GENETIC SCREENS IDENTIFY NOVEL REGULATORS OF SLEEP AND METABOLISM IN *DROSOPHILA* MELANOGASTER

by

Kazuma N. Murakami

A Dissertation Submitted to the Faculty of

The Charles E. Schmidt College of Science

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Florida Atlantic University

Boca Raton, FL

May 2021

GENETIC SCREENS IDENTIFY NOVEL REGULATORS OF SLEEP AND METABOLISM IN *DROSOPHILA* MELANOGASTER

by

Kazuma Murakami

This dissertation was prepared under the direction of the candidate's dissertation advisor, Dr. Alex C. Keene, Department of Biological Sciences, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

SUPERVISORY COMMITTEE:

Alex Carl Keene
Alex Carl Keene (Apr 14, 2021 09:19 EDT)

Alex C. Keene, Ph.D.
Dissertation Advisor

T. A, Godenschwege
T. A, Godenschwege (Apr 15, 2021 13:20 EDT)

Tanja Godenschwege, Ph.D.

Brenda Claiborne
Brenda Claiborne (Apr 15, 2021 10:38 PDT)

Brenda J. Claiborne, Ph.D.

G.T.Macleod
G.T.Macleod (Apr 15, 2021 14:48 EDT)

Gregory Macleod, Ph.D.

SL Milton
Sarah Milton (Apr 15, 2021 14:56 EDT)

Sarah Milton, Ph.D.
Chair, Department of Biological Sciences

Teresa Wilcox

Teresa Wilcox, Ph.D.
Interim Dean, Charles E. Schmidt College of Science

Robert W. Stackman Jr., Ph.D.
Dean, Graduate College

April 15th, 2021
Date

ACKNOWLEDGEMENTS

I am very thankful to all my committee members for their tremendous support. I would like to thank my PI and mentor, Dr. Alex Keene, for giving me the opportunity to pursue my PhD at Florida Atlantic University. I started in his lab at the University of Nevada, Reno as a Master's student and I moved to FAU to pursue my PhD. He has been my advocate of the sciences. I am also grateful for Dr. Tanja Godenschwege for helping me to become a better scientist. She has become one of my good friends. I also would like to thank Dr. Greg Macleod for making me think outside my field. Also, I would like to thank Dr. Brenda Claiborne for being so kind to me over the years.

I am thankful for my friend/colleagues at UNR. Dr. Pavel Masek and Dr. Masato Yoshizawa. To Dr Rebecca Heidker and Dr. Brielle Bjork for their friendship and support. At FAU, Dr. Bethany Stanhope, Dr. Elizabeth Brown and Dr. James Jaggard. In particular, to Bethany for her amazing support over the years. Also, I would like to thank Dr. Maria Yurgel and Dr. Paloma Amaral for their continuing support and friendship.

To all the graduate students and staff members of MC-19 that have been there for me over the years. I would like to thank the members of the Macleod, Godenschwege, Duboue, Stackman, and Murphey Labs. In particular, I would like to thank Dr. David Crerilli and Clarie Kuchira, JD. Finally, I would like to thank Silvana Jaramillo, Dr. Cathy Trivino, and Dr. Stacee Caplan for helping me with administrative issues throughout my PhD.

I would like to thank my family for their love and support. I am particularly grateful to the Kauffman's, who are my Fallon family. Especially to my second mother, Christina Kauffman, for her love and support over the past +20 years. Lastly, I would like to acknowledge all the fur friends that got me through the difficult times.

ABSTRACT

Author:	Kazuma N. Murakami
Title:	GENETIC SCREENS IDENTIFY NOVEL REGULATORS OF SLEEP AND METABOLISM IN *DROSOPHILA* MELANOGASTER
Institution:	Florida Atlantic University
Dissertation Advisor:	Dr. Alex C. Keene
Degree:	Doctor of Philosophy
Year:	2021

Proper regulation of sleep and metabolism are critical to the survival of all organisms. In humans, dysregulation of sleep is linked to metabolic syndrome, including hypertension, hyperglycemia and hyperlipidemia. However, the mechanisms regulating interactions between sleep and metabolism are poorly understood. Although the fruit fly, *Drosophila melanogaster*, bears little anatomical resemblance to humans, it shares similar genetics essential in understanding normal development and disease in humans. From humans to flies, many disease-related genes and pathways are highly conserved, rendering the fruit fly ideal to understanding the interactions between sleep and metabolism. Therefore, using the fruit fly provides a framework for understanding how genes function between sleep and metabolism. During starvation, both humans and rats reduce their sleep. Similarly, previous studies have shown that fruit flies also suppress sleep to forage for food, further showing that sleep and metabolism are intricately tied to

one another and that they are highly conserved across species. To further explore the interactions between sleep and metabolism, I have conducted multiple genetic screens to identify novel regulators of sleep-metabolism interactions. These experiments led to the identification of the mRNA binding protein *translin (trsn)* as being required for starvation-induced sleep suppression. A second screen that targeted metabolic genes from a genome-wide association study identified the ion channel accessory protein *uncoordinated 79* (*unc79)* as a critical regulator of both sleep duration and starvation resistance. The genes function in different regions of the brain and suggest complex neural circuitry is likely to underlie regulation of sleep metabolism interactions. Taken together, a mechanistic understanding of how different genes function to regulate sleep in flies will further our understanding of how sleep and metabolism is regulated in humans.

GENETIC SCREENS IDENTIFY NOVEL REGULATORS OF SLEEP AND METABOLISM IN *DROSOPHILA* MELANOGASTER

LIST OF FIGURES

CHAPTER 1. LITERATURE REVIEW

Health implications in sleep-metabolism interactions

A considerable portion of our life is dedicated to sleep. Sleep has many documented functions including improving in concentration (Aggarwal *et al.* 2013), memory consolidation (Mesarwi *et al.* 2013), metabolic clearance (Xie *et al.* 2013), and immunity restoration (Zager and Andersen 2007). Sleep is regulated by homeostatic mechanisms that control sleep need as well as circadian components that regulate the timing of sleep (Borbely 1982; Borbély *et al.* 2016). Sleep modifies gene expression, body temperature, activity patterns, energy expenditure, and release of various hormones (Zimmet *et al.* 2019). Sleep also controls weight gain by altering hormone levels that affect appetite (Spiegel *et al.* 1999; Anafi *et al.* 2013). In extreme cases, sleep deprivation has been shown to result in death (Shaw *et al.* 2002). According to data collected from the NHIS, close to 30% of adults reported an average of ≤6 hours of sleep per day in 2005-2007 (Schoenborn and Adams 2010) and only 31% of high school students attain 8 hours of sleep on an average school night (Ford *et al.* 2002). A 2002 study by NHNES describes metabolic disease as highly prevalent and estimated that it will continue to rise and may result in important implications for health care providers(Healy 2016). Among shift workers, circadian misalignment contributes to obesity caused by sedentary lifestyle and excessive food intake (Touitou *et al.* 2017). Thus, sleep and metabolism are tightly interconnected, and disruption of either process is associated with metabolic disorders. Acute sleep loss

correlates with increased appetite and insulin insensitivity, while chronically sleep-deprived individuals are more likely to develop obesity, type II diabetes, and cardiovascular disease (Grandner *et al.* 2014a). In another study, the opposite habit of sleeping too much can cause metabolic disorders (Leng *et al.* 2016). In a recent study, increased variability in sleep duration and sleep onset was associated with higher prevalence and incidence of metabolic abnormalities; which include: obesity, waist circumference, hypertriglyceridemia, low HDL cholesterol, high blood pressure, and hyperglycemia (Huang and Redline 2019). Despite the widespread evidence for interactions between sleep and metabolic dysfunction (Knutson and Van Cauter 2008; McDonald and Keene 2010), little is known about the mechanisms the regulate these processes.

Mammalian sleep and metabolism interactions

Research in mice and rats has been developed to understand sleep/metabolism interactions (Luan *et al.* 2012; Petit *et al.* 2014; Varin *et al.* 2015). In the 1979, a study examined EEG recordings in lean or large rats, as well Ventromedial Hypothalamic (VMH) lesioned obese rats during fed and starvation conditions. Slow Wave Sleep (SWS) and Paradoxical Sleep (PS) were reduced in starved lean rats, but unchanged in large and VMH obese rats, suggesting that the interaction between sleep and metabolic state is dependent on energy stores(Danguir and Nicