression have also been shown to have sleep deficits with increased age (Tabuchi *et al.*, 2015; Dissel *et al.*, 2017).

Tau is most notably associated with Alzheimer's disease, but tau pathology also characterizes corticobasal degeneration, Pick's disease, progressive supranuclear palsy, and forms of frontotemporal dementia, a group collectively referred to as tauopathies. Like APP, *Drosophila* have an endogenous copy of a tau gene (Iijima-Ando and Iijima, 2010). Overexpression of this endogenous gene produced a more severe eye degeneration phenotype than the human tau gene (Chen *et al.*, 2007). Overexpression of human tau is sufficient for shortened lifespan and neurodegeneration (Wittmann *et al.*, 2001). Many tau mutations have been discovered in human patients and found to be associated with early onset and severe nature of disease. Similarly, these mutated peptides cause hastened neurodegeneration when expressed in *Drosophila* brains as well (Wittmann *et al.*, 2001). Surprisingly, these models do not feature intracellular inclusions characteristic of human disease (Wittmann *et al.*, 2001). Expression of tau in

mushroom bodies, the site of olfactory memory in the fly, produces memory deficits before the onset of neurodegeneration (Merishin *et al.*, 2004). This diverse array of models of Alzheimer's disease in *Drosophila* enables further study into the mechanisms of disease and potential therapies in a genetically tractable system.

Amyotrophic Lateral Sclerosis

In contrast to the overexpression models that predominate in the study of Alzheimer's disease, *Drosophila* models of amyotrophic lateral sclerosis (ALS) have utilized both knockdown and overexpression models to study disease progression (Zhang, Coyne and Lloyd, 2018). Some of the known ALS-causing mutations cause no obvious phenotype in *Drosophila* (Chang and Morton, 2017), perhaps a function of the condensed lifespan of flies compared to the time to disease onset in humans. It is for these reasons that overexpression models have again been embraced in the study of this disease. In nearly all cases of ALS, regardless of genetic etiology, brains show a mislocalization of TDP-43 protein out of the nucleus and into cytoplasmic accumulations (Neumann *et al.*, 2006).

The most compelling models of ALS in the fly are therefore overexpression models of TDP-43. These models produce a remarkable deterioration of photoreceptors and retinal neurons when overexpressed in the eye (Elden *et al.*, 2010; Hanson *et al.*, 2010; Li *et al.*, 2010). Importantly, neuronal overexpression of TDP-43 confers truncated lifespan, neuronal degradation, and motor deficits as well (Elden *et al.*, 2010). On a cellular level, these models critically confer similar pathologic findings to human brains: a redistribution of TDP-43 to cytoplasmic stress granules (Kim *et al.*, 2014). This overexpression paradigm has also resulted in the discovery of novel modifiers of TDP-43 toxicity (Kim *et al.*, 2014; Berson *et al.*, 2017; C. Y. Chung *et al.*, 2018; McGurk *et al.*,

2018). Mutations in endogenous *Drosophila* TDP-43 similarly cause shortened lifespan and motor deficits (Feiguin *et al.*, 2009). Intriguingly, depletion and overexpression of TDP-43 cause similar gene expression changes (Vanden Broeck *et al.*, 2013), implying that manipulation of TDP-43 levels in either direction may be activating similar pathways (McGurk, Berson and Bonini, 2015). ALS models in *Drosophila* therefore provide compelling data that promotes a deeper understanding of disease processes.

Concluding Remarks

With a vast array of genetic tools, the fruit fly has become one of the most powerful model systems for studying how brain circuits give rise to complex behaviors; research in the fly has repeatedly led to novel insights into basic principles that scale to mammalian systems. For this reason, *Drosophila* has tremendous potential to elucidate the relationship between sleep and neurodegenerative diseases. The diverse models of disease in flies presents an opportunity to exploit these tools to gain insight into disease pathogenesis and novel treatment strategies.

References

- Acharyar, T. M. *et al.* (2017) 'Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation', *Molecular neurodegeneration*. doi: 10.1186/s13024-016-0147-7.
- Agrawal, P. *et al.* (2017) 'Drosophila CRY Entrain Clocks in Body Tissues to Light and Maintains Passive Membrane Properties in a Non-clock Body Tissue Independent of Light', *Current Biology*, 27(16), pp. 2431-2441.e3. doi: 10.1016/j.cub.2017.06.064.
- Aldrich, M. S. *et al.* (1989) 'Sleep abnormalities in progressive supranuclear palsy', *Annals of Neurology*. doi: 10.1002/ana.410250609.
- Allada, R. and Siegel, J. M. (2008) 'Unearthing the Phylogenetic Roots of Sleep', *Current Biology*. doi: 10.1016/j.cub.2008.06.033.
- Altintas, O., Park, S. and Lee, S. J. V. (2016) 'The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*', *BMB Reports*. doi: 10.5483/BMBRep.2016.49.2.261.
- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders (5th Edition)*, *American Journal of Psychiatry*. doi: 10.1176/appi.books.9780890425596.744053.
- Anafi, R. C., Kayser, M. S. and Raizen, D. M. (2019) 'Exploring phylogeny to find the function of sleep', *Nature Reviews Neuroscience*. doi: 10.1038/s41583-018-0098-9.
- Apergis-Schoute, A. M. *et al.* (2005) 'Auditory fear conditioning and long-term potentiation in the lateral amygdala require ERK/MAP kinase signaling in the auditory thalamus: A role for presynaptic plasticity in the fear system', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0096-05.2005.
- Artiushin, G. *et al.* (2018) 'Endocytosis at the drosophila blood– brain barrier as a function for sleep', *eLife*. doi: 10.7554/eLife.43326.
- Ascherio, A. and Schwarzschild, M. A. (2016) 'The epidemiology of Parkinson's disease: risk factors and prevention', *The Lancet Neurology*. doi: 10.1016/S1474-4422(16)30230-7.
- Babačić, H. *et al.* (2019) 'CRISPR-cas gene-editing as plausible treatment of neuromuscular and nucleotide-repeat-expansion diseases: A systematic review', *PLoS ONE*. doi: 10.1371/journal.pone.0212198.
- Baillargeon, L., Demers, M. and Ladouceur, R. (1998) 'Stimulus-control: Nonpharmacologic treatment for insomnia', *Canadian Family Physician*.
- Baines, R. A. *et al.* (2001) 'Altered Electrical Properties in Drosophila Neurons Developing without Synaptic Transmission', *The Journal of Neuroscience*. doi: 10.1523/jneurosci.21-05-01523.2001.
- Bathgate, C. J., Edinger, J. D. and Krystal, A. D. (2017) 'Insomnia patients with objective short sleep duration have a blunted response to cognitive behavioral therapy for Insomnia', *Sleep*, 40(1). doi: 10.1093/sleep/zsw012.
- Becker, L. A. *et al.* (2017) 'Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice', *Nature*. doi: 10.1038/nature22038.

- Becnel, J. *et al.* (2013) 'DREADDs in drosophila: A pharmacogenetic approach for controlling behavior, neuronal signaling, and physiology in the fly', *Cell Reports*. doi: 10.1016/j.celrep.2013.08.003.
- Belfer, S. J. *et al.* (2019) 'A Drosophila model of sleep restriction therapy for insomnia', *Molecular Psychiatry*. doi: 10.1038/s41380-019-0376-6.
- Bellen, H. J., Tong, C. and Tsuda, H. (2010) '100 years of Drosophila research and its impact on vertebrate neuroscience: A history lesson for the future', *Nature Reviews Neuroscience*. doi: 10.1038/nrn2839.
- Berlivet, S. *et al.* (2019) 'Readers of the m 6 A epitranscriptomic code', *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*. doi: 10.1016/j.bbagr.2018.12.008.
- Berry, J. A. *et al.* (2015) 'Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting', *Cell*. doi: 10.1016/j.cell.2015.05.027.
- Berson, A. *et al.* (2017) 'TDP-43 Promotes Neurodegeneration by Impairing Chromatin Remodeling', *Current Biology*. doi: 10.1016/j.cub.2017.10.024.
- Bliwise, D. L. (1993) 'Sleep in normal aging and dementia', *Sleep*. doi: 10.1093/sleep/16.1.40.
- Bloom, G. S. (2014) 'Amyloid- β and tau: The trigger and bullet in Alzheimer disease pathogenesis', *JAMA Neurology*. doi: 10.1001/jamaneurol.2013.5847.
- Boland, E. *et al.* (2019) 'Precision Medicine for Insomnia', *Sleep Medicine Clinics*. doi: 10.1016/j.jsmc.2019.04.001.
- Borbély, A. A. (1982) 'A two process model of sleep regulation.', *Human neurobiology*.
- Borbély, A. A., Neuhaus, H. U. and Tobler, I. (1981) 'Effect of p-chlorophenylalanine and tryptophan on sleep, EEG and motor activity in the rat', *Behavioural Brain Research*. doi: 10.1016/0166-4328(81)90035-8.
- Brenes, G. A. *et al.* (2016) 'Effects of Telephone-Delivered Cognitive-Behavioral Therapy and Nondirective Supportive Therapy on Sleep, Health-Related Quality of Life, and Disability', *American Journal of Geriatric Psychiatry*. doi: 10.1016/j.jagp.2016.04.002.
- Vanden Broeck, L. *et al.* (2013) 'TDP-43 Loss-of-Function Causes Neuronal Loss Due to Defective Steroid Receptor-Mediated Gene Program Switching In Drosophila', *Cell Reports*. doi: 10.1016/j.celrep.2012.12.014.
- Brown, M. K. *et al.* (2014) 'Aging induced endoplasmic reticulum stress alters sleep and sleep homeostasis', *Neurobiology of Aging*, 35(6), pp. 1431–1441. doi: 10.1016/j.neurobiolaging.2013.12.005.
- Brummett, B. H. *et al.* (2007) 'Sleep quality varies as a function of 5-HTTLPR genotype and stress', *Psychosomatic Medicine*. doi: 10.1097/PSY.0b013e31814b8de6.
- Buhl, E., Higham, J. P. and Hodge, J. J. L. (2019) 'Alzheimer's disease-associated tau alters Drosophila circadian activity, sleep and clock neuron electrophysiology', *Neurobiology of Disease*. doi: 10.1016/j.nbd.2019.104507.
- Bushey, D. *et al.* (2007) 'Drosophila Hyperkinetic mutants have reduced sleep and impaired memory.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(20), pp. 5384–5393. doi: 10.1523/JNEUROSCI.0108-07.2007.
- Bushey, D. *et al.* (2010) 'Sleep, aging, and lifespan in Drosophila.', *BMC neuroscience*, 11, p. 56. doi: 10.1186/1471-

2202-11-56.

- Bushey, D., Tononi, G. and Cirelli, C. (2011) 'Sleep and synaptic homeostasis: Structural evidence in *Drosophila*', *Science*. doi: 10.1126/science.1202839.
- De Carlo, V., Calati, R. and Serretti, A. (2016) 'Socio-demographic and clinical predictors of non-response/non-remission in treatment resistant depressed patients: A systematic review', *Psychiatry Research*. doi: 10.1016/j.psychres.2016.04.034.
- Cassidy-Eagle, E. *et al.* (2018) 'Cognitive Behavioral Treatment for Insomnia in Older Adults with Mild Cognitive Impairment in Independent Living Facilities : A Pilot Study', *Journal of Sleep Disorders and Medical Care*, 1(1). doi: 10.16966/jsdmc.105.
- Cavuto, M. G. *et al.* (2016) 'Objective but not subjective sleep predicts memory in community-dwelling older adults', *Journal of Sleep Research*. doi: 10.1111/jsr.12391.
- Chakravarti, L., Moscato, E. H. and Kayser, M. S. (2017) 'Unraveling the Neurobiology of Sleep and Sleep Disorders Using *Drosophila*', in *Current Topics in Developmental Biology*. doi: 10.1016/bs.ctdb.2016.07.010.
- Chang, J. C. and Morton, D. B. (2017) 'Drosophila lines with mutant and wild type human TDP-43 replacing the endogenous gene reveals phosphorylation and ubiquitination in mutant lines in the absence of viability or lifespan defects', *PLoS ONE*. doi: 10.1371/journal.pone.0180828.
- Chen, K. S. *et al.* (2018) 'A Hypothalamic Switch for REM and Non-REM Sleep', *Neuron*. doi: 10.1016/j.neuron.2018.02.005.
- Chen, X. *et al.* (2007) 'Study of tauopathies by comparing *Drosophila* and human tau in *Drosophila*', *Cell and Tissue Research*. doi: 10.1007/s00441-007-0401-y.
- Choi, Y. K. *et al.* (2018) 'Smartphone applications to support sleep self-management: Review and evaluation', *Journal of Clinical Sleep Medicine*. doi: 10.5664/jcsm.7396.
- Chung, C. Y. *et al.* (2018) 'Aberrant activation of non-coding RNA targets of transcriptional elongation complexes contributes to TDP-43 toxicity', *Nature Communications*. doi: 10.1038/s41467-018-06543-0.
- Chung, K. F. *et al.* (2018) 'Sleep hygiene education as a treatment of insomnia: A systematic review and meta-analysis', *Family Practice*. doi: 10.1093/fampra/cmz122.
- Cirelli, C. *et al.* (2005) 'Reduced sleep in *Drosophila* Shaker mutants.', *Nature*, 434(7037), pp. 1087–1092. doi: 10.1038/nature03486.
- Congiu, P. *et al.* (2017) 'Impairment of autonomic nervous system in amyotrophic lateral sclerosis', *Sleep Medicine*. doi: 10.1016/j.sleep.2017.11.795.
- Congiu, P. *et al.* (2019) 'Sleep cardiac dysautonomia and EEG oscillations in Amyotrophic Lateral Sclerosis', *Sleep*, epub ahead.
- Crocker, A. *et al.* (2010) 'Identification of a Neural Circuit that Underlies the Effects of Octopamine on Sleep:Wake Behavior', *Neuron*. doi: 10.1016/j.neuron.2010.01.032.

- Crocker, A. and Sehgal, A. (2010) 'Genetic analysis of sleep', *Genes and Development*. doi: 10.1101/gad.1913110.
- Crowther, D. C. *et al.* (2005) 'Intraneuronal A β , non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease', *Neuroscience*, 132(1), pp. 123–135. doi: 10.1016/j.neuroscience.2004.12.025.
- Cunningham, J. E. A. and Shapiro, C. M. (2018) 'Cognitive Behavioural Therapy for Insomnia (CBT-I) to treat depression: A systematic review', *Journal of Psychosomatic Research*. doi: 10.1016/j.jpsychores.2017.12.012.
- Curtsinger, J. W. (2013) 'Late-life fecundity plateaus in *Drosophila melanogaster* can be explained by variation in reproductive life spans', *Experimental Gerontology*, 48(11), pp. 1338–1342. doi: 10.1016/j.exger.2013.08.016.
- Daley, M. *et al.* (2009) 'Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents', *Sleep Medicine*, 10(4), pp. 427–438. doi: 10.1016/j.sleep.2008.04.005.
- van Dalen, J. H. and Markus, C. R. (2019) 'The serotonin transporter 5-HTTLPR polymorphism and the risk for insomnia: a non-replication', *Sleep Medicine*, 53, pp. 195–196.
- Davis, M. *et al.* (2006) 'Effects of D-Cycloserine on Extinction: Translation From Preclinical to Clinical Work', *Biological Psychiatry*. doi: 10.1016/j.biopsych.2006.03.084.
- Dement, W. and Pelayo, R. (1997) 'Public health impact and treatment of insomnia', *European Psychiatry*, 12((Suppl 1)), pp. 31s-39s.
- Depner, C. M., Stothard, E. R. and Wright, K. P. (2014) 'Metabolic consequences of sleep and circadian disorders', *Current Diabetes Reports*. doi: 10.1007/s11892-014-0507-z.
- Dijk, D. J. *et al.* (2010) 'Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep', *Sleep*. doi: 10.1093/sleep/33.2.211.
- Dijk, D. J., Duffy, J. F. and Czeisler, C. A. (2000) 'Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep', *Chronobiology International*. doi: 10.1081/CBI-100101049.
- Dijk, D. J., Duffy, J. F. and Czeisler, C. A. (2001) 'Age-related increase in awakenings: Impaired consolidation of nonREM sleep at all circadian phases', *Sleep*. doi: 10.1093/sleep/24.5.565.
- Dilley, L. C. *et al.* (2018) 'Behavioral and genetic features of sleep ontogeny in *Drosophila*', *Sleep*. doi: 10.1093/sleep/zsy086.
- Dissel, S. *et al.* (2015) 'Sleep restores behavioral plasticity to *drosophila* mutants', *Current Biology*. doi: 10.1016/j.cub.2015.03.027.
- Dissel, S. *et al.* (2017) 'Enhanced sleep reverses memory deficits and underlying pathology in *drosophila* models of Alzheimer's disease', *Neurobiology of Sleep and Circadian Rhythms*. doi: 10.1016/j.nbscr.2016.09.001.
- Donelson, N. *et al.* (2012) 'High-resolution positional tracking for long-term analysis of *Drosophila* sleep and locomotion using the "tracker" program.', *PLoS one*. doi: 10.1371/journal.pone.0037250.
- Donlea, J. M., Pimentel, D. and Miesenböck, G. (2014) 'Neuronal machinery of sleep homeostasis in *Drosophila*', *Neuron*. doi: 10.1016/j.neuron.2013.12.013.
- Dubowy, C. *et al.* (2016) 'Genetic Dissociation of Daily Sleep and Sleep Following Thermogenetic Sleep Deprivation in

- Drosophila*, *Sleep*. doi: 10.5665/sleep.5760.
- Dubowy, C. and Sehgal, A. (2017) 'Circadian rhythms and sleep in *Drosophila melanogaster*', *Genetics*. doi: 10.1534/genetics.115.185157.
- Eban-Rothschild, A., Appelbaum, L. and De Lecea, L. (2018) 'Neuronal Mechanisms for Sleep/Wake Regulation and Modulatory Drive', *Neuropsychopharmacology*. doi: 10.1038/npp.2017.294.
- Eddinger, J. D. *et al.* (2001) 'Cognitive behavioral therapy for treatment of chronic primary insomnia: a randomized controlled trial.', *JAMA : the journal of the American Medical Association*, 285(14), pp. 1856–64.
- Eddinger, J. D. *et al.* (2007) 'Dose-response effects of cognitive-behavioral insomnia therapy: A randomized clinical trial', *Sleep*, 30(2), pp. 203–212. doi: 10.1093/sleep/30.2.203.
- Elden, A. C. *et al.* (2010) 'Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS', *Nature*. doi: 10.1038/nature09320.
- Ellis, J. G., Cushing, T. and Germain, A. (2015) 'Treating Acute Insomnia: A Randomized Controlled Trial of a "Single-Shot" of Cognitive Behavioral Therapy for Insomnia', *SLEEP*. doi: 10.5665/sleep.4752.
- Emery, P. *et al.* (1998) 'Cry, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity', *Cell*, 95(5), pp. 669–679. doi: 10.1016/S0092-8674(00)81637-2.
- Emery, P. *et al.* (2000) '*Drosophila* CRY is a deep brain circadian photoreceptor', *Neuron*, 26(2), pp. 493–504. doi: 10.1016/S0896-6273(00)81181-2.
- Engel, M. *et al.* (2018) 'The Role of m6A/m-RNA Methylation in Stress Response Regulation', *Neuron*. doi: 10.1016/j.neuron.2018.07.009.
- Espie, C. A. *et al.* (2012) 'A Randomized, Placebo-Controlled Trial of Online Cognitive Behavioral Therapy for Chronic Insomnia Disorder Delivered via an Automated Media-Rich Web Application', *Sleep*. doi: 10.5665/sleep.1872.
- Estes, P. S. *et al.* (2013) 'Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a *Drosophila* model of amyotrophic lateral sclerosis', *Disease Models & Mechanisms*. doi: 10.1242/dmm.010710.
- Falls, W. A., Miserendino, M. J. D. and Davis, M. (1992) 'Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala', *Journal of Neuroscience*.
- Farajnia, S. *et al.* (2012) 'Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0469-12.2012.
- Farca Luna, A. J., Perier, M. and Seugnet, L. (2017) 'Amyloid Precursor Protein in *Drosophila* Glia Regulates Sleep and Genes Involved in Glutamate Recycling', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. doi: 10.1523/JNEUROSCI.2826-16.2017.
- Feiguin, F. *et al.* (2009) 'Depletion of TDP-43 affects *Drosophila* motoneurons terminal synapsis and locomotive behavior', *FEBS Letters*. doi: 10.1016/j.febslet.2009.04.019.
- Feld, G. B. *et al.* (2013) 'Slow Wave Sleep Induced by GABA Agonist Tiagabine Fails to Benefit Memory Consolidation', *Sleep*. doi: 10.5665/sleep.2954.

- Figard, L. *et al.* (2019) 'Cofilin-Mediated Actin Stress Response Is Maladaptive in Heat-Stressed Embryos', *Cell Reports*. doi: 10.1016/j.celrep.2019.02.092.
- Filion, A. J. *et al.* (2015) 'Examining the influence of a text message-based sleep and physical activity intervention among young adult smokers in the United States', *BMC Public Health*. doi: 10.1186/s12889-015-2045-2.
- Finelli, A. *et al.* (2004) 'A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*', *Molecular and Cellular Neuroscience*, 26(3), pp. 365–375. doi: 10.1016/j.mcn.2004.03.001.
- Foley, D. J. *et al.* (2007) 'Frequent napping is associated with excessive daytime sleepiness, depression, pain, and nocturia in older adults: Findings from the national sleep foundation 2003 sleep in america poll', *American Journal of Geriatric Psychiatry*. doi: 10.1097/01.JGP.0000249385.50101.67.
- Furini, C., Myskiw, J. and Izquierdo, I. (2014) 'The learning of fear extinction', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2014.10.016.
- Gagnon, J. F. *et al.* (2009) 'Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease', *Annals of Neurology*. doi: 10.1002/ana.21680.
- Gagnon, J. F. *et al.* (2010) 'The Montreal cognitive assessment: A screening tool for mild cognitive impairment in REM sleep behavior disorder', *Movement Disorders*. doi: 10.1002/mds.23079.
- Gama, R. L. *et al.* (2010) 'Sleep disturbances and brain MRI morphometry in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy - a comparative study', *Parkinsonism and Related Disorders*. doi: 10.1016/j.parkreldis.2010.01.002.
- Ganguly-Fitzgerald, I., Donlea, J. and Shaw, P. J. (2006) 'Waking experience affects sleep need in *Drosophila*', *Science*. doi: 10.1126/science.1130408.
- Gao, X. *et al.* (2019) 'The bidirectional causal relationships of insomnia with five major psychiatric disorders: A Mendelian randomization study', *European Psychiatry*. doi: 10.1016/j.eurpsy.2019.05.004.
- Garcia, R. (2017) 'Neurobiology of fear and specific phobias', *Learning and Memory*. doi: 10.1101/lm.044115.116.
- Gehrman, P. R., Pfeiffenberger, C. and Byrne, E. M. (2013) 'The role of genes in the insomnia phenotype', *Sleep Medicine Clinics*, pp. 323–331. doi: 10.1016/j.jsmc.2013.04.005.
- Gilestro, G. F. (2012) 'Video tracking and analysis of sleep in *drosophila melanogaster*', *Nature Protocols*. doi: 10.1038/nprot.2012.041.
- Gilestro, G. F. and Cirelli, C. (2009) 'PySolo: A complete suite for sleep analysis in *Drosophila*', *Bioinformatics*. doi: 10.1093/bioinformatics/btp237.
- Van Gool, W. A. and Mirmiran, M. (1983) 'Age-related changes in the sleep pattern of male adult rats', *Brain Research*. doi: 10.1016/0006-8993(83)90217-2.
- Guo, F. *et al.* (2016) 'Circadian neuron feedback controls the *Drosophila* sleep-activity profile', *Nature*. doi: 10.1038/nature19097.
- Hagatun, S. *et al.* (2019) 'The Short-Term Efficacy of an Unguided Internet-Based Cognitive-Behavioral Therapy for

- Insomnia: A Randomized Controlled Trial With a Six-Month Nonrandomized Follow-Up', *Behavioral Sleep Medicine*. doi: 10.1080/15402002.2017.1301941.
- Halberg, J. *et al.* (1981) 'Changes with age characterize circadian rhythm in telemetered core temperature of stroke-prone rats', *Journals of Gerontology*. doi: 10.1093/geronj/36.1.28.
- Hamada, F. N. *et al.* (2008) 'An internal thermal sensor controlling temperature preference in *Drosophila*', *Nature*. doi: 10.1038/nature07001.
- Hanson, K. A. *et al.* (2010) 'Ubiquitin modifies TDP-43 toxicity in a *Drosophila* model of amyotrophic lateral sclerosis (ALS)', *Journal of Biological Chemistry*. doi: 10.1074/jbc.C109.078527.
- Harbison, S. T., McCoy, L. J. and Mackay, T. F. C. (2013) 'Genome-wide association study of sleep in *Drosophila melanogaster*', *BMC Genomics*, 14(1). doi: 10.1186/1471-2164-14-281.
- Hasan, S. *et al.* (2012) 'Age-related changes in sleep in inbred mice are genotype dependent', *Neurobiology of Aging*, 33(1). doi: 10.1016/j.neurobiolaging.2010.05.010.
- Hatano, M. *et al.* (2018) 'Assessment of switching to suvorexant versus the use of add-on suvorexant in combination with benzodiazepine receptor agonists in insomnia patients: A retrospective study', *Clinical Psychopharmacology and Neuroscience*. doi: 10.9758/cpn.2018.16.2.184.
- Head, L. M. *et al.* (2015) 'The influence of light on temperature preference in *Drosophila*', *Current Biology*. doi: 10.1016/j.cub.2015.02.038.
- Helfrich-Förster, C. *et al.* (2001) 'The circadian clock of fruit flies is blind after elimination of all known photoreceptors', *Neuron*, 30(1), pp. 249–261. doi: 10.1016/S0896-6273(01)00277-X.
- Hendricks, J. C. *et al.* (2000) 'Rest in *Drosophila* Is a Sleep-like State', *Neuron*, 25(1), pp. 129–138. doi: 10.1016/S0896-6273(00)80877-6.
- Hill, A. J. *et al.* (2014) 'Cellular stress induces a protective sleep-like state in *C. elegans*', *Current Biology*. doi: 10.1016/j.cub.2014.08.040.
- Ho, F. Y. Y., Chan, C. S. and Tang, K. N. S. (2016) 'Cognitive-behavioral therapy for sleep disturbances in treating posttraumatic stress disorder symptoms: A meta-analysis of randomized controlled trials', *Clinical Psychology Review*. doi: 10.1016/j.cpr.2015.09.005.
- Hofmann, S. G. *et al.* (2012) 'The efficacy of cognitive behavioral therapy: a review of meta-analyses', *Cognitive Therapy Research*. NIH Public Access, 36(5), pp. 427–440. doi: 10.1007/s10608-012-9476-1.The.
- Hohagen, F. *et al.* (1994) 'Sleep onset insomnia, sleep maintaining insomnia and insomnia with early morning awakening—temporal stability of subtypes in a longitudinal study on general practice attenders', *Sleep*, 17, pp. 551–554.
- Holth, J. K. *et al.* (2019) 'The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans', *Science*. doi: 10.1126/science.aav2546.
- Horsch, C. H. G. *et al.* (2017) 'Mobile phone-delivered cognitive behavioral therapy for insomnia: A randomized waitlist

- controlled trial', *Journal of Medical Internet Research*. doi: 10.2196/jmir.6524.
- Hotta, Y. and Benzer, S. (1972) 'Mapping of behaviour in *Drosophila* mosaics', *Nature*. doi: 10.1038/240527a0.
- Iijima-Ando, K. and Iijima, K. (2010) 'Transgenic *drosophila* models of Alzheimer's disease and tauopathies', *Brain Structure and Function*. doi: 10.1007/s00429-009-0234-4.
- Iijima, K. *et al.* (2004) 'Dissecting the pathological effects of human A 40 and A 42 in *Drosophila*: A potential model for Alzheimer's disease', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0400895101.
- Iijima, Koichi *et al.* (2004) 'Dissecting the pathological effects of human A β 40 and A β 42 in *Drosophila*: A potential model for Alzheimer's disease', *Proceedings of the National Academy of Sciences of the United States of America*, 101(17), pp. 6623–6628. doi: 10.1073/pnas.0400895101.
- Iijima, K. *et al.* (2008) 'A β 42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0001703.
- Insel, T. R. (2012) 'Next-generation treatments for mental disorders.', *Science translational medicine*, 4(155), p. 155ps19. doi: 10.1126/scitranslmed.3004873.
- Iranzo, A. *et al.* (2006) 'Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study', *Lancet Neurology*. doi: 10.1016/S1474-4422(06)70476-8.
- Iranzo, A. (2016) 'Sleep in Neurodegenerative Diseases', *Sleep Medicine Clinics*, pp. 1–18. doi: 10.1016/j.jsmc.2015.10.011.
- Ishimoto, H., Lark, A. and Kitamoto, T. (2012) 'Factors that differentially affect daytime and nighttime sleep in *Drosophila melanogaster*', *Frontiers in Neurology*, FEB, pp. 1–5. doi: 10.3389/fneur.2012.00024.
- Ito, K. *et al.* (2017) 'Effects of Kamikihito and Unkei-to on sleep behavior of wild type and Parkinson model in *Drosophila*', *Frontiers in Psychiatry*. doi: 10.3389/fpsy.2017.00132.
- Jansen, P. R. *et al.* (2019) 'Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways', *Nature Genetics*. doi: 10.1038/s41588-018-0333-3.
- Javaheri, S. and Redline, S. (2017) 'Insomnia and Risk of Cardiovascular Disease', *Chest*. doi: 10.1016/j.chest.2017.01.026.
- Johansen, J. P. *et al.* (2011) 'Molecular mechanisms of fear learning and memory.', *Cell*.
- Ju, Y.-E. S., Lucey, B. P. and Holtzman, D. M. (2014) 'Sleep and Alzheimer disease pathology—a bidirectional relationship', *Nature Reviews Neurology*, 10(2), pp. 115–119. doi: 10.1038/nrneurol.2013.269.
- Ju, Y. E. S., Lucey, B. P. and Holtzman, D. M. (2014) 'Sleep and Alzheimer disease pathology—a bidirectional relationship', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2013.269.
- Kallman, B. R., Kim, H. and Scott, K. (2015) 'Excitation and inhibition onto central courtship neurons biases *drosophila* mate choice', *eLife*. doi: 10.7554/eLife.11188.
- Kaneko, H. *et al.* (2012) 'Circadian rhythm of temperature preference and its neural control in *Drosophila*', *Current Biology*, 22(19), pp. 1851–1857. doi: 10.1016/j.cub.2012.08.006.

- Kang, J. E. *et al.* (2009) 'Amyloid- β dynamics are regulated by orexin and the sleep-wake cycle', *Science*. doi: 10.1126/science.1180962.
- Kaplan, K. A. and Harvey, A. G. (2013) 'Behavioral treatment of insomnia in bipolar disorder', *American Journal of Psychiatry*. doi: 10.1176/appi.ajp.2013.12050708.
- Kathol, R. G. and Arnedt, J. T. (2016) 'Cognitive behavioral therapy for chronic insomnia: Confronting the challenges to implementation', *Annals of Internal Medicine*, pp. 149–150. doi: 10.7326/M16-0359.
- Kautzky, A. *et al.* (2017) 'A new prediction model for evaluating treatment-resistant depression', *Journal of Clinical Psychiatry*. doi: 10.4088/JCP.15m10381.
- Kautzky, A. *et al.* (2018) 'Refining prediction in treatment-resistant depression: Results of machine learning analyses in the TRD III sample', *Journal of Clinical Psychiatry*. doi: 10.4088/JCP.16m11385.
- Kawakami, F. *et al.* (1997) 'Loss of day-night differences in VIP mRNA levels in the suprachiasmatic nucleus of aged rats', *Neuroscience Letters*. doi: 10.1016/S0304-3940(97)13355-9.
- Kayser, M. S. and Biron, D. (2016) 'Sleep and development in genetically tractable model organisms', *Genetics*. doi: 10.1534/genetics.116.189589.
- Kayser, M. S., Yue, Z. and Sehgal, A. (2014) 'A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*.', *Science*, 344(6181), pp. 269–274. doi: 10.1126/science.1250553.
- Keene, A. C. and Joiner, W. J. (2015) 'Neurodegeneration: Paying it off with sleep', *Current Biology*. doi: 10.1016/j.cub.2015.02.003.
- Kim, H. J. *et al.* (2014) 'Therapeutic modulation of eIF2 α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models', *Nature Genetics*. doi: 10.1038/ng.2853.
- Kishi, T., Matsunaga, S. and Iwata, N. (2015) 'Suvorexant for primary insomnia: A systematic review and meta-analysis of randomized placebo-controlled trials', *PLoS ONE*. doi: 10.1371/journal.pone.0136910.
- Kitamoto, T. (2001) 'Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive *shibire* allele in defined neurons', *Journal of Neurobiology*. doi: 10.1002/neu.1018.
- Kivipelto, M. *et al.* (2013) 'The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): Study design and progress', *Alzheimer's and Dementia*, 9(6), pp. 657–665. doi: 10.1016/j.jalz.2012.09.012.
- Klapoetke, N. C. *et al.* (2014) 'Independent optical excitation of distinct neural populations', *Nature Methods*. doi: 10.1038/nmeth.2836.
- Koffel, E. *et al.* (2018) 'A randomized controlled pilot study of CBT-I Coach: Feasibility, acceptability, and potential impact of a mobile phone application for patients in cognitive behavioral therapy for insomnia', *Health Informatics Journal*. doi: 10.1177/1460458216656472.
- Koffel, E. A., Koffel, J. B. and Gehrman, P. R. (2015) 'A meta-analysis of group cognitive behavioral therapy for insomnia', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2014.05.001.

- Koh, K. *et al.* (2006) 'A *Drosophila* model for age-associated changes in sleep:wake cycles.', *Proceedings of the National Academy of Sciences of the United States of America*, 103(37), pp. 13843–13847. doi: 10.1073/pnas.0605903103.
- Koh, K. *et al.* (2008) 'Identification of SLEEPLESS, a Sleep-Promoting Factor', *Science*, 321(5887), pp. 372–376. doi: 10.1126/science.1155942.
- Konopka, R. J. and Benzer, S. (1971) 'Clock mutants of *Drosophila melanogaster*.', *Proceedings of the National Academy of Sciences of the United States of America*, 68(9), pp. 2112–6. doi: 10.1073/pnas.68.9.2112.
- Kripke, D. F., Langer, R. D. and Kline, L. E. (2012) 'Hypnotics' association with mortality or cancer: A matched cohort study', *BMJ Open*. doi: 10.1136/bmjopen-2012-000850.
- Krystal, A. D. and Edinger, J. D. (2010) 'Sleep EEG predictors and correlates of the response to cognitive behavioral therapy for insomnia.', *Sleep*, 33(5), pp. 669–77.
- Kume, K. *et al.* (2005) 'Dopamine Is a Regulator of Arousal in the Fruit Fly', 25(32), pp. 7377–7384. doi: 10.1523/JNEUROSCI.2048-05.2005.
- Kumru, H. *et al.* (2007) 'Relation between subtype of Parkinson's disease and REM sleep behavior disorder', *Sleep Medicine*. doi: 10.1016/j.sleep.2007.02.005.
- Kuriyama, A. and Tabata, H. (2017) 'Suvorexant for the treatment of primary insomnia: A systematic review and meta-analysis', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2016.09.004.
- Kyle, S. D. *et al.* (2015) 'Towards standardisation and improved understanding of sleep restriction therapy for insomnia disorder: A systematic examination of CBT-I trial content', *Sleep Medicine Reviews*, pp. 83–88. doi: 10.1016/j.smr.2015.02.003.
- Lamaze, A. *et al.* (2017) 'Regulation of sleep plasticity by a thermo-sensitive circuit in *Drosophila*', *Scientific Reports*. doi: 10.1038/srep40304.
- Lancaster, C. *et al.* (2016) 'Posttraumatic Stress Disorder: Overview of Evidence-Based Assessment and Treatment', *Journal of Clinical Medicine*. doi: 10.3390/jcm5110105.
- Lancee, J. *et al.* (2013) 'Motivational support provided via email improves the effectiveness of internet-delivered self-help treatment for insomnia: A randomized trial', *Behaviour Research and Therapy*. doi: 10.1016/j.brat.2013.09.004.
- LeBlanc, M. *et al.* (2009) 'Incidence and risk factors of insomnia in a population-based sample', *Sleep*. doi: 10.1093/sleep/32.8.1027.
- Li, Y. *et al.* (2010) 'A *Drosophila* model for TDP-43 proteinopathy', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.0913602107.
- Liang, X., Holy, T. E. and Taghert, P. H. (2016) 'Synchronous *drosophila* circadian pacemakers display nonsynchronous Ca²⁺ rhythms in vivo', *Science*. doi: 10.1126/science.aad3997.
- Liguori, C. *et al.* (2014) 'Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease', *JAMA Neurology*. doi: 10.1001/jamaneurol.2014.2510.

- Lin, Y. Q. and Chen, S. Di (2018) 'RBD: a red flag for cognitive impairment in Parkinson's disease?', *Sleep Medicine*. doi: 10.1016/j.sleep.2018.01.006.
- Lind, M. J. and Gehrman, P. R. (2016) 'Genetic pathways to insomnia', *Brain Sciences*. doi: 10.3390/brainsci6040064.
- Liu, Q. *et al.* (2012) 'Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in *Drosophila*', *Current Biology*. doi: 10.1016/j.cub.2012.09.008.
- Liu, S. *et al.* (2014) 'WIDE AWAKE mediates the circadian timing of sleep onset', *Neuron*, 82(1), pp. 151–166. doi: 10.1016/j.neuron.2014.01.040.
- Liu, S. *et al.* (2016) 'Sleep drive is encoded by neural plastic changes in a dedicated circuit', *Cell*. doi: 10.1016/j.cell.2016.04.013.
- Lone, S. R. *et al.* (2016) 'Social experience is sufficient to modulate sleep need of *Drosophila* without increasing wakefulness', *PLoS ONE*. doi: 10.1371/journal.pone.0150596.
- Low, D., Wu, M. and Spira, A. (2019) 'Sleep Duration and Cognition in a Nationally Representative Sample of U.S. Older Adults.', *American Journal of Geriatric Psychiatry*.
- Luan, H. *et al.* (2006) 'Functional Dissection of a Neuronal Network Required for Cuticle Tanning and Wing Expansion in *Drosophila*', *Journal of Neuroscience*. doi: 10.1523/jneurosci.3916-05.2006.
- Luo, L., Tully, T. and White, K. (1992) 'Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for *appl* gene', *Neuron*. doi: 10.1016/0896-6273(92)90024-8.
- Luo, W. *et al.* (2012) 'Old flies have a robust central oscillator but weaker behavioral rhythms that can be improved by genetic and environmental manipulations', *Aging Cell*, 11(3), pp. 428–438. doi: 10.1111/j.1474-9726.2012.00800.x.
- Madeira, M. D. *et al.* (1995) 'Age and sex do not affect the volume, cell numbers, or cell size of the suprachiasmatic nucleus of the rat: An unbiased stereological study', *Journal of Comparative Neurology*. doi: 10.1002/cne.903610404.
- Majercak, J. *et al.* (1999) 'How a circadian clock adapts to seasonal decreases in temperature and day length', *Neuron*, 24(1), pp. 219–230. doi: 10.1016/S0896-6273(00)80834-X.
- Malhotra, R. K. (2018) 'Neurodegenerative Disorders and Sleep', *Sleep Medicine Clinics*. doi: 10.1016/j.jsmc.2017.09.006.
- Mander, B. A., Winer, J. R. and Walker, M. P. (2017a) 'Sleep and Human Aging', *Neuron*, pp. 19–36. doi: 10.1016/j.neuron.2017.02.004.
- Mander, B. A., Winer, J. R. and Walker, M. P. (2017b) 'Sleep and Human Aging', *Neuron*. doi: 10.1016/j.neuron.2017.02.004.
- Mann, J. R. *et al.* (2019) 'RNA Binding Antagonizes Neurotoxic Phase Transitions of TDP-43', *Neuron*. doi: 10.1016/j.neuron.2019.01.048.
- Mathis, S. *et al.* (2019) 'Genetics of amyotrophic lateral sclerosis: A review', *Journal of the Neurological Sciences*. doi:

10.1016/j.jns.2019.02.030.

- Matthews, E. E. *et al.* (2013) 'Adherence to cognitive behavioral therapy for insomnia: A systematic review', *Sleep Medicine Reviews*, pp. 453–464. doi: 10.1016/j.smrv.2013.01.001.
- McCurry, S. M. *et al.* (1999) 'Characteristics of sleep disturbance in community-dwelling Alzheimer's disease patients', *Journal of Geriatric Psychiatry and Neurology*. doi: 10.1177/089198879901200203.
- McGurk, L. *et al.* (2018) 'Poly(ADP-Ribose) Prevents Pathological Phase Separation of TDP-43 by Promoting Liquid Demixing and Stress Granule Localization', *Molecular Cell*. doi: 10.1016/j.molcel.2018.07.002.
- McGurk, L., Berson, A. and Bonini, N. M. (2015) 'Drosophila as an in vivo model for human neurodegenerative disease', *Genetics*. doi: 10.1534/genetics.115.179457.
- McShane, B. B. *et al.* (2010) 'Characterization of the bout durations of sleep and wakefulness', *Journal of Neuroscience Methods*. doi: 10.1016/j.jneumeth.2010.08.024.
- Mershin, A. *et al.* (2004) 'Learning and memory deficits upon TAU accumulation in Drosophila mushroom body neurons', *Learning and Memory*. doi: 10.1101/lm.70804.
- Mhatre, S. D. *et al.* (2014) 'Synaptic abnormalities in a Drosophila model of Alzheimer's disease', *DMM Disease Models and Mechanisms*. doi: 10.1242/dmm.012104.
- Miller, C. B. *et al.* (2014) 'The evidence base of sleep restriction therapy for treating insomnia disorder', *Sleep Medicine Reviews*. Elsevier Ltd, pp. 415–424. doi: 10.1016/j.smrv.2014.01.006.
- Mitchell, M. D. *et al.* (2012) 'Comparative effectiveness of cognitive behavioral therapy for insomnia: A systematic review', *BMC Family Practice*. doi: 10.1186/1471-2296-13-40.
- Molliex, A. *et al.* (2015) 'Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization', *Cell*. doi: 10.1016/j.cell.2015.09.015.
- Moran, M. *et al.* (2005) 'Sleep disturbance in mild to moderate Alzheimer's disease', *Sleep Medicine*. doi: 10.1016/j.sleep.2004.12.005.
- Morin, C. M. *et al.* (2006) 'Epidemiology of insomnia: Prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors', *Sleep Medicine*, 7(2), pp. 123–130. doi: 10.1016/j.sleep.2005.08.008.
- Morin, C. M., Vallières, A., *et al.* (2009) 'Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: A randomized controlled trial', *JAMA - Journal of the American Medical Association*. doi: 10.1001/jama.2009.682.
- Morin, C. M., Bélanger, L., *et al.* (2009) 'The natural history of insomnia a population-based 3-year longitudinal study', *Archives of Internal Medicine*. doi: 10.1001/archinternmed.2008.610.
- Münch, M. *et al.* (2004) 'The frontal predominance in human EEG delta activity after sleep loss decreases with age', *European Journal of Neuroscience*. doi: 10.1111/j.1460-9568.2004.03580.x.
- Musiek, E. S. and Holtzman, D. M. (2016) 'Mechanisms linking circadian clocks, sleep, and neurodegeneration', *Science*. doi: 10.1126/science.aah4968.

- Naidoo, N. *et al.* (2012) 'Role of homer proteins in the maintenance of sleep-wake states', *PLoS ONE*. doi: 10.1371/journal.pone.0035174.
- Nall, A. H. *et al.* (2016) 'Caffeine promotes wakefulness via dopamine signaling in *Drosophila*', *Scientific Reports*. doi: 10.1038/srep20938.
- Nath, R. D. *et al.* (2017) 'The Jellyfish *Cassiopea* Exhibits a Sleep-like State', *Current Biology*. doi: 10.1016/j.cub.2017.08.014.
- Néric, N. and Desplan, C. (2016) 'From the Eye to the Brain. Development of the *Drosophila* Visual System', in *Current Topics in Developmental Biology*. doi: 10.1016/bs.ctdb.2015.11.032.
- Neumann, M. *et al.* (2006) 'Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis', *Science*. doi: 10.1126/science.1134108.
- Ngandu, T. *et al.* (2015) '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', *The Lancet*, 385(9984), pp. 2255–2263. doi: 10.1016/S0140-6736(15)60461-5.
- Nilsberth, C. *et al.* (2001) 'The " Arctic " APP mutation (E693G) causes Alzheimer ' s disease by enhanced A β protofibril formation', pp. 18–23.
- Okamoto-Mizuno, K. and Mizuno, K. (2012) 'Effects of thermal environment on sleep and circadian rhythm', *Journal of Physiological Anthropology*, pp. 1–9. doi: 10.1186/1880-6805-31-14.
- Ozminkowski, R. J., Wang, S. and Walsh, J. K. (2007) 'The direct and indirect costs of untreated insomnia in adults in the United States', *Sleep*, 30(3), pp. 263–273.
- Palesh, O. *et al.* (2018) 'Feasibility and acceptability of brief behavioral therapy for cancer-related insomnia: effects on insomnia and circadian rhythm during chemotherapy: a phase II randomised multicentre controlled trial', *British Journal of Cancer*. doi: 10.1038/s41416-018-0154-2.
- Pallesen, S. *et al.* (2019) 'The 5-HTTLPR rs25531 LALA-genotype increases the risk of insomnia symptoms among shift workers', *Sleep Medicine*. doi: 10.1016/j.sleep.2019.04.009.
- Panda, S., Gourie-Devi, M. and Sharma, A. (2019) 'Sleep disorders in amyotrophic lateral sclerosis: A questionnaire-based study from India', *Neurology India*. doi: 10.4103/0028-3886.232327.
- Pandi-Perumal, S. R. *et al.* (2002) 'Senescence, sleep, and circadian rhythms', *Ageing Research Reviews*, pp. 559–604. doi: 10.1016/S1568-1637(02)00014-4.
- Parisky, K. M. *et al.* (2008) 'PDF Cells Are a GABA-Responsive Wake-Promoting Component of the *Drosophila* Sleep Circuit', *Neuron*. doi: 10.1016/j.neuron.2008.10.042.
- Parisky, K. M. *et al.* (2016) 'Reorganization of Sleep by Temperature in *Drosophila* Requires Light, the Homeostat, and the Circadian Clock', *Current Biology*, 26(7), pp. 882–892. doi: 10.1016/j.cub.2016.02.011.
- Passos, G. S. *et al.* (2017) 'Chronotype and anxiety are associated in patients with chronic primary insomnia', *Revista Brasileira de Psiquiatria*. doi: 10.1590/1516-4446-2016-2007.

- Pawlyk, A. C. *et al.* (2008) 'Stress-induced changes in sleep in rodents: Models and mechanisms', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2007.06.001.
- Perkins, L. A. *et al.* (2015) 'The transgenic RNAi project at Harvard medical school: Resources and validation', *Genetics*. doi: 10.1534/genetics.115.180208.
- Perlis, M. L. *et al.* (2014) 'Models of Insomnia', pp. 850–866.
- Perlis, M. L., Corbitt, C. B. and Kloss, J. D. (2014) 'Insomnia research: 3Ps and beyond', *Sleep Medicine Reviews*, pp. 191–193. doi: 10.1016/j.smrv.2014.01.003.
- Peter-Derex, L. *et al.* (2015) 'Sleep and Alzheimer's disease', *Sleep Medicine Reviews*. doi: 10.1016/j.smrv.2014.03.007.
- Petrovsky, D. V. *et al.* (2018) 'Sleep disruption and quality of life in persons with dementia: A state-of-the-art review', *Geriatric Nursing*. doi: 10.1016/j.gerinurse.2018.04.014.
- Pigeon, W. R. *et al.* (2017) 'Brief cognitive behavioral therapy for insomnia delivered to depressed veterans receiving primary care services: A pilot study', *Journal of Affective Disorders*. doi: 10.1016/j.jad.2017.04.003.
- Piper, M. D. W. and Partridge, L. (2018) 'Drosophila as a model for ageing', *Biochimica et Biophysica Acta - Molecular Basis of Disease*. doi: 10.1016/j.bbdis.2017.09.016.
- Pons, M. *et al.* (2017) 'Splicing factors act as genetic modulators of TDP-43 production in a new autoregulatory TDP-43 Drosophila model', *Human Molecular Genetics*. doi: 10.1093/hmg/ddx229.
- Postuma, R. B. *et al.* (2008) 'REM sleep behaviour disorder in Parkinson's disease is associated with specific motor features', *Journal of Neurology, Neurosurgery and Psychiatry*. doi: 10.1136/jnnp.2008.149195.
- Postuma, R. B. *et al.* (2009) 'Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder', *Neurology*. doi: 10.1212/01.wnl.0000340980.19702.6e.
- Potdar, S. *et al.* (2018) 'Sleep deprivation negatively impacts reproductive output in *Drosophila melanogaster*', *The Journal of Experimental Biology*, 221(6), p. jeb174771. doi: 10.1242/jeb.174771.
- Qaseem, A. *et al.* (2016) 'Management of chronic insomnia disorder in adults: A clinical practice guideline from the American college of physicians', *Annals of Internal Medicine*, 165(2), pp. 125–133. doi: 10.7326/M15-2175.
- Qiu, J. and Hardin, P. E. (1996) 'per mRNA cycling is locked to lights-off under photoperiodic conditions that support circadian feedback loop function.', *Molecular and cellular biology*, 16(8), pp. 4182–8. doi: 10.1128/MCB.16.8.4182.
- Raizen, D. M. *et al.* (2008) 'Lethargus is a Caenorhabditis elegans sleep-like state', *Nature*. doi: 10.1038/nature06535.
- Rauch, S. A. M. *et al.* (2009) 'Prolonged exposure for PTSD in a Veterans Health Administration PTSD clinic', *Journal of Traumatic Stress*. doi: 10.1002/jts.20380.
- Reitz, C., Brayne, C. and Mayeux, R. (2011) 'Epidemiology of Alzheimer disease', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2011.2.
- Rera, M., Azizi, M. J. and Walker, D. W. (2013) 'Organ-specific mediation of lifespan extension: More than a gut feeling?', *Ageing Research Reviews*. doi: 10.1016/j.arr.2012.05.003.

- Revel, F. G. *et al.* (2009) 'Rodent models of insomnia: A review of experimental procedures that induce sleep disturbances', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2009.03.002.
- Reynold, A. *et al.* (2014) 'Negative Effects of Time in Bed Extension : A Pilot Study', *Journal of sleep medicine and disorders*, 1(1), pp. 1–13.
- Riedel, N. *et al.* (2012) 'Insomnia and urban neighbourhood contexts - Are associations modified by individual social characteristics and change of residence? Results from a population-based study using residential histories', *BMC Public Health*. doi: 10.1186/1471-2458-12-810.
- Riemann, D. *et al.* (2015) 'The neurobiology, investigation, and treatment of chronic insomnia', *The Lancet Neurology*. doi: 10.1016/S1474-4422(15)00021-6.
- Riemann, D. *et al.* (2019) 'Sleep, insomnia, and depression', *Neuropsychopharmacology*. doi: 10.1038/s41386-019-0411-y.
- Riemann, D. and Perlis, M. L. (2009) 'The treatments of chronic insomnia: A review of benzodiazepine receptor agonists and psychological and behavioral therapies', *Sleep Medicine Reviews*, pp. 205–214. doi: 10.1016/j.smrv.2008.06.001.
- Ritterband, L. M. *et al.* (2017) 'Effect of a web-based cognitive behavior therapy for insomnia intervention with 1-year follow-up: A randomized clinical trial', *JAMA Psychiatry*. doi: 10.1001/jamapsychiatry.2016.3249.
- Robertson, M. and Keene, A. C. (2013) 'Molecular Mechanisms of Age-Related Sleep Loss in the Fruit Fly - A Mini-Review', *Gerontology*, 59(4), pp. 334–339. doi: 10.1159/000348576.
- Roffwarg, H. P., Muzio, J. N. and Dement, W. C. (1966) 'Ontogenetic development of the human sleep-dream cycle', *Science*. doi: 10.1126/science.152.3722.604.
- Roh, Jee Hoon *et al.* (2012) 'Disruption of the sleep-wake cycle and diurnal fluctuation of amyloid- β in mice with Alzheimer's disease pathology', *Science Translational Medicine*. doi: 10.1126/scitranslmed.3004291.
- Roh, J H *et al.* (2012) 'Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology', *Sci Transl Med*, 4(150), p. 150ra122. doi: 10.1126/scitranslmed.3004291.
- Roost, C. *et al.* (2015) 'Structure and thermodynamics of N⁶-methyladenosine in RNA: A spring-loaded base modification', *Journal of the American Chemical Society*. doi: 10.1021/ja513080v.
- Roth, T. (2007) 'Insomnia: Definition, prevalence, etiology, and consequences', *Journal of Clinical Sleep Medicine*.
- Ruff, R. R. *et al.* (2018) 'Neighborhood Stigma and Sleep: Findings from a Pilot Study of Low-Income Housing Residents in New York City', *Behavioral Medicine*. doi: 10.1080/08964289.2016.1203754.
- Saarelainen, L. *et al.* (2018) 'Risk of death associated with new benzodiazepine use among persons with Alzheimer disease: A matched cohort study.', *Int J Geriatr Psychiatry*, 33(4), pp. 583–590.
- Sarsour, K. *et al.* (2010) 'Association of insomnia severity and comorbid medical and psychiatric disorders in a health plan-based sample: Insomnia severity and comorbidities', *Sleep Medicine*, 11(1), pp. 69–74. doi: 10.1016/j.sleep.2009.02.008.

- Sateia, M. J. *et al.* (2000) 'Evaluation of chronic insomnia. An American Academy of Sleep Medicine review.', *Sleep*, 23(2), pp. 243–308.
- Schenck, C. H., Boeve, B. F. and Mahowald, M. W. (2013) 'Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: A 16-year update on a previously reported series', *Sleep Medicine*. doi: 10.1016/j.sleep.2012.10.009.
- Schenck, C. H., Bundlie, S. R. and Mahowald, M. W. (1996) 'Delayed emergence of a parkinsonian disorder in 38% of 29 older, men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder', *Neurology*. doi: 10.1212/WNL.46.2.388.
- Schlichting, M. *et al.* (2019) 'Light-mediated circuit switching in the *Drosophila* neuronal clock network', *BioRxiv*.
- Schmidt, C., Peigneux, P. and Cajochen, C. (2012) 'Age-related changes in sleep and circadian rhythms: Impact on cognitive performance and underlying neuroanatomical networks', *Frontiers in Neurology*. doi: 10.3389/fneur.2012.00118.
- Schwabe, T. *et al.* (2005) 'GPCR signaling is required for blood-brain barrier formation in *Drosophila*', *Cell*. doi: 10.1016/j.cell.2005.08.037.
- Scott, K. (2018) 'Gustatory Processing in *Drosophila melanogaster*', *Annual Review of Entomology*. doi: 10.1146/annurev-ento-020117-043331.
- Seelig, J. D. *et al.* (2010) 'Two-photon calcium imaging from head-fixed *Drosophila* during optomotor walking behavior', *Nature Methods*. doi: 10.1038/nmeth.1468.
- Seidner, G. *et al.* (2015) 'Identification of Neurons with a Privileged Role in Sleep Homeostasis in *Drosophila melanogaster*', *Current Biology*. doi: 10.1016/j.cub.2015.10.006.
- Seugnet, L. *et al.* (2009) 'Identifying Sleep Regulatory Genes Using a *Drosophila* Model of Insomnia', *Journal of Neuroscience*, 29(22), pp. 7148–7157. doi: 10.1523/JNEUROSCI.5629-08.2009.
- Shaw, P. J. *et al.* (2000) 'Correlates of Sleep and Waking in *Drosophila melanogaster*', 287(January).
- Shi, M. *et al.* (2014) 'Identification of Redeye, a new sleep-regulating protein whose expression is modulated by sleep amount', pp. 1–17. doi: 10.7554/eLife.01473.
- Sivertsen, B., Vedaa, Ø. and Nordgreen, T. (2013) 'The Future of Insomnia Treatment—the Challenge of Implementation', *Sleep*, 36(3), pp. 303–304. doi: 10.5665/sleep.2432.
- Slocumb, M. E. *et al.* (2015) 'Enhanced sleep is an evolutionarily adaptive response to starvation stress in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0131275.
- Smith, M. T. *et al.* (2002) 'Comparative meta-analysis of pharmacotherapy and behavior therapy for persistent insomnia', *American Journal of Psychiatry*, pp. 5–11. doi: 10.1176/appi.ajp.159.1.5.
- Spielman, A. J. (1986) 'Assessment of insomnia', *Clinical Psychology Review*, 6(1), pp. 11–25. doi: 10.1016/0272-7358(86)90015-2.
- Spielman, A. J., Caruso, L. S. and Glovinsky, P. B. (1987) 'A Behavioral Perspective on Insomnia Treatment', *Psychiatric*

- Clinics of North America*, 10(4), pp. 541–553. doi: 10.1016/s0193-953x(18)30532-x.
- Spielman, A., Saskin, P. and Thorpy, M. J. (1987) 'Treatment of Chronic Insomnia by Restriction of Time in Bed', *Sleep*, 10(1), pp. 45–56. doi: 10.1093/sleep/10.1.45.
- Spira, A. P. *et al.* (2013) 'Self-reported sleep and β -amyloid deposition in community-dwelling older adults', *JAMA Neurology*. doi: 10.1001/jamaneurol.2013.4258.
- Sprecher, K. E. *et al.* (2015) 'Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults', *Neurobiology of Aging*. doi: 10.1016/j.neurobiolaging.2015.05.004.
- Stavropoulos, N. and Young, M. W. (2011) 'Article insomnia and Cullin-3 Regulate Sleep and Wakefulness in *Drosophila*', *Neuron*. Elsevier Inc., 72(6), pp. 964–976. doi: 10.1016/j.neuron.2011.12.003.
- Sterniczuk, R. *et al.* (2013) 'Sleep Disturbance is Associated with Incident Dementia and Mortality', *Current Alzheimer Research*. doi: 10.2174/15672050113109990134.
- Stork, T. *et al.* (2008) 'Organization and Function of the Blood Brain Barrier in *Drosophila*', *Journal of Neuroscience*. doi: 10.1523/jneurosci.4367-07.2008.
- Sun, X. *et al.* (2016) 'Melatonin attenuates hLRRK2-induced Sleep disturbances and synaptic dysfunction in a *Drosophila* model of Parkinson's disease', *Molecular Medicine Reports*. doi: 10.3892/mmr.2016.4991.
- Sun, Z. *et al.* (2011) 'Molecular determinants and genetic modifiers of aggregation and toxicity for the als disease protein fus/tls', *PLoS Biology*. doi: 10.1371/journal.pbio.1000614.
- Sutton, E. L. (2014) 'Insomnia', *Med Clin North Am*, 98(3), pp. 565–581.
- Swaab, D. F., Fliers, E. and Partiman, T. S. (1985) 'The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia', *Brain Research*. doi: 10.1016/0006-8993(85)91350-2.
- Tabuchi, M. *et al.* (2015) 'Sleep interacts with $\alpha\beta$ to modulate intrinsic neuronal excitability', *Current Biology*. doi: 10.1016/j.cub.2015.01.016.
- Talay, M. *et al.* (2017) 'Transsynaptic Mapping of Second-Order Taste Neurons in Flies by trans-Tango', *Neuron*. doi: 10.1016/j.neuron.2017.10.011.
- Taub, J. M. and Berger, R. J. (1973) 'Performance and Mood Following Variations in the Length and Timing of Sleep', *Psychophysiology*, 10(6), pp. 559–570. doi: 10.1111/j.1469-8986.1973.tb00805.x.
- Taylor, D. J., Lichstein, K. L. and Durrence, H. H. (2003) 'Insomnia as a health risk factor.', *Behavioral sleep medicine*. doi: 10.1207/S15402010BSM0104_5.
- Thase, M. E. (2014) 'Using biomarkers to predict treatment response in major depressive disorder: Evidence from past and present studies', *Dialogues in Clinical Neuroscience*.
- Thorpy, M. (2017) 'International classification of sleep disorders', in *Sleep Disorders Medicine: Basic Science, Technical Considerations and Clinical Aspects: Fourth Edition*. doi: 10.1007/978-1-4939-6578-6_27.
- Tononi, G. and Cirelli, C. (2006) 'Sleep function and synaptic homeostasis', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2005.05.002.

- Toth, L. A. and Bhargava, P. (2013) 'Animal models of sleep disorders', *Comparative Medicine*, 63(2), pp. 91–104. doi: 10.1300/J094v06n03_16.
- Trauer, J. M. *et al.* (2015) 'Cognitive behavioral therapy for chronic insomnia: A systematic review and meta-analysis', *Annals of Internal Medicine*, 163(3), pp. 191–204. doi: 10.7326/M14-2841.
- Tsukahara, S. *et al.* (2005) 'Age-related change and its sex differences in histoarchitecture of the hypothalamic suprachiasmatic nucleus of F344/N rats', *Experimental Gerontology*. doi: 10.1016/j.exger.2004.10.003.
- Udan-Johns, M. *et al.* (2014) 'Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones', *Human Molecular Genetics*. doi: 10.1093/hmg/ddt408.
- Ueno, T. *et al.* (2012) 'Identification of a dopamine pathway that regulates sleep and arousal in *Drosophila*', *Nature Neuroscience*. doi: 10.1038/nn.3238.
- Veatch, O. J. *et al.* (2017) 'Pleiotropic genetic effects influencing sleep and neurological disorders', *The Lancet Neurology*, pp. 158–170. doi: 10.1016/S1474-4422(16)30339-8.
- Vienne, J. *et al.* (2016) 'Age-Related Reduction of Recovery Sleep and Arousal Threshold in *Drosophila*', *Sleep*, 39(8), pp. 1613–1624. doi: 10.5665/sleep.6032.
- de Vivo, L. *et al.* (2019) 'Evidence for sleep-dependent synaptic renormalization in mouse pups.', *Sleep*.
- Wagley, J. N. *et al.* (2013) 'Effectiveness of abbreviated CBT for insomnia in psychiatric outpatients: Sleep and depression outcomes', *Journal of Clinical Psychology*. doi: 10.1002/jclp.21927.
- Walker, D. L. *et al.* (2002) 'Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats', *Journal of Neuroscience*.
- Wang, F. *et al.* (2014) 'Meta-analysis on night shift work and risk of metabolic syndrome', *Obesity Reviews*. doi: 10.1111/obr.12194.
- Weber, F. *et al.* (2018) 'Regulation of REM and Non-REM Sleep by Periaqueductal GABAergic Neurons', *Nature Communications*. doi: 10.1038/s41467-017-02765-w.
- Wheaton, A. G. *et al.* (2016) 'Sleep Duration and Injury-Related Risk Behaviors Among High School Students — United States, 2007–2013', *MMWR. Morbidity and Mortality Weekly Report*. doi: 10.15585/mmwr.mm6513a1.
- Wheeler, J. R. *et al.* (2016) 'Distinct stages in stress granule assembly and disassembly', *eLife*. doi: 10.7554/eLife.18413.
- Wimmer, M. E. *et al.* (2013) 'Aging in mice reduces the ability to sustain sleep/wake states', *PLoS ONE*. doi: 10.1371/journal.pone.0081880.
- Wittmann, C. W. *et al.* (2001) 'Tauopathy in *Drosophila*: Neurodegeneration without neurofibrillary tangles', *Science*. doi: 10.1126/science.1062382.
- Wu, J. Q. *et al.* (2015) 'Cognitive behavioral therapy for insomnia comorbid with psychiatric and medical conditions a meta-analysis', *JAMA Internal Medicine*, 175(9), pp. 1461–1472. doi: 10.1001/jamainternmed.2015.3006.
- Wu, M. N. *et al.* (2010) 'SLEEPLESS, a *Ly-6/neurotoxin* family member, regulates the levels, localization and activity of Shaker', *Nature Neuroscience*. doi: 10.1038/nn.2454.

- Xie, L. *et al.* (2013) 'Sleep drives metabolite clearance from the adult brain', *Science*. doi: 10.1126/science.1241224.
- Yang, C. M., Lin, S. C. and Cheng, C. P. (2013) 'Transient insomnia versus chronic insomnia: A comparison study of sleep-related psychological/behavioral characteristics', *Journal of Clinical Psychology*. doi: 10.1002/jclp.22000.
- Yoshii, T., Hermann-Luibl, C. and Helfrich-Förster, C. (2016) 'Circadian light-input pathways in *Drosophila*', *Communicative and Integrative Biology*. doi: 10.1080/19420889.2015.1102805.
- Zhan, L. *et al.* (2013) 'Identification of Genetic Modifiers of TDP-43 Neurotoxicity in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0057214.
- Zhang, K., Coyne, A. N. and Lloyd, T. E. (2018) '*Drosophila* models of amyotrophic lateral sclerosis with defects in RNA metabolism', *Brain Research*. doi: 10.1016/j.brainres.2018.04.043.
- Zhang, S. *et al.* (2008) 'Increased dopaminergic signaling impairs aversive olfactory memory retention in *Drosophila*', *Biochemical and Biophysical Research Communications*, 370(1), pp. 82–86. doi: 10.1016/j.bbrc.2008.03.015.
- Zhang, S. L. *et al.* (2018) 'A Circadian Clock in the Blood-Brain Barrier Regulates Xenobiotic Efflux', *Cell*. doi: 10.1016/j.cell.2018.02.017.
- Zhou, J. *et al.* (2015) 'Dynamic m6 A mRNA methylation directs translational control of heat shock response', *Nature*. doi: 10.1038/nature15377.
- Zhou, J. *et al.* (2018) 'N6-Methyladenosine Guides mRNA Alternative Translation during Integrated Stress Response', *Molecular Cell*. doi: 10.1016/j.molcel.2018.01.019.
- Zhu, Y. *et al.* (2018) 'Chronic sleep disruption advances the temporal progression of tauopathy in P301S mutant mice', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0275-18.2018.
- Ziehm, M., Piper, M. D. and Thornton, J. M. (2013) 'Analysing variation in *Drosophila* aging across independent experimental studies: A meta-analysis of survival data', *Aging Cell*. doi: 10.1111/accel.12123.

Chapter 2:

A *Drosophila* Model of Sleep Restriction Therapy for Insomnia

Samuel J. Belfer^{1,2}, Alexander G. Bashaw², Michael L. Perlis²⁻⁴ and

Matthew S. Kayser^{1,2,4,5}

¹Department of Neuroscience,

²Department of Psychiatry,

³Behavioral Sleep Medicine Program,

⁴Center for Sleep and Circadian Neurobiology, and

⁵Chronobiology Program,

Perelman School of Medicine at the University of Pennsylvania

Acknowledgements

We thank Kyunghye 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).

This chapter was published in *Molecular Psychiatry* (2019).

DOI: 10.1038/s41380-019-0376-6

AGB analyzed data for this chapter.

MLP provided conceptual guidance and edited this manuscript.

MSK provided experimental and conceptual guidance and edited this manuscript.

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 animal 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.

Introduction

Insomnia is the most common sleep disorder among adults, with significant public health and economic consequences (Dement and Pelayo, 1997; Ozminkowski, Wang and Walsh, 2007; Daley *et al.*, 2009; Sarsour *et al.*, 2010). Cognitive Behavioral Therapy for Insomnia (CBT-I) is the first-line intervention for treatment of insomnia (Qaseem *et al.*, 2016). 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 (Miller *et al.*, 2014). 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 (Spielman, 1986). 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 (Morin *et al.*, 2006; Trauer *et al.*, 2015; Wu *et al.*, 2015), limited accessibility of practitioners and long duration of therapy are obstacles to broad implementation (Mitchell *et al.*, 2012; Sivertsen, Vedaa and Nordgreen, 2013; Kathol and Arnedt, 2016).

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 impairment (Roth, 2007). 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 (Toth and Bhargava, 2013), 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 (Cirelli *et al.*, 2005; Kume *et al.*, 2005; Bushey *et al.*, 2007; Koh *et al.*, 2008; Stavropoulos and Young, 2011). It is unlikely that these short sleepers simply do not need sleep, as mutants exhibit shortened lifespan and/or memory deficits (Cirelli *et al.*, 2005; Bushey *et al.*, 2007, 2010; Koh *et al.*, 2008; Zhang *et al.*, 2008; Stavropoulos and Young, 2011). In addition, a fly line generated by laboratory selection for insomnia-like traits (Seugnet *et al.*, 2009) 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 (Hasan *et al.*, 2012; Robertson and Keene, 2013; Mander, Winer and Walker, 2017a). 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 ($A\beta$) worsens sleep while poor sleep accelerates $A\beta$ accumulation (Kang *et al.*, 2009; J H Roh *et al.*, 2012; Y.-E. S. Ju, Lucey and Holtzman, 2014). Indeed, in *Drosophila*, $A\beta$ accumulation in the brain leads to reduced and fragmented sleep (Tabuchi *et al.*, 2015) and shortened lifespan (Koichi Iijima *et al.*, 2004; Tabuchi *et al.*, 2015). Related lines of work also suggest that sleep might serve as a modifiable risk factor in AD progression (Kang *et al.*, 2009; J H Roh *et al.*, 2012; Y.-E. S. Ju, Lucey and Holtzman, 2014; Tabuchi *et al.*, 2015; Dissel *et al.*, 2017). While hypnotic use is associated with increased morbidity/mortality in individuals with AD (Saarelainen *et al.*, 2018), behavioral therapies show promise for improving sleep (Riemann and Perlis, 2009; Krystal and Edinger, 2010; Kivipelto *et al.*, 2013; Ngandu *et al.*, 2015). 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 $A\beta$ 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* (Crowther *et al.*, 2005) 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 x 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 inactivity (Gilestro, 2012). Data was processed using PySolo software (Gilestro and Cirelli, 2009). 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 described (Kayser, Yue and Sehgal, 2014). Briefly, a 685g rubber weight was dropped onto a rack supporting small DAMs monitors (Trikinetics, *wide awake*) or MultiBeam Activity Monitors (Trikinetics, A 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 4E; 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: * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$. 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 (Kayser, Yue and Sehgal, 2014; Dilley *et al.*, 2018).

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 (Hendricks *et al.*, 2000; Shaw *et al.*, 2000). 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 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 deficits (Sateia *et al.*, 2000). 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 *per⁰¹* (Konopka and Benzer, 1971). 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, Kume et al. 2005). 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 (Kyle *et al.*, 2015). 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 (Hendricks *et al.*, 2000; Shaw *et al.*, 2000). 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 (Majercak *et al.*, 1999; Hendricks *et al.*, 2000; Shaw *et al.*,

2000). 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 ($24.05 \pm 0.74\%$ 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 hours of dark potentiated daytime sleep efficiency even more during the 6 hour 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 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)* (Koh *et al.*, 2008; Liu *et al.*, 2014; Shi *et al.*, 2014). 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 (Edinger *et al.*, 2001, 2007; Smith *et al.*, 2002). 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 (Qiu and Hardin, 1996). To determine whether enhanced sleep following sleep opportunity restriction requires molecular circadian rhythms, we generated *per⁰¹;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 (Kaneko *et al.*, 2012; Luo *et al.*, 2012; Okamoto-Mizuno and Mizuno, 2012), and under constant dark conditions, flies exhibit consolidated sleep at subjective night with lower temperature (Ishimoto, Lark and Kitamoto, 2012). 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 (Parisky *et al.*, 2016). Under 12:12 LD conditions, *fmn* 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 *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 dark period from 4 to 10 hours in comparison to direct initiation of sleep opportunity restriction at either 6, 8, or 10 hours 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 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 *fmn* 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 outcome (Matthews *et al.*, 2013). We asked whether enhanced sleep with dark period compression persists with termination of sleep restriction. We restricted sleep opportunity in *fmn* 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, Yoshii et al. 2016); genetic disruption of both of these pathways renders *Drosophila* insensitive to LD cycling and behavioral arrhythmicity (Helfrich-Förster et al., 2001; Yoshii, Hermann-Luibl and Helfrich-Förster, 2016). 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 (Emery et al., 1998, 2000; Agrawal et al., 2017). To test whether CRY plays a role in the response to sleep opportunity restriction, we generated *cry⁰²;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* (Koh et al., 2006; Brown et al., 2014; Vienne et al., 2016) and humans (Pandi-Perumal et al., 2002). 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 (Luo et al., 2012), 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 (Luo *et al.*, 2012). 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 output (Curtsinger, 2013), and reproductive output is also impaired with sleep deprivation (Potdar *et al.*, 2018). 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, Ju *et al.* 2014). Several models of AD have been described in *Drosophila*, including

those based on expression of aggregating β -amyloid ($A\beta$) peptides (Finelli *et al.*, 2004; Koichi Iijima *et al.*, 2004); $A\beta$ accumulation results in decreased and fragmented sleep, while sleep deprivation increases $A\beta$ burden (Tabuchi *et al.*, 2015). We examined sleep following pan-neuronal expression of $A\beta$ Arctic, which carries a mutation to induce enhanced aggregation (Nilsberth *et al.*, 2001; Crowther *et al.*, 2005). Consistent with previous work (Tabuchi *et al.*, 2015), 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 $A\beta$ 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 $A\beta$ Arctic expression, though SL was unaffected (Fig. 5G,H).

We next examined whether sleep degradation related to $A\beta$ 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 $A\beta$ 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 $A\beta$ 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 $A\beta$ 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 $A\beta$ 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 lifespan (Tabuchi *et al.*, 2015), 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 response (Riemann and Perlis, 2009). However, CBT-I is limited by obstacles to broad implementation (Mitchell *et al.*, 2012; Sivertsen, Vedaas and Nordgreen, 2013; Kathol and Arnedt, 2016). 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 (Seugnet *et al.* 2009; Perlis *et al.* 2014). Single gene mutants such as those tested here (Kume *et al.*, 2005; Koh *et al.*, 2008; Liu *et al.*, 2014; Shi *et al.*, 2014), as well as a line generated by laboratory selection over many generations (Seugnet *et al.*, 2009), 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 (Gehrman, Pfeiffenberger and Byrne, 2013; Lind and Gehrman, 2016), and while this disease is likely multigenic in nature (Veatch *et al.*, 2017), 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 (Dubowy and Sehgal, 2017), 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, A 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 duration (Harbison, McCoy and Mackay, 2013).

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 (Bathgate, Edinger and Krystal, 2017). 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 (Taub and Berger, 1973; Reynold *et al.*, 2014). 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 (Spielman et al. 1987; Perlis et al. 2014). 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.

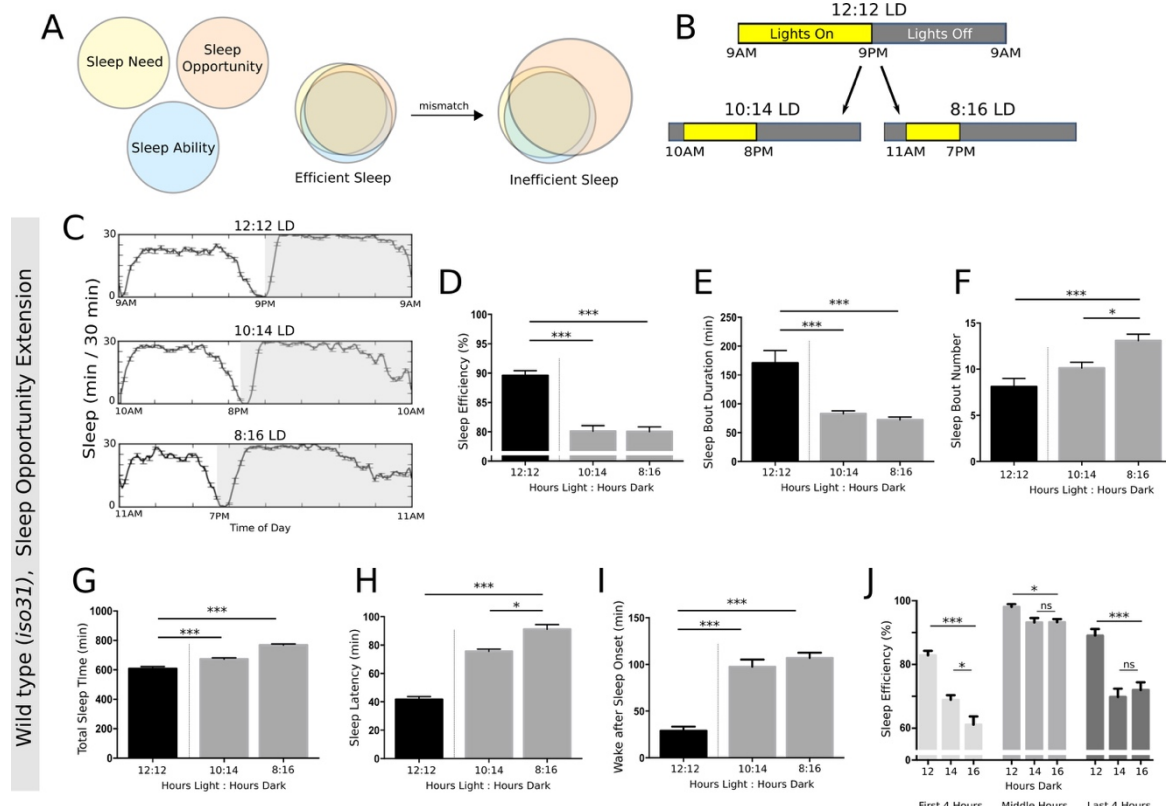
Modeling SRT in neurodegenerative and psychiatric disorders

Poor sleep has long been appreciated as a comorbidity of aging and neurodegeneration (Mander et al. 2017; Ju et al. 2014), but more recently identified as a potential modifiable risk factor for neurodegenerative disease progression (Kang et al. 2009; Roh et al. 2012; Ju et al. 2014). In flies, pharmacologic and genetic approaches to improve sleep have been shown to ameliorate memory deficits in an Alzheimer’s Disease model (Dissel *et al.*, 2017); similarly, altering the sleep-A β interaction by modulating neuronal excitability with a pharmacotherapy prolongs lifespan in A β -

expressing flies (Tabuchi *et al.*, 2015). 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 function (Cassidy-Eagle *et al.*, 2018).

Most pharmacological treatments in psychiatry are based on drugs discovered serendipitously over a half century ago (Insel, 2012). In recent years, significant advances in treating mental illness have been behavioral interventions (Hofmann *et al.*, 2012), 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.

Figures



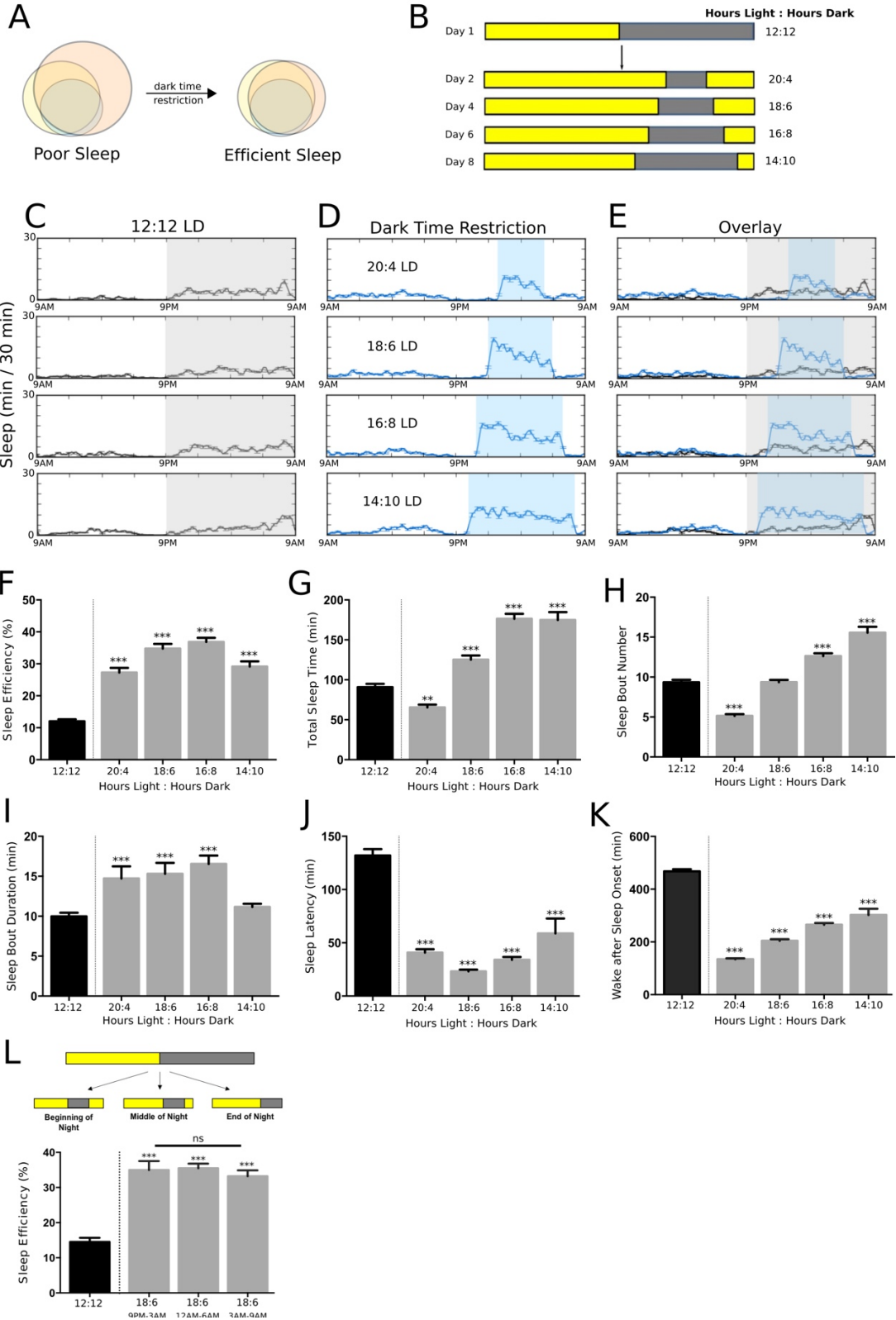


Figure 2. Sleep opportunity restriction enhances sleep in *fumin* mutants.

(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).

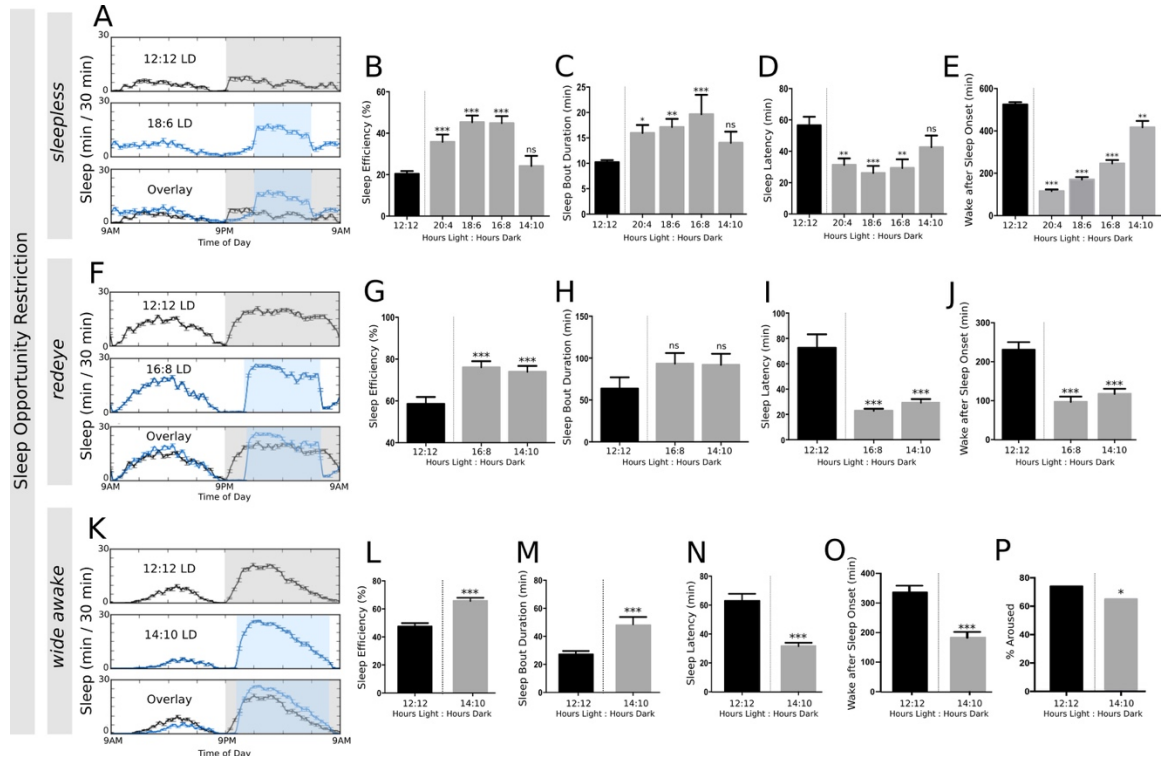


Figure 3. Sleep opportunity restriction improves sleep in multiple short-sleeping mutants.

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).

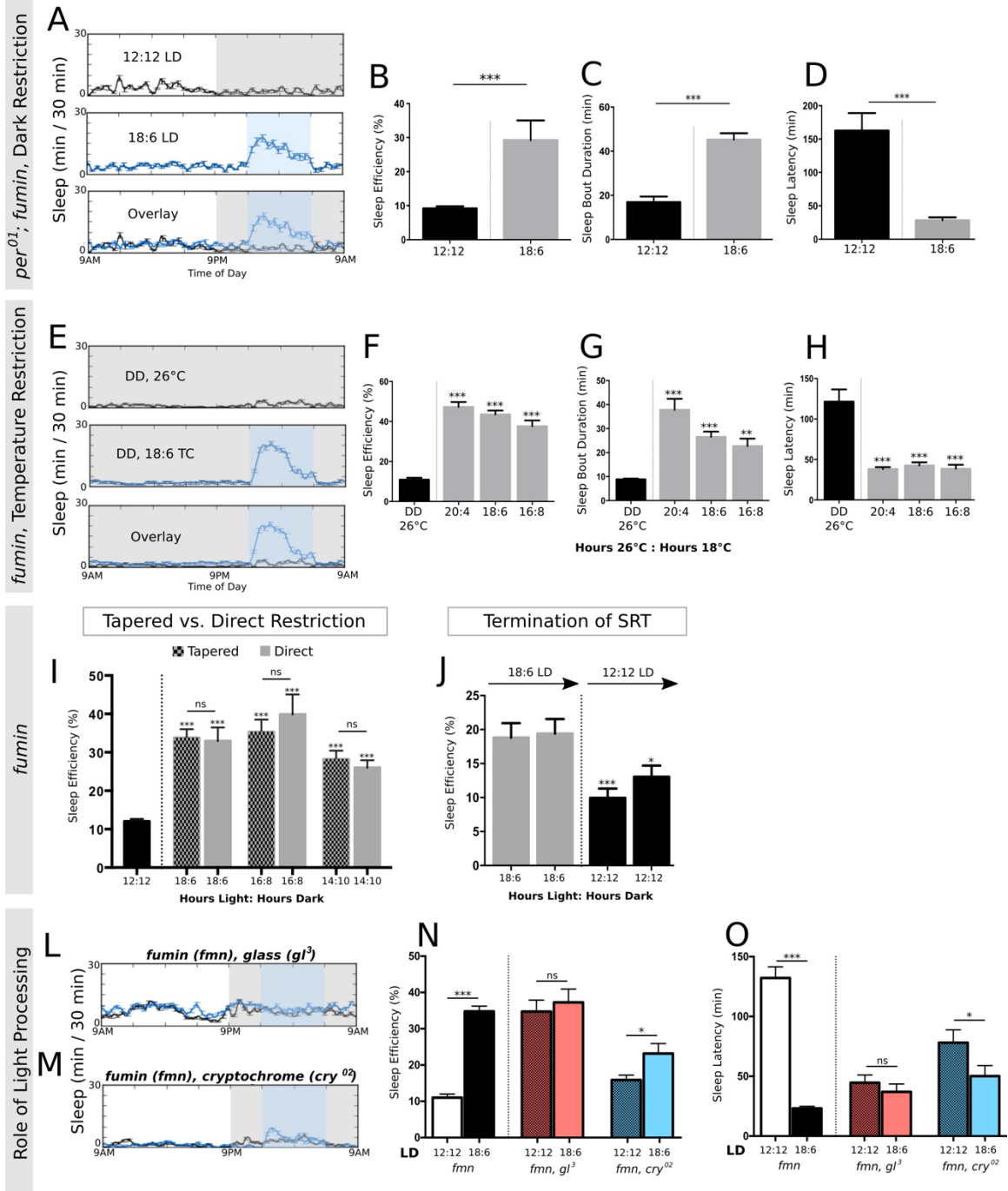
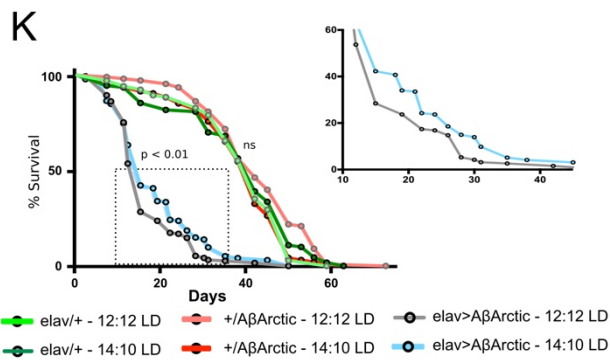
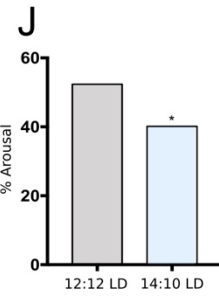
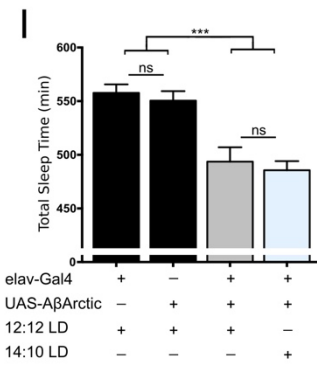
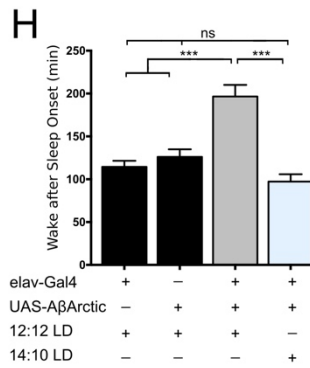
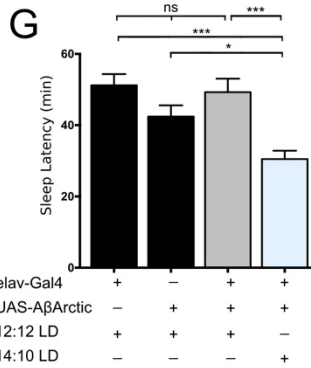
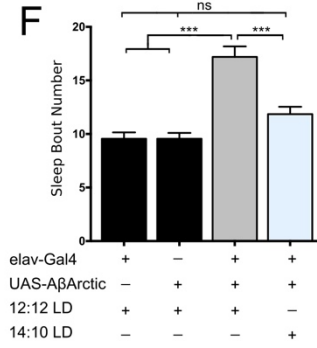
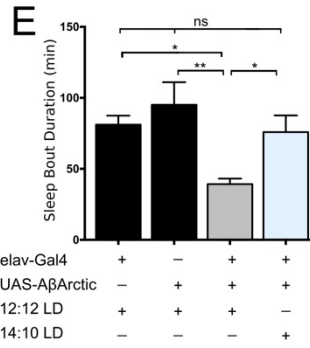
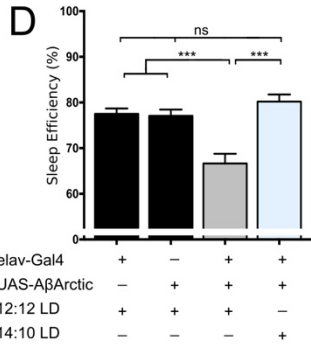
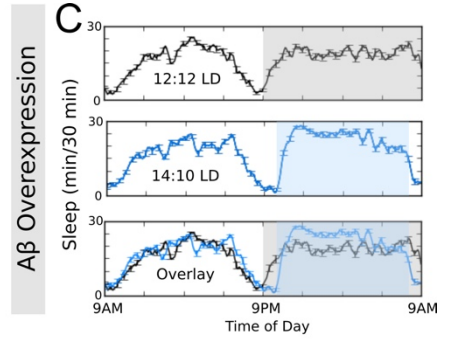
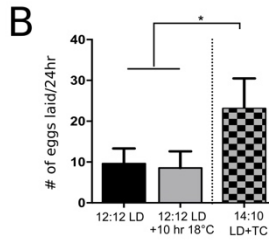
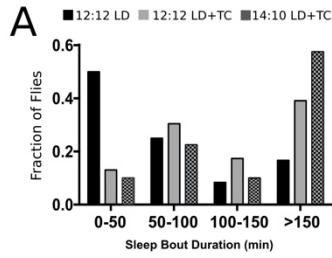


Figure 4. Response to sleep restriction requires ongoing environmental cues.

(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)

(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*).

iso31, Aged 53 days

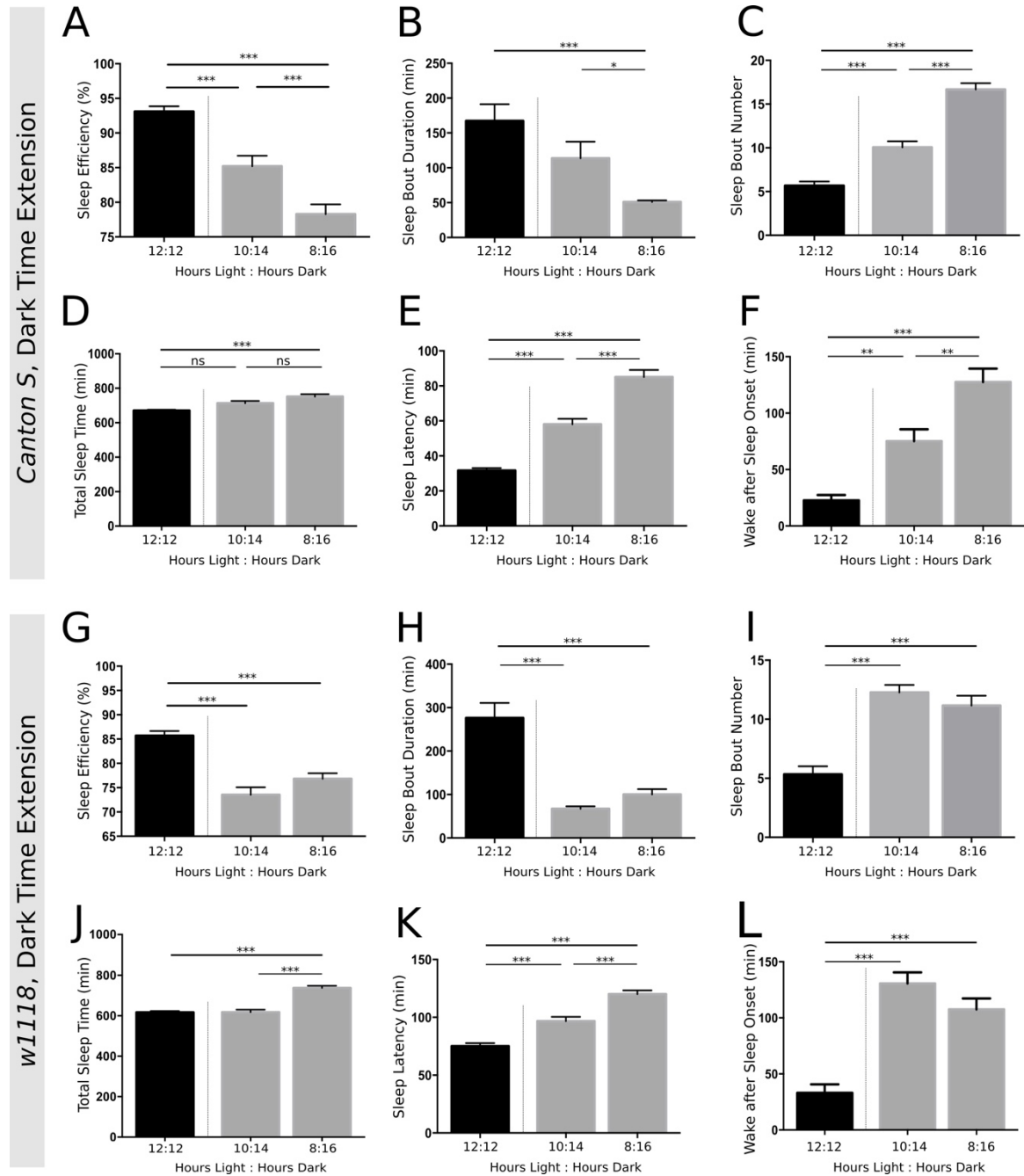


A β Overexpression, Sleep Opportunity Restriction (Dark)

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

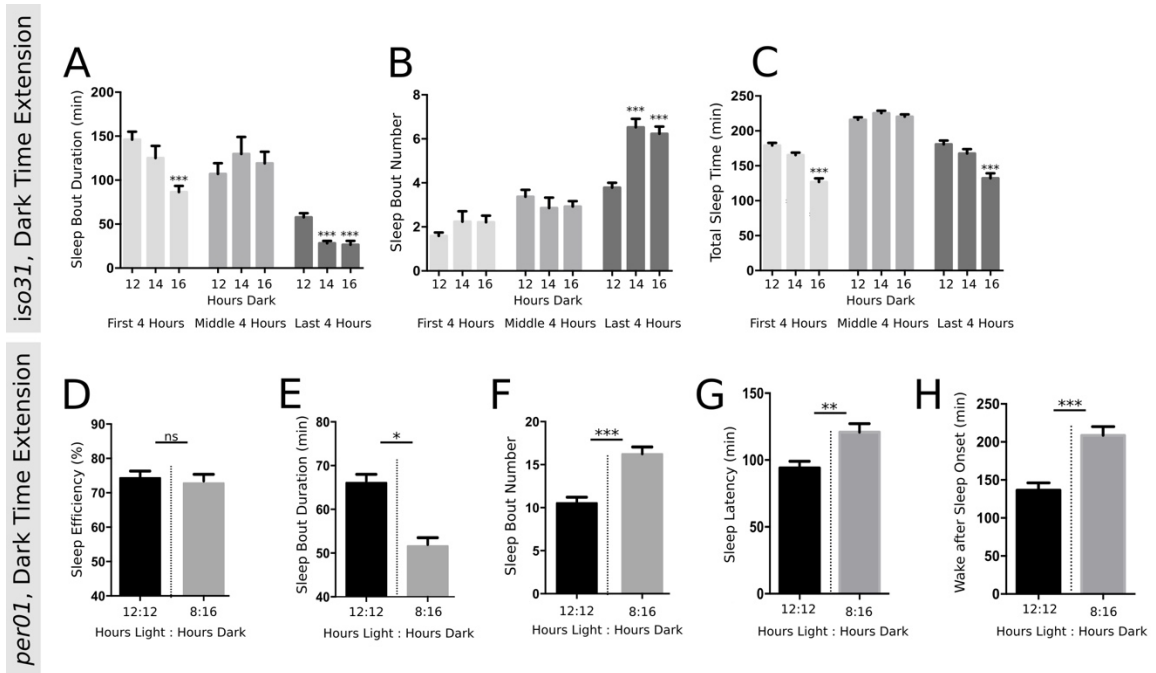
(A) Histogram of sleep bout durations of aged flies (53 days old) under 12:12 LD (black bars, n = 75), 12:12 LD+TC (26°C:18°C, gray bars, n = 78), or 14:10 LD+TC conditions (blue bars, n = 77). (B) Number of eggs laid by aged female flies under 12:12 LD, 12:12 LD plus 10 hours of low temperature during 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-A β Arctic*: 12:12 LD (gray) and 14:10 LD restriction (blue). Inset shows enlarged survival curves of *elav-Gal4>UAS-A β Arctic* flies under each condition.

Supplementary Figures



Supplementary Figure 1. Sleep opportunity extension in wild type flies and *period* mutants.

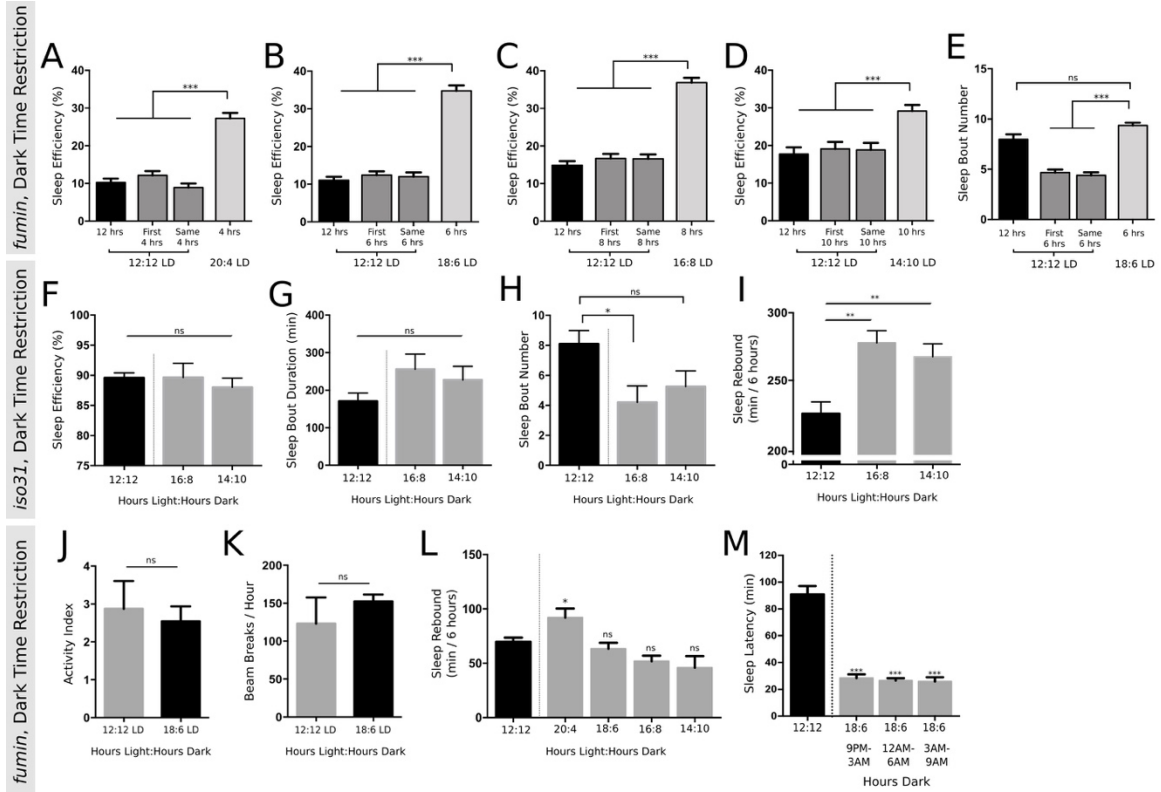
Quantification of sleep efficiency, sleep bout duration, number of sleep bouts, total sleep time, sleep latency, and wake after sleep onset for *Canton S* (A-F, n = 48 flies) and *w1118* (G-L, n = 48) under 12:12 LD conditions (black bars) or sleep opportunity extension (gray bars).



Supplementary Figure 2. Binned time-of-day analysis of sleep opportunity extension in wild type flies.

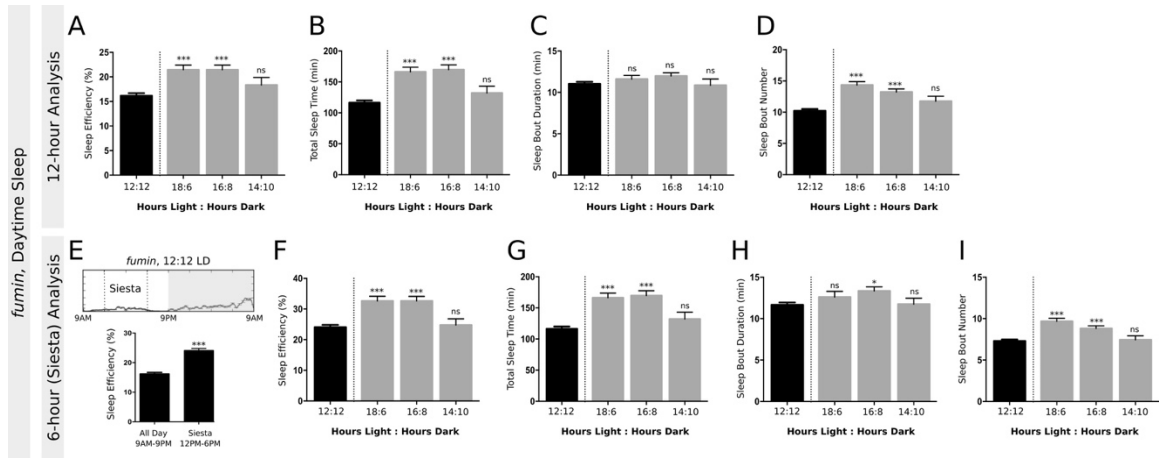
Comparison of sleep measures in 4-hour windows at different times of night for *iso31* (A-C, n = 48 flies) under 12:12 LD conditions or with sleep opportunity extension (14 or 16 hour dark period). (D-H)

Quantification of sleep efficiency, sleep bout duration, number of sleep bouts, sleep latency, and wake after sleep onset for *per⁰¹* under 12:12 LD conditions (black bars) or sleep opportunity extension (gray bars, n = 48).



Supplementary Figure 3. Response to sleep opportunity restriction in *fumin* and wild type flies.

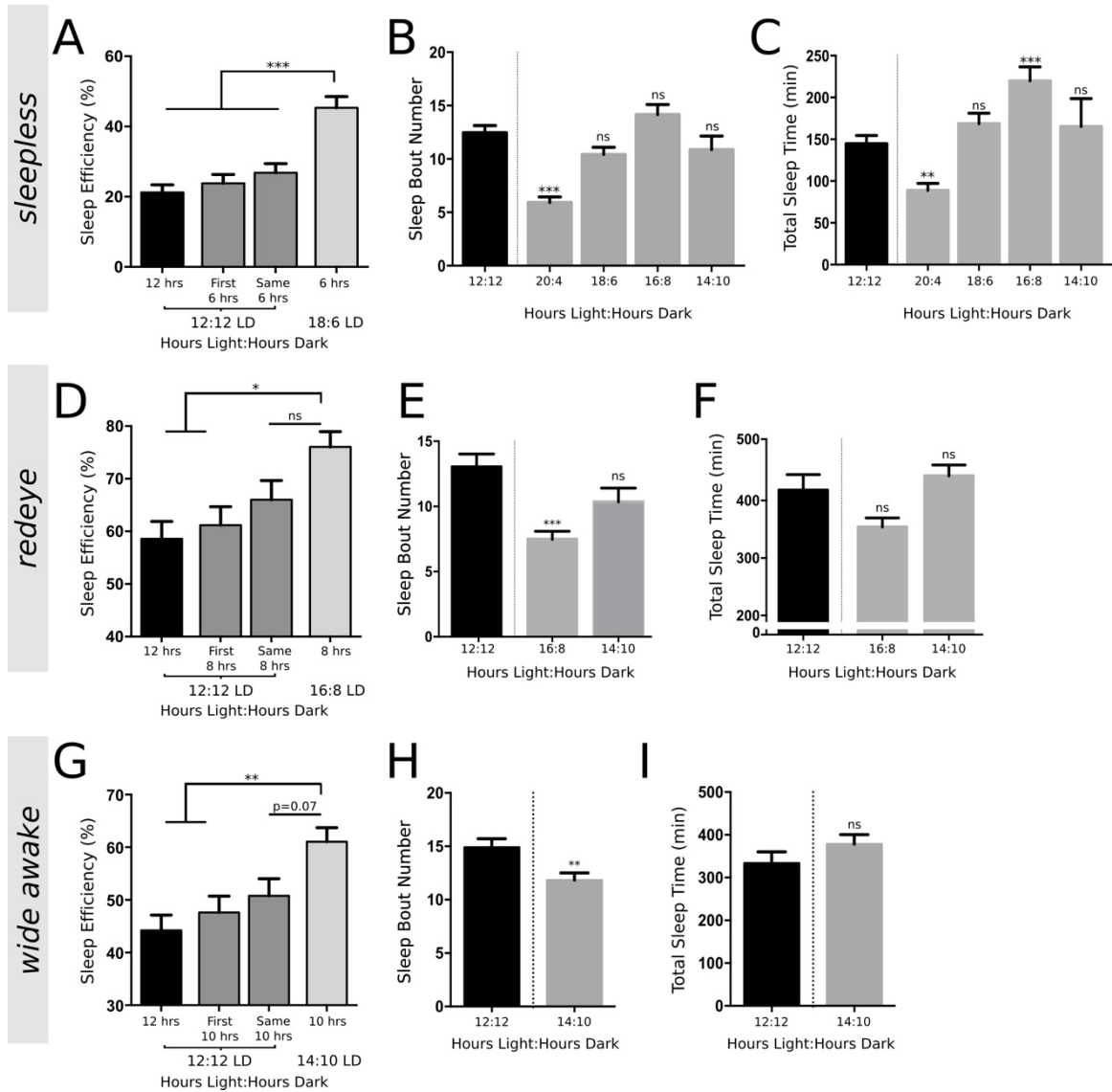
Sleep efficiency (A-D) and sleep bout number (E) with sleep opportunity restriction (light gray bars) comparing equivalent time period under 12:12 LD conditions (dark gray bars) or entire 12-hour night (black bars) in *fumin* mutants (n = 551 for 12:12 LD, n = 192 for 20:4 LD, n = 204 for 18:6 LD, n = 199 for 16:8 L, and n = 55 for 14:10 LD). Quantification of sleep efficiency (F), sleep bout duration (G), and number of sleep bouts in *iso31* wild-type flies (n = 32 for each condition) with restriction of sleep opportunity. (I) Rebound sleep in 6 hours after lights on in *iso31* flies (n = 32) following sleep restriction (gray bars). Activity index (J), total beam breaks per hour (K), and rebound sleep in 6 hours after lights on (L) in *fumin* flies (n = 551 for 12:12 LD, n = 192 for 20:4 LD, n = 204 for 18:6 LD, n = 199 for 16:8 L, and n = 55 for 14:10 LD). (M) Sleep latency in *fumin* mutants under 18:6 LD conditions with the dark period occurring at different times (n = 53 for 9p-3a, n = 172 for 12a-6a, n = 105 for 3a-9a).



Supplementary Figure 4. Daytime changes to sleep with nocturnal sleep opportunity restriction.

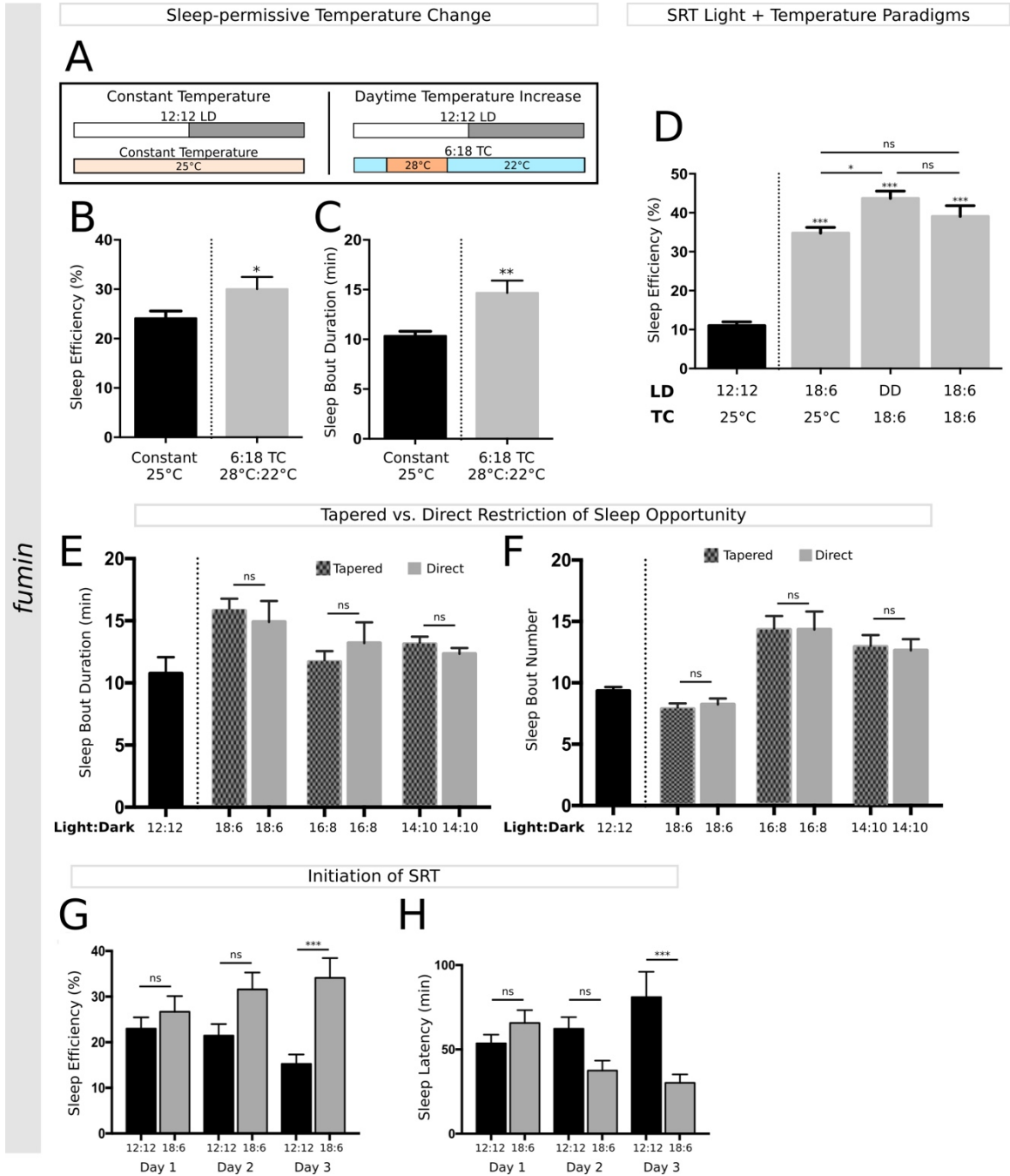
Quantification of sleep efficiency (A), total sleep time (B), sleep bout duration (C), and number of sleep bouts (D) during a 12-hour daytime period (9AM-9PM) in *fumin* flies. (E) Representative sleep trace and comparison of sleep efficiency between 12-hour daytime period and 6-hour siesta period in *fumin* flies.

Quantification of sleep measures during siesta period (12p-6p) in *fumin* flies with nocturnal sleep restriction (n = 551 for 12:12 LD, n = 204 for 18:6 LD, n = 199 for 16:8 L, and n = 55 for 14:10 LD).



Supplementary Figure 5. Response to sleep opportunity restriction in short-sleeping mutants.

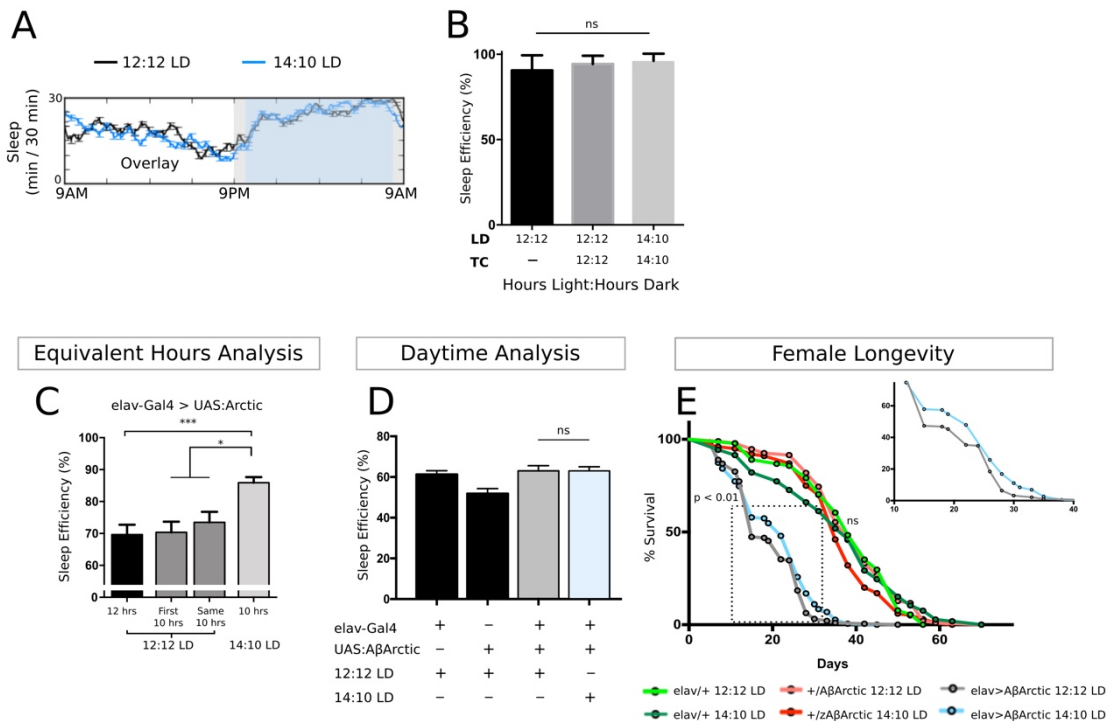
Comparison of sleep efficiency with sleep opportunity restriction (light gray bars), equivalent time in 12:12 LD (dark gray bars), or entire 12-hour night (black bars) in *sleepless* (A; n = 210 for 12:12 LD, 64 for 20:4 LD, 69 for 18:6 LD, 68 for 16:8 LD, and 33 for 14:10 LD), *redeye* (D; n = 63 for 12:12 LD, 60 for 16:8 LD, 58 for 14:10 LD), and *wide awake* mutants (G, n = 62 for 12:12 LD, n = 62 for 14:10 LD). Quantification of number of sleep bouts (B,E,H), and total sleep time during the dark period (C,F,I) for each mutant.



Supplementary Figure 6. Sleep opportunity restriction with alternative sleep-promoting paradigms and temporal dynamics of sleep response.

(A) Schematic of experimental paradigm of sleep-permissive temperature increase during daytime and control at constant temperature. Sleep efficiency (B) and sleep bout duration (C) of *fumin* mutants with

restricted high temperature exposure during daytime under 12:12 LD conditions (n = 60 for constant temperature, n = 56 for high temperature). (D) Quantification of sleep efficiency for sleep restriction therapy (SRT) using coincident light and temperature cues (n = 551 for 12:12 LD, n = 204 for 18:6 LD, n = 53 for 18:6 TC, and n = 52 for 18:6 LD+TC). Sleep bout duration (E) and number of sleep bouts (F) in *fumin* mutants with sleep restriction via tapered protocol (Fig. 2B) 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). Day-by-day quantification of sleep efficiency (G) and sleep latency (H) following initiation of sleep restriction in *fumin* mutants (n = 32).



Supplementary Figure 7. Sleep opportunity restriction in aged flies and AβArctic females.

(A) Representative sleep traces of aged *iso*³¹ female flies under 12:12 LD conditions (black line, gray shading indicates dark phase) and 14:10 LD dark time restriction (blue line, blue shading indicates dark phase). (B) Sleep efficiency of aged *iso*³¹ wild type flies under 12:12 LD (black bar, n = 75), 12:12 LD+TC (26°C:18°C, dark gray bar, n = 78), or 14:10 LD+TC conditions (light gray bar, n = 77). (C) Sleep efficiency with sleep opportunity restriction (light gray bar) comparing equivalent time period under 12:12 LD conditions (dark gray bars) or entire 12-hour night (black bar) with overexpression of AβArctic (n = 59 for 12:12 LD and n = 60 for 14:10 LD). (D) Quantification of daytime sleep efficiency for overexpression of AβArctic or genetic controls (n = 60 for *elav-Gal4*+/+, n = 53 for *UAS-AβArctic*+/+, n = 59 for *elav-Gal4/UAS-AβArctic* flies under 12:12 LD, and n = 60 for *elav-Gal4/UAS-AβArctic* under 14:10 LD conditions (n = 60). (E) Survival curves with pan-neuronal overexpression of AβArctic or genetic controls under 12:12 LD or 14:10 LD conditions (n=100 females 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.

References

- Agrawal, Parul, Jerry H. Houl, Kushan L. Gunawardhana, Tianxin Liu, Jian Zhou, Mark J. Zoran, and Paul E. Hardin. 2017. "Drosophila CRY Entrain Clocks in Body Tissues to Light and Maintains Passive Membrane Properties in a Non-Clock Body Tissue Independent of Light." *Current Biology* 27 (16): 2431-2441.e3. <https://doi.org/10.1016/j.cub.2017.06.064>.
- Bathgate, Christina J., Jack D. Edinger, and Andrew D. Krystal. 2017. "Insomnia Patients with Objective Short Sleep Duration Have a Blunted Response to Cognitive Behavioral Therapy for Insomnia." *Sleep* 40 (1). <https://doi.org/10.1093/sleep/zsw012>.
- Brown, Marishka K., May T. Chan, John E. Zimmerman, Allan I. Pack, Nicholas E. Jackson, and Nirinjini Naidoo. 2014. "Aging Induced Endoplasmic Reticulum Stress Alters Sleep and Sleep Homeostasis." *Neurobiology of Aging* 35 (6): 1431–41. <https://doi.org/10.1016/j.neurobiolaging.2013.12.005>.
- Bushey, Daniel, Reto Huber, Giulio Tononi, and Chiara Cirelli. 2007. "Drosophila Hyperkinetic Mutants Have Reduced Sleep and Impaired Memory." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 27 (20): 5384–93. <https://doi.org/10.1523/JNEUROSCI.0108-07.2007>.
- Bushey, Daniel, Kimberly a Hughes, Giulio Tononi, and Chiara Cirelli. 2010. "Sleep, Aging, and Lifespan in Drosophila." *BMC Neuroscience* 11: 56. <https://doi.org/10.1186/1471-2202-11-56>.
- Cassidy-Eagle, E, A Siebern, L Unti, J Glassman, O R Hara, Fayetteville Veterans, and Affairs Medical. 2018. "Cognitive Behavioral Treatment for Insomnia in Older Adults with Mild Cognitive Impairment in Independent Living Facilities : A Pilot Study." *Journal of Sleep Disorders and Medical Care* 1 (1). <https://doi.org/10.16966/jsdmc.105>.
- Cirelli, Chiara, Daniel Bushey, Sean Hill, Reto Huber, Robert Kreber, Barry Ganetzky, and Giulio Tononi. 2005. "Reduced Sleep in Drosophila Shaker Mutants." *Nature*. <https://doi.org/10.1038/nature03486>.
- Crowther, D. C., K. J. Kinghorn, E. Miranda, R. Page, J. A. Curry, F. A I Duthie, D. C. Gubb, and D. A. Lomas. 2005. "Intraneuronal A β , Non-Amyloid Aggregates and Neurodegeneration in a Drosophila Model of Alzheimer's Disease." *Neuroscience* 132 (1): 123–35. <https://doi.org/10.1016/j.neuroscience.2004.12.025>.
- Curtsinger, James W. 2013. "Late-Life Fecundity Plateaus in Drosophila Melanogaster Can Be Explained by Variation in Reproductive Life Spans." *Experimental Gerontology* 48 (11): 1338–42. <https://doi.org/10.1016/j.exger.2013.08.016>.
- Daley, M., C. M. Morin, M. LeBlanc, J. P. Grégoire, J. Savard, and L. Baillargeon. 2009. "Insomnia and Its Relationship to Health-Care Utilization, Work Absenteeism, Productivity and Accidents." *Sleep Medicine* 10 (4): 427–38. <https://doi.org/10.1016/j.sleep.2008.04.005>.
- Dement, W, and R Pelayo. 1997. "Public Health Impact and Treatment of Insomnia." *European Psychiatry* 12 ((Suppl 1)): 31s-39s.
- Dilley, Leela C., Abigail Vigderman, Charlette E. Williams, and Matthew S. Kayser. 2018. "Behavioral and Genetic

- Features of Sleep Ontogeny in *Drosophila*." *Sleep*. <https://doi.org/10.1093/sleep/zsy086>.
- Dissel, Stephane, Markus Klose, Jeff Donlea, Lijuan Cao, Denis English, Raphaelle Winsky-Sommerer, Bruno van Swinderen, and Paul J. Shaw. 2017. "Enhanced Sleep Reverses Memory Deficits and Underlying Pathology in *Drosophila* Models of Alzheimer's Disease." *Neurobiology of Sleep and Circadian Rhythms*. <https://doi.org/10.1016/j.nbscr.2016.09.001>.
- Dubowy, Christine, and Amita Sehgal. 2017. "Circadian Rhythms and Sleep in *Drosophila Melanogaster*." *Genetics*. <https://doi.org/10.1534/genetics.115.185157>.
- Edinger, J D, W K Wohlgemuth, R a Radtke, G R Marsh, and R E Quillian. 2001. "Cognitive Behavioral Therapy for Treatment of Chronic Primary Insomnia: A Randomized Controlled Trial." *JAMA : The Journal of the American Medical Association* 285 (14): 1856–64.
- Edinger, Jack D., William K. Wohlgemuth, Rodney A. Radtke, Cynthia J. Coffman, and Colleen E. Carney. 2007. "Dose-Response Effects of Cognitive-Behavioral Insomnia Therapy: A Randomized Clinical Trial." *Sleep* 30 (2): 203–12. <https://doi.org/10.1093/sleep/30.2.203>.
- Emery, Patrick, W. Venus So, Maki Kaneko, Jeffrey C. Hall, and Michael Rosbash. 1998. "Cry, a *Drosophila* Clock and Light-Regulated Cryptochrome, Is a Major Contributor to Circadian Rhythm Resetting and Photosensitivity." *Cell* 95 (5): 669–79. [https://doi.org/10.1016/S0092-8674\(00\)81637-2](https://doi.org/10.1016/S0092-8674(00)81637-2).
- Emery, Patrick, Ralf Stanewsky, Charlotte Helfrich-Förster, Myai Emery-Le, Jeffrey C. Hall, and Michael Rosbash. 2000. "Drosophila CRY Is a Deep Brain Circadian Photoreceptor." *Neuron* 26 (2): 493–504. [https://doi.org/10.1016/S0896-6273\(00\)81181-2](https://doi.org/10.1016/S0896-6273(00)81181-2).
- Finelli, Alyce, Anju Kelkar, Ho Juhn Song, Haidi Yang, and Mary Konsolaki. 2004. "A Model for Studying Alzheimer's Abeta42-Induced Toxicity in *Drosophila Melanogaster*." *Molecular and Cellular Neuroscience* 26 (3): 365–75. <https://doi.org/10.1016/j.mcn.2004.03.001>.
- Gehrman, Philip R., Cory Pfeiffenberger, and Enda M. Byrne. 2013. "The Role of Genes in the Insomnia Phenotype." *Sleep Medicine Clinics*. <https://doi.org/10.1016/j.jsmc.2013.04.005>.
- Gilestro, Giorgio F. 2012. "Video Tracking and Analysis of Sleep in *Drosophila Melanogaster*." *Nature Protocols*. <https://doi.org/10.1038/nprot.2012.041>.
- Gilestro, Giorgio F., and Chiara Cirelli. 2009. "PySolo: A Complete Suite for Sleep Analysis in *Drosophila*." *Bioinformatics*. <https://doi.org/10.1093/bioinformatics/btp237>.
- Harbison, Susan T., Lenovia J. McCoy, and Trudy F.C. Mackay. 2013. "Genome-Wide Association Study of Sleep in *Drosophila Melanogaster*." *BMC Genomics* 14 (1). <https://doi.org/10.1186/1471-2164-14-281>.
- Hasan, Sibah, Yves Dauvilliers, Valérie Mongrain, Paul Franken, and Mehdi Tafti. 2012. "Age-Related Changes in Sleep in Inbred Mice Are Genotype Dependent." *Neurobiology of Aging* 33 (1). <https://doi.org/10.1016/j.neurobiolaging.2010.05.010>.
- Helfrich-Förster, Charlotte, Christine Winter, Alois Hofbauer, Jeffrey C. Hall, and Ralf Stanewsky. 2001. "The Circadian

- Clock of Fruit Flies Is Blind after Elimination of All Known Photoreceptors." *Neuron* 30 (1): 249–61.
[https://doi.org/10.1016/S0896-6273\(01\)00277-X](https://doi.org/10.1016/S0896-6273(01)00277-X).
- Hendricks, Joan C, Stefanie M Finn, Karen A Panckeri, Jessica Chavkin, Julie A Williams, Amita Sehgal, and Allan I Pack. 2000. "Rest in *Drosophila* Is a Sleep-like State." *Neuron* 25 (1): 129–38. [https://doi.org/10.1016/S0896-6273\(00\)80877-6](https://doi.org/10.1016/S0896-6273(00)80877-6).
- Hofmann, Stefan G, Anu Asnaani, Imke J J Vonk, Alice T Sawyer, and Angela Fang. 2012. "The Efficacy of Cognitive Behavioral Therapy: A Review of Meta-Analyses." *Cognitive Therapy Research* 36 (5): 427–40.
<https://doi.org/10.1007/s10608-012-9476-1>.
- Iijima, Koichi, Hsin-Ping Liu, Ann-Shyn Chiang, Stephen a. Hearn, Mary Konsolaki, and Yi Zhong. 2004. "Dissecting the Pathological Effects of Human A β 40 and A β 42 in *Drosophila*: A Potential Model for Alzheimer's Disease." *Proceedings of the National Academy of Sciences of the United States of America* 101 (17): 6623–28.
<https://doi.org/10.1073/pnas.0400895101>.
- Insel, Thomas R. 2012. "Next-Generation Treatments for Mental Disorders." *Science Translational Medicine* 4 (155): 155ps19. <https://doi.org/10.1126/scitranslmed.3004873>.
- Ishimoto, Hiroshi, Arianna Lark, and Toshihiro Kitamoto. 2012. "Factors That Differentially Affect Daytime and Nighttime Sleep in *Drosophila Melanogaster*." *Frontiers in Neurology* FEB: 1–5. <https://doi.org/10.3389/fneur.2012.00024>.
- Ju, Yo-Ei S., Brendan P. Lucey, and David M. Holtzman. 2014. "Sleep and Alzheimer Disease Pathology—a Bidirectional Relationship." *Nature Reviews Neurology* 10 (2): 115–19. <https://doi.org/10.1038/nrneuro.2013.269>.
- Kaneko, Haruna, Lauren M. Head, Jinli Ling, Xin Tang, Yilin Liu, Paul E. Hardin, Patrick Emery, and Fumika N. Hamada. 2012. "Circadian Rhythm of Temperature Preference and Its Neural Control in *Drosophila*." *Current Biology* 22 (19): 1851–57. <https://doi.org/10.1016/j.cub.2012.08.006>.
- Kang, Jae Eun, Miranda M. Lim, Randall J. Bateman, James J. Lee, Liam P. Smyth, John R. Cirrito, Nobuhiro Fujiki, Seiji Nishino, and David M. Holtzman. 2009. "Amyloid- β Dynamics Are Regulated by Orexin and the Sleep-Wake Cycle." *Science*. <https://doi.org/10.1126/science.1180962>.
- Kathol, Roger G., and J. Todd Arnedt. 2016. "Cognitive Behavioral Therapy for Chronic Insomnia: Confronting the Challenges to Implementation." *Annals of Internal Medicine*, 2016. <https://doi.org/10.7326/M16-0359>.
- Kayser, Matthew S, Zhifeng Yue, and Amita Sehgal. 2014. "A Critical Period of Sleep for Development of Courtship Circuitry and Behavior in *Drosophila*." *Science* 344 (6181): 269–74. <https://doi.org/10.1126/science.1250553>.
- Kivipelto, Miia, Alina Solomon, Satu Ahtiluoto, Tiia Ngandu, Jenni Lehtisalo, Riitta Antikainen, Lars Bäckman, et al. 2013. "The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): Study Design and Progress." *Alzheimer's and Dementia* 9 (6): 657–65. <https://doi.org/10.1016/j.jalz.2012.09.012>.
- Koh, Kyunghye, Joshua M Evans, Joan C Hendricks, and Amita Sehgal. 2006. "A *Drosophila* Model for Age-Associated Changes in Sleep:Wake Cycles." *Proceedings of the National Academy of Sciences of the United States of America* 103 (37): 13843–47. <https://doi.org/10.1073/pnas.0605903103>.

- Koh, Kyunghye, William J Joiner, Mark N Wu, Zhifeng Yue, Corinne J Smith, and Amita Sehgal. 2008. "Identification of SLEEPLESS, a Sleep-Promoting Factor." *Science* 321 (5887): 372–76. <https://doi.org/10.1126/science.1155942>.
- Konopka, R J, and S Benzer. 1971. "Clock Mutants of *Drosophila Melanogaster*." *Proceedings of the National Academy of Sciences of the United States of America* 68 (9): 2112–16. <https://doi.org/10.1073/pnas.68.9.2112>.
- Krystal, Andrew D, and Jack D Edinger. 2010. "Sleep EEG Predictors and Correlates of the Response to Cognitive Behavioral Therapy for Insomnia." *Sleep* 33 (5): 669–77.
- Kume, Kazuhiko, Shoen Kume, Sang Ki Park, Jay Hirsh, and F Rob Jackson. 2005. "Dopamine Is a Regulator of Arousal in the Fruit Fly" 25 (32): 7377–84. <https://doi.org/10.1523/JNEUROSCI.2048-05.2005>.
- Kyle, Simon D., Maria Raisa Jessica Aquino, Christopher B. Miller, Alasdair L. Henry, Megan R. Crawford, Colin A. Espie, and Arthur J. Spielman. 2015. "Towards Standardisation and Improved Understanding of Sleep Restriction Therapy for Insomnia Disorder: A Systematic Examination of CBT-I Trial Content." *Sleep Medicine Reviews*. <https://doi.org/10.1016/j.smr.2015.02.003>.
- Lind, Mackenzie J., and Philip R. Gehrman. 2016. "Genetic Pathways to Insomnia." *Brain Sciences*. <https://doi.org/10.3390/brainsci6040064>.
- Liu, Sha, Angelique Lamaze, Qili Liu, Masashi Tabuchi, Yong Yang, Melissa Fowler, Rajnish Bharadwaj, et al. 2014. "WIDE AWAKE Mediates the Circadian Timing of Sleep Onset." *Neuron* 82 (1): 151–66. <https://doi.org/10.1016/j.neuron.2014.01.040>.
- Luo, Wenyu, Wen Feng Chen, Zhifeng Yue, Dechun Chen, Mallory Sowcik, Amita Sehgal, and Xiangzhong Zheng. 2012. "Old Flies Have a Robust Central Oscillator but Weaker Behavioral Rhythms That Can Be Improved by Genetic and Environmental Manipulations." *Aging Cell* 11 (3): 428–38. <https://doi.org/10.1111/j.1474-9726.2012.00800.x>.
- Majercak, John, David Sidote, Paul E. Hardin, and Isaac Edery. 1999. "How a Circadian Clock Adapts to Seasonal Decreases in Temperature and Day Length." *Neuron* 24 (1): 219–30. [https://doi.org/10.1016/S0896-6273\(00\)80834-X](https://doi.org/10.1016/S0896-6273(00)80834-X).
- Mander, Bryce A., Joseph R. Winer, and Matthew P. Walker. 2017. "Sleep and Human Aging." *Neuron*. <https://doi.org/10.1016/j.neuron.2017.02.004>.
- Matthews, Ellyn E., J. Todd Arnedt, Michaela S. McCarthy, Leisha J. Cuddihy, and Mark S. Aloia. 2013. "Adherence to Cognitive Behavioral Therapy for Insomnia: A Systematic Review." *Sleep Medicine Reviews*. <https://doi.org/10.1016/j.smr.2013.01.001>.
- Miller, Christopher B., Colin A. Espie, Dana R. Epstein, Leah Friedman, Charles M. Morin, Wilfred R. Pigeon, Arthur J. Spielman, and Simon D. Kyle. 2014. "The Evidence Base of Sleep Restriction Therapy for Treating Insomnia Disorder." *Sleep Medicine Reviews*. <https://doi.org/10.1016/j.smr.2014.01.006>.
- Mitchell, Matthew D., Philip Gehrman, Michael Perlis, and Craig A. Umscheid. 2012. "Comparative Effectiveness of Cognitive Behavioral Therapy for Insomnia: A Systematic Review." *BMC Family Practice*. <https://doi.org/10.1186/1471-2296-13-40>.

- Morin, C. M., M. LeBlanc, M. Daley, J. P. Gregoire, and C. Mérette. 2006. "Epidemiology of Insomnia: Prevalence, Self-Help Treatments, Consultations, and Determinants of Help-Seeking Behaviors." *Sleep Medicine* 7 (2): 123–30. <https://doi.org/10.1016/j.sleep.2005.08.008>.
- Ngandu, Tiia, Jenni Lehtisalo, Alina Solomon, Esko Levälähti, Satu Ahtiluoto, Riitta Antikainen, Lars Bäckman, et al. 2015. "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." *The Lancet* 385 (9984): 2255–63. [https://doi.org/10.1016/S0140-6736\(15\)60461-5](https://doi.org/10.1016/S0140-6736(15)60461-5).
- Nilsberth, Camilla, Anita Westlind-danielsson, Christopher B Eckman, Margaret M Condron, Karin Axelman, Charlotte Forsell, Charlotte Stenh, et al. 2001. "The ' Arctic ' APP Mutation (E693G) Causes Alzheimer ' s Disease by Enhanced A β Protofibril Formation," 18–23.
- Okamoto-Mizuno, Kazue, and Koh Mizuno. 2012. "Effects of Thermal Environment on Sleep and Circadian Rhythm." *Journal of Physiological Anthropology*. <https://doi.org/10.1186/1880-6805-31-14>.
- Ozminkowski, R J, S Wang, and J K Walsh. 2007. "The Direct and Indirect Costs of Untreated Insomnia in Adults in the United States." *Sleep* 30 (3): 263–73.
- Pandi-Perumal, S. R., L. K. Seils, L. Kayumov, M. R. Ralph, A. Lowe, H. Moller, and D. F. Swaab. 2002. "Senescence, Sleep, and Circadian Rhythms." *Ageing Research Reviews*. [https://doi.org/10.1016/S1568-1637\(02\)00014-4](https://doi.org/10.1016/S1568-1637(02)00014-4).
- Parisky, Katherine M, José L. Agosto Rivera, Nathan C Donelson, Sejal Kotecha, and Leslie C Griffith. 2016. "Reorganization of Sleep by Temperature in *Drosophila* Requires Light, the Homeostat, and the Circadian Clock." *Current Biology* 26 (7): 882–92. <https://doi.org/10.1016/j.cub.2016.02.011>.
- Perlis, Michael L., Charles B. Corbitt, and Jacqueline D. Kloss. 2014. "Insomnia Research: 3Ps and Beyond." *Sleep Medicine Reviews*, 2014. <https://doi.org/10.1016/j.smr.2014.01.003>.
- Perlis, Michael L, Paul J Shaw, Georgina Cano, and Colin A Espie. 2014. "Models of Insomnia," 850–66.
- Potdar, Sheetal, Danita K. Daniel, Femi A. Thomas, Shraddha Lall, and Vasu Sheeba. 2018. "Sleep Deprivation Negatively Impacts Reproductive Output in *Drosophila Melanogaster*." *The Journal of Experimental Biology* 221 (6): jeb174771. <https://doi.org/10.1242/jeb.174771>.
- Qaseem, Amir, Devan Kansagara, Mary Ann Forciea, Molly Cooke, Thomas D. Denberg, Michael J. Barry, Cynthia Boyd, et al. 2016. "Management of Chronic Insomnia Disorder in Adults: A Clinical Practice Guideline from the American College of Physicians." *Annals of Internal Medicine* 165 (2): 125–33. <https://doi.org/10.7326/M15-2175>.
- Qiu, J, and P E Hardin. 1996. "Per mRNA Cycling Is Locked to Lights-off under Photoperiodic Conditions That Support Circadian Feedback Loop Function." *Molecular and Cellular Biology* 16 (8): 4182–88. <https://doi.org/10.1128/MCB.16.8.4182>.
- Reynold, AM, BR Emily, A Saxena, R Rayad, and SD Youngstedt. 2014. "Negative Effects of Time in Bed Extension : A Pilot Study." *Journal of Sleep Medicine and Disorders* 1 (1): 1–13.
- Riemann, Dieter, and Michael L. Perlis. 2009. "The Treatments of Chronic Insomnia: A Review of Benzodiazepine

- Receptor Agonists and Psychological and Behavioral Therapies." *Sleep Medicine Reviews*.
<https://doi.org/10.1016/j.smr.2008.06.001>.
- Robertson, Meagan, and Alex C. Keene. 2013. "Molecular Mechanisms of Age-Related Sleep Loss in the Fruit Fly - A Mini-Review." *Gerontology* 59 (4): 334–39. <https://doi.org/10.1159/000348576>.
- Roh, J H, Y Huang, A W Bero, T Kasten, F R Stewart, R J Bateman, and D M Holtzman. 2012. "Disruption of the Sleep-Wake Cycle and Diurnal Fluctuation of Beta-Amyloid in Mice with Alzheimer's Disease Pathology." *Sci Transl Med* 4 (150): 150ra122. <https://doi.org/10.1126/scitranslmed.3004291>.
- Roth, Thomas. 2007. "Insomnia: Definition, Prevalence, Etiology, and Consequences." *Journal of Clinical Sleep Medicine*.
- Saarelainen, L, AM Tolppanen, M Koponen, A Tanskanen, J Tiihonen, S Hartikainen, and H Taipale. 2018. "Risk of Death Associated with New Benzodiazepine Use among Persons with Alzheimer Disease: A Matched Cohort Study." *Int J Geriatr Psychiatry* 33 (4): 583–90.
- Sarsour, Khaled, Charles M. Morin, Kathleen Foley, Anupama Kalsekar, and James K. Walsh. 2010. "Association of Insomnia Severity and Comorbid Medical and Psychiatric Disorders in a Health Plan-Based Sample: Insomnia Severity and Comorbidities." *Sleep Medicine* 11 (1): 69–74. <https://doi.org/10.1016/j.sleep.2009.02.008>.
- Sateia, M J, K Doghramji, P J Hauri, and C M Morin. 2000. "Evaluation of Chronic Insomnia. An American Academy of Sleep Medicine Review." *Sleep* 23 (2): 243–308.
- Seugnet, L., Y. Suzuki, M. Thimgan, J. Donlea, S. I. Gimbel, L. Gottschalk, S. P. Duntley, and P. J. Shaw. 2009. "Identifying Sleep Regulatory Genes Using a Drosophila Model of Insomnia." *Journal of Neuroscience* 29 (22): 7148–57. <https://doi.org/10.1523/JNEUROSCI.5629-08.2009>.
- Shaw, Paul J, Chiara Cirelli, Ralph J Greenspan, and Giulio Tononi. 2000. "Correlates of Sleep and Waking in Drosophila Melanogaster" 287 (January).
- Shi, Mi, Zhifeng Yue, Alexandre Kuryatov, Jon M Lindstrom, and Amita Sehgal. 2014. "Identification of Redeye , a New Sleep- Regulating Protein Whose Expression Is Modulated by Sleep Amount," 1–17.
<https://doi.org/10.7554/eLife.01473>.
- Sivertsen, Børge, Øystein Vedaa, and Tine Nordgreen. 2013. "The Future of Insomnia Treatment—the Challenge of Implementation." *Sleep* 36 (3): 303–4. <https://doi.org/10.5665/sleep.2432>.
- Smith, Michael T., Michael L. Perlis, Amy Park, Michelle S. Smith, JaeMi Pennington, Donna E. Giles, and Daniel J. Buysse. 2002. "Comparative Meta-Analysis of Pharmacotherapy and Behavior Therapy for Persistent Insomnia." *American Journal of Psychiatry*. <https://doi.org/10.1176/appi.ajp.159.1.5>.
- Spielman, Arthur J. 1986. "Assessment of Insomnia." *Clinical Psychology Review* 6 (1): 11–25.
[https://doi.org/10.1016/0272-7358\(86\)90015-2](https://doi.org/10.1016/0272-7358(86)90015-2).
- Spielman, Arthur, Paul Saskin, and Michael J Thorpy. 1987. "Treatment of Chronic Insomnia by Restriction of Time in Bed." *Sleep* 10 (1): 45–56. <https://doi.org/10.1093/sleep/10.1.45>.
- Stavropoulos, Nicholas, and Michael W Young. 2011. "Article Insomniac and Cullin-3 Regulate Sleep and Wakefulness in

- Drosophila." *Neuron* 72 (6): 964–76. <https://doi.org/10.1016/j.neuron.2011.12.003>.
- Tabuchi, Masashi, Shahnaz R. Lone, Sha Liu, Qili Liu, Julia Zhang, Adam P. Spira, and Mark N. Wu. 2015. "Sleep Interacts with A β to Modulate Intrinsic Neuronal Excitability." *Current Biology*. <https://doi.org/10.1016/j.cub.2015.01.016>.
- Taub, John M., and Ralph J. Berger. 1973. "Performance and Mood Following Variations in the Length and Timing of Sleep." *Psychophysiology* 10 (6): 559–70. <https://doi.org/10.1111/j.1469-8986.1973.tb00805.x>.
- Toth, Linda A., and Pavan Bhargava. 2013. "Animal Models of Sleep Disorders." *Comparative Medicine* 63 (2): 91–104. https://doi.org/10.1300/J094v06n03_16.
- Trauer, James M., Mary Y. Qian, Joseph S. Doyle, Shantha M W Rajaratnam, and David Cunnington. 2015. "Cognitive Behavioral Therapy for Chronic Insomnia: A Systematic Review and Meta-Analysis." *Annals of Internal Medicine* 163 (3): 191–204. <https://doi.org/10.7326/M14-2841>.
- Veatch, Olivia J., Brendan T. Keenan, Philip R. Gehrman, Beth A. Malow, and Allan I. Pack. 2017. "Pleiotropic Genetic Effects Influencing Sleep and Neurological Disorders." *The Lancet Neurology*. [https://doi.org/10.1016/S1474-4422\(16\)30339-8](https://doi.org/10.1016/S1474-4422(16)30339-8).
- Vienne, Julie, Ryanne Spann, Fang Guo, and Michael Rosbash. 2016. "Age-Related Reduction of Recovery Sleep and Arousal Threshold in Drosophila ." *Sleep*. <https://doi.org/10.5665/sleep.6032>.
- Wu, Jade Q., Erica R. Appleman, Robert D. Salazar, and Jason C. Ong. 2015. "Cognitive Behavioral Therapy for Insomnia Comorbid with Psychiatric and Medical Conditions a Meta-Analysis." *JAMA Internal Medicine* 175 (9): 1461–72. <https://doi.org/10.1001/jamainternmed.2015.3006>.
- Yoshii, Taishi, Christiane Hermann-Luibl, and Charlotte Helfrich-Förster. 2016. "Circadian Light-Input Pathways in Drosophila." *Communicative and Integrative Biology*. <https://doi.org/10.1080/19420889.2015.1102805>.
- Zhang, Shixing, Yan Yin, Huimin Lu, and Aike Guo. 2008. "Increased Dopaminergic Signaling Impairs Aversive Olfactory Memory Retention in Drosophila." *Biochemical and Biophysical Research Communications* 370 (1): 82–86. <https://doi.org/10.1016/j.bbrc.2008.03.015>.

Chapter 3:

A *Drosophila* Model of Sleep Restriction Therapy for Neurodegenerative Disease

Samuel J. Belfer^{1,2}, Anyara Rodriguez², Alexandra Pergelos^{1,3}, Nancy Bonini³
and Matthew S. Kayser^{1,2,4,5}

¹Department of Neuroscience,

²Department of Psychiatry,

³Department of Biology,

⁴Center for Sleep and Circadian Neurobiology, and

⁵Chronobiology Program,

Perelman School of Medicine at the University of Pennsylvania

Acknowledgements

We thank David Raizen and members of the Kayser Lab for helpful discussions. This work was supported by NIH grants F30AG058409 (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 Burroughs Wellcome Career Award for Medical Scientists, March of Dimes Basil O'Connor Scholar Award, and Sloan Research Fellowship (MSK).

AR performed longevity and climbing assays.

AP performed Western blotting experiments.

NB provided experimental and conceptual guidance for this chapter.

MSK provided experimental and conceptual guidance and edited this chapter.

Abstract

With an aging world population, the rate of neurodegenerative disease diagnoses is steadily increasing. Surprisingly, despite an array of more prominent cognitive symptoms, sleep disturbances are a large contributor to the decreased quality of life of both patients and caregivers. Sleep disturbances have been well-characterized in some neurodegenerative diseases; sleep disturbance can even be used as a predictor of disease in some cases, as sleep symptoms can precede the onset of cognitive symptoms by a decade. Here, we find that overexpression of human TDP-43, the protein deposited in intracellular inclusions in ALS and FTD, causes profound sleep disturbances. Overexpression of the *Drosophila* homolog of TDP-43 similarly shows sleep deficits. We demonstrate that Ataxin 2, a known modifier of TDP-43 toxicity, also modifies the sleep phenotype. A candidate-based RNAi screen for modifiers of TDP-43 toxicity identified YT5-21B, a nuclear reader of m6a modifications on RNA, as a novel suppressor of TDP-43 toxicity. YT5-21B knockdown increases sleep time and improves sleep continuity, while extending longevity and improving climbing ability in TDP-43 overexpressing flies. Finally, we demonstrate that a paradigm of Sleep Restriction Therapy that improves sleep in *Drosophila* models of insomnia serves to consolidate sleep and improve sleep quality in TDP-43 overexpressing flies. Improving sleep causes extension of longevity as well in these flies. This work describes novel sleep phenotypes in a model of ALS/FTD, establishes this platform for identifying novel modifiers of TDP-43 toxicity, and demonstrates benefits of improving sleep these models.

Introduction

With an aging world population, the rate of neurodegenerative disease diagnoses is steadily increasing (Reitz, Brayne and Mayeux, 2011; Ascherio and Schwarzschild, 2016). Despite an array of more prominent cognitive symptoms, sleep disturbances are a large contributor to the decreased quality of life of both patients and caregivers (McCurry *et al.*, 1999; Petrovsky *et al.*, 2018). Sleep disturbances have been well-characterized in some neurodegenerative diseases; idiopathic REM Behavior Disorder is considered a risk factor for the development of an α -synucleinopathy, including Parkinson's disease (PD), dementia with Lewy Bodies, and multiple system atrophy (Schenck, Bundlie and Mahowald, 1996; Iranzo *et al.*, 2006; Postuma *et al.*, 2009; Schenck, Boeve and Mahowald, 2013). In fact, diagnosis with REM Behavior Disorder can be used as a predictor of PD subtype and disease course, as REM Behavior Disorder can precede the onset of cognitive symptoms by a decade (Kumru *et al.*, 2007; Postuma *et al.*, 2008; Lin and Chen, 2018). Significant sleep disturbances and EEG abnormalities have also been reported in progressive supranuclear palsy (Aldrich *et al.*, 1989; Gama *et al.*, 2010; Malhotra, 2018). Moreover, recent work suggests a bidirectional relationship between sleep and Alzheimer's disease (AD) pathology, where accumulation of the pathogenic proteins tau and β -amyloid ($A\beta$) worsen sleep while poor sleep accelerates tau and $A\beta$ accumulation (Kang *et al.*, 2009; Jee Hoon Roh *et al.*, 2012; Y. E. S. Ju, Lucey and Holtzman, 2014; Holth *et al.*, 2019). This association has been linked to the clearance of misfolded or aggregating proteins preferentially during sleep, via a process called glymphatic flow (Achariyar *et al.*, 2017). Illustrating the close relationship between sleep features and dementia, serial cognitive testing is common in the workup of parasomnias, and is often responsible for early diagnosis of

neurodegenerative diseases (Gagnon *et al.*, 2009, 2010). Additionally, sleep disturbances are beginning to be recognized in the disease course of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). Long considered a disease that solely affected motor neurons, recent work has implicated cognitive and autonomic dysfunction as being involved in the disease (Congiu *et al.*, 2017). Moreover, recent work indicates sleep is significantly disrupted in ALS/FTD patients, despite subjective reports of no sleep disturbance (Congiu *et al.*, 2019; Panda, Gourie-Devi and Sharma, 2019). Therefore, elucidating the cellular mechanisms that couple neurodegenerative diseases to sleep disruption could reveal new treatment avenues.

Many neurodegenerative diseases feature the aberrant accumulation of proteins in cellular compartments that induce toxicity to neurons and glia. Animal models should recapitulate these cellular phenomena, while also displaying the behavioral sequelae experienced by patients suffering from disease. *Drosophila* models of neurodegenerative diseases have been well-established (K. Iijima *et al.*, 2004; Estes *et al.*, 2013; Zhan *et al.*, 2013; Tabuchi *et al.*, 2015; Sun *et al.*, 2016; Dissel *et al.*, 2017; Ito *et al.*, 2017; Buhl, Higham and Hodge, 2019), and their genetic tractability has enabled large-scale screens that have identified novel modifiers of disease (Zhan *et al.*, 2013; Kim *et al.*, 2014; Pons *et al.*, 2017; C. Y. Chung *et al.*, 2018; McGurk *et al.*, 2018). The robust sleep behavior of flies makes them an excellent system to explore the relationship between sleep and neurodegeneration (Keene and Joiner, 2015; Tabuchi *et al.*, 2015; Chakravarti, Moscato and Kayser, 2017). Previous work has identified sleep deficits in *Drosophila* models of Alzheimer's disease (Tabuchi *et al.*, 2015; Dissel *et al.*, 2017), tauopathy (Buhl, Higham and Hodge, 2019), ALS/FTD (Estes *et al.*, 2013), and Parkinson's disease (Sun *et al.*, 2016; Ito *et al.*, 2017).

Furthermore, several groups have demonstrated that sleep improvement has positive behavioral outcomes in neurodegeneration models, whether by pharmacotherapy (Dissel *et al.*, 2017) or behavioral sleep therapy adapted from human techniques (Belfer *et al.*, 2019). Here, we took a candidate-based approach to analyze sleep in protein overexpression models of neurodegenerative disease, and found that TDP-43 overexpression causes severe sleep deficits. Some known modifiers of TDP-43 toxicity also modified sleep behavior, while sleep restriction therapy, a behavioral approach modeled after human techniques (Belfer *et al.*, 2019), was capable of modifying sleep despite accumulation of TDP-43. We used this approach to conduct a screen for genetic modifiers of the TDP-43 overexpression sleep phenotype. We discovered that knockdown of m6a nuclear reader protein YT5-21B significantly improved sleep, concurrently rescuing locomotor deficits and extending lifespan. Our findings demonstrate that sleep disturbances are closely associated with models of ALS/FTD, and utilizes this platform to identify novel modifiers of TDP-43 toxicity.

Methods

Fly Strains

*Iso*³¹, pdf-Gal4, and Daughterless-GS flies were obtained from A. Sehgal. *UAS:AβArctic* flies were obtained from M. Wu. *UAS:APP*, *UAS:BACE* flies were obtained from D. Marenda. *UAS-dAtx2.1B* and *UAS-dAtx2.4* were obtained from T. Satterfield. All of these lines were outcrossed at least 5x into the *iso*³¹ background. Elav-Gal4 (#458) and all RNAi lines were obtained from the Bloomington Drosophila Stock Center. Flies were maintained on standard yeast/cornmeal-based medium at 25 degrees on a 12hr:12hr light:dark cycle.

RU486 Food Preparation

To prepare a 50mM stock solution, 215mg RU486 (Fisher) was dissolved in 10mL 100% Ethanol. 100mL of standard food or sucrose agar was melted, and 1mL of RU486 or EtOH was added and mixed once cooled. Molten solution was poured into empty Polystyrene vials (Genessee Scientific) or used to fill DAM tubes (Trikinetics).

Sleep Analysis

Male and female flies were collected at 1-3 days old and aged in group housing. Flies were then loaded at the appropriate age into glass tubes containing 5% sucrose and 2% agar and RU486 or EtOH as described above. Locomotor activity was monitored using the Drosophila Activity Monitoring (DAM) system (Trikinetics, Waltham MA). Activity was measured in 1 min bins and sleep was defined as 5 minutes of consolidated inactivity (Gilestro, 2012). Data was processed using PySolo software (Gilestro and Cirelli, 2009). Sleep latency (SL) was determined by time (minutes) until first sleep episode following lights off. 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.

Sleep Restriction Therapy

SRT was performed as previously described (Belfer *et al.*, 2019). Briefly, *Da-GS>UAS-TDP-43^{37M}* male flies were collected at day 1-3 post eclosion, aged 6 days on RU486, and loaded into incubators with light schedules of 12:12 LD (9AM-9PM), 18:6 LD (12AM-6AM), 16:8 LD (11PM-7AM), or 14:10 LD (10PM-8AM, Figure 3A). Days 2-3 day of the new LD cycle was used for analysis.

Longevity Assay

Ten replicate vials, each containing 10 male and 5 female flies, were established for each condition. Female flies were not assessed for longevity. Flies were transferred to fresh standard food containing RU486 or EtOH vials daily (Figure 3), or every other day (Figure 4), at which time dead flies were removed and recorded. Assays were conducted at least twice per genotype.

Negative Geotaxis Assay

Five replicate vials, containing 10 male flies, were established on RU486 or EtOH vials and aged for 5 days. Flies were transferred to empty vials, and gently tapped down to the bottom after which they were video recorded. After 5 seconds, videos were scored such that each fly received a score of 0-6 corresponding to centimeters climbed.

Individual fly scores were averaged for each vial and three trials were averaged to obtain a performance index. Assays were conducted at least twice per genotype.

Western Blotting

Adult male fly heads (6-7 days old, 5 days on RU486) were homogenized in Laemmli sample buffer (Bio-Rad) with β ME, boiled and centrifuged to remove debris. NuPAGE 4–12% Bis-Tris gel (ThermoFisher) was used to run the samples. Proteins were transferred to nitrocellulose membrane by the iBlot blotting system (ThermoFisher). Primary antibodies used were anti-TDP-43 rabbit polyclonal antibody (1:5000; Proteintech; #10782-2-AP) and anti- α -tubulin rabbit polyclonal antibody conjugated with horseradish peroxidase (HRP) (1:1000; Cell Signaling; #9099). The secondary antibodies used were goat anti-rabbit IgG-HRP (1:5000; Milipore, #AP307P) and goat anti-mouse (1:5000; Jackson ImmunoResearch; #115-035-146). All blocking and antibody incubations were

done in 5% milk in phosphate-buffered saline (PBS) overnight (O/N) at 4 °C for primary and 1 h at room temperature (RT) for secondary. Signals were developed by ECL plus (ThermoFisher) or ECL prime (GE healthcare) western blotting reagents. The images were scanned by Amersham Imager 600 (GE healthcare), and quantification was performed using ImageJ.

Statistical Analysis and Data Reproducibility

Analysis was done using Prism (GraphPad Software). ANOVA with Tukey's test was used in Figure 1A, B, I, J; Figure 2B, C; Figure 3H, I, K; Figure 4C, D; Supplementary Figure 1F; Supplementary Figure 2A; Supplementary Figure 3L; and Supplementary Figure 4C. Student's t-test was used in Figure 1G, H; Figure 3D, E, F; Supplementary Figure 1A, C, D, E; Supplementary Figure 3F, H, I, J; and Supplementary Figure 4A. Log-rank test was used in Figure 3G and Figure 4F. Fisher's Exact Test was used in Figure 4E; and Supplementary Figure 4D. For significance: * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$. Each experiment was generated from a minimum of 3 independent biological replicates. Samples were allocated based on genotype or experimental manipulation and statistics performed on aggregated data. 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 (Kayser, Yue and Sehgal, 2014; Dilley *et al.*, 2018).

Results

TDP-43 overexpression impairs sleep in *Drosophila*

In aiming to elucidate the relationship between neurodegeneration and sleep impairment, we examined sleep in a number of established *Drosophila* models of neurodegenerative diseases. To avoid developmental effects of these lines and to ensure broad expression, we utilized the inducible GeneSwitch driver under control of the daughterless promoter (Da-GS). We examined sleep after 7 days of Da-GS activation on RU486 in adults. We did not observe significant changes in total sleep time or sleep bout duration for most models of neurodegeneration (Figure 1A,B). However, ectopic expression of TDP-43, the major pathological protein in ALS/FTD, caused a loss of ~200 minutes of sleep during the dark period (542.6 ± 10.1 min for Da-GS/+ vs. 316.2 ± 23.66 min for Da-GS>UAS-TDP-43, Figure 1A), and increased sleep fragmentation as evidenced by a nearly 3-fold reduction in the length of the average sleep bout (44.94 ± 2.53 min for EtOH control vs. 17.37 ± 1.97 min for RU486, Figure 1D). The finding that increased TDP-43 levels can induce sleep changes is consistent with previous work in *Drosophila* models with mutations in TDP-43 (Estes *et al.*, 2013). To confirm the result of this initial screen, we measured sleep in fly lines with two separate insertions of UAS-TDP-43: one line with medium-strength expression (UAS-TDP-43^{37M}) and one line with strong expression (UAS-TDP-43^{52S}). Both lines demonstrated similar sleep deficits; overexpression of UAS-TDP-43^{37M} caused reduction of sleep by days 7-10 on RU486, whereas overexpression of UAS-TDP-43^{52S} caused decreased sleep by days 4-6 on RU486, while much of the population was dead by days 7-10 (Figure 1 D-E). Both lines showed significant decreases in total sleep time and sleep continuity on

RU486 when compared to EtOH control (Figure 1G-H, Supplementary Figure 1A). We also confirmed the sleep phenotype of Da-GS>UAS-TDP-43^{37M} flies using a higher spatial resolution sleep assay (Supplementary Figure 1B-D). This assay uses 17 distinct infrared beams to more finely detect position and movement of flies in the monitoring apparatus. As TDP-43 overexpressing flies have been demonstrated to have motor deficits (Kim *et al.*, 2014), we were concerned that a locomotor phenotype could account for the observed sleep changes. Importantly, locomotor impairments would likely result in less movement and therefore be interpreted as increased sleep in our system, yet our TDP-43 overexpression model demonstrates reduced sleep. Moreover, no change to the waking activity of flies was observed in our analysis (Supplementary Figure 1E). This indicates that flies are able to move normally in the *Drosophila* Activity Monitoring system, and that previously described motor deficits are not responsible for sleep changes.

We asked if overexpression of TBPH, the *Drosophila* ortholog of human TDP-43, in adult flies would mirror the sleep changes observed with overexpression of TDP-43. TBPH overexpression induced severe toxicity, and most flies were dead by day 5 (unpublished observations). However, flies began to exhibit sleep changes by the second day on RU486, and prominent decreases in total sleep time and sleep fragmentation could be observed by day 3 (Figure 1E,G-H). Mutations in the fused in sarcoma (FUS) gene is one of the most common causes of familial ALS (Mathis *et al.*, 2019). Importantly, FUS protein aggregates in inclusions that resemble pathologic findings in human ALS brains (Sun *et al.*, 2011). Overexpression of FUS in flies also caused decreased TST, while fragmenting sleep as well (Figure 1F-H). Together, these results suggest that overexpression models of ALS/FTD in *Drosophila* cause decreased total sleep and sleep fragmentation.

To determine whether the sleep deficits observed were a result of neuronal functions, we expressed UAS-TDP-43^{52S} using the pan-neuronal drivers *elav-Gal4* and *nsyb-Gal4*. *Elav-Gal4*, but not *nsyb-Gal4*, caused a decrease in total sleep time, but both neuronal drivers caused significant fragmentation of sleep during the dark period (Figure 1I-J). We next asked if motor neuron toxicity was responsible for sleep changes, as motor neuron degeneration is the primary mechanism of pathology in human ALS, as well as locomotor deficits in *Drosophila* models (Estes *et al.*, 2013). We actually observed a slight increase in total sleep time, and no change to sleep fragmentation using motor neuron driver *D42-Gal4* to drive UAS-TDP-43^{52S} (Figure 1I-J), suggesting sleep changes were not a direct result of motor neuron toxicity. These results demonstrate that neuronal overexpression of TDP-43, but not motor neuron overexpression, is sufficient to induce sleep changes.

Ataxin 2, a known modifier of TDP-43 toxicity, modifies sleep phenotype

To examine whether impaired sleep with TDP-43 overexpression is directly tied to TDP-43 cellular toxicity, we asked whether sleep is changed with modulation of Ataxin 2, a known modifier of TDP-43 (Elden *et al.*, 2010). Coincident upregulation of *Atx2* and TDP-43 in *Drosophila* worsens retinal degeneration and severely truncates lifespan, beyond that of expressing *Atx2* or TDP-43 alone (Elden *et al.*, 2010). Moreover, genetic knockouts of *Atx2* ameliorate TDP-43 toxicity and cellular inclusions in mouse models (Becker *et al.*, 2017). Therefore, we asked if modulation of *Atx2* levels in *Drosophila* would affect sleep in a TDP-43 overexpression background. To test whether overexpression of both TDP-43 and *Atx2* would worsen sleep, we used the *Da-GS* driver to express both UAS-TDP-43^{52S} and UAS-*Atx2.1B*. All flies died within 2 days of being

placed on RU486, preventing sleep analysis. We then asked if RNAi-mediated knockdown of *Atx2* would ameliorate sleep deficits observed in *Da-GS>UAS-TDP-43^{52S}*. Using this approach, we observed a twofold increase in total sleep time during the dark period in each of three distinct RNAi lines when compared to mCherry RNAi (Figure 2A-B). Moreover, *Atx2* knockdown caused increases in sleep bout duration in most lines and decreases in the number of sleep bouts in all lines, indicating consolidation of sleep (Figure 2B, Supplementary Fig 2A). We next asked what effect *Atx2* knockdown has on sleep in the absence of TDP-43 overexpression. Driving *Atx2* RNAi expression with *Da-GS* did not change total sleep time, nor was any significant change observed to sleep fragmentation (Supplementary Figure 2B). Taken together, this suggests *Atx2* knockdown suppresses sleep deficits caused by TDP-43.

Sleep screen for genetic modifiers of TDP-43 toxicity reveals novel hits

Following the finding that TDP-43 overexpression decreased sleep time and continuity, we initiated a screen to search for modifiers of TDP-43 sleep degradation (Supplementary Fig 3A). We utilized the *Drosophila* TRiP RNAi collection to disrupt expression of selected genes (Perkins *et al.*, 2015). Using *Da-GS* as a driver, our screen was designed to detect modifiers of sleep in adults, as activation of the inducible driver occurred after eclosion. We initially selected RNAi lines for genes previously identified as enhancers or suppressors of TDP-43 neurotoxicity in the eye (Elden *et al.*, 2010; Kim *et al.*, 2014; C. Y. Chung *et al.*, 2018; McGurk *et al.*, 2018). Surprisingly, most of these lines demonstrated no enhancement or suppression of sleep degradation when compared to control mCherry RNAi expression (Supplementary Figure 3B-E). This finding was intriguing, as perhaps a novel behavioral output would reveal novel

modifiers of TDP-43 pathology. We next decided to broaden our screen by selecting RNAi transgenic lines by gene ontology searches of terms of interest (Supplementary Table 1). These terms were selected by associations to sleep or to TDP-43 pathology. Of the 825 RNAi transgenes assayed, 141 produced significant changes to sleep upon the initial screening compared to mCherry RNAi controls (Figure 3A). Of these initial screen hits, 86 RNAi lines subsequently reproduced sleep effects upon rescreening (Figure 3B).

m6a nuclear reader YT5-21B is a novel suppressor of TDP-43 toxicity

One of the surprising results of the screen was that knockdown of YT5-21B, a reader of N⁶-methyl adenosine (m⁶A) RNA modifications that is found only in the nucleus, significantly suppressed TDP-43 sleep deficits (Figure 3C). m⁶A modifications are the most abundant and evolutionarily-conserved of post-transcriptionally added modifications to RNA (Berlivet *et al.*, 2019). m⁶A readers bind to RNA; this can increase affinity of RNA-binding proteins for particular RNA residues, or can alter the RNA structure to promote binding in what has been called a “m⁶A switch.” (Roost *et al.*, 2015) *Drosophila* has one m⁶A reader protein in the nucleus, called YT5-21B, and another in the cytoplasm, CG6422. In our screen YT5-21B RNAi transgene expression in the setting of TDP-43 overexpression produced a twofold increase in total sleep time over the 24 hour day, including an increase of nearly 200 minutes during the dark period (Figure 5D-E). Moreover, YT5-21B knockdown consolidated sleep via a threefold increase in sleep bout duration and corresponding decrease in total bouts (Figure 3F, Supplementary Figure 3F). No sleep change was observed when YT5-21B knockdown occurred in the absence of TDP-43 overexpression, indicating that sleep improvement is

likely a result of the interaction between these proteins (Supplementary Figure 3G). Conversely, RNAi knockdown of cytoplasmic m⁶A reader protein CG6422 in flies overexpressing TDP-43 showed only a small increase in total sleep time. This was a result of trying to initiate more sleep bouts, not due to increased bout duration (Supplementary Figure 3H-J). Together, these findings indicating a unique role of the nuclear m⁶A reader YT5-21B in affecting sleep.

Is YT5-21B knockdown causing reversal of the sleep deficit by reducing levels of TDP-43? To test this possibility, we performed western blotting for TDP-43 protein, and observed no change in total TDP-43 compared to control mCherry RNAi levels (Figure 3J-K). We next asked whether rescue of sleep due to RNAi knockdown of YT5-21B would have other beneficial behavioral effects. TDP-43 overexpression models exhibit severely truncated lifespan (Kim *et al.*, 2014; Berson *et al.*, 2017; McGurk *et al.*, 2018), so we tested whether YT5-21B knockdown would affect longevity in this model. Comparing flies expressing YT5-21B RNAi transgene to controls expressing mCherry RNAi, we found that knockdown of YT5-21B significantly extended lifespan of male flies (Figure 3G). Median survival was extended by approximately 2 days, a 20% extension compared to control flies. Disturbances in climbing ability have been previously characterized in TDP-43 overexpression models (Kim *et al.*, 2014). We wondered if knockdown of YT5-21B would rescue climbing dysfunction as well. As expected, we observed severe deficits in climbing performance of Da-GS>UAS-TDP-43 flies after 5 days on RU486 compared to controls of the same genetic background on EtOH (Figure 3H). Climbing ability was partially rescued by YT5-21B RNAi transgene expression, showing a threefold increase in both performance index and percentage of flies climbing 6 cm in a 5-second window. Taken together, YT5-21B knockdown improves sleep, while rescuing other behavioral deficits incurred by TDP-43 overexpression.

While our data suggest a nuclear role of TDP-43 in its degradation of sleep, cytoplasmic stress granules have been commonly associated with neurotoxicity (Mann *et al.*, 2019). Stress granules are membraneless organelles that sequester mRNA, ribosomal subunits, and RNA-binding proteins, including TDP-43 (Molliex *et al.*, 2015). Stress granule formation is activated during cellular stress, so we asked whether stress due to sleep deprivation was sufficient to induce further SG formation. Levels of eIF2 α phosphorylation are directly correlated with SG amount, as these phosphorylated proteins accumulate in cytoplasmic granules (Kim *et al.*, 2014). Therefore, we extracted protein from the heads of TDP-43 overexpressing flies under 12:12 LD control conditions or 12- or 24-hr mechanical sleep deprivation, and measured phosphorylated eIF2 α levels. We found that sleep deprivation of either duration did not significantly affect the amount of phosphorylated eIF2 α protein by Western blot (Supplementary Fig 3K-L), arguing against a cytoplasmic stress granule etiology coupling sleep and TDP-43. Taken together, these results propose a nuclear role for TDP-43-mediated sleep degradation.

Sleep Restriction Therapy improves sleep in *Drosophila* model of ALS/FTD

Cognitive Behavioral Therapy for Insomnia is the first-line therapy for insomnia in human patients, a key component of which is Sleep Restriction Therapy (SRT) (Riemann *et al.*, 2015). SRT enables poor sleepers to effectively match limited sleep ability to sleep opportunity by reducing the amount of time spent in bed, thereby maximizing sleep drive. Previous work has demonstrated that SRT can be adapted to improve sleep in *Drosophila* models of insomnia and neurodegeneration (Belfer *et al.*, 2019). In humans undergoing SRT, the amount of sleep opportunity is determined

based on an individual's total sleep time (TST); further compression can result in sleep rebound during the subsequent day. Applying this approach to *Da-GS>UAS-TDP-43^{37M}*, whose sleep time is 300-400 minutes (Figure 1H), dark time was compressed from the baseline 12 hours light:12 hours dark (12:12 LD) cycle by initially contracting dark time to 6 hours (18:6 LD, Figure 4A-B). Importantly, no homeostatic rebound sleep was observed in the 2 hours following lights on in flies under 18:6 LD conditions (Supplementary Figure 4A). These flies are capable of rebound sleep following sleep deprivation (Supplementary Figure 4B), indicating that the sleep homeostat is intact in these animals. Next, we compared three different durations of SRT (18:6 LD, 16:8 LD, and 14:10 LD) to determine the optimal matching of sleep opportunity to the sleep ability of TDP-43 overexpressing flies (Figure 4A). Previous work has demonstrated that optimal matching maximizes sleep continuity (long bout duration, few bouts), while not affecting total sleep time (Belfer *et al.*, 2019). We observed that flies under 18:6 LD cycles had less total sleep during the dark period than under 12:12 LD conditions, but no change in sleep amount was observed under either 16:8 LD or 14:10 LD conditions (Figure 4C). This indicates that flies were able to compress their typical sleep amount into a smaller time window, increasing sleep efficiency. The enhancement of sleep efficiency was coupled with an increase in sleep consolidation under 18:6 LD and 16:8 LD conditions, evidenced by an increase in sleep bout duration and decrease in number of total bouts (Figure 4D, Supplementary Figure 4A). Consolidation of sleep in flies under 14:10 LD conditions was unchanged from 12:12 LD controls. These results suggest that 16:8 LD presents the optimal matching of sleep opportunity and sleep ability in flies with TDP-43 overexpression, as flies at this LD cycle show increased sleep continuity while demonstrating no change to total sleep time.

Do SRT-induced changes to sleep consolidation in TDP-43 overexpressing flies correspond to increased depth of sleep? To answer this question, we exposed flies under 12:12 LD or 16:8 LD cycles to mechanical stimuli designed to arouse flies from sleep. While 69% of flies under 12:12 LD conditions were aroused from this stimulus, significantly fewer sleeping flies under SRT were aroused (49%, Figure 4E). This indicates that improvements in sleep quality due to SRT correspond with increased sleep depth.

Discussion

The link between sleep and neurodegeneration has been well-established, but the exact molecular mechanisms that underly effects to cellular toxicity have not been fully established. Adequate sleep is increasingly viewed as a modifiable risk factor for many neurodegenerative diseases, as sleep begins to deteriorate decades before the onset of cognitive decline. Increasingly, sleep's role in disease is being elucidated; flow through glymphatic fluid transport system robustly increases during sleep versus wake (Acharyar *et al.*, 2017). This finding proposes sleep as a privileged time for increased flow and clearance of accumulated toxins that localize to the extracellular space. While Alzheimer's disease feature extracellular A β plaques in brain pathology that can flow out of the brain through the glymphatic system, ALS/FTD pathological findings are predominantly intracellular. What effects might sleep be having on this different etiology of neurodegeneration?

Research in *Drosophila* has yielded numerous insights into basic sleep neurobiology as well as an understanding of molecular mechanisms of neurodegenerative disease. Here, we have leveraged this model system to develop a

tractable model to study sleep deficits associated with TDP-43 accumulation, common to ALS and FTD. We observe that sleep deteriorated with broad overexpression of TDP-43, and toxicity is suppressed by knockdown Ataxin 2, consistent with previously identified mechanisms of disease (Elden *et al.*, 2010). We utilized this platform for discovery of novel modifiers of TDP-43 toxicity to sleep. Using a candidate-based approach, we identified that YT5-21B, a nuclear reader of the RNA modification m⁶a was a modifier of TDP-43 toxicity. Knockdown of this protein was sufficient to improve sleep in a TDP-43 overexpression background. Finally, we previously developed a paradigm that adapts tenets of human behavioral therapy to improve sleep in flies with short sleep phenotypes. Application of this paradigm to TDP-43 overexpressing flies served to improve sleep and extend lifespan in this *Drosophila* model of ALS/FTD. These data establish a novel platform for identifying modifiers of TDP-43 cellular toxicity, as well as identifying a behavioral output that reflects the progression of disease.

Toward understanding the effect of TDP-43 on sleep

In recent years, sleep deficits have been characterized in human patients with ALS (Congiu *et al.*, 2019; Panda, Gourie-Devi and Sharma, 2019). These patients feature sleep fragmentation and nighttime awakenings as prominent symptoms, and these studies suggest that sleep disturbances are underreported in this population. In *Drosophila*, previous work has identified that mutated forms of TDP-43 in motor neurons can induce small changes in sleep (Estes *et al.*, 2013). In contrast, our overexpression model shows a near 40% reduction in total sleep time, as well as dramatic degradation in sleep continuity. Additionally, our work indicates that neuronal TDP-43 expression is sufficient to induce a sleep phenotype, but expression in motor neurons alone has no effect on total sleep time or sleep continuity.

Intriguingly, previous work has shown that depletion and overexpression of TDP-43 cause similar gene expression changes (Vanden Broeck *et al.*, 2013), implying that manipulation of TDP-43 levels in either direction may be activating similar pathways (McGurk, Berson and Bonini, 2015). Our data supports this notion, showing that overexpression of TDP-43 produces significant short sleeping phenotypes, and adding to previous work with TDP-43 mutations. How then might TDP-43 affect sleep levels? There is evidence from other neurodegenerative models that accumulation of aggregating proteins serves to promote hyperexcitability in neurons. Do TDP-43 accumulations cause these same intracellular effects? Further investigation into the mechanisms of TDP-43 accumulation on *in vivo* neurons in flies is necessary to elucidate these potential effects.

A novel screening platform for modifiers of disease

Modifiers of TDP-43 toxicity have been previously identified by looking for suppressors and enhancers of neurodegeneration in the *Drosophila* eye (Kim *et al.*, 2014; Berson *et al.*, 2017; C. Y. Chung *et al.*, 2018; McGurk *et al.*, 2018). While this method has been fruitful in identifying modifiers, behavioral outputs provide an additional high-throughput way to discover novel interactions. To this end, Ataxin 2 knockdown ameliorated sleep deficits in our assay, recapitulating its effects on eye degeneration. However, other previously-identified modifiers of TDP-43 toxicity produced no sleep changes in our assay. Further inquiry is necessary to determine why known modifiers of eye toxicity do not manifest in sleep changes. This screening platform identified that YT5-21B, a nuclear reader of m6a modifications on RNA, is a suppressor of TDP-43 toxicity. Surprisingly, the cytoplasmic m6a reader produced limited effects on sleep. While the leading hypotheses support cytoplasmic stress granules driving TDP-43

toxicity, our findings suggest a nuclear etiology may also be contributing to sleep changes. Further work will isolate RNA binding proteins downstream of YT5-21B interactions with m6a-tagged RNA, seeking to elucidate the mechanism by which this protein improves sleep.

Importantly, sleep improvement correlates with increased climbing ability and extended lifespan with knockdown of YT5-21B. This again supports a powerful role of improved sleep in ameliorating neurodegenerative processes occurring with TDP-43 overexpression. Future work will include broadening our screen to identify additional suppressors and enhancers of TDP-43 toxicity, hoping to identify new treatment targets for this disease.

Behavioral Sleep Therapy for the treatment of neurodegenerative disease

We have previously demonstrated that by applying the tenets of human Cognitive Behavioral Therapy for Insomnia to *Drosophila* models of neurodegeneration that feature short sleeping phenotypes, we could improve sleep and extend longevity of these animals (Belfer *et al.*, 2019). Here, we demonstrate in another model of neurodegenerative disease that sleep is modifiable via sleep restriction therapy. Furthermore, rescuing sleep continuity improves lifespan, indicating that there are beneficial effects of improved sleep for the entire organism.

This fly model of behavioral sleep modification can be used to generate granular insights into the dynamic interplay between sleep and neurodegenerative disease. 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 treatment targets that are conceptually based on the established efficacy of CBT-I.

Figures

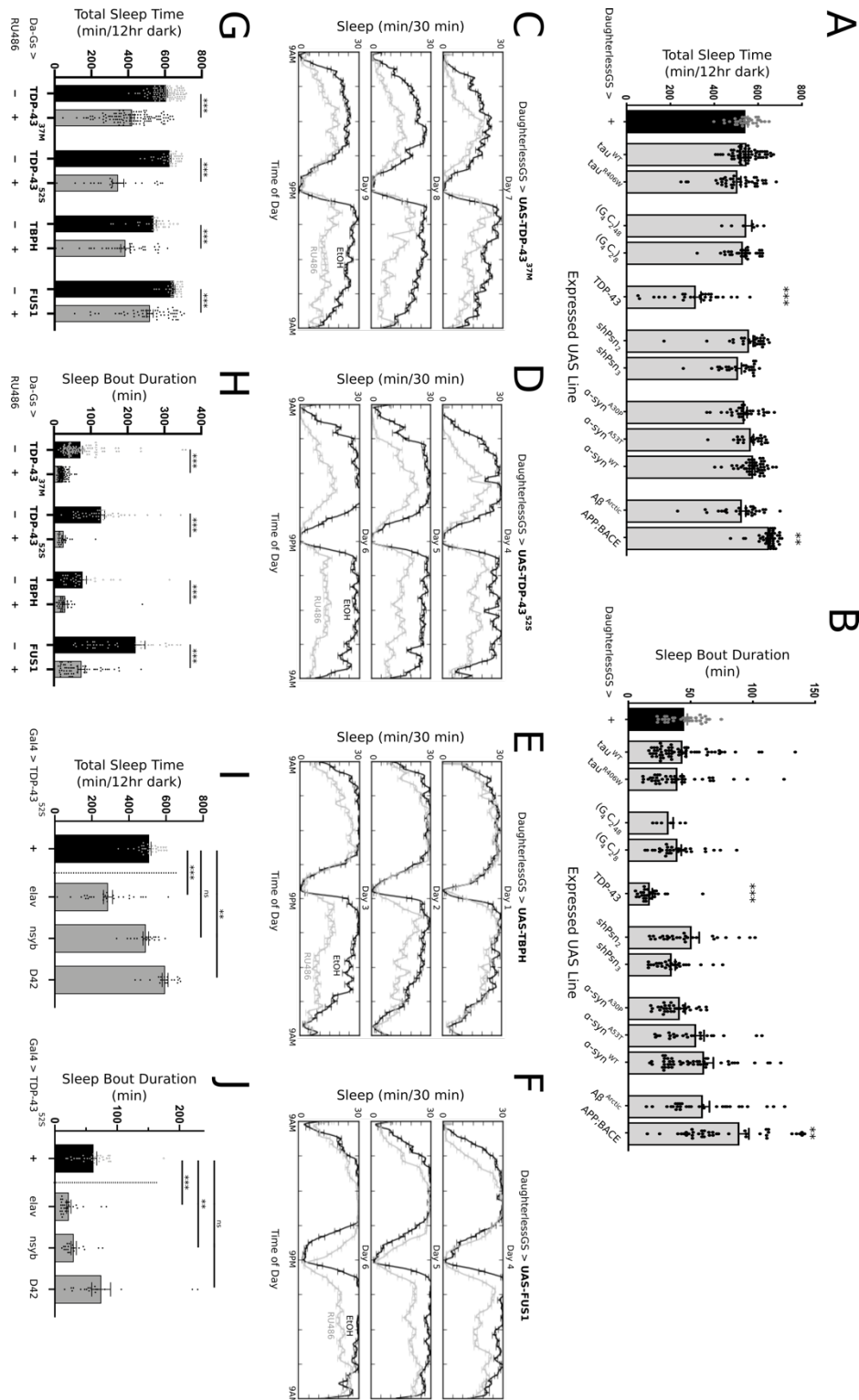


Figure 1. TDP-43 overexpression causes sleep deterioration.

Screen of sleep phenotypes of overexpression models of neurodegenerative diseases. Quantification of total sleep time (A) and sleep bout duration (B, $n > 32$ flies per condition). (C-F) Representative sleep traces of ALS models on ethanol (gray traces) or RU486 (gray traces). Day of assay was determined based on toxicity of transgene expression. Quantification of total sleep time (G), and sleep bout duration (H) for ALS models ($n > 32$ flies per condition). (H-I) Sleep analysis with limited overexpression of UAS-TDP-43 to all neurons or neuronal subpopulation. Quantification of total sleep time (H) and sleep bout duration (I). $n > 32$ flies. For all figures, error bars represent SEM; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

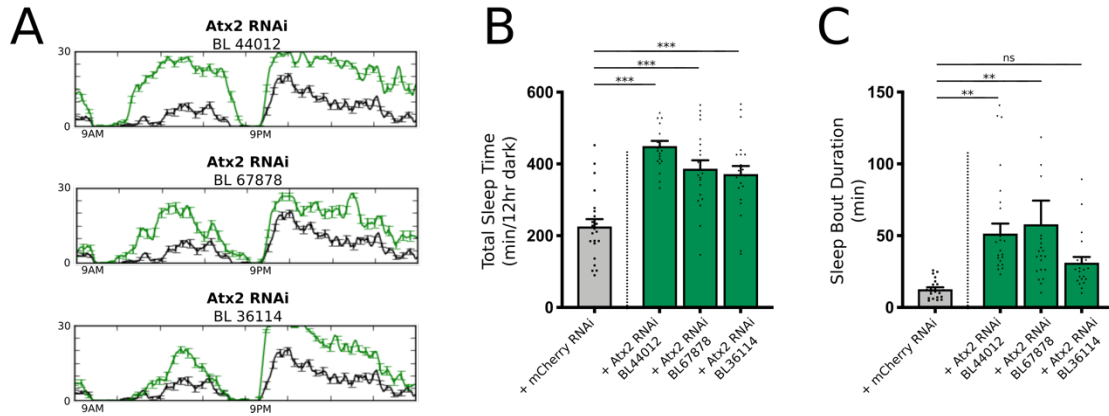


Figure 2. Ataxin 2 knockdown modifies sleep phenotype with TDP-43 overexpression.

(A-C) Representative sleep traces of Da-GS>UAS-TDP-43^{52S} flies, also expressing Ataxin2 RNAi after 5 days on RU486 food. Quantification of total sleep time (B) and sleep bout duration (C) with Ataxin2 RNAi expression (n = 24 flies).

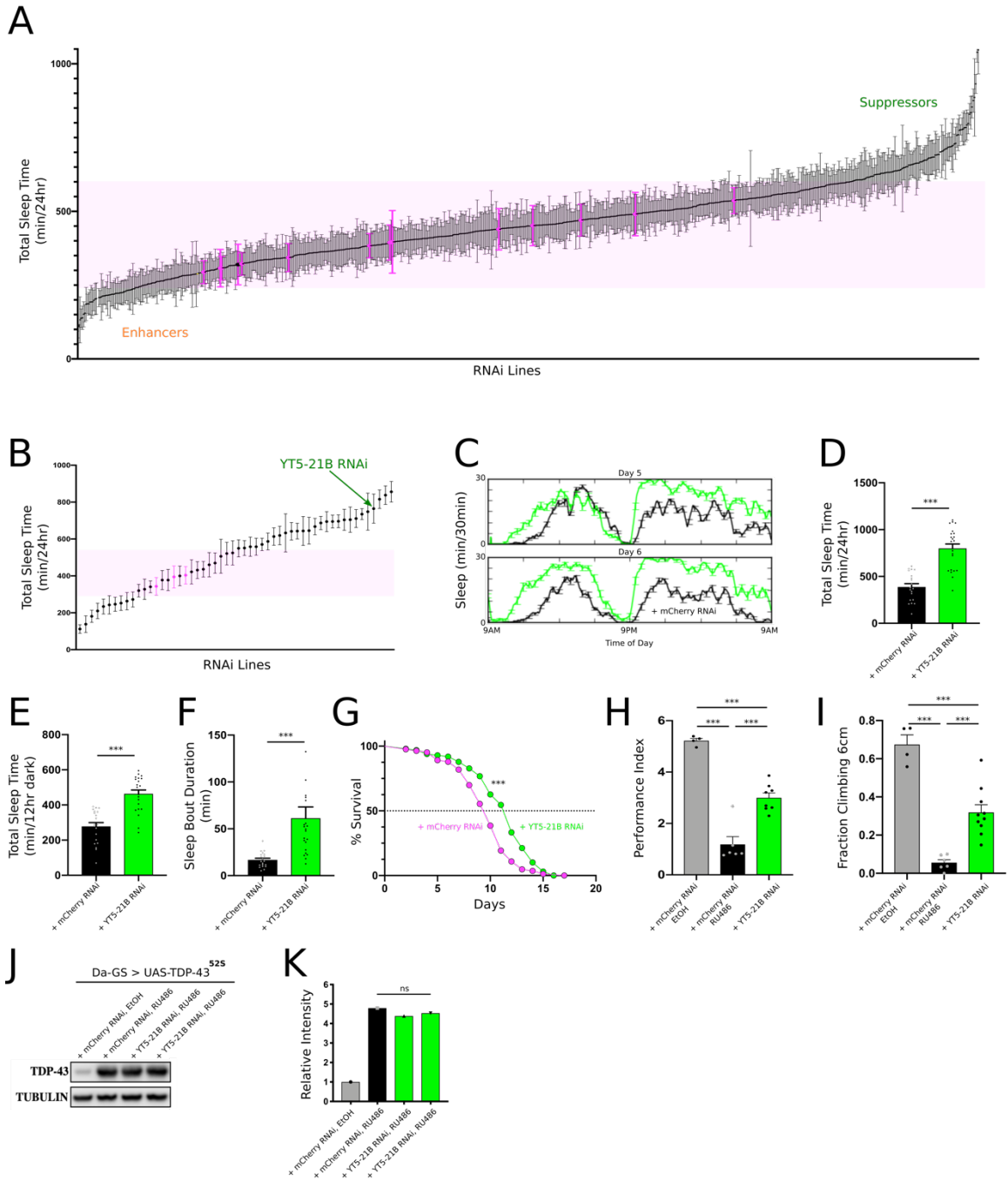


Figure 3. TDP-43 modifier screen identifies m6a nuclear reader YT5-21B as a suppressor of toxicity.

(A) Summary of total sleep time distribution of 825 screened RNAi lines. Pink points represent mCherry RNAi controls for each day of screening. Pink shading indicates range of control sleep. Suppressors and

enhancers identified from primary screen were rescreened to ensure durability of sleep phenotype (B). (C) Representative sleep trace of Da-GS>UAS-TDP-43^{52S}, YT5-21B RNAi after 4 days on RU486. Quantification of total sleep time in 24 hours (D), during 12 hours of night (E), and sleep bout duration at night (F). (G) Survival curves of Da-GS>UAS-TDP-43^{52S}, mCherry RNAi (pink, n = 200 males) and Da-GS>UAS-TDP-43^{52S}, YT5-21B RNAi (green, n = 200 males). Measurements of negative geotaxis performance index (H) and % of flies climbing 6cm in 5 seconds after tapping down. Western blot (J) and total protein quantification (K) of TDP-43 in Da-GS>UAS-TDP-43^{52S}, mCherry RNAi or Da-GS>UAS-TDP-43^{52S}, YT5-21B RNAi heads (n = 20 male heads).

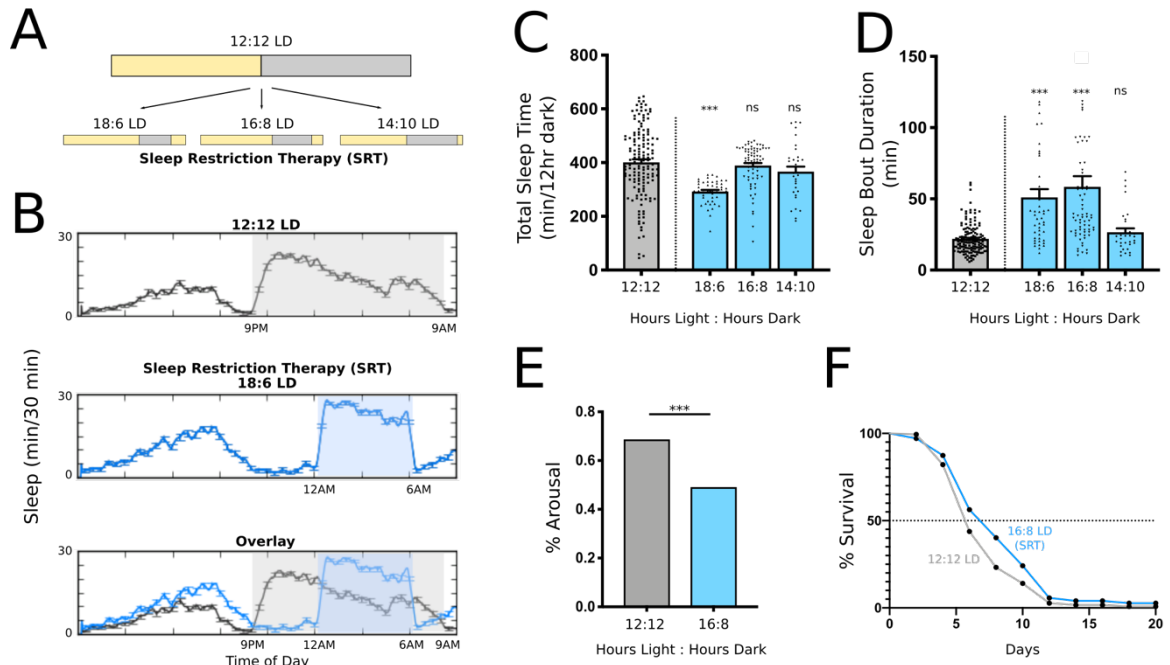
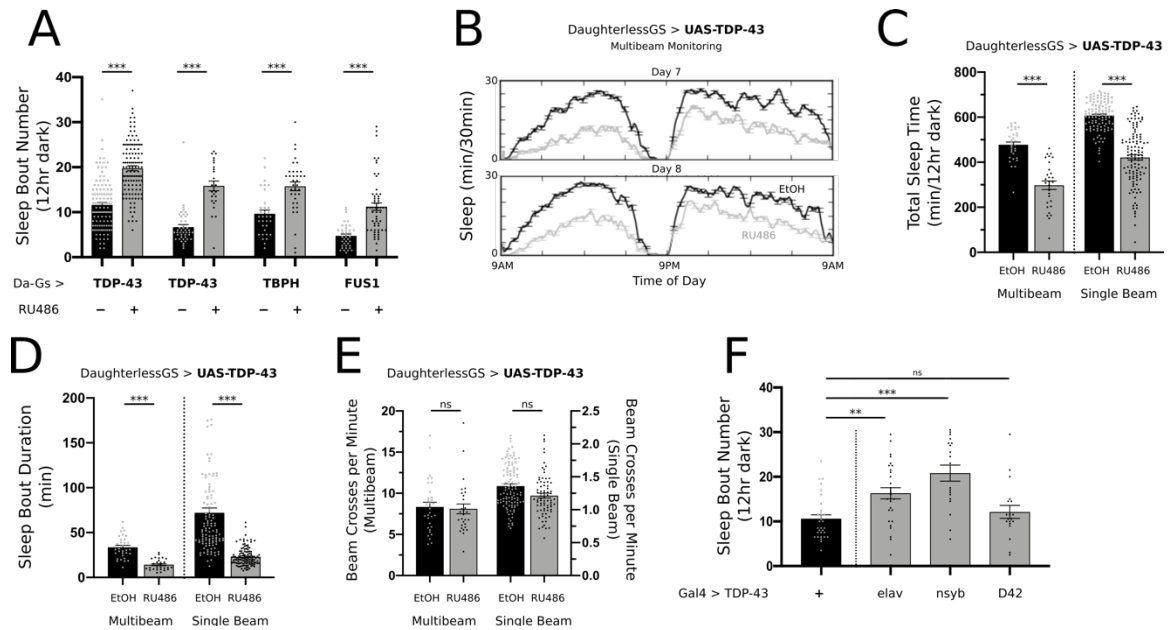


Figure 4. Sleep Restriction Therapy improves sleep and longevity in TDP-43 overexpression model of ALS.

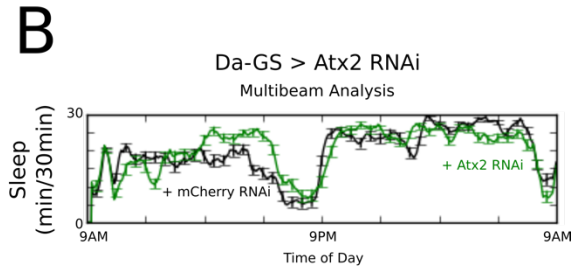
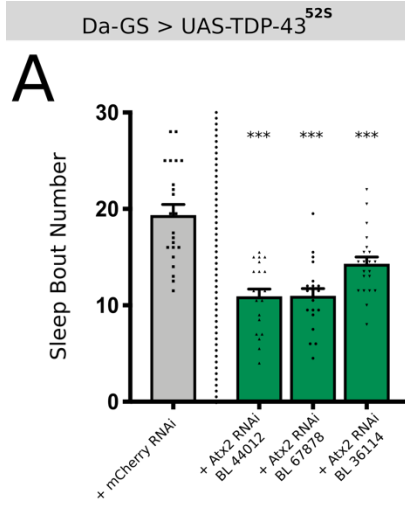
(A) Schematic of experimental protocol for determining optimal light:dark cycle for Sleep Restriction Therapy by manipulating the dark period. (B) Representative sleep traces of Da-GS>UAS-TDP-43^{37M} flies under 12:12 LD conditions (top, gray shading indicates dark), 18:6 LD SRT conditions (middle, blue shading indicates dark), and overlaid plots (bottom). Quantification of total sleep time (D), and sleep bout duration (D, n > 32 flies) for different light:dark cycle durations of SRT. (E) Percentage of flies aroused from a mechanical stimulus delivered during dark period. (F) Survival of male flies on RU486 under 12:12 LD conditions (gray) or 16:8 LD SRT conditions (blue, n = 100 flies).

Supplementary Figures



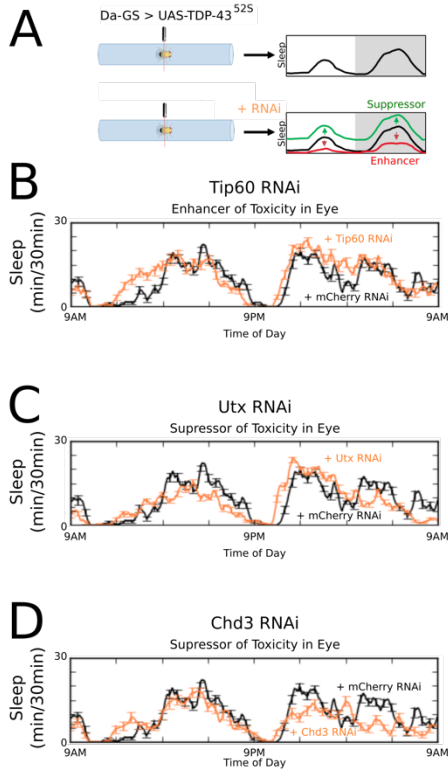
Supplementary Figure 1. TDP-43 overexpression models of ALS cause sleep deficits.

(A) Quantification of number of sleep bouts for several ALS models. (B) Representative trace of multibeam sleep analysis of Da-GS>UAS-TDP-43^{37M}. Quantification of total sleep time (C), sleep bout duration (D), and number of beam crosses per minute of wake time (E), for Da-GS>UAS-TDP-43^{37M} under single beam and multibeam analysis. (F) Quantification of number of sleep bouts upon restriction of expression of UAS-TDP-43 to neurons or neuronal subpopulations.



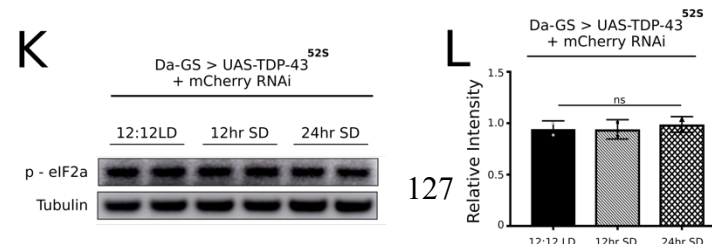
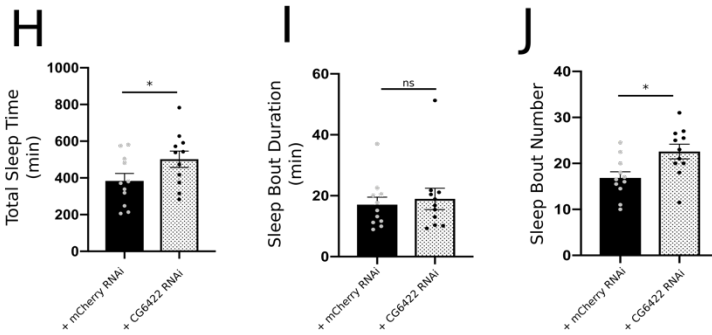
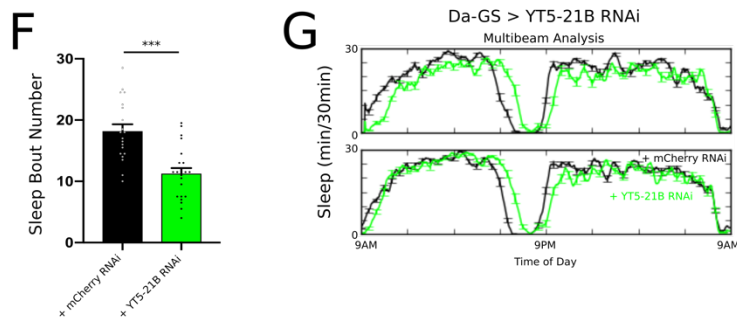
Supplementary Figure 2. Ataxin 2 knockdown improves sleep only in TDP-43 overexpression.

(A) Quantification of number of sleep bouts for Da-GS>UAS-TDP-43^{52S} flies, also expressing Ataxin2 RNAi after 5 days on RU486 food. (B) Representative trace of Da-GS>Ataxin2 RNAi^{BL44012} in multibeam analysis (without expression of UAS-TDP-43).



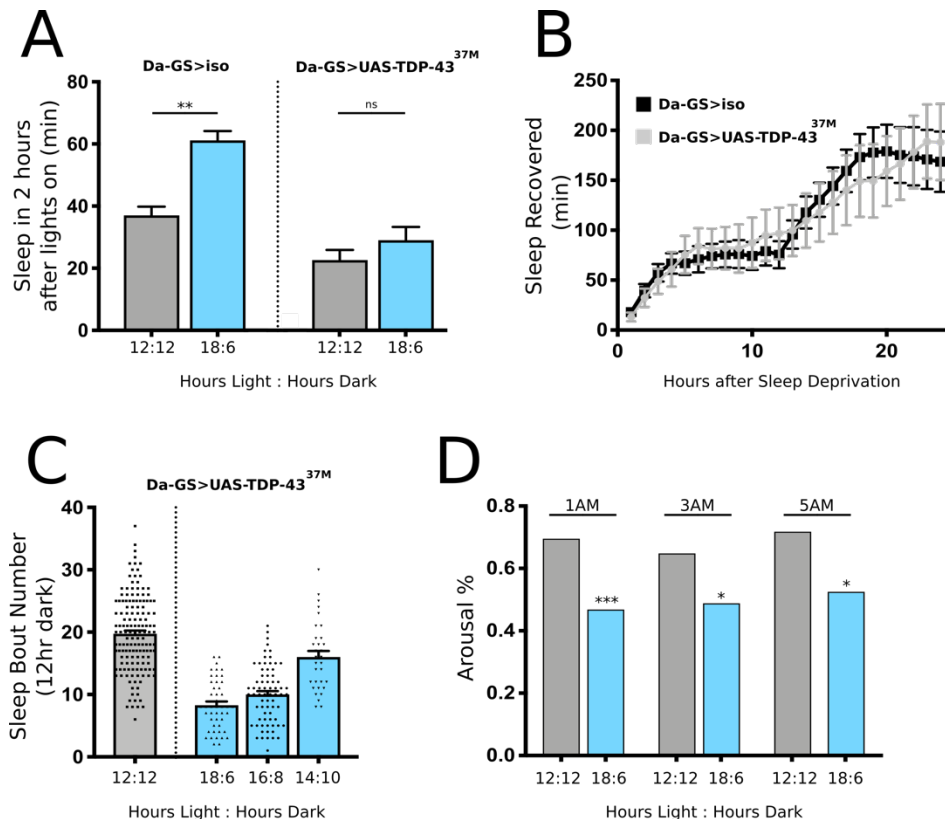
E

Gene Name	Total Sleep Time Fold Change
lilli	0.495
Snr1	0.605
Wdr82	0.799
Utx	0.894
Set1	0.919
Bap60	0.967
Tip60	0.968
Scm	0.981
HDAC4	1.102
Su(Tpl)	1.104
cad	1.118
Sirt4	1.125
Bap111	1.126
Spt6	1.140
MBD-R2	1.191
dom	1.205
Chd3	1.223
ear	1.244
Ice1	1.275
Rox8	1.413
Su(z)2	1.457



Supplementary Figure 3. Results of modifier screen.

(A) Schematic of screening approach. (B-D) Representative sleep traces RNAi lines found to be modifiers of TDP-43 toxicity in measures of eye neurodegeneration. (E) Table of fold change of total sleep time of known modifiers of TDP-43. Pink shading indicates significant enhancer and green shading indicates significant suppressor of TDP-43 sleep toxicity. (F) Quantification of number of sleep bouts for Da-GS>UAS-TDP-43^{52S}, mCherry RNAi (black) and Da-GS>UAS-TDP-43^{52S}, YT5-21B RNAi (green). Quantification of m6a cytoplasmic reader CG6422 RNAi total sleep time (H), sleep bout duration (I), and number of sleep bouts (J). Western blots (K) and total protein quantification (L) of phosphorylated eIF2a.



Supplementary Figure 4. SRT improves sleep in TDP-43 overexpression model of ALS.

(A) Quantification of sleep in 2 hours after lights on to assess rebound sleep following 12:12 LD cycle or 16:8 LD SRT cycle in Da-GS/+ (left) or Da-GS>UAS-TDP-43^{37M} (right). (B) Recovered minutes of sleep after sleep deprivation in Da-GS/+ (black) or Da-GS>UAS-TDP-43^{37M} (gray). (C) Quantification of number of sleep bouts for different light:dark cycle durations of SRT. (D) Percentage of flies aroused from a mechanical stimulus delivered during dark period, broken down by time of stimulation.

Supplementary Table 1 - Table of TRIP RNAi Lines Screened for Modifiers of TDP-43 Toxicity				
Flybase GO Term	"Sleep" + Adult Stage, "circadian sleep/wake cycle"	"autophagy"	"synapse organization"	"nucleocytoplasmic transport"
Genes	Sh	EndoA	Top3β	aKap4
	Ilp2	Wdr24	fz2	Kap-α1
	Ktl	PEK	dlp	Kap-α3
	wake	Dpr1	Tbce	Pen
	TfAP-2	sti	Nlg1	Cse1
	Clk	Ras85D	HSPC300	emb
	In	Traf-like	p120ctn	RanGAP
	gvr	Traf6	ns1	Fs(2)Ket
	Fbxl4	Rheb	Tip60	Nxt1
	CanA1	GlyS	wrapper	Rcc1
	Hk	Hsc70-4	RtGEF	tho2
	Nipped-B	It	DIP-α	Ntf-2
	EcR	Snap29	caz	mxf2
	per	Uvrag	plum	Hel25E
	Pp2B-14D	park	Nf1	sbr
	bgm	scnv	Sec8	ZC3H3
	homer	RagA-B	beag	Nup58
	CanB	Ate4b	Cul2	Mtor
	5-HT1A	hep	Ten-a	Nup62
	CrebB	Fer2	smt3	Ns3
	Cul3	Vps16A	Cals	Nup93-1
	Shab	comt	Liprin-α	e(v)2
	Pdf	Sec61α	dbo	Arts
	Dh31	Doa	Lar	RanBP3
	Qamb	wrd	atl	Nup214
	InR	Ubc6	DIP-y	Nup93-2
	cry	RagC-D	RhoGAP100F	Nup98-96
	Hsc70-3	daw	dum	cdm
	SIFa	sima	Mob4	Moe
	Gabat	Nprl2	Thor	edl
	sra	sesB	gbb	mbo
	cvc	Acn	Src42A	Impβ11
	VGAT	raptor	TBPH	Clbn
	Hsp83	Vha100-1	Rab11	Torsin
	cv-e	Cdk8	Ulp1	Aladin
	Nlg4	Atg2	mGluR	Vhl
	Pka-C1	srp	ena	Nup153
	ITP	Atp1	tkv	sqd
	Ilp3	Pi3K92E	Nedd4	ITV1
	Acer	Pink1	dpr20	Nup154
	Shaw	Pten	Smn	swm
	rut	Rae1	DIP-β	Tnpo
	Dop1R2	Atg16	nmo	SF2
	Ilp5	Raf	Prosap	Nup50
	cue	Atg18a	aPKC	Sul(fu)
	Pdfr	wts	cin	Nup160
	DAT	Vps25	mr	msk
	tara	Fkbp39	DIP-θ	fu
	Rdl	Buffy	ND-20	Ran-like
	Men	Smox	wnd	pix
	inc	Atg8a	Src64B	thoc7
	Fmr1	Trpml	spin	Mapmodulin
	Trh	Vps15	Med	sta
	tim	Atg18b	velo	xmas
	mld	Chc	SGkl	Gp210
	AANAT1	Cpsf6	mys	Sem1
	Ets96B	Atg14	dpr12	chinmo
	BTBD9	TER94	unc-13	Nup35
	sand	Dronc	dpr8	Gle1
	Sik3	Toll-7	DIP-ζ	ebo
		Rab21	Pak	Nup54
		Lrrk	dpr9	Sec13
		Fis1	Liprin-y	hh
		shrb	drpr	Nup358
		AP-2α	Adf1	Ranbp9
		Tango5	Imp	thoc5
		cac	OctB1R	U2af50
		Desat1	RhoGAP92B	Ntf-2r
		lqf	Yip1d1	Nup133
		Gyf	wg	Ran
		br	Akt1	Tnpo-SR
		ri	OctB2R	cos
		sigmar	CASK	
		Atg6	Gas	
		DOR	Fsn	
		Sod2	BOD1	
		p53	GluRIIA	
		Rab32	cpx	
		hiw	Svnd	

	Pax	sp1ct	
	Sec61B	SiaT	
	Rack1	brat	
	Sec61y	Nrx-IV	
	Sesn	SrpK79D	
	Sod1	Rac1	
	ctd	Ank2	
	mask	SkpA	
	Rab23	dpr14	
	Vps13D	cpb	
	Ccz1	Smx16	
	PI3K59F	Act57B	
	ca	nos	
	gig	futsch	
	I(3)76BDm	α-Spec	
	sga	Aro3	
	Myc	sff	
	Syx17	AGO1	
	Atg9	C1GalTA	
	mts	Svn2	
	Svj	Ten-m	
	Atg3	Nrx-1	
	Tsc1	Cdk5	
	Tak1	nwk	
	car	unc-104	
	bsk	Sin1	
	lft	baz	
	Vamp7	DIP-1	
	foxo	dlg1	
	Sbf	par-1	
	Atg8b	Syx18	
	babo	Nup75	
	Nup44A	comm	
	Npr13	slo	
	ago	Abi	
	Egg5	CCKLR-17D1	
	Atg17	Dad	
	Aduk	Aplip1	
	Atg13	WASp	
	CycC	Acsi	
	CstF64	Glt	
	Pp2A-29B	Flp3	
	SNF4Ay	Tl	
	Atg12	dpr18	
	Ubi-p63E	par-6	
	Atg101	cup	
	SREBP	Kat60	
	Atg4a	veli	
	Hsp67Bc	Spn	
	ref(2)P	Fps-15	
	Lerp	Dvsb	
	Dcp-1	Sac1	
	mio	Hem	
	S6k	Nlg3	
	Debcl	brp	
	Mitf	Gs2	
	wdb	repo	
	Eip74EF	LIMK1	
	Vps39	simi	
	dor	Appl	
	Bruce	ewg	
	Atg10	DIP-n	
	fab1	metro	
	Tor	Neto	
	Vps4	Svp	
	shi	Nrg	
	AMPKα	β-Spec	
	Cpsf160	Cip4	
	AP-1-2β	Jeb	
	Uba1	Frg2	
	Atg7	caps	
	Atg5	stau	
	Utx	Sema1a	
	SH3PX1	Fas2	
		mtg	
		Rab8	
		spas	
		ben	
		Nmnat	
		Gem3	
		Jra	
		PI4KIIIα	

			Rab3	
			pros	
			trio	
			Sra-1	
			kay	
			Gad1	
			rictor	
			CaMKII	
			JCA69	
			Sar1	
			rg	
			bchs	
			Upf2	
			Nlg2	
			Syn1	
			shv	
			nonC	
			Atg9	
			scb	
			Ibm	
			Dsk	
			RhoGAP68F	
			Itgbn	
			sgg	
			Pak3	
			mnb	
			Ada2b	
			PICK1	
			dpr1	
			stat	
			Goo	
			dpr10	
			Lrp4	
			cmpv	
			pod1	
			Abp1	
			DIP-ε	
			LanA	
			Syx4	
			drl	
			sax	
			Grip	
			Vap33	
			Svn	
			Fur1	
			Ca-β	
			Fak	
			Patr-1	
			Cdc42	
			dpr17	
			stan	
			kis	
			Pdk1	
			Rab2	
			Frg1	
			Cul5	
			spartin	
			Ube3a	
			Rab1	
			Nsf2	
			Smu1	
			nej	
			Mical	
			Blos1	
			ppk30	
			PIP5K59B	
			Sdc	
			dpr5	
			Rbp	
			Abi	
			Nos	
			wit	
			kig	
			Strip	
			dpr19	
			p38b	
			Mad	
			Arp2	
			Got2	
			Liprin-β	
			Ion	
			miro	

References

- Acharyar, T. M. *et al.* (2017) 'Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation', *Molecular neurodegeneration*. doi: 10.1186/s13024-016-0147-7.
- Aldrich, M. S. *et al.* (1989) 'Sleep abnormalities in progressive supranuclear palsy', *Annals of Neurology*. doi: 10.1002/ana.410250609.
- Ascherio, A. and Schwarzschild, M. A. (2016) 'The epidemiology of Parkinson's disease: risk factors and prevention', *The Lancet Neurology*. doi: 10.1016/S1474-4422(16)30230-7.
- Becker, L. A. *et al.* (2017) 'Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice', *Nature*. doi: 10.1038/nature22038.
- Belfer, S. J. *et al.* (2019) 'A Drosophila model of sleep restriction therapy for insomnia', *Molecular Psychiatry*. doi: 10.1038/s41380-019-0376-6.
- Berlivet, S. *et al.* (2019) 'Readers of the m6A epitranscriptomic code', *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*. doi: 10.1016/j.bbagr.2018.12.008.
- Berson, A. *et al.* (2017) 'TDP-43 Promotes Neurodegeneration by Impairing Chromatin Remodeling', *Current Biology*. doi: 10.1016/j.cub.2017.10.024.
- Vanden Broeck, L. *et al.* (2013) 'TDP-43 Loss-of-Function Causes Neuronal Loss Due to Defective Steroid Receptor-Mediated Gene Program Switching In Drosophila', *Cell Reports*. doi: 10.1016/j.celrep.2012.12.014.
- Buhl, E., Higham, J. P. and Hodge, J. J. L. (2019) 'Alzheimer's disease-associated tau alters Drosophila circadian activity, sleep and clock neuron electrophysiology', *Neurobiology of Disease*. doi: 10.1016/j.nbd.2019.104507.
- Chakravarti, L., Moscato, E. H. and Kayser, M. S. (2017) 'Unraveling the Neurobiology of Sleep and Sleep Disorders Using Drosophila', in *Current Topics in Developmental Biology*. doi: 10.1016/bs.ctdb.2016.07.010.
- Chung, C. Y. *et al.* (2018) 'Aberrant activation of non-coding RNA targets of transcriptional elongation complexes contributes to TDP-43 toxicity', *Nature Communications*. doi: 10.1038/s41467-018-06543-0.
- Congiu, P. *et al.* (2017) 'Impairment of autonomic nervous system in amyotrophic lateral sclerosis', *Sleep Medicine*. doi: 10.1016/j.sleep.2017.11.795.
- Congiu, P. *et al.* (2019) 'Sleep cardiac dysautonomia and EEG oscillations in Amyotrophic Lateral Sclerosis', *Sleep*, epub ahead.
- Dilley, L. C. *et al.* (2018) 'Behavioral and genetic features of sleep ontogeny in Drosophila', *Sleep*. doi: 10.1093/sleep/zsy086.
- Dissel, S. *et al.* (2017) 'Enhanced sleep reverses memory deficits and underlying pathology in drosophila models of Alzheimer's disease', *Neurobiology of Sleep and Circadian Rhythms*. doi: 10.1016/j.nbscr.2016.09.001.
- Elden, A. C. *et al.* (2010) 'Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS', *Nature*. doi: 10.1038/nature09320.

- Estes, P. S. *et al.* (2013) 'Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a *Drosophila* model of amyotrophic lateral sclerosis', *Disease Models & Mechanisms*. doi: 10.1242/dmm.010710.
- Gagnon, J. F. *et al.* (2009) 'Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease', *Annals of Neurology*. doi: 10.1002/ana.21680.
- Gagnon, J. F. *et al.* (2010) 'The Montreal cognitive assessment: A screening tool for mild cognitive impairment in REM sleep behavior disorder', *Movement Disorders*. doi: 10.1002/mds.23079.
- Gama, R. L. *et al.* (2010) 'Sleep disturbances and brain MRI morphometry in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy - a comparative study', *Parkinsonism and Related Disorders*. doi: 10.1016/j.parkreldis.2010.01.002.
- Gilestro, G. F. (2012) 'Video tracking and analysis of sleep in *Drosophila melanogaster*', *Nature Protocols*. doi: 10.1038/nprot.2012.041.
- Gilestro, G. F. and Cirelli, C. (2009) 'PySolo: A complete suite for sleep analysis in *Drosophila*', *Bioinformatics*. doi: 10.1093/bioinformatics/btp237.
- Holth, J. K. *et al.* (2019) 'The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans', *Science*. doi: 10.1126/science.aav2546.
- Iijima, K. *et al.* (2004) 'Dissecting the pathological effects of human A40 and A42 in *Drosophila*: A potential model for Alzheimer's disease', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0400895101.
- Iranzo, A. *et al.* (2006) 'Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study', *Lancet Neurology*. doi: 10.1016/S1474-4422(06)70476-8.
- Ito, K. *et al.* (2017) 'Effects of Kamikihito and Unkei-to on sleep behavior of wild type and Parkinson model in *Drosophila*', *Frontiers in Psychiatry*. doi: 10.3389/fpsy.2017.00132.
- Ju, Y. E. S., Lucey, B. P. and Holtzman, D. M. (2014) 'Sleep and Alzheimer disease pathology—a bidirectional relationship', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2013.269.
- Kang, J. E. *et al.* (2009) 'Amyloid- β dynamics are regulated by orexin and the sleep-wake cycle', *Science*. doi: 10.1126/science.1180962.
- Kayser, M. S., Yue, Z. and Sehgal, A. (2014) 'A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*.', *Science*, 344(6181), pp. 269–274. doi: 10.1126/science.1250553.
- Keene, A. C. and Joiner, W. J. (2015) 'Neurodegeneration: Paying it off with sleep', *Current Biology*. doi: 10.1016/j.cub.2015.02.003.
- Kim, H. J. *et al.* (2014) 'Therapeutic modulation of eIF2 α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models', *Nature Genetics*. doi: 10.1038/ng.2853.
- Kumru, H. *et al.* (2007) 'Relation between subtype of Parkinson's disease and REM sleep behavior disorder', *Sleep Medicine*. doi: 10.1016/j.sleep.2007.02.005.
- Lin, Y. Q. and Chen, S. Di (2018) 'RBD: a red flag for cognitive impairment in Parkinson's disease?', *Sleep Medicine*. doi:

- 10.1016/j.sleep.2018.01.006.
- Malhotra, R. K. (2018) 'Neurodegenerative Disorders and Sleep', *Sleep Medicine Clinics*. doi: 10.1016/j.jsmc.2017.09.006.
- Mann, J. R. *et al.* (2019) 'RNA Binding Antagonizes Neurotoxic Phase Transitions of TDP-43', *Neuron*. doi: 10.1016/j.neuron.2019.01.048.
- Mathis, S. *et al.* (2019) 'Genetics of amyotrophic lateral sclerosis: A review', *Journal of the Neurological Sciences*. doi: 10.1016/j.jns.2019.02.030.
- McCurry, S. M. *et al.* (1999) 'Characteristics of sleep disturbance in community-dwelling Alzheimer's disease patients', *Journal of Geriatric Psychiatry and Neurology*. doi: 10.1177/089198879901200203.
- McGurk, L. *et al.* (2018) 'Poly(ADP-Ribose) Prevents Pathological Phase Separation of TDP-43 by Promoting Liquid Demixing and Stress Granule Localization', *Molecular Cell*. doi: 10.1016/j.molcel.2018.07.002.
- McGurk, L., Berson, A. and Bonini, N. M. (2015) 'Drosophila as an in vivo model for human neurodegenerative disease', *Genetics*. doi: 10.1534/genetics.115.179457.
- Molliex, A. *et al.* (2015) 'Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization', *Cell*. doi: 10.1016/j.cell.2015.09.015.
- Panda, S., Gourie-Devi, M. and Sharma, A. (2019) 'Sleep disorders in amyotrophic lateral sclerosis: A questionnaire-based study from India', *Neurology India*. doi: 10.4103/0028-3886.232327.
- Perkins, L. A. *et al.* (2015) 'The transgenic RNAi project at Harvard medical school: Resources and validation', *Genetics*. doi: 10.1534/genetics.115.180208.
- Petrovsky, D. V. *et al.* (2018) 'Sleep disruption and quality of life in persons with dementia: A state-of-the-art review', *Geriatric Nursing*. doi: 10.1016/j.gerinurse.2018.04.014.
- Pons, M. *et al.* (2017) 'Splicing factors act as genetic modulators of TDP-43 production in a new autoregulatory TDP-43 Drosophila model', *Human Molecular Genetics*. doi: 10.1093/hmg/ddx229.
- Postuma, R. B. *et al.* (2008) 'REM sleep behaviour disorder in Parkinson's disease is associated with specific motor features', *Journal of Neurology, Neurosurgery and Psychiatry*. doi: 10.1136/jnnp.2008.149195.
- Postuma, R. B. *et al.* (2009) 'Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder', *Neurology*. doi: 10.1212/01.wnl.0000340980.19702.6e.
- Reitz, C., Brayne, C. and Mayeux, R. (2011) 'Epidemiology of Alzheimer disease', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2011.2.
- Riemann, D. *et al.* (2015) 'The neurobiology, investigation, and treatment of chronic insomnia', *The Lancet Neurology*. doi: 10.1016/S1474-4422(15)00021-6.
- Roh, J. H. *et al.* (2012) 'Disruption of the sleep-wake cycle and diurnal fluctuation of amyloid- β in mice with Alzheimer's disease pathology', *Science Translational Medicine*. doi: 10.1126/scitranslmed.3004291.
- Roost, C. *et al.* (2015) 'Structure and thermodynamics of N⁶-methyladenosine in RNA: A spring-loaded base modification',

Journal of the American Chemical Society. doi: 10.1021/ja513080v.

- Schenck, C. H., Boeve, B. F. and Mahowald, M. W. (2013) 'Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: A 16-year update on a previously reported series', *Sleep Medicine*. doi: 10.1016/j.sleep.2012.10.009.
- Schenck, C. H., Bundlie, S. R. and Mahowald, M. W. (1996) 'Delayed emergence of a parkinsonian disorder in 38% of 29 older, men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder', *Neurology*. doi: 10.1212/WNL.46.2.388.
- Sun, X. *et al.* (2016) 'Melatonin attenuates hLRRK2-induced Sleep disturbances and synaptic dysfunction in a *Drosophila* model of Parkinson's disease', *Molecular Medicine Reports*. doi: 10.3892/mmr.2016.4991.
- Sun, Z. *et al.* (2011) 'Molecular determinants and genetic modifiers of aggregation and toxicity for the als disease protein fus/tls', *PLoS Biology*. doi: 10.1371/journal.pbio.1000614.
- Tabuchi, M. *et al.* (2015) 'Sleep interacts with $\alpha\beta$ to modulate intrinsic neuronal excitability', *Current Biology*. doi: 10.1016/j.cub.2015.01.016.
- Zhan, L. *et al.* (2013) 'Identification of Genetic Modifiers of TDP-43 Neurotoxicity in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0057214.

Chapter 4: Conclusions and Future Directions

Samuel J. Belfer

Departments of Neuroscience and Psychiatry,
Perelman School of Medicine at the University of Pennsylvania

Conclusions and Future Directions

והאמר רבי יוחנן שבועה שלא אישן שלשה ימים מלקין אותו וישן לאלתר

“But didn’t Rabbi Yoḥanan say that if one says: ‘I hereby take an oath that I will not sleep for three days,’ the court flogs him for taking an oath in vain ... as he is *incapable of fulfilling his oath?*”

- Babylonian Talmud, Tractate Nedarim 15a

Sleep is an essential behavior to humans, such that swearing off of sleep has always been an impossible feat. In all species studied, sleep is present in organisms’ behavioral repertoires, and regulation of sleep is conducted according to the same broad set of rules. While jellyfish and humans might have disparate cellular and molecular functions that occur with sleep, the unanimity of sleep across the animal kingdom points to a critical underlying function. Studying the mechanisms by which sleep becomes disturbed is therefore imperative; if sleep becomes inefficient, so too might the cellular processes occurring during this privileged time, leading to physiological sequelae. Supporting this notion, the millions of people affected by insomnia have increased rates of mental illness, diabetes, and cardiovascular disease (Taylor, Lichstein and Durrence, 2003; Depner, Stothard and Wright, 2014; Javaheri and Redline, 2017). Cognitive Behavioral Therapy for Insomnia (CBT-I) is remarkably effective at treating disrupted sleep, and there is some evidence to suggest that it may be effective at improving comorbid depression, PTSD, and bipolar disorder as well (Kaplan and Harvey, 2013; Ho,

Chan and Tang, 2016; Cunningham and Shapiro, 2018). Despite its broad efficacy, CBT-I classically depends upon availability of trained practitioners, and therapy can last longer than 12 weeks. This places an outsized burden on the patient; behavioral therapy requires a significant commitment of time and financial resources that most patients cannot afford. Sleep restriction therapy (SRT) – a key component of CBT-I – addresses mismatch between sleep opportunity and ability by restricting time in bed to an amount equal to average sleep ability, leading to enhanced sleep drive and consolidation. Remarkably, studies show that SRT alone is sufficient to gain most of the benefits of CBT-I (Miller *et al.*, 2014). Using innovative methods that apply principles of human sleep biology to *Drosophila*, I have taken steps toward delineating the molecular and neural basis of SRT.

Modeling Behavioral Sleep Therapy in *Drosophila*

How effective is our model at mirroring the effects of human CBT-I? There are numerous features of *Drosophila* SRT that mirror tenets of human behavioral therapy for insomnia. This model, like its human therapy counterpart, is effective across various etiologies of short sleep, and can be tailored to address the severity of each genotype's sleep deficit (Chapter 2, Figure 3). Furthermore, its kinetics are similar; maximal improvements in sleep are not observed immediately upon restricting sleep opportunity. Rather, sleep efficiency increases as sleep drive mounts (Chapter 2, Supplementary Figure 6G-H).

Limitations of Drosophila model

Not all elements of human therapy are replicated by the *Drosophila* model. In human patients, SRT begins by restricting patients to their total amount of time slept, which could be as few as 6 hours spent in bed. Over several weeks, sleep opportunity is slowly titrated to increase sleep time, while maintaining consolidated sleep. In *Drosophila*, a therapy of this duration would be unwieldy. Furthermore, our work indicates that restriction of opportunity to 6 hours of dark time achieves the same treatment response as restricting to 4 hours and titrating to 6 hours of dark time after several days (Chapter 2, Figure 4I). There are several potential reasons for this discrepancy. First, it is possible that our measurement of the effect of titration in *Drosophila* lacked the resolution to observe changes to sleep. Our model extended sleep amount by 2 hours every other day; perhaps this was too drastic of a change to assess the effects of titration. To test this theory, I could further examine the results I obtained from *fumin* mutants under 16:8 LD cycles, where I observed significantly increased sleep efficiency and decreased fragmentation, and 14:10 cycles, which demonstrated no change in sleep metrics (Chapter 2, Figure 2). I propose starting with 8 hours dark time and extending dark time by only 20 min every other day to assess for changes to sleep. If this slower titration technique allows sleep to remain efficient when dark time reaches 10 hours, I would conclude that titration is necessary to maximize sleep time in a given window of opportunity. It is possible, however, that upon reaching 10 hours, flies are unable to consolidate their sleep given the abundance of opportunity, regardless of the slow titration. One way this could be explained is by the undeniable differences between human and fly longevity. The need for sleep titration over weeks for an organism whose life spans many decades may not be necessary in a model system that must adapt in much shorter windows of time, with a lifespan of only 60-90 days.

Another possibility is that fly sleep is studied in an environment idealized for sleep and devoid of outside influences that are known to significantly alter sleep behavior (fear of predation, limited food availability, social interaction). Therefore, “true” sleep ability is easy to assess, and titration to obtain appropriate matching of sleep opportunity is unnecessary. While human sleep behavioral studies are also conducted in a controlled environment, it is impossible to eliminate stressors that affect sleep. Perhaps it is the amelioration of acute stressors over several weeks of titration, in addition to the elimination of perpetuating factors, that allows for the measurement of a patients’ “true” sleep ability, and appropriate matching of sleep opportunity. While this hypothesis is difficult to carefully assess, if studies were designed to limit outside influences as is done in flies (several consecutive nights of observation, ideal environmental settings for sleep, the absence of confounding medications/substances, limiting anxiety wherever possible) perhaps the need for titration of sleep opportunity in human patients would become less critical. Might sleep titration in humans be possible on a shorter time scale? While this question has not been addressed by formal studies, piloting this technique in people suffering from chronic insomnia would significantly reduce the duration of treatment, alleviating some of the burden for patients.

The contribution of SRT to efficacy of CBT-I

The simplification of the *Drosophila* SRT model poses an intriguing question: is sleep restriction therapy the only component of human CBT-I necessary to achieve efficient sleep? There is some evidence in human patients that SRT is sufficient to achieve the effectiveness of full course of CBT-I (Miller *et al.*, 2014). In contrast, stimulus control therapy alone has shown modest effects in decreasing sleep onset latency (Baillargeon, Demers and Ladouceur, 1998). Furthermore, sleep hygiene

education alone shows a small positive effect in treating of chronic insomnia, but is less effective than complete CBT-I (K. F. Chung *et al.*, 2018). The fact that these monotherapies, with the notable exception of SRT, are less effective than comprehensive CBT-I, indicates that they contribute to, but are not responsible for, CBT-I's efficacy in treating insomnia. Is there a way to unify the observation that SRT is as effective as CBT-I, but still value the modest effects of other elements of CBT-I? I propose that the mounting physiological drive to sleep associated with SRT induces broader effects on other maladaptive sleep behaviors. An insomnia patient with increased sleep drive may be less likely to embrace ingrained habits of doing other activities during bed time and practice elements of good sleep hygiene even if not part of their typical bedtime routine. Experiencing improved sleep may serve to restructure negative emotions surrounding sleep as well. In this way, SRT may induce effects of other parts of CBT-I even without explicit therapy. To assess this hypothesis, I propose measuring the persistence of habits associated with poor sleep hygiene and stimulus dysregulation, as well as the emotions surrounding sleep before and after a course of SRT monotherapy. If these maladaptive habits ameliorate with the improvement in sleep quality, I would conclude that these effects were the result of behavioral change associated with increased sleep drive, and therefore could be attributed to SRT.

Technology and CBT-I: Impact on future therapy

One of the great strengths of CBT-I treatment is that it can be carefully tailored to a patient's individual needs. For example, patients who have anxiety about a large decrease in the amount of time in bed required for SRT can utilize a compression technique which slowly restricts sleep opportunity over several weeks (Boland *et al.*, 2019). For patients who prefer group therapy, CBT-I in group settings has showed

durable treatment responses (Koffel, Koffel and Gehrman, 2015). Technology-assisted behavioral therapy regimens are poised to add another dimension how behavioral therapy is administered. The trend toward utilizing mobile applications, internet modules, and online telecommunication for therapy has opened CBT-I to new patients who previously could not access care. Digital platforms now can be utilized to track daily activity, administer serial sleep surveys, record sleep diaries, and pair with at-home polysomnography kits (Choi *et al.*, 2018). Furthermore, initial trials of individualized digital CBT-I platforms have shown efficacy for long durations after treatment (Espie *et al.*, 2012; Horsch *et al.*, 2017; Ritterband *et al.*, 2017; Hagatun *et al.*, 2019). Traditional CBT-I, while effective, is limited by the availability of trained practitioners and long duration of therapy that requires a significant commitment of time and financial resources. Digital CBT-I can bridge this gap and make treatment affordable and accessible. Will this mean the end of CBT-I in traditional therapy settings? The evidence supports a lasting role of trained practitioners even in predominantly-digital therapy. The inclusion of weekly emails from a practitioner in otherwise-unguided digital CBT-I significantly increased improvement in most sleep measures (Lancee *et al.*, 2013). Integrating practitioners with ever-evolving digital applications to maximize therapeutic gains remains an ongoing challenge for this field.

Clearly, technological advances are enabling CBT-I to reach previously inaccessible patients. Why, then, is it critical to model CBT-I in model organisms in an attempt to understand this therapy on a mechanistic level? I posit that there is tremendous power in cellular and molecular insight into mechanisms underlying behavioral sleep therapy. Sleep disturbances are nearly ubiquitous across diseases, and development of novel therapies should be considered a priority given the breadth of patients who might use them. Additionally, insight how behavioral therapies can affect

neural circuits is an exciting open area of research. Modeling this therapy in a model system with tractable genetic tools enables the careful dissection of circuit-level mechanics, and the findings can be extrapolated to more complex systems.

Revisiting the 3P behavioral model of insomnia

Our model of SRT in the *Drosophila* model system beckons a reanalysis of traditional models of insomnia. The 3P behavioral model states that predisposing, precipitating, and perpetuating factors are the underlying causes of chronic insomnia (Spielman, 1986; Figure 1-1). Foremost, our results support the universality of the 3P behavioral model across species. However, in the *Drosophila* model of SRT, there are no precipitating factors of insomnia. Instead, the short sleeping phenotype is caused by

a combination of predisposing factors that consist of genetic, epigenetic, and environmental conditions unique to a given population of isogenic flies. Additionally, I have shown that amount of sleep opportunity is an important contributor to sleep efficiency, thereby demonstrating that perpetuating factors of insomnia contribute to fly sleep disturbance. Furthermore, in addition to treating the perpetuating factors associated

with sleep extension, our results raise the possibility that sleep restriction therapy 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. I posit that restriction of

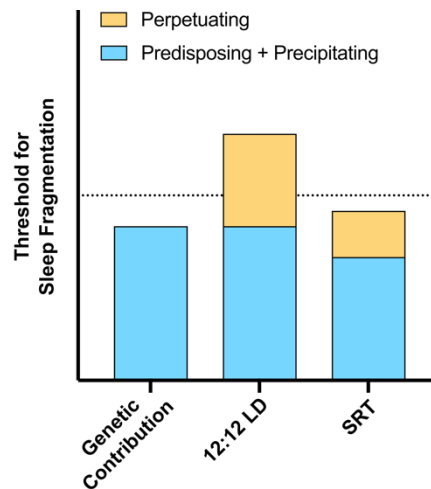


Figure 4-1 - Adapted *Drosophila* 3P Behavioral Model
Perpetuating and precipitating factors combine to form genetic contribution. Mismatch of inherent sleep ability with sleep opportunity produces fragmented, inefficient sleep, and can be considered a perpetuating factor as in human patients. Appropriate matching of opportunity and ability through SRT promote efficient, consolidated sleep.

sleep opportunity therefore increases sleep efficiency and potentiates sleep ability. In this way, SRT targets not only perpetuating factors of chronic insomnia, but the underlying predisposing factors that previous iterations of the 3P model had viewed as static and immutable (Perlis et al. 2014). I have incorporated these changes to contrast with the traditional 3P model in Figure 4-1.

Sleep homeostasis and SRT

The *Drosophila* SRT model corroborates the importance of building homeostatic drive for efficient sleep. Flies undergoing SRT do not reach maximal sleep efficiency until day 3 of restricted opportunity, indicating that several sleep/wake cycles are needed to increase overall sleep drive (Chapter 2, Supplementary Figure 6G-H). Additionally, timing of the dark period – beginning, middle, or end of the night – is inconsequential, indicating that it is the amount of total sleep opportunity, and not when that opportunity is presented, that is critical to efficient sleep (Chapter 2, Figure 2L). With homeostatic sleep drive serving such a pivotal role in producing consolidated sleep, what cellular and molecular mechanisms govern the homeostatic regulation of sleep remains an important open question. Previous work has identified several cellular loci and molecular targets that are involved in the functioning of a putative sleep homeostat (Naidoo *et al.*, 2012; Donlea, Pimentel and Miesenböck, 2014; Liu *et al.*, 2016). This work has primarily leveraged total sleep deprivation to activate the homeostatic drive to sleep. Other work in rodents has interrogated homeostatic mechanisms by using a chronic sleep restriction paradigm that limits healthy animals to fewer-than-necessary sleep hours (Zhu *et al.*, 2018). I speculate that the mechanisms underlying homeostatic response to total sleep deprivation are different than those governing response to chronic sleep restriction or our *therapeutic* SRT model. Supporting this idea, there is evidence that even within

studies of total sleep deprivation, whether animals are kept awake by manual stimulation or by genetically activating wake-promoting centers produces homeostatic responses of different magnitudes (Seidner *et al.*, 2015; Dubowy *et al.*, 2016). If two modalities of total sleep deprivation elicit different responses from sleep homeostat circuitry, it is likely that there are other discoverable differences between sleep deprivation and therapeutic SRT. I propose that our model of TDP-43 overexpression is ideally suited to search for similarities and discrepancies within these circuits. Using the robust literature in *Drosophila* to generate a list of candidate genes, I propose a candidate-based RNAi modifier screen of homeostatic responses in TDP-43 overexpressing flies. This screen will concurrently measure sleep responses following total sleep deprivation by manual stimulation, and response to SRT. I hypothesize that I will uncover genes that modulate both homeostatic responses, and others that will modulate one sleep response, but leave the other unaffected. In this way, it will be possible to untangle the differences in response to different means of activating the homeostatic drive for sleep, while simultaneously revealing conserved molecular targets that govern all output from the sleep homeostat.

Overcoming wake-promoting cues using SRT

While I argue that SRT is an accurate model of CBT-I in humans, there remains outstanding work to truly understand the conditions upon which this *Drosophila* model is effective. Is SRT sufficient to overcome continuous exposure to hyperarousal and allow animals to sleep despite these conditions? While mutants with chronic dopamine activation may begin to answer this question, other manipulations could give a more complete picture. Caffeine intake is carefully considered when treating humans with insomnia, and can be fed directly to flies to cause wakefulness (Hendricks *et al.*, 2000;

Shaw *et al.*, 2000; Nall *et al.*, 2016). Furthermore, recent work has identified small subgroups of cells in the fly brain whose activity control arousal states. Experimental activation of dopaminergic neurons, a small cluster of octopaminergic neurons, or well-described populations of neuropeptidergic cells each induces sustained wakefulness (Parisky *et al.*, 2008; Crocker *et al.*, 2010; Liu *et al.*, 2012; Ueno *et al.*, 2012). I hypothesize that while constitutive activation of these wake-promoting centers produce strong short-sleeping phenotypes, SRT can overcome this activation to allow for consolidated sleep. SRT's ability to produce consolidated sleep despite hyperarousal via pharmacology or constitutive activation of wake-promoting neurons would lend even more evidence to the broad application of CBT-I to all etiologies of insomnia.

Alternative stimuli as cues for sleep

I have demonstrated that restriction of opportunity of sleep-permissive cues is sufficient to induce changes to sleep, whether with darkness or low temperature. However, other cues may also be sleep-permitting. Food availability has been shown to modulate sleep in flies (Slocumb *et al.*, 2015), and adult flies show a place preference of sleeping near a food source during daytime sleep (Hendricks *et al.*, 2000; Donelson *et al.*, 2012; Dilley *et al.*, 2018). Furthermore, social interaction between flies has been found to have an impact on subsequent sleep (Ganguly-Fitzgerald, Donlea and Shaw, 2006; Lone *et al.*, 2016). I propose that utilizing temporally-restricted opportunities of food availability and social interaction can cue flies to consolidate sleep during these windows, and hypothesize that these manipulations can be optimized to produce similar effect sizes to those observed with light and temperature manipulation.

Understanding cellular circuits governing SRT

The diversity in pro-sleep cues is interesting on a phenomenological level, but also holds a critical function in understanding the circuits governing SRT's effects. Our results suggesting dark and cold temperature cues are each sufficient to produce consolidated sleep has led us to hypothesize that sensory information is collected in the peripheral sensory neurons and communicated to central brain regulators of sleep/wake states. I hypothesize that SRT requires 4 intact sites of signaling: 1) sensory input centers, 2) an integrator of sensory input that determines whether the environment is conducive to sleep or wakefulness, 3) central sleep-regulating centers that consolidate information about environment, circadian timing, and homeostatic drive for sleep and determine whether to sleep or promote wakefulness, 4) motor output neurons that dictate rest or activity. The processing of sensory information has been well-described for several sensory modalities in *Drosophila* (Néric and Desplan, 2016; Scott, 2018). I hypothesize that this sensory information converges on an integrator that relays information about environmental suitability for sleep. One compelling candidate for an integrator of sensory information is a subset of dorsal clock neurons, the DN1s. These clock neurons are thought to be wake promoting in the morning, while sleep promoting during midday siesta and at night (Guo *et al.*, 2016; Lamaze *et al.*, 2017). DN1s also demonstrate direct contacts with other core pacemaker neurons, inhibiting sLNv and LNd cells that modify sleep (Guo *et al.*, 2016). This is critical as DN1s are therefore capable of promoting or preventing sleep. Furthermore, DN1 neurons have been found to form direct synaptic connections with two distinct sets of temperature-sensitive neurons (Lamaze *et al.*, 2017), while its own activity increases with warm temperatures (Guo *et al.*, 2016). DN1s also are capable of integrating light information, as they are necessary for modulating behavior in response to acute changes in light/dark state

(Head *et al.*, 2015). Finally, the integration of information in DN1 neurons is not limited to external cues, as DN1 activity differs based on circadian timing and sex (Guo *et al.*, 2016; Liang, Holy and Taghert, 2016). The input of several internal and external sensory cues onto DN1s, as well as its privileged position to exert influence on sleep/wake state, leads us to hypothesize that DN1 neurons are critically involved in this circuit. Because the TDP-43 overexpression model features such a robust response to SRT, this fly provides a perfect platform upon which to interrogate the integrator function of DN1 neurons. I propose the use of two different binary expression systems: the Gal4/UAS system to express TDP-43 in all somatic cells causing short sleep, and the LexA/LexAop system to block synaptic transmission from DN1 cells by expressing a dominant negative allele of *shibire*. I hypothesize that if DN1 neurons are indeed an integrator of sensory information critical for SRT, blocking signaling from these neurons will prevent consolidation of sleep. If DN1 silencing does not prevent sleep improvement, recent evidence has linked other clock neurons to adaptation in response to changing light:dark schedules (Schlichting *et al.*, 2019). I therefore propose silencing small populations of other core clock and sleep-controlling neurons, searching for an occluded response to SRT.

Another approach to identifying integrators of sensory cues that govern SRT response is to identify common neurons downstream of both visual and temperature-sensing pathways. GFP Reconstitution Across Synaptic Partners (GRASP) is a technique that has been useful in interrogating connections between neurons involved in sleep and sensation of sensory cues (Lamaze *et al.*, 2017; Schlichting *et al.*, 2019). GRASP involves the targeting of two split-GFP fragments to separate neuronal populations. If these neurons form synaptic connections, GFP will be reconstituted, and be visible with fluorescent imaging. I propose labelling 2nd order visual system neurons

with one GFP fragment, and PDF-positive clock neurons with the other fragment, and carefully recording the anatomic loci of fluorescence. Repeating this procedure with temperature-sensing neurons replacing visual system neurons will potentially reveal anatomical loci that are downstream of both sensory input modalities. These cells would represent good candidates for being integrators in this system. If these potential integrator cells could be isolated genetically, they could be interrogated for their ability to occlude SRT response via the neuron silencing approach described above.

If no core clock neurons were confirmed as integrators, trans-Tango labeling could be used to identify all post-synaptic targets in an unbiased way (Talay *et al.*, 2017). This approach could be utilized to create complete anatomical maps of neurons downstream of visual or temperature-sensing systems, thereby expanding the search for integrators beyond known circadian clock or sleep-related neurons.

The Purpose of Sleep

There have been many proposed hypotheses to explain sleep's importance across phylogeny. These hypotheses take four general forms: (1) that sleep is controlled by neurons for neuronal purposes, that (2) sleep is controlled by neurons for overall bodily health, (3) that sleep is controlled by the body for neuronal purposes, and that (4) the body controls sleep for its own sake. While categorization of the role of sleep in this manner is inherently reductive, it allows for distillation of core functions of this behavior.

One widely-debated idea that falls into the first category above is the synaptic homeostasis hypothesis (SHY). SHY posits that the number of synaptic connections increases with experiences during wake. Sleep, as a respite from waking experience, is

a privileged time where homeostatic processes at the synapse can occur. This includes the pruning of some synapses and the strengthening of others, plasticity that enables learning of important phenomena while preventing neuronal resources from being wasted on trivial ones (Tononi and Cirelli, 2006). Evidence for this process includes increased synapse number and size with wake duration in flies (Bushey, Tononi and Cirelli, 2011), and recent electron microscopy findings of decreased size of the axon spine interface in cortical synapses of mice, indicative of downscaling (de Vivo *et al.*, 2019). This hypothesis has clear implications for learning and memory, as long-term potentiation of synapses that characterize learning occurs over periods of hours to days, a window that often includes sleep. But the evidence for sleep as a time of consolidation of memories is not purely theoretical. While many studies indicate sleep loss is detrimental to memory formation and retention, studies have also shown that sleep induction is essential for the restoring memory function to mutants (Dissel *et al.*, 2015), or to block forgetting (Berry *et al.*, 2015). Our work has built on findings in short sleeping mutants that have known memory deficits (Kume *et al.*, 2005; Koh *et al.*, 2008; Liu *et al.*, 2014; Shi *et al.*, 2014), demonstrating that it is possible to consolidate sleep in these flies. I hypothesize that improved sleep vis SRT also improves memory function in these mutants. Technical hurdles have prevented work on this question; interrogating memory following sleep improvement requires an assay with sensitivity to small changes, while also having the ability to correlate an individual fly's memory with its sleep behavior on the previous night. I posit that short sleeping mutants and neurodegenerative models that feature progressively worsening sleep provide fertile ground to test this hypothesis and lend support to synaptic restructuring as a core function of sleep.

Another proposed function of sleep is as a time of low energy expenditure. Wake time is expensive in terms of energy use, and having a period of inactivity could serve as

an energy-conserving mechanism. A corollary to this energy homeostasis theory is that once organisms had optimized their expenditure of energy across the day (expend lots of energy during wake, and feature sleep for many hours per day as a way to compensate), other behaviors became attached to sleep once energy production and use were curtailed. For example, in humans, parasympathetic processes like digestion occur at all times of day, but activity in this system peaks overnight. Alternatively, net energy use may remain constant throughout the day, but the onset of sleep allocates energy to different functions than during wake (Schmidt *et al.*, 2017; Anafi, Kayser and Raizen, 2019). In either case, disruption of this rhythm when overnight shift workers consume meals and are active throughout the night are potential explanations for the high rates of metabolic syndrome and microbiome composition in this population (Wang *et al.*, 2014). To this end, I expect that since short-sleeping animals display greater activity over the course of the day, this is likely reflected in greater energy expenditure. Further, there is evidence to suggest that metabolic signatures in flies change in response to sleep amount. Therefore, I propose profiling of the metabolome of short sleeping flies and neurodegenerative disease models to look for common signatures of short sleep. Additionally, improving sleep with SRT may restore some, but not all, of the changed metabolites. Understanding how metabolic profiles reflect acute sleep need or reflect past sleep behavior could be important in understanding the dynamic interplay of sleep and metabolism.

Another compelling hypothesis for a role of sleep is as a time for clearance of toxins from the brain. Recent work in mammals indicates that the brain is optimized to increase clearance of damaging proteins during sleep (Holth *et al.*, 2019). Can flux of toxic protein accumulations from neurons into blood (or hemolymph) be observed in *Drosophila*? The blood-brain barrier of the fly is well described (Schwabe *et al.*, 2005;

Stork *et al.*, 2008), and glia that form or associate with the blood brain barrier have been shown to modulate sleep amount (Farca Luna, Perier and Seugnet, 2017). Further connecting sleep with flow across the blood-brain barrier, disturbance of vesicular trafficking at this site increases sleep, while sleep promotes endocytosis in barrier cells (Artiushin *et al.*, 2018). Moreover, efflux in transporters located at the blood-brain barrier are under circadian control, such that xenobiotic permeability to the brain is increased at night (Zhang *et al.*, 2018). However, observing aggregated protein efflux from *Drosophila* neurons has been elusive. Recent work has described fluorescence-labeled proteins, such as TDP-43, accumulating in brain tissues (Estes *et al.*, 2013), while head-fixed imaging from behaving flies has become possible in recent years (Seelig *et al.*, 2010; Kallman, Kim and Scott, 2015). I hypothesize that, harnessing these tools in combination, it is possible to visualize accumulated proteins *in vivo*. Furthermore, similar to published findings in animal models and of xenobiotics in *Drosophila*, I hypothesize that these proteins will be shuttled through the blood-brain barrier during sleep at higher rates than wake. Finally, I propose that clearance efficiency will change based on the quality of sleep of that organism. Specifically, I predict that short sleeping flies will have decreased clearance of accumulating extracellular proteins and that improving sleep will improve flow. I thereby speculate that sleep depth will correlate with toxic protein clearance in flies.

Behavioral Therapies in Animal Models

Lessons from fear extinction

Virtually all pharmacological treatments in psychiatry are based on drugs discovered serendipitously over a half century ago, standing in stark contrast to

treatment in other areas of medicine (Insel, 2012). In recent years, many of the greatest advances in treating mental illness have been behavioral interventions, yet little is known regarding the mechanistic basis of such interventions (Hofmann *et al.*, 2012). How can behavioral therapies be studied at a molecular level? While new pharmacotherapies are developed and easily tested in animal models, whether it is possible to assess novel behavioral therapies in animal models is much less clear. There are some examples, however, when animal models of behavioral therapy have informed mechanisms of human disease, such as in PTSD and specific phobia. In humans with these diseases, persistent fears of an object, situation, or salient memory causes behavioral change that impacts everyday life (Garcia, 2017). Symptoms can include avoidance of new or unpredictable environments that impact interpersonal relationships and negatively affect career trajectory in those affected. Like insomnia, the preferred treatment for this disorder is a form of behavioral therapy, namely prolonged exposure therapy. The focus of this therapeutic tactic is fear deconditioning, in which repeated exposures to a fear-inducing stimulus will eventually ameliorate the strong fear response associate with that stimulus. Along with relaxation exercises and education about PTSD symptoms, prolonged exposure therapy requires patients to recount the memory in detail (imaginal therapy), while also approaching situations that remind the patient of their trauma (Lancaster *et al.*, 2016). By exposing themselves to these reminders of trauma in a safe context, a majority of patients experience reliable reductions in PTSD symptoms (Rauch *et al.*, 2009).

The mechanisms underlying the efficacy of prolonged exposure therapy have been elucidated by decades of work on acquisition, consolidation and maintenance of fear memories in model systems. Rodent models can be conditioned to experience fear by pairing a neutral cue (auditory tone) with an aversive stimulus (foot shock). By

continuously repeating the pairing, the cue takes on the fear-inducing properties of the aversive stimulus, and animals will fear the cue as if it was the aversive stimulus itself. By using rodent models to study fear, the lateral amygdala has been identified as a locus for formation and storage of fear memories. Intriguingly, the lateral amygdala can influence behavior by initiating fear responses only after a memory is acquired; it has no role in the learning of fear responses (Johansen *et al.*, 2011). Memories are stored via synaptic changes at this site as well (Apergis-Schoute *et al.*, 2005). In the majority of cases, the site of fear extinction is not the same as the sites of memory acquisition or consolidation (Furini, Myskiw and Izquierdo, 2014). While a cellular locus has not been isolated, NMDA receptors have been known to affect fear extinction for many years (Falls, Miserendino and Davis, 1992). The success of this rodent model has been highlighted by the discovery that NMDA blockade by an FDA-approved drug D-cycloserine enhanced extinction of fear in rats (Walker *et al.*, 2002). Due to its already approved status, human studies followed and demonstrated enhanced extinction in patients as well (Davis *et al.*, 2006). D-cycloserine is one of the fastest-translated central nervous system therapeutics on record (Furini, Myskiw and Izquierdo, 2014), and this is solely due to mechanistic work modeling an existing behavioral therapy in a robust model of disease.

Lessons from SRT

Using innovative methods that apply principles of human sleep biology to *Drosophila* models of insomnia, I too have contributed to the understanding of behavioral therapies, by uncovering a path towards delineating the molecular and neural basis of behavioral sleep modification. Short sleeping *Drosophila* mutants discovered through screens are arguably the best animal models of insomnia. Flies recapitulate not only

reductions in sleep, but also changes to sleep continuity common to patients with insomnia. These deficits are coupled with memory problems, and in some cases truncated lifespan. Modeling SRT in fly models of insomnia has already yielded important insights; I conclude that sleep improvement by SRT does not rely on a molecular circadian clock, can be regulated by light:dark and temperature rhythms, and is effective at improving sleep in numerous etiologies of short-sleeping phenotypes (Chapter 2). I have also demonstrated that it can be used to improve sleep in *Drosophila* models of neurodegenerative disease (Chapters 2 & 3). This platform is poised for further contributions to the understanding of CBT-I. Above, I describe an experimental approach for mapping the circuits controlling SRT. Below, I detail a forward genetic screen protocol for identifying molecular modifiers of SRT. I posit that modeling behavioral therapies that have already demonstrated their efficacy in human patients in simpler animal models is a way to develop new techniques and pharmacotherapeutics in psychiatry. Compelling models of human disease have been developed in both invertebrates and vertebrates. Recent innovations in gene editing have brought the ability to introduce individual polymorphisms of unknown clinical significance into animal models to demonstrate how they affect behavior (Babačić *et al.*, 2019). With these innovations, animal modeling of human behavioral therapies becomes even more critical to assess the potential efficacy of existing therapies particularly when several options exist.

Future outlook

While I propose that the benefits of CBT-I are primarily derived from sleep restriction therapy, by what mechanisms is Cognitive Behavioral Therapy effective for treating generalized anxiety, depression, and other psychiatric diseases in which there is

compelling evidence for its efficacy? I anticipate that the mechanisms of these behavioral therapies are capable of being understood by modeling these diseases in *Drosophila*, rodent models, or any other models that appropriately recapitulate treatment strategies. As these therapies have large cognitive components, creative paradigms will be necessary to effectively model these interventions. By extrapolating the conclusions to human patients, psychiatry will benefit with new understanding, and potentially new targets to direct efforts of drug development.

Sleep and Neurodegeneration

Our work contributes to mounting evidence that neurodegenerative processes can alter sleep quality in *Drosophila* (Tabuchi *et al.*, 2015; Dissel *et al.*, 2017; Buhl, Higham and Hodge, 2019). This corroborates evidence from human patients that sleep disturbance is a major factor in decreased quality of life due to neurodegenerative disease in patients and their caretakers. The question of how these processes affect sleep remains an open one. One hypothesis for how sleep changes with progression of disease is that pathogenic proteins aggregate in sleep-controlling neurons in the brain. As damage occurs to these cells and neuronal death follows, sleep becomes unregulated and disordered, leading to the observed sleep phenotypes in *Drosophila* models of Alzheimer's disease and ALS/FTD. Surprisingly, preliminary data indicates that overexpression of TDP-43 in wake-promoting dopaminergic, octopaminergic, peptidergic, or PDF-positive clock neurons is insufficient to produce sleep degradation. Similarly, overexpression in sleep-promoting fan-shaped body, ellipsoid body, or mushroom body neurons alone does not have significant impacts on sleep. Furthermore, in a *Drosophila* Alzheimer's disease model, Tabuchi *et. al.* failed to observe sleep

changes when UAS-A β Arctic was overexpressed using a sleep-promoting mushroom body driver (Tabuchi *et al.*, 2015). This work also found that neurons expressing A β Arctic are hyperexcitable (Tabuchi *et al.*, 2015). Is global hyperexcitability responsible for the sleep disruption associated with models of neurodegenerative disease? As more wake-promoting neurons than sleep promoting neurons have been discovered in flies and mammals (Eban-Rothschild, Appelbaum and De Lecea, 2018), perhaps brain-wide excitability pushes the sleep/wake balance in favor of wakefulness? To test this hypothesis, I propose expressing A β Arctic in large populations of wake-promoting neurons, namely dopaminergic and mushroom body neurons. If hyperexcitability in these wake-promoting centers is responsible for sleep degradation, I expect that this should recapitulate pan-neuronal expression (Chapter 2, Figure 5C-I).

If global hyperexcitability is not responsible for sleep deterioration, perhaps the brain has mechanisms of detecting the level of burden of accumulating proteins. When the burden of protein aggregation reaches a certain threshold brain-wide, changes in sleep centers would result in sleep disruption. To examine this hypothesis, I could test the level of sleep disruption by inducing TDP-43 overexpression using drivers that express in progressively larger populations of neurons. If sleep disturbance was reflective of brain-wide protein burden, I would expect that sleep disruption should scale with broadness of the driver used. When there is protein aggregation throughout the brain, and therefore sufficient burden to produce a sleep response, must there also be pathology specifically in sleep centers to observe sleep deterioration? To test this theory, I will broadly express TDP-43 in all somatic cells, but exclude wake-promoting populations of neurons (dorsal fan-shaped body, ellipsoid body, and mushroom body) using a Gal80 to block Gal4/UAS expression in only those neurons. If exclusion of these

known sleep centers does not cause sleep deterioration, it would indicate that these neurons are necessary, but not sufficient, for TDP-43 induced sleep changes.

Cellular localization of pathology

In which cellular or extracellular compartments might protein accumulations be acting to affect neuronal integrity? This question may not have a consistent answer, but rather may vary with the specific nature of the accumulating protein. In human pathology, tau forms intracellular tangles, enriched in axonal segments as they interact with microtubules involved in cellular transport, while amyloid forms extracellular plaques. However, to think of these processes as independent is erroneous; toxic tau enhances A β accumulation via a feedback loop, intertwining the mechanisms of cellular disturbance of tau and A β (Bloom, 2014). TDP-43 is most well-known to form cytoplasmic inclusions called stress granules, accumulating RNA, misfolded protein products, and other cellular waste. Stress granules are then walled off from other cytoplasmic components by liquid-liquid phase separation (Wheeler *et al.*, 2016). While RNA-bound TDP-43 forms protective cytoplasmic stress granules, RNA-deficient TDP-43 form neurotoxic cytoplasmic inclusions (Mann *et al.*, 2019). Recent work, however, has pointed to nuclear accumulations as the site of some pathology related to ALS (Udan-Johns *et al.*, 2014). Our work supports a nuclear role of TDP-43 accumulation that affects sleep. A screen for modifiers of TDP-43 toxicity identified that knockdown of YT5-21B, a reader of N⁶-methyl adenosine (m⁶A) RNA modifications that is found only in the nucleus, significantly suppressed sleep deficits (Chapter 3, Figure 3C). Conversely, the cytoplasmic m⁶A reader had a limited effect on sleep. m⁶A modifications are the

most abundant and evolutionarily-conserved of post-transcriptionally added modifications to RNA (Berlivet *et al.*, 2019). m⁶A readers bind to RNA; this can increase affinity of RNA-binding proteins for particular RNA residues, or can alter the RNA structure to promote binding in what has been called a “m⁶A switch” (Roost *et al.*, 2015). Knockdown of m⁶A reader protein YT5-21B in a TDP-43 overexpression line does not just rescue sleep behavior, but extends longevity and improves negative geotaxis behavior, indicating broad effects on the health of animals. This finding argues that a change in the processing of m⁶A-tagged RNAs in the nucleus leads to suppression of TDP-43 toxicity. m⁶A markers on RNAs are known to be a critical component of the response to stress (Zhou *et al.*, 2015, 2018; Engel *et al.*, 2018), and stress signals have occasionally been found to be maladaptive to survival in flies (Figard *et al.*, 2019). Could transcription of stress-related RNAs be attributing to TDP-43 toxicity, and knockdown of YT5-21B prevent the appropriate localization of these RNAs? If this hypothesis were correct, I would expect the toxicity-suppressing effects of YT5-21B knockdown to be apparent in a variety of stressful stimuli. I plan to assess this hypothesis by exposing TDP-43 overexpressing flies to other forms of cellular stress, including heat shock, paraquat exposure, and UV irradiation, with and without expression of YT5-21B RNAi. If YT5-21B knockdown was repressing stress signal transcription I would expect increased survival following all of these stressors.

m⁶A readers permit binding by RNA-binding proteins to previously inaccessible residues on RNA (Roost *et al.*, 2015). Knockdown of appropriate RNA-binding proteins may therefore be able to recapitulate suppression of TDP-43 toxicity to sleep. To test this hypothesis, I will screen RNAi transgenes to RNA-binding protein genes. If there are RNAi lines that cause improvement of sleep, I will see if dual knockdown of YT5-21B and the RNA-binding protein further improves sleep. I hypothesize that knockdown of

these two genes will act in the same pathway, and therefore not be able to further improve sleep. Further work is necessary to clarify the association between m6a and sleep in the context of TDP-43 overexpression.

The screen I conducted used a candidate-based approach to identify modifiers of TDP-43 toxicity. The robust response to SRT of TDP-43 overexpressing flies poses an intriguing possibility: could modifiers of sleep restriction therapy be found by using this same RNAi transgene approach? I propose using this platform to identify RNAi lines that occlude or enhance the robustness of response to SRT. I hypothesize that proteins that have known roles in homeostatic regulation of sleep will alter the effectiveness of SRT, but I hope to identify novel pathways involved in modifying SRT as well.

Concluding Remarks

The work presented here describes the efficacy of a *Drosophila* model for sleep restriction therapy, an important component of human Cognitive Behavioral Therapy for Insomnia. I have demonstrated that this model is effective for broad use in numerous etiologies of short sleep, and demonstrated far-reaching behavioral effects of improved sleep in models of insomnia and aging. Our work also elucidates the relationship between degraded sleep and neurodegenerative decline; improving sleep serves to improve behavior and longevity in *Drosophila* models of Alzheimer's disease and ALS/FTD. Using this platform, I have identified novel modifiers of sleep behavior in a TDP-43 overexpression background that also modify disease course. Future work is poised to use this model for further insights into how TDP-43 pathology affects sleep function, while also elucidating cellular loci and molecular mechanisms of SRT. Our work, building on existing data, places sleep disturbance at the forefront of important

symptoms to address in human patients, while highlighting potential therapeutic gains upon restoring consolidated and efficient sleep.

References

- Acharyar, T. M. *et al.* (2017) 'Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation', *Molecular neurodegeneration*. doi: 10.1186/s13024-016-0147-7.
- Agrawal, P. *et al.* (2017) 'Drosophila CRY Entrains Clocks in Body Tissues to Light and Maintains Passive Membrane Properties in a Non-clock Body Tissue Independent of Light', *Current Biology*, 27(16), pp. 2431-2441.e3. doi: 10.1016/j.cub.2017.06.064.
- Aldrich, M. S. *et al.* (1989) 'Sleep abnormalities in progressive supranuclear palsy', *Annals of Neurology*. doi: 10.1002/ana.410250609.
- Allada, R. and Siegel, J. M. (2008) 'Unearthing the Phylogenetic Roots of Sleep', *Current Biology*. doi: 10.1016/j.cub.2008.06.033.
- Altintas, O., Park, S. and Lee, S. J. V. (2016) 'The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*', *BMB Reports*. doi: 10.5483/BMBRep.2016.49.2.261.
- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders (5th Edition)*, *American Journal of Psychiatry*. doi: 10.1176/appi.books.9780890425596.744053.
- Anafi, R. C., Kayser, M. S. and Raizen, D. M. (2019) 'Exploring phylogeny to find the function of sleep', *Nature Reviews Neuroscience*. doi: 10.1038/s41583-018-0098-9.
- Apergis-Schoute, A. M. *et al.* (2005) 'Auditory fear conditioning and long-term potentiation in the lateral amygdala require ERK/MAP kinase signaling in the auditory thalamus: A role for presynaptic plasticity in the fear system', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0096-05.2005.
- Artiushin, G. *et al.* (2018) 'Endocytosis at the drosophila blood– brain barrier as a function for sleep', *eLife*. doi: 10.7554/eLife.43326.
- Ascherio, A. and Schwarzschild, M. A. (2016) 'The epidemiology of Parkinson's disease: risk factors and prevention', *The Lancet Neurology*. doi: 10.1016/S1474-4422(16)30230-7.
- Babačić, H. *et al.* (2019) 'CRISPR-cas gene-editing as plausible treatment of neuromuscular and nucleotide-repeat-expansion diseases: A systematic review', *PLoS ONE*. doi: 10.1371/journal.pone.0212198.
- Baillargeon, L., Demers, M. and Ladouceur, R. (1998) 'Stimulus-control: Nonpharmacologic treatment for insomnia', *Canadian Family Physician*.
- Baines, R. A. *et al.* (2001) 'Altered Electrical Properties in Drosophila Neurons Developing without Synaptic Transmission', *The Journal of Neuroscience*. doi: 10.1523/jneurosci.21-05-01523.2001.
- Bathgate, C. J., Edinger, J. D. and Krystal, A. D. (2017) 'Insomnia patients with objective short sleep duration have a blunted response to cognitive behavioral therapy for Insomnia', *Sleep*, 40(1). doi: 10.1093/sleep/zsw012.
- Becker, L. A. *et al.* (2017) 'Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice', *Nature*. doi: 10.1038/nature22038.

- Becnel, J. *et al.* (2013) 'DREADDs in drosophila: A pharmacogenetic approach for controlling behavior, neuronal signaling, and physiology in the fly', *Cell Reports*. doi: 10.1016/j.celrep.2013.08.003.
- Belfer, S. J. *et al.* (2019) 'A Drosophila model of sleep restriction therapy for insomnia', *Molecular Psychiatry*. doi: 10.1038/s41380-019-0376-6.
- Bellen, H. J., Tong, C. and Tsuda, H. (2010) '100 years of Drosophila research and its impact on vertebrate neuroscience: A history lesson for the future', *Nature Reviews Neuroscience*. doi: 10.1038/nrn2839.
- Berlivet, S. *et al.* (2019) 'Readers of the m 6 A epitranscriptomic code', *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*. doi: 10.1016/j.bbagr.2018.12.008.
- Berry, J. A. *et al.* (2015) 'Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting', *Cell*. doi: 10.1016/j.cell.2015.05.027.
- Berson, A. *et al.* (2017) 'TDP-43 Promotes Neurodegeneration by Impairing Chromatin Remodeling', *Current Biology*. doi: 10.1016/j.cub.2017.10.024.
- Bliwise, D. L. (1993) 'Sleep in normal aging and dementia', *Sleep*. doi: 10.1093/sleep/16.1.40.
- Bloom, G. S. (2014) 'Amyloid- β and tau: The trigger and bullet in Alzheimer disease pathogenesis', *JAMA Neurology*. doi: 10.1001/jamaneurol.2013.5847.
- Boland, E. *et al.* (2019) 'Precision Medicine for Insomnia', *Sleep Medicine Clinics*. doi: 10.1016/j.jsmc.2019.04.001.
- Borbély, A. A. (1982) 'A two process model of sleep regulation.', *Human neurobiology*.
- Borbély, A. A., Neuhaus, H. U. and Tobler, I. (1981) 'Effect of p-chlorophenylalanine and tryptophan on sleep, EEG and motor activity in the rat', *Behavioural Brain Research*. doi: 10.1016/0166-4328(81)90035-8.
- Brenes, G. A. *et al.* (2016) 'Effects of Telephone-Delivered Cognitive-Behavioral Therapy and Nondirective Supportive Therapy on Sleep, Health-Related Quality of Life, and Disability', *American Journal of Geriatric Psychiatry*. doi: 10.1016/j.jagp.2016.04.002.
- Vanden Broeck, L. *et al.* (2013) 'TDP-43 Loss-of-Function Causes Neuronal Loss Due to Defective Steroid Receptor-Mediated Gene Program Switching In Drosophila', *Cell Reports*. doi: 10.1016/j.celrep.2012.12.014.
- Brown, M. K. *et al.* (2014) 'Aging induced endoplasmic reticulum stress alters sleep and sleep homeostasis', *Neurobiology of Aging*, 35(6), pp. 1431–1441. doi: 10.1016/j.neurobiolaging.2013.12.005.
- Brummett, B. H. *et al.* (2007) 'Sleep quality varies as a function of 5-HTTLPR genotype and stress', *Psychosomatic Medicine*. doi: 10.1097/PSY.0b013e31814b8de6.
- Buhl, E., Higham, J. P. and Hodge, J. J. L. (2019) 'Alzheimer's disease-associated tau alters Drosophila circadian activity, sleep and clock neuron electrophysiology', *Neurobiology of Disease*. doi: 10.1016/j.nbd.2019.104507.
- Bushey, D. *et al.* (2007) 'Drosophila Hyperkinetic mutants have reduced sleep and impaired memory.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(20), pp. 5384–5393. doi: 10.1523/JNEUROSCI.0108-07.2007.
- Bushey, D. *et al.* (2010) 'Sleep, aging, and lifespan in Drosophila.', *BMC neuroscience*, 11, p. 56. doi: 10.1186/1471-

2202-11-56.

- Bushey, D., Tononi, G. and Cirelli, C. (2011) 'Sleep and synaptic homeostasis: Structural evidence in *Drosophila*', *Science*. doi: 10.1126/science.1202839.
- De Carlo, V., Calati, R. and Serretti, A. (2016) 'Socio-demographic and clinical predictors of non-response/non-remission in treatment resistant depressed patients: A systematic review', *Psychiatry Research*. doi: 10.1016/j.psychres.2016.04.034.
- Cassidy-Eagle, E. *et al.* (2018) 'Cognitive Behavioral Treatment for Insomnia in Older Adults with Mild Cognitive Impairment in Independent Living Facilities : A Pilot Study', *Journal of Sleep Disorders and Medical Care*, 1(1). doi: 10.16966/jsdmc.105.
- Cavuoto, M. G. *et al.* (2016) 'Objective but not subjective sleep predicts memory in community-dwelling older adults', *Journal of Sleep Research*. doi: 10.1111/jsr.12391.
- Chakravarti, L., Moscato, E. H. and Kayser, M. S. (2017) 'Unraveling the Neurobiology of Sleep and Sleep Disorders Using *Drosophila*', in *Current Topics in Developmental Biology*. doi: 10.1016/bs.ctdb.2016.07.010.
- Chang, J. C. and Morton, D. B. (2017) 'Drosophila lines with mutant and wild type human TDP-43 replacing the endogenous gene reveals phosphorylation and ubiquitination in mutant lines in the absence of viability or lifespan defects', *PLoS ONE*. doi: 10.1371/journal.pone.0180828.
- Chen, K. S. *et al.* (2018) 'A Hypothalamic Switch for REM and Non-REM Sleep', *Neuron*. doi: 10.1016/j.neuron.2018.02.005.
- Chen, X. *et al.* (2007) 'Study of tauopathies by comparing *Drosophila* and human tau in *Drosophila*', *Cell and Tissue Research*. doi: 10.1007/s00441-007-0401-y.
- Choi, Y. K. *et al.* (2018) 'Smartphone applications to support sleep self-management: Review and evaluation', *Journal of Clinical Sleep Medicine*. doi: 10.5664/jcsm.7396.
- Chung, C. Y. *et al.* (2018) 'Aberrant activation of non-coding RNA targets of transcriptional elongation complexes contributes to TDP-43 toxicity', *Nature Communications*. doi: 10.1038/s41467-018-06543-0.
- Chung, K. F. *et al.* (2018) 'Sleep hygiene education as a treatment of insomnia: A systematic review and meta-analysis', *Family Practice*. doi: 10.1093/fampra/cmz122.
- Cirelli, C. *et al.* (2005) 'Reduced sleep in *Drosophila* Shaker mutants.', *Nature*, 434(7037), pp. 1087–1092. doi: 10.1038/nature03486.
- Congiu, P. *et al.* (2017) 'Impairment of autonomic nervous system in amyotrophic lateral sclerosis', *Sleep Medicine*. doi: 10.1016/j.sleep.2017.11.795.
- Congiu, P. *et al.* (2019) 'Sleep cardiac dysautonomia and EEG oscillations in Amyotrophic Lateral Sclerosis', *Sleep*, epub ahead.
- Crocker, A. *et al.* (2010) 'Identification of a Neural Circuit that Underlies the Effects of Octopamine on Sleep:Wake Behavior', *Neuron*. doi: 10.1016/j.neuron.2010.01.032.

- Crocker, A. and Sehgal, A. (2010) 'Genetic analysis of sleep', *Genes and Development*. doi: 10.1101/gad.1913110.
- Crowther, D. C. *et al.* (2005) 'Intraneuronal A β , non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease', *Neuroscience*, 132(1), pp. 123–135. doi: 10.1016/j.neuroscience.2004.12.025.
- Cunningham, J. E. A. and Shapiro, C. M. (2018) 'Cognitive Behavioural Therapy for Insomnia (CBT-I) to treat depression: A systematic review', *Journal of Psychosomatic Research*. doi: 10.1016/j.jpsychores.2017.12.012.
- Curtsinger, J. W. (2013) 'Late-life fecundity plateaus in *Drosophila melanogaster* can be explained by variation in reproductive life spans', *Experimental Gerontology*, 48(11), pp. 1338–1342. doi: 10.1016/j.exger.2013.08.016.
- Daley, M. *et al.* (2009) 'Insomnia and its relationship to health-care utilization, work absenteeism, productivity and accidents', *Sleep Medicine*, 10(4), pp. 427–438. doi: 10.1016/j.sleep.2008.04.005.
- van Dalfsen, J. H. and Markus, C. R. (2019) 'The serotonin transporter 5-HTTLPR polymorphism and the risk for insomnia: a non-replication', *Sleep Medicine*, 53, pp. 195–196.
- Davis, M. *et al.* (2006) 'Effects of D-Cycloserine on Extinction: Translation From Preclinical to Clinical Work', *Biological Psychiatry*. doi: 10.1016/j.biopsych.2006.03.084.
- Dement, W. and Pelayo, R. (1997) 'Public health impact and treatment of insomnia', *European Psychiatry*, 12((Suppl 1)), pp. 31s-39s.
- Depner, C. M., Stothard, E. R. and Wright, K. P. (2014) 'Metabolic consequences of sleep and circadian disorders', *Current Diabetes Reports*. doi: 10.1007/s11892-014-0507-z.
- Dijk, D. J. *et al.* (2010) 'Age-related reduction in daytime sleep propensity and nocturnal slow wave sleep', *Sleep*. doi: 10.1093/sleep/33.2.211.
- Dijk, D. J., Duffy, J. F. and Czeisler, C. A. (2000) 'Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep', *Chronobiology International*. doi: 10.1081/CBI-100101049.
- Dijk, D. J., Duffy, J. F. and Czeisler, C. A. (2001) 'Age-related increase in awakenings: Impaired consolidation of nonREM sleep at all circadian phases', *Sleep*. doi: 10.1093/sleep/24.5.565.
- Dilley, L. C. *et al.* (2018) 'Behavioral and genetic features of sleep ontogeny in *Drosophila*', *Sleep*. doi: 10.1093/sleep/zsy086.
- Dissel, S. *et al.* (2015) 'Sleep restores behavioral plasticity to *drosophila* mutants', *Current Biology*. doi: 10.1016/j.cub.2015.03.027.
- Dissel, S. *et al.* (2017) 'Enhanced sleep reverses memory deficits and underlying pathology in *drosophila* models of Alzheimer's disease', *Neurobiology of Sleep and Circadian Rhythms*. doi: 10.1016/j.nbscr.2016.09.001.
- Donelson, N. *et al.* (2012) 'High-resolution positional tracking for long-term analysis of *Drosophila* sleep and locomotion using the "tracker" program.', *PLoS one*. doi: 10.1371/journal.pone.0037250.
- Donlea, J. M., Pimentel, D. and Miesenböck, G. (2014) 'Neuronal machinery of sleep homeostasis in *Drosophila*', *Neuron*. doi: 10.1016/j.neuron.2013.12.013.
- Dubowy, C. *et al.* (2016) 'Genetic Dissociation of Daily Sleep and Sleep Following Thermogenetic Sleep Deprivation in

- Drosophila*, *Sleep*. doi: 10.5665/sleep.5760.
- Dubowy, C. and Sehgal, A. (2017) 'Circadian rhythms and sleep in *Drosophila melanogaster*', *Genetics*. doi: 10.1534/genetics.115.185157.
- Eban-Rothschild, A., Appelbaum, L. and De Lecea, L. (2018) 'Neuronal Mechanisms for Sleep/Wake Regulation and Modulatory Drive', *Neuropsychopharmacology*. doi: 10.1038/npp.2017.294.
- Eddinger, J. D. *et al.* (2001) 'Cognitive behavioral therapy for treatment of chronic primary insomnia: a randomized controlled trial.', *JAMA : the journal of the American Medical Association*, 285(14), pp. 1856–64.
- Eddinger, J. D. *et al.* (2007) 'Dose-response effects of cognitive-behavioral insomnia therapy: A randomized clinical trial', *Sleep*, 30(2), pp. 203–212. doi: 10.1093/sleep/30.2.203.
- Elden, A. C. *et al.* (2010) 'Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS', *Nature*. doi: 10.1038/nature09320.
- Ellis, J. G., Cushing, T. and Germain, A. (2015) 'Treating Acute Insomnia: A Randomized Controlled Trial of a "Single-Shot" of Cognitive Behavioral Therapy for Insomnia', *SLEEP*. doi: 10.5665/sleep.4752.
- Emery, P. *et al.* (1998) 'Cry, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity', *Cell*, 95(5), pp. 669–679. doi: 10.1016/S0092-8674(00)81637-2.
- Emery, P. *et al.* (2000) '*Drosophila* CRY is a deep brain circadian photoreceptor', *Neuron*, 26(2), pp. 493–504. doi: 10.1016/S0896-6273(00)81181-2.
- Engel, M. *et al.* (2018) 'The Role of m6A/m-RNA Methylation in Stress Response Regulation', *Neuron*. doi: 10.1016/j.neuron.2018.07.009.
- Espie, C. A. *et al.* (2012) 'A Randomized, Placebo-Controlled Trial of Online Cognitive Behavioral Therapy for Chronic Insomnia Disorder Delivered via an Automated Media-Rich Web Application', *Sleep*. doi: 10.5665/sleep.1872.
- Estes, P. S. *et al.* (2013) 'Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a *Drosophila* model of amyotrophic lateral sclerosis', *Disease Models & Mechanisms*. doi: 10.1242/dmm.010710.
- Falls, W. A., Miserendino, M. J. D. and Davis, M. (1992) 'Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala', *Journal of Neuroscience*.
- Farajnia, S. *et al.* (2012) 'Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0469-12.2012.
- Farca Luna, A. J., Perier, M. and Seugnet, L. (2017) 'Amyloid Precursor Protein in *Drosophila* Glia Regulates Sleep and Genes Involved in Glutamate Recycling', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. doi: 10.1523/JNEUROSCI.2826-16.2017.
- Feiguin, F. *et al.* (2009) 'Depletion of TDP-43 affects *Drosophila* motoneurons terminal synapsis and locomotive behavior', *FEBS Letters*. doi: 10.1016/j.febslet.2009.04.019.
- Feld, G. B. *et al.* (2013) 'Slow Wave Sleep Induced by GABA Agonist Tiagabine Fails to Benefit Memory Consolidation', *Sleep*. doi: 10.5665/sleep.2954.

- Figard, L. *et al.* (2019) 'Cofilin-Mediated Actin Stress Response Is Maladaptive in Heat-Stressed Embryos', *Cell Reports*. doi: 10.1016/j.celrep.2019.02.092.
- Filion, A. J. *et al.* (2015) 'Examining the influence of a text message-based sleep and physical activity intervention among young adult smokers in the United States', *BMC Public Health*. doi: 10.1186/s12889-015-2045-2.
- Finelli, A. *et al.* (2004) 'A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*', *Molecular and Cellular Neuroscience*, 26(3), pp. 365–375. doi: 10.1016/j.mcn.2004.03.001.
- Foley, D. J. *et al.* (2007) 'Frequent napping is associated with excessive daytime sleepiness, depression, pain, and nocturia in older adults: Findings from the national sleep foundation 2003 sleep in america poll', *American Journal of Geriatric Psychiatry*. doi: 10.1097/01.JGP.0000249385.50101.67.
- Furini, C., Myskiw, J. and Izquierdo, I. (2014) 'The learning of fear extinction', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2014.10.016.
- Gagnon, J. F. *et al.* (2009) 'Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease', *Annals of Neurology*. doi: 10.1002/ana.21680.
- Gagnon, J. F. *et al.* (2010) 'The Montreal cognitive assessment: A screening tool for mild cognitive impairment in REM sleep behavior disorder', *Movement Disorders*. doi: 10.1002/mds.23079.
- Gama, R. L. *et al.* (2010) 'Sleep disturbances and brain MRI morphometry in Parkinson's disease, multiple system atrophy and progressive supranuclear palsy - a comparative study', *Parkinsonism and Related Disorders*. doi: 10.1016/j.parkreldis.2010.01.002.
- Ganguly-Fitzgerald, I., Donlea, J. and Shaw, P. J. (2006) 'Waking experience affects sleep need in *Drosophila*', *Science*. doi: 10.1126/science.1130408.
- Gao, X. *et al.* (2019) 'The bidirectional causal relationships of insomnia with five major psychiatric disorders: A Mendelian randomization study', *European Psychiatry*. doi: 10.1016/j.eurpsy.2019.05.004.
- Garcia, R. (2017) 'Neurobiology of fear and specific phobias', *Learning and Memory*. doi: 10.1101/lm.044115.116.
- Gehrman, P. R., Pfeiffenberger, C. and Byrne, E. M. (2013) 'The role of genes in the insomnia phenotype', *Sleep Medicine Clinics*, pp. 323–331. doi: 10.1016/j.jsmc.2013.04.005.
- Gilestro, G. F. (2012) 'Video tracking and analysis of sleep in *drosophila melanogaster*', *Nature Protocols*. doi: 10.1038/nprot.2012.041.
- Gilestro, G. F. and Cirelli, C. (2009) 'PySolo: A complete suite for sleep analysis in *Drosophila*', *Bioinformatics*. doi: 10.1093/bioinformatics/btp237.
- Van Gool, W. A. and Mirmiran, M. (1983) 'Age-related changes in the sleep pattern of male adult rats', *Brain Research*. doi: 10.1016/0006-8993(83)90217-2.
- Guo, F. *et al.* (2016) 'Circadian neuron feedback controls the *Drosophila* sleep-activity profile', *Nature*. doi: 10.1038/nature19097.
- Hagatun, S. *et al.* (2019) 'The Short-Term Efficacy of an Unguided Internet-Based Cognitive-Behavioral Therapy for

- Insomnia: A Randomized Controlled Trial With a Six-Month Nonrandomized Follow-Up', *Behavioral Sleep Medicine*. doi: 10.1080/15402002.2017.1301941.
- Halberg, J. *et al.* (1981) 'Changes with age characterize circadian rhythm in telemetered core temperature of stroke-prone rats', *Journals of Gerontology*. doi: 10.1093/geronj/36.1.28.
- Hamada, F. N. *et al.* (2008) 'An internal thermal sensor controlling temperature preference in *Drosophila*', *Nature*. doi: 10.1038/nature07001.
- Hanson, K. A. *et al.* (2010) 'Ubiquilin modifies TDP-43 toxicity in a *Drosophila* model of amyotrophic lateral sclerosis (ALS)', *Journal of Biological Chemistry*. doi: 10.1074/jbc.C109.078527.
- Harbison, S. T., McCoy, L. J. and Mackay, T. F. C. (2013) 'Genome-wide association study of sleep in *Drosophila melanogaster*', *BMC Genomics*, 14(1). doi: 10.1186/1471-2164-14-281.
- Hasan, S. *et al.* (2012) 'Age-related changes in sleep in inbred mice are genotype dependent', *Neurobiology of Aging*, 33(1). doi: 10.1016/j.neurobiolaging.2010.05.010.
- Hatano, M. *et al.* (2018) 'Assessment of switching to suvorexant versus the use of add-on suvorexant in combination with benzodiazepine receptor agonists in insomnia patients: A retrospective study', *Clinical Psychopharmacology and Neuroscience*. doi: 10.9758/cpn.2018.16.2.184.
- Head, L. M. *et al.* (2015) 'The influence of light on temperature preference in *Drosophila*', *Current Biology*. doi: 10.1016/j.cub.2015.02.038.
- Helfrich-Förster, C. *et al.* (2001) 'The circadian clock of fruit flies is blind after elimination of all known photoreceptors', *Neuron*, 30(1), pp. 249–261. doi: 10.1016/S0896-6273(01)00277-X.
- Hendricks, J. C. *et al.* (2000) 'Rest in *Drosophila* Is a Sleep-like State', *Neuron*, 25(1), pp. 129–138. doi: 10.1016/S0896-6273(00)80877-6.
- Hill, A. J. *et al.* (2014) 'Cellular stress induces a protective sleep-like state in *C. elegans*', *Current Biology*. doi: 10.1016/j.cub.2014.08.040.
- Ho, F. Y. Y., Chan, C. S. and Tang, K. N. S. (2016) 'Cognitive-behavioral therapy for sleep disturbances in treating posttraumatic stress disorder symptoms: A meta-analysis of randomized controlled trials', *Clinical Psychology Review*. doi: 10.1016/j.cpr.2015.09.005.
- Hofmann, S. G. *et al.* (2012) 'The efficacy of cognitive behavioral therapy: a review of meta-analyses', *Cognitive Therapy Research*. NIH Public Access, 36(5), pp. 427–440. doi: 10.1007/s10608-012-9476-1.The.
- Hohagen, F. *et al.* (1994) 'Sleep onset insomnia, sleep maintaining insomnia and insomnia with early morning awakening—temporal stability of subtypes in a longitudinal study on general practice attenders', *Sleep*, 17, pp. 551–554.
- Holth, J. K. *et al.* (2019) 'The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans', *Science*. doi: 10.1126/science.aav2546.
- Horsch, C. H. G. *et al.* (2017) 'Mobile phone-delivered cognitive behavioral therapy for insomnia: A randomized waitlist

- controlled trial', *Journal of Medical Internet Research*. doi: 10.2196/jmir.6524.
- Hotta, Y. and Benzer, S. (1972) 'Mapping of behaviour in *Drosophila* mosaics', *Nature*. doi: 10.1038/240527a0.
- Iijima-Ando, K. and Iijima, K. (2010) 'Transgenic *drosophila* models of Alzheimer's disease and tauopathies', *Brain Structure and Function*. doi: 10.1007/s00429-009-0234-4.
- Iijima, K. *et al.* (2004) 'Dissecting the pathological effects of human A 40 and A 42 in *Drosophila*: A potential model for Alzheimer's disease', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0400895101.
- Iijima, Koichi *et al.* (2004) 'Dissecting the pathological effects of human A β 40 and A β 42 in *Drosophila*: A potential model for Alzheimer's disease', *Proceedings of the National Academy of Sciences of the United States of America*, 101(17), pp. 6623–6628. doi: 10.1073/pnas.0400895101.
- Iijima, K. *et al.* (2008) 'A β 42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0001703.
- Insel, T. R. (2012) 'Next-generation treatments for mental disorders.', *Science translational medicine*, 4(155), p. 155ps19. doi: 10.1126/scitranslmed.3004873.
- Iranzo, A. *et al.* (2006) 'Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study', *Lancet Neurology*. doi: 10.1016/S1474-4422(06)70476-8.
- Iranzo, A. (2016) 'Sleep in Neurodegenerative Diseases', *Sleep Medicine Clinics*, pp. 1–18. doi: 10.1016/j.jsmc.2015.10.011.
- Ishimoto, H., Lark, A. and Kitamoto, T. (2012) 'Factors that differentially affect daytime and nighttime sleep in *Drosophila melanogaster*', *Frontiers in Neurology*, FEB, pp. 1–5. doi: 10.3389/fneur.2012.00024.
- Ito, K. *et al.* (2017) 'Effects of Kamikihito and Unkei-to on sleep behavior of wild type and Parkinson model in *Drosophila*', *Frontiers in Psychiatry*. doi: 10.3389/fpsy.2017.00132.
- Jansen, P. R. *et al.* (2019) 'Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways', *Nature Genetics*. doi: 10.1038/s41588-018-0333-3.
- Javaheri, S. and Redline, S. (2017) 'Insomnia and Risk of Cardiovascular Disease', *Chest*. doi: 10.1016/j.chest.2017.01.026.
- Johansen, J. P. *et al.* (2011) 'Molecular mechanisms of fear learning and memory.', *Cell*.
- Ju, Y.-E. S., Lucey, B. P. and Holtzman, D. M. (2014) 'Sleep and Alzheimer disease pathology—a bidirectional relationship', *Nature Reviews Neurology*, 10(2), pp. 115–119. doi: 10.1038/nrneurol.2013.269.
- Ju, Y. E. S., Lucey, B. P. and Holtzman, D. M. (2014) 'Sleep and Alzheimer disease pathology—a bidirectional relationship', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2013.269.
- Kallman, B. R., Kim, H. and Scott, K. (2015) 'Excitation and inhibition onto central courtship neurons biases *drosophila* mate choice', *eLife*. doi: 10.7554/eLife.11188.
- Kaneko, H. *et al.* (2012) 'Circadian rhythm of temperature preference and its neural control in *Drosophila*', *Current Biology*, 22(19), pp. 1851–1857. doi: 10.1016/j.cub.2012.08.006.

- Kang, J. E. *et al.* (2009) 'Amyloid- β dynamics are regulated by orexin and the sleep-wake cycle', *Science*. doi: 10.1126/science.1180962.
- Kaplan, K. A. and Harvey, A. G. (2013) 'Behavioral treatment of insomnia in bipolar disorder', *American Journal of Psychiatry*. doi: 10.1176/appi.ajp.2013.12050708.
- Kathol, R. G. and Arnedt, J. T. (2016) 'Cognitive behavioral therapy for chronic insomnia: Confronting the challenges to implementation', *Annals of Internal Medicine*, pp. 149–150. doi: 10.7326/M16-0359.
- Kautzky, A. *et al.* (2017) 'A new prediction model for evaluating treatment-resistant depression', *Journal of Clinical Psychiatry*. doi: 10.4088/JCP.15m10381.
- Kautzky, A. *et al.* (2018) 'Refining prediction in treatment-resistant depression: Results of machine learning analyses in the TRD III sample', *Journal of Clinical Psychiatry*. doi: 10.4088/JCP.16m11385.
- Kawakami, F. *et al.* (1997) 'Loss of day-night differences in VIP mRNA levels in the suprachiasmatic nucleus of aged rats', *Neuroscience Letters*. doi: 10.1016/S0304-3940(97)13355-9.
- Kayser, M. S. and Biron, D. (2016) 'Sleep and development in genetically tractable model organisms', *Genetics*. doi: 10.1534/genetics.116.189589.
- Kayser, M. S., Yue, Z. and Sehgal, A. (2014) 'A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*.', *Science*, 344(6181), pp. 269–274. doi: 10.1126/science.1250553.
- Keene, A. C. and Joiner, W. J. (2015) 'Neurodegeneration: Paying it off with sleep', *Current Biology*. doi: 10.1016/j.cub.2015.02.003.
- Kim, H. J. *et al.* (2014) 'Therapeutic modulation of eIF2 α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models', *Nature Genetics*. doi: 10.1038/ng.2853.
- Kishi, T., Matsunaga, S. and Iwata, N. (2015) 'Suvorexant for primary insomnia: A systematic review and meta-analysis of randomized placebo-controlled trials', *PLoS ONE*. doi: 10.1371/journal.pone.0136910.
- Kitamoto, T. (2001) 'Conditional modification of behavior in *Drosophila* by targeted expression of a temperature-sensitive *shibire* allele in defined neurons', *Journal of Neurobiology*. doi: 10.1002/neu.1018.
- Kivipelto, M. *et al.* (2013) 'The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER): Study design and progress', *Alzheimer's and Dementia*, 9(6), pp. 657–665. doi: 10.1016/j.jalz.2012.09.012.
- Klapoetke, N. C. *et al.* (2014) 'Independent optical excitation of distinct neural populations', *Nature Methods*. doi: 10.1038/nmeth.2836.
- Koffel, E. *et al.* (2018) 'A randomized controlled pilot study of CBT-I Coach: Feasibility, acceptability, and potential impact of a mobile phone application for patients in cognitive behavioral therapy for insomnia', *Health Informatics Journal*. doi: 10.1177/1460458216656472.
- Koffel, E. A., Koffel, J. B. and Gehrman, P. R. (2015) 'A meta-analysis of group cognitive behavioral therapy for insomnia', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2014.05.001.

- Koh, K. *et al.* (2006) 'A *Drosophila* model for age-associated changes in sleep:wake cycles.', *Proceedings of the National Academy of Sciences of the United States of America*, 103(37), pp. 13843–13847. doi: 10.1073/pnas.0605903103.
- Koh, K. *et al.* (2008) 'Identification of SLEEPLESS, a Sleep-Promoting Factor', *Science*, 321(5887), pp. 372–376. doi: 10.1126/science.1155942.
- Konopka, R. J. and Benzer, S. (1971) 'Clock mutants of *Drosophila melanogaster*.', *Proceedings of the National Academy of Sciences of the United States of America*, 68(9), pp. 2112–6. doi: 10.1073/pnas.68.9.2112.
- Kripke, D. F., Langer, R. D. and Kline, L. E. (2012) 'Hypnotics' association with mortality or cancer: A matched cohort study', *BMJ Open*. doi: 10.1136/bmjopen-2012-000850.
- Krystal, A. D. and Edinger, J. D. (2010) 'Sleep EEG predictors and correlates of the response to cognitive behavioral therapy for insomnia.', *Sleep*, 33(5), pp. 669–77.
- Kume, K. *et al.* (2005) 'Dopamine Is a Regulator of Arousal in the Fruit Fly', 25(32), pp. 7377–7384. doi: 10.1523/JNEUROSCI.2048-05.2005.
- Kumru, H. *et al.* (2007) 'Relation between subtype of Parkinson's disease and REM sleep behavior disorder', *Sleep Medicine*. doi: 10.1016/j.sleep.2007.02.005.
- Kuriyama, A. and Tabata, H. (2017) 'Suvorexant for the treatment of primary insomnia: A systematic review and meta-analysis', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2016.09.004.
- Kyle, S. D. *et al.* (2015) 'Towards standardisation and improved understanding of sleep restriction therapy for insomnia disorder: A systematic examination of CBT-I trial content', *Sleep Medicine Reviews*, pp. 83–88. doi: 10.1016/j.smr.2015.02.003.
- Lamaze, A. *et al.* (2017) 'Regulation of sleep plasticity by a thermo-sensitive circuit in *Drosophila*', *Scientific Reports*. doi: 10.1038/srep40304.
- Lancaster, C. *et al.* (2016) 'Posttraumatic Stress Disorder: Overview of Evidence-Based Assessment and Treatment', *Journal of Clinical Medicine*. doi: 10.3390/jcm5110105.
- Lancee, J. *et al.* (2013) 'Motivational support provided via email improves the effectiveness of internet-delivered self-help treatment for insomnia: A randomized trial', *Behaviour Research and Therapy*. doi: 10.1016/j.brat.2013.09.004.
- LeBlanc, M. *et al.* (2009) 'Incidence and risk factors of insomnia in a population-based sample', *Sleep*. doi: 10.1093/sleep/32.8.1027.
- Li, Y. *et al.* (2010) 'A *Drosophila* model for TDP-43 proteinopathy', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.0913602107.
- Liang, X., Holy, T. E. and Taghert, P. H. (2016) 'Synchronous *drosophila* circadian pacemakers display nonsynchronous Ca²⁺ rhythms in vivo', *Science*. doi: 10.1126/science.aad3997.
- Liguori, C. *et al.* (2014) 'Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease', *JAMA Neurology*. doi: 10.1001/jamaneurol.2014.2510.

- Lin, Y. Q. and Chen, S. Di (2018) 'RBD: a red flag for cognitive impairment in Parkinson's disease?', *Sleep Medicine*. doi: 10.1016/j.sleep.2018.01.006.
- Lind, M. J. and Gehrman, P. R. (2016) 'Genetic pathways to insomnia', *Brain Sciences*. doi: 10.3390/brainsci6040064.
- Liu, Q. *et al.* (2012) 'Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in *Drosophila*', *Current Biology*. doi: 10.1016/j.cub.2012.09.008.
- Liu, S. *et al.* (2014) 'WIDE AWAKE mediates the circadian timing of sleep onset', *Neuron*, 82(1), pp. 151–166. doi: 10.1016/j.neuron.2014.01.040.
- Liu, S. *et al.* (2016) 'Sleep drive is encoded by neural plastic changes in a dedicated circuit', *Cell*. doi: 10.1016/j.cell.2016.04.013.
- Lone, S. R. *et al.* (2016) 'Social experience is sufficient to modulate sleep need of *Drosophila* without increasing wakefulness', *PLoS ONE*. doi: 10.1371/journal.pone.0150596.
- Low, D., Wu, M. and Spira, A. (2019) 'Sleep Duration and Cognition in a Nationally Representative Sample of U.S. Older Adults.', *American Journal of Geriatric Psychiatry*.
- Luan, H. *et al.* (2006) 'Functional Dissection of a Neuronal Network Required for Cuticle Tanning and Wing Expansion in *Drosophila*', *Journal of Neuroscience*. doi: 10.1523/jneurosci.3916-05.2006.
- Luo, L., Tully, T. and White, K. (1992) 'Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for *appl* gene', *Neuron*. doi: 10.1016/0896-6273(92)90024-8.
- Luo, W. *et al.* (2012) 'Old flies have a robust central oscillator but weaker behavioral rhythms that can be improved by genetic and environmental manipulations', *Aging Cell*, 11(3), pp. 428–438. doi: 10.1111/j.1474-9726.2012.00800.x.
- Madeira, M. D. *et al.* (1995) 'Age and sex do not affect the volume, cell numbers, or cell size of the suprachiasmatic nucleus of the rat: An unbiased stereological study', *Journal of Comparative Neurology*. doi: 10.1002/cne.903610404.
- Majercak, J. *et al.* (1999) 'How a circadian clock adapts to seasonal decreases in temperature and day length', *Neuron*, 24(1), pp. 219–230. doi: 10.1016/S0896-6273(00)80834-X.
- Malhotra, R. K. (2018) 'Neurodegenerative Disorders and Sleep', *Sleep Medicine Clinics*. doi: 10.1016/j.jsmc.2017.09.006.
- Mander, B. A., Winer, J. R. and Walker, M. P. (2017a) 'Sleep and Human Aging', *Neuron*, pp. 19–36. doi: 10.1016/j.neuron.2017.02.004.
- Mander, B. A., Winer, J. R. and Walker, M. P. (2017b) 'Sleep and Human Aging', *Neuron*. doi: 10.1016/j.neuron.2017.02.004.
- Mann, J. R. *et al.* (2019) 'RNA Binding Antagonizes Neurotoxic Phase Transitions of TDP-43', *Neuron*. doi: 10.1016/j.neuron.2019.01.048.
- Mathis, S. *et al.* (2019) 'Genetics of amyotrophic lateral sclerosis: A review', *Journal of the Neurological Sciences*. doi:

10.1016/j.jns.2019.02.030.

- Matthews, E. E. *et al.* (2013) 'Adherence to cognitive behavioral therapy for insomnia: A systematic review', *Sleep Medicine Reviews*, pp. 453–464. doi: 10.1016/j.smrv.2013.01.001.
- McCurry, S. M. *et al.* (1999) 'Characteristics of sleep disturbance in community-dwelling Alzheimer's disease patients', *Journal of Geriatric Psychiatry and Neurology*. doi: 10.1177/089198879901200203.
- McGurk, L. *et al.* (2018) 'Poly(ADP-Ribose) Prevents Pathological Phase Separation of TDP-43 by Promoting Liquid Demixing and Stress Granule Localization', *Molecular Cell*. doi: 10.1016/j.molcel.2018.07.002.
- McGurk, L., Berson, A. and Bonini, N. M. (2015) 'Drosophila as an in vivo model for human neurodegenerative disease', *Genetics*. doi: 10.1534/genetics.115.179457.
- McShane, B. B. *et al.* (2010) 'Characterization of the bout durations of sleep and wakefulness', *Journal of Neuroscience Methods*. doi: 10.1016/j.jneumeth.2010.08.024.
- Mershin, A. *et al.* (2004) 'Learning and memory deficits upon TAU accumulation in Drosophila mushroom body neurons', *Learning and Memory*. doi: 10.1101/lm.70804.
- Mhatre, S. D. *et al.* (2014) 'Synaptic abnormalities in a Drosophila model of Alzheimer's disease', *DMM Disease Models and Mechanisms*. doi: 10.1242/dmm.012104.
- Miller, C. B. *et al.* (2014) 'The evidence base of sleep restriction therapy for treating insomnia disorder', *Sleep Medicine Reviews*. Elsevier Ltd, pp. 415–424. doi: 10.1016/j.smrv.2014.01.006.
- Mitchell, M. D. *et al.* (2012) 'Comparative effectiveness of cognitive behavioral therapy for insomnia: A systematic review', *BMC Family Practice*. doi: 10.1186/1471-2296-13-40.
- Molliex, A. *et al.* (2015) 'Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization', *Cell*. doi: 10.1016/j.cell.2015.09.015.
- Moran, M. *et al.* (2005) 'Sleep disturbance in mild to moderate Alzheimer's disease', *Sleep Medicine*. doi: 10.1016/j.sleep.2004.12.005.
- Morin, C. M. *et al.* (2006) 'Epidemiology of insomnia: Prevalence, self-help treatments, consultations, and determinants of help-seeking behaviors', *Sleep Medicine*, 7(2), pp. 123–130. doi: 10.1016/j.sleep.2005.08.008.
- Morin, C. M., Vallières, A., *et al.* (2009) 'Cognitive behavioral therapy, singly and combined with medication, for persistent insomnia: A randomized controlled trial', *JAMA - Journal of the American Medical Association*. doi: 10.1001/jama.2009.682.
- Morin, C. M., Bélanger, L., *et al.* (2009) 'The natural history of insomnia a population-based 3-year longitudinal study', *Archives of Internal Medicine*. doi: 10.1001/archinternmed.2008.610.
- Münch, M. *et al.* (2004) 'The frontal predominance in human EEG delta activity after sleep loss decreases with age', *European Journal of Neuroscience*. doi: 10.1111/j.1460-9568.2004.03580.x.
- Musiek, E. S. and Holtzman, D. M. (2016) 'Mechanisms linking circadian clocks, sleep, and neurodegeneration', *Science*. doi: 10.1126/science.aah4968.

- Naidoo, N. *et al.* (2012) 'Role of homer proteins in the maintenance of sleep-wake states', *PLoS ONE*. doi: 10.1371/journal.pone.0035174.
- Nall, A. H. *et al.* (2016) 'Caffeine promotes wakefulness via dopamine signaling in *Drosophila*', *Scientific Reports*. doi: 10.1038/srep20938.
- Nath, R. D. *et al.* (2017) 'The Jellyfish *Cassiopea* Exhibits a Sleep-like State', *Current Biology*. doi: 10.1016/j.cub.2017.08.014.
- Néric, N. and Desplan, C. (2016) 'From the Eye to the Brain. Development of the *Drosophila* Visual System', in *Current Topics in Developmental Biology*. doi: 10.1016/bs.ctdb.2015.11.032.
- Neumann, M. *et al.* (2006) 'Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis', *Science*. doi: 10.1126/science.1134108.
- Ngandu, T. *et al.* (2015) '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', *The Lancet*, 385(9984), pp. 2255–2263. doi: 10.1016/S0140-6736(15)60461-5.
- Nilsberth, C. *et al.* (2001) 'The " Arctic " APP mutation (E693G) causes Alzheimer ' s disease by enhanced A β protofibril formation', pp. 18–23.
- Okamoto-Mizuno, K. and Mizuno, K. (2012) 'Effects of thermal environment on sleep and circadian rhythm', *Journal of Physiological Anthropology*, pp. 1–9. doi: 10.1186/1880-6805-31-14.
- Ozminkowski, R. J., Wang, S. and Walsh, J. K. (2007) 'The direct and indirect costs of untreated insomnia in adults in the United States', *Sleep*, 30(3), pp. 263–273.
- Palesh, O. *et al.* (2018) 'Feasibility and acceptability of brief behavioral therapy for cancer-related insomnia: effects on insomnia and circadian rhythm during chemotherapy: a phase II randomised multicentre controlled trial', *British Journal of Cancer*. doi: 10.1038/s41416-018-0154-2.
- Pallesen, S. *et al.* (2019) 'The 5-HTTLPR rs25531 LALA-genotype increases the risk of insomnia symptoms among shift workers', *Sleep Medicine*. doi: 10.1016/j.sleep.2019.04.009.
- Panda, S., Gourie-Devi, M. and Sharma, A. (2019) 'Sleep disorders in amyotrophic lateral sclerosis: A questionnaire-based study from India', *Neurology India*. doi: 10.4103/0028-3886.232327.
- Pandi-Perumal, S. R. *et al.* (2002) 'Senescence, sleep, and circadian rhythms', *Ageing Research Reviews*, pp. 559–604. doi: 10.1016/S1568-1637(02)00014-4.
- Parisky, K. M. *et al.* (2008) 'PDF Cells Are a GABA-Responsive Wake-Promoting Component of the *Drosophila* Sleep Circuit', *Neuron*. doi: 10.1016/j.neuron.2008.10.042.
- Parisky, K. M. *et al.* (2016) 'Reorganization of Sleep by Temperature in *Drosophila* Requires Light, the Homeostat, and the Circadian Clock', *Current Biology*, 26(7), pp. 882–892. doi: 10.1016/j.cub.2016.02.011.
- Passos, G. S. *et al.* (2017) 'Chronotype and anxiety are associated in patients with chronic primary insomnia', *Revista Brasileira de Psiquiatria*. doi: 10.1590/1516-4446-2016-2007.

- Pawlyk, A. C. *et al.* (2008) 'Stress-induced changes in sleep in rodents: Models and mechanisms', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2007.06.001.
- Perkins, L. A. *et al.* (2015) 'The transgenic RNAi project at Harvard medical school: Resources and validation', *Genetics*. doi: 10.1534/genetics.115.180208.
- Perlis, M. L. *et al.* (2014) 'Models of Insomnia', pp. 850–866.
- Perlis, M. L., Corbitt, C. B. and Kloss, J. D. (2014) 'Insomnia research: 3Ps and beyond', *Sleep Medicine Reviews*, pp. 191–193. doi: 10.1016/j.smrv.2014.01.003.
- Peter-Derex, L. *et al.* (2015) 'Sleep and Alzheimer's disease', *Sleep Medicine Reviews*. doi: 10.1016/j.smrv.2014.03.007.
- Petrovsky, D. V. *et al.* (2018) 'Sleep disruption and quality of life in persons with dementia: A state-of-the-art review', *Geriatric Nursing*. doi: 10.1016/j.gerinurse.2018.04.014.
- Pigeon, W. R. *et al.* (2017) 'Brief cognitive behavioral therapy for insomnia delivered to depressed veterans receiving primary care services: A pilot study', *Journal of Affective Disorders*. doi: 10.1016/j.jad.2017.04.003.
- Piper, M. D. W. and Partridge, L. (2018) 'Drosophila as a model for ageing', *Biochimica et Biophysica Acta - Molecular Basis of Disease*. doi: 10.1016/j.bbdis.2017.09.016.
- Pons, M. *et al.* (2017) 'Splicing factors act as genetic modulators of TDP-43 production in a new autoregulatory TDP-43 Drosophila model', *Human Molecular Genetics*. doi: 10.1093/hmg/ddx229.
- Postuma, R. B. *et al.* (2008) 'REM sleep behaviour disorder in Parkinson's disease is associated with specific motor features', *Journal of Neurology, Neurosurgery and Psychiatry*. doi: 10.1136/jnnp.2008.149195.
- Postuma, R. B. *et al.* (2009) 'Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder', *Neurology*. doi: 10.1212/01.wnl.0000340980.19702.6e.
- Potdar, S. *et al.* (2018) 'Sleep deprivation negatively impacts reproductive output in *Drosophila melanogaster*', *The Journal of Experimental Biology*, 221(6), p. jeb174771. doi: 10.1242/jeb.174771.
- Qaseem, A. *et al.* (2016) 'Management of chronic insomnia disorder in adults: A clinical practice guideline from the American college of physicians', *Annals of Internal Medicine*, 165(2), pp. 125–133. doi: 10.7326/M15-2175.
- Qiu, J. and Hardin, P. E. (1996) 'per mRNA cycling is locked to lights-off under photoperiodic conditions that support circadian feedback loop function.', *Molecular and cellular biology*, 16(8), pp. 4182–8. doi: 10.1128/MCB.16.8.4182.
- Raizen, D. M. *et al.* (2008) 'Lethargus is a Caenorhabditis elegans sleep-like state', *Nature*. doi: 10.1038/nature06535.
- Rauch, S. A. M. *et al.* (2009) 'Prolonged exposure for PTSD in a Veterans Health Administration PTSD clinic', *Journal of Traumatic Stress*. doi: 10.1002/jts.20380.
- Reitz, C., Brayne, C. and Mayeux, R. (2011) 'Epidemiology of Alzheimer disease', *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2011.2.
- Rera, M., Azizi, M. J. and Walker, D. W. (2013) 'Organ-specific mediation of lifespan extension: More than a gut feeling?', *Ageing Research Reviews*. doi: 10.1016/j.arr.2012.05.003.

- Revel, F. G. *et al.* (2009) 'Rodent models of insomnia: A review of experimental procedures that induce sleep disturbances', *Neuroscience and Biobehavioral Reviews*. doi: 10.1016/j.neubiorev.2009.03.002.
- Reynold, A. *et al.* (2014) 'Negative Effects of Time in Bed Extension : A Pilot Study', *Journal of sleep medicine and disorders*, 1(1), pp. 1–13.
- Riedel, N. *et al.* (2012) 'Insomnia and urban neighbourhood contexts - Are associations modified by individual social characteristics and change of residence? Results from a population-based study using residential histories', *BMC Public Health*. doi: 10.1186/1471-2458-12-810.
- Riemann, D. *et al.* (2015) 'The neurobiology, investigation, and treatment of chronic insomnia', *The Lancet Neurology*. doi: 10.1016/S1474-4422(15)00021-6.
- Riemann, D. *et al.* (2019) 'Sleep, insomnia, and depression', *Neuropsychopharmacology*. doi: 10.1038/s41386-019-0411-y.
- Riemann, D. and Perlis, M. L. (2009) 'The treatments of chronic insomnia: A review of benzodiazepine receptor agonists and psychological and behavioral therapies', *Sleep Medicine Reviews*, pp. 205–214. doi: 10.1016/j.smrv.2008.06.001.
- Ritterband, L. M. *et al.* (2017) 'Effect of a web-based cognitive behavior therapy for insomnia intervention with 1-year follow-up: A randomized clinical trial', *JAMA Psychiatry*. doi: 10.1001/jamapsychiatry.2016.3249.
- Robertson, M. and Keene, A. C. (2013) 'Molecular Mechanisms of Age-Related Sleep Loss in the Fruit Fly - A Mini-Review', *Gerontology*, 59(4), pp. 334–339. doi: 10.1159/000348576.
- Roffwarg, H. P., Muzio, J. N. and Dement, W. C. (1966) 'Ontogenetic development of the human sleep-dream cycle', *Science*. doi: 10.1126/science.152.3722.604.
- Roh, Jee Hoon *et al.* (2012) 'Disruption of the sleep-wake cycle and diurnal fluctuation of amyloid- β in mice with Alzheimer's disease pathology', *Science Translational Medicine*. doi: 10.1126/scitranslmed.3004291.
- Roh, J H *et al.* (2012) 'Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology', *Sci Transl Med*, 4(150), p. 150ra122. doi: 10.1126/scitranslmed.3004291.
- Roost, C. *et al.* (2015) 'Structure and thermodynamics of N⁶-methyladenosine in RNA: A spring-loaded base modification', *Journal of the American Chemical Society*. doi: 10.1021/ja513080v.
- Roth, T. (2007) 'Insomnia: Definition, prevalence, etiology, and consequences', *Journal of Clinical Sleep Medicine*.
- Ruff, R. R. *et al.* (2018) 'Neighborhood Stigma and Sleep: Findings from a Pilot Study of Low-Income Housing Residents in New York City', *Behavioral Medicine*. doi: 10.1080/08964289.2016.1203754.
- Saarelainen, L. *et al.* (2018) 'Risk of death associated with new benzodiazepine use among persons with Alzheimer disease: A matched cohort study.', *Int J Geriatr Psychiatry*, 33(4), pp. 583–590.
- Sarsour, K. *et al.* (2010) 'Association of insomnia severity and comorbid medical and psychiatric disorders in a health plan-based sample: Insomnia severity and comorbidities', *Sleep Medicine*, 11(1), pp. 69–74. doi: 10.1016/j.sleep.2009.02.008.

- Sateia, M. J. *et al.* (2000) 'Evaluation of chronic insomnia. An American Academy of Sleep Medicine review.', *Sleep*, 23(2), pp. 243–308.
- Schenck, C. H., Boeve, B. F. and Mahowald, M. W. (2013) 'Delayed emergence of a parkinsonian disorder or dementia in 81% of older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder: A 16-year update on a previously reported series', *Sleep Medicine*. doi: 10.1016/j.sleep.2012.10.009.
- Schenck, C. H., Bundlie, S. R. and Mahowald, M. W. (1996) 'Delayed emergence of a parkinsonian disorder in 38% of 29 older, men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder', *Neurology*. doi: 10.1212/WNL.46.2.388.
- Schlichting, M. *et al.* (2019) 'Light-mediated circuit switching in the *Drosophila* neuronal clock network', *BioRxiv*.
- Schmidt, C., Peigneux, P. and Cajochen, C. (2012) 'Age-related changes in sleep and circadian rhythms: Impact on cognitive performance and underlying neuroanatomical networks', *Frontiers in Neurology*. doi: 10.3389/fneur.2012.00118.
- Schwabe, T. *et al.* (2005) 'GPCR signaling is required for blood-brain barrier formation in *Drosophila*', *Cell*. doi: 10.1016/j.cell.2005.08.037.
- Scott, K. (2018) 'Gustatory Processing in *Drosophila melanogaster*', *Annual Review of Entomology*. doi: 10.1146/annurev-ento-020117-043331.
- Seelig, J. D. *et al.* (2010) 'Two-photon calcium imaging from head-fixed *Drosophila* during optomotor walking behavior', *Nature Methods*. doi: 10.1038/nmeth.1468.
- Seidner, G. *et al.* (2015) 'Identification of Neurons with a Privileged Role in Sleep Homeostasis in *Drosophila melanogaster*', *Current Biology*. doi: 10.1016/j.cub.2015.10.006.
- Seugnet, L. *et al.* (2009) 'Identifying Sleep Regulatory Genes Using a *Drosophila* Model of Insomnia', *Journal of Neuroscience*, 29(22), pp. 7148–7157. doi: 10.1523/JNEUROSCI.5629-08.2009.
- Shaw, P. J. *et al.* (2000) 'Correlates of Sleep and Waking in *Drosophila melanogaster*', 287(January).
- Shi, M. *et al.* (2014) 'Identification of Redeye, a new sleep-regulating protein whose expression is modulated by sleep amount', pp. 1–17. doi: 10.7554/eLife.01473.
- Sivertsen, B., Vedaa, Ø. and Nordgreen, T. (2013) 'The Future of Insomnia Treatment—the Challenge of Implementation', *Sleep*, 36(3), pp. 303–304. doi: 10.5665/sleep.2432.
- Slocumb, M. E. *et al.* (2015) 'Enhanced sleep is an evolutionarily adaptive response to starvation stress in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0131275.
- Smith, M. T. *et al.* (2002) 'Comparative meta-analysis of pharmacotherapy and behavior therapy for persistent insomnia', *American Journal of Psychiatry*, pp. 5–11. doi: 10.1176/appi.ajp.159.1.5.
- Spielman, A. J. (1986) 'Assessment of insomnia', *Clinical Psychology Review*, 6(1), pp. 11–25. doi: 10.1016/0272-7358(86)90015-2.
- Spielman, A. J., Caruso, L. S. and Glovinsky, P. B. (1987) 'A Behavioral Perspective on Insomnia Treatment', *Psychiatric*

- Clinics of North America*, 10(4), pp. 541–553. doi: 10.1016/s0193-953x(18)30532-x.
- Spielman, A., Saskin, P. and Thorpy, M. J. (1987) 'Treatment of Chronic Insomnia by Restriction of Time in Bed', *Sleep*, 10(1), pp. 45–56. doi: 10.1093/sleep/10.1.45.
- Spira, A. P. *et al.* (2013) 'Self-reported sleep and β -amyloid deposition in community-dwelling older adults', *JAMA Neurology*. doi: 10.1001/jamaneurol.2013.4258.
- Sprecher, K. E. *et al.* (2015) 'Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults', *Neurobiology of Aging*. doi: 10.1016/j.neurobiolaging.2015.05.004.
- Stavropoulos, N. and Young, M. W. (2011) 'Article insomnia and Cullin-3 Regulate Sleep and Wakefulness in *Drosophila*', *Neuron*. Elsevier Inc., 72(6), pp. 964–976. doi: 10.1016/j.neuron.2011.12.003.
- Sterniczuk, R. *et al.* (2013) 'Sleep Disturbance is Associated with Incident Dementia and Mortality', *Current Alzheimer Research*. doi: 10.2174/15672050113109990134.
- Stork, T. *et al.* (2008) 'Organization and Function of the Blood Brain Barrier in *Drosophila*', *Journal of Neuroscience*. doi: 10.1523/jneurosci.4367-07.2008.
- Sun, X. *et al.* (2016) 'Melatonin attenuates hLRRK2-induced Sleep disturbances and synaptic dysfunction in a *Drosophila* model of Parkinson's disease', *Molecular Medicine Reports*. doi: 10.3892/mmr.2016.4991.
- Sun, Z. *et al.* (2011) 'Molecular determinants and genetic modifiers of aggregation and toxicity for the als disease protein fus/tls', *PLoS Biology*. doi: 10.1371/journal.pbio.1000614.
- Sutton, E. L. (2014) 'Insomnia', *Med Clin North Am*, 98(3), pp. 565–581.
- Swaab, D. F., Fliers, E. and Partiman, T. S. (1985) 'The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia', *Brain Research*. doi: 10.1016/0006-8993(85)91350-2.
- Tabuchi, M. *et al.* (2015) 'Sleep interacts with $\alpha\beta$ to modulate intrinsic neuronal excitability', *Current Biology*. doi: 10.1016/j.cub.2015.01.016.
- Talay, M. *et al.* (2017) 'Transsynaptic Mapping of Second-Order Taste Neurons in Flies by trans-Tango', *Neuron*. doi: 10.1016/j.neuron.2017.10.011.
- Taub, J. M. and Berger, R. J. (1973) 'Performance and Mood Following Variations in the Length and Timing of Sleep', *Psychophysiology*, 10(6), pp. 559–570. doi: 10.1111/j.1469-8986.1973.tb00805.x.
- Taylor, D. J., Lichstein, K. L. and Durrence, H. H. (2003) 'Insomnia as a health risk factor.', *Behavioral sleep medicine*. doi: 10.1207/S15402010BSM0104_5.
- Thase, M. E. (2014) 'Using biomarkers to predict treatment response in major depressive disorder: Evidence from past and present studies', *Dialogues in Clinical Neuroscience*.
- Thorpy, M. (2017) 'International classification of sleep disorders', in *Sleep Disorders Medicine: Basic Science, Technical Considerations and Clinical Aspects: Fourth Edition*. doi: 10.1007/978-1-4939-6578-6_27.
- Tononi, G. and Cirelli, C. (2006) 'Sleep function and synaptic homeostasis', *Sleep Medicine Reviews*. doi: 10.1016/j.smr.2005.05.002.

- Toth, L. A. and Bhargava, P. (2013) 'Animal models of sleep disorders', *Comparative Medicine*, 63(2), pp. 91–104. doi: 10.1300/J094v06n03_16.
- Trauer, J. M. *et al.* (2015) 'Cognitive behavioral therapy for chronic insomnia: A systematic review and meta-analysis', *Annals of Internal Medicine*, 163(3), pp. 191–204. doi: 10.7326/M14-2841.
- Tsukahara, S. *et al.* (2005) 'Age-related change and its sex differences in histoarchitecture of the hypothalamic suprachiasmatic nucleus of F344/N rats', *Experimental Gerontology*. doi: 10.1016/j.exger.2004.10.003.
- Udan-Johns, M. *et al.* (2014) 'Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones', *Human Molecular Genetics*. doi: 10.1093/hmg/ddt408.
- Ueno, T. *et al.* (2012) 'Identification of a dopamine pathway that regulates sleep and arousal in *Drosophila*', *Nature Neuroscience*. doi: 10.1038/nn.3238.
- Veatch, O. J. *et al.* (2017) 'Pleiotropic genetic effects influencing sleep and neurological disorders', *The Lancet Neurology*, pp. 158–170. doi: 10.1016/S1474-4422(16)30339-8.
- Vienne, J. *et al.* (2016) 'Age-Related Reduction of Recovery Sleep and Arousal Threshold in *Drosophila*', *Sleep*, 39(8), pp. 1613–1624. doi: 10.5665/sleep.6032.
- de Vivo, L. *et al.* (2019) 'Evidence for sleep-dependent synaptic renormalization in mouse pups.', *Sleep*.
- Wagley, J. N. *et al.* (2013) 'Effectiveness of abbreviated CBT for insomnia in psychiatric outpatients: Sleep and depression outcomes', *Journal of Clinical Psychology*. doi: 10.1002/jclp.21927.
- Walker, D. L. *et al.* (2002) 'Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats', *Journal of Neuroscience*.
- Wang, F. *et al.* (2014) 'Meta-analysis on night shift work and risk of metabolic syndrome', *Obesity Reviews*. doi: 10.1111/obr.12194.
- Weber, F. *et al.* (2018) 'Regulation of REM and Non-REM Sleep by Periaqueductal GABAergic Neurons', *Nature Communications*. doi: 10.1038/s41467-017-02765-w.
- Wheaton, A. G. *et al.* (2016) 'Sleep Duration and Injury-Related Risk Behaviors Among High School Students — United States, 2007–2013', *MMWR. Morbidity and Mortality Weekly Report*. doi: 10.15585/mmwr.mm6513a1.
- Wheeler, J. R. *et al.* (2016) 'Distinct stages in stress granule assembly and disassembly', *eLife*. doi: 10.7554/eLife.18413.
- Wimmer, M. E. *et al.* (2013) 'Aging in mice reduces the ability to sustain sleep/wake states', *PLoS ONE*. doi: 10.1371/journal.pone.0081880.
- Wittmann, C. W. *et al.* (2001) 'Tauopathy in *Drosophila*: Neurodegeneration without neurofibrillary tangles', *Science*. doi: 10.1126/science.1062382.
- Wu, J. Q. *et al.* (2015) 'Cognitive behavioral therapy for insomnia comorbid with psychiatric and medical conditions a meta-analysis', *JAMA Internal Medicine*, 175(9), pp. 1461–1472. doi: 10.1001/jamainternmed.2015.3006.
- Wu, M. N. *et al.* (2010) 'SLEEPLESS, a *Ly-6/neurotoxin* family member, regulates the levels, localization and activity of Shaker', *Nature Neuroscience*. doi: 10.1038/nn.2454.

- Xie, L. *et al.* (2013) 'Sleep drives metabolite clearance from the adult brain', *Science*. doi: 10.1126/science.1241224.
- Yang, C. M., Lin, S. C. and Cheng, C. P. (2013) 'Transient insomnia versus chronic insomnia: A comparison study of sleep-related psychological/behavioral characteristics', *Journal of Clinical Psychology*. doi: 10.1002/jclp.22000.
- Yoshii, T., Hermann-Luibl, C. and Helfrich-Förster, C. (2016) 'Circadian light-input pathways in *Drosophila*', *Communicative and Integrative Biology*. doi: 10.1080/19420889.2015.1102805.
- Zhan, L. *et al.* (2013) 'Identification of Genetic Modifiers of TDP-43 Neurotoxicity in *Drosophila*', *PLoS ONE*. doi: 10.1371/journal.pone.0057214.
- Zhang, K., Coyne, A. N. and Lloyd, T. E. (2018) '*Drosophila* models of amyotrophic lateral sclerosis with defects in RNA metabolism', *Brain Research*. doi: 10.1016/j.brainres.2018.04.043.
- Zhang, S. *et al.* (2008) 'Increased dopaminergic signaling impairs aversive olfactory memory retention in *Drosophila*', *Biochemical and Biophysical Research Communications*, 370(1), pp. 82–86. doi: 10.1016/j.bbrc.2008.03.015.
- Zhang, S. L. *et al.* (2018) 'A Circadian Clock in the Blood-Brain Barrier Regulates Xenobiotic Efflux', *Cell*. doi: 10.1016/j.cell.2018.02.017.
- Zhou, J. *et al.* (2015) 'Dynamic m6 A mRNA methylation directs translational control of heat shock response', *Nature*. doi: 10.1038/nature15377.
- Zhou, J. *et al.* (2018) 'N6-Methyladenosine Guides mRNA Alternative Translation during Integrated Stress Response', *Molecular Cell*. doi: 10.1016/j.molcel.2018.01.019.
- Zhu, Y. *et al.* (2018) 'Chronic sleep disruption advances the temporal progression of tauopathy in P301S mutant mice', *Journal of Neuroscience*. doi: 10.1523/JNEUROSCI.0275-18.2018.
- Ziehm, M., Piper, M. D. and Thornton, J. M. (2013) 'Analysing variation in *Drosophila* aging across independent experimental studies: A meta-analysis of survival data', *Aging Cell*. doi: 10.1111/accel.12123.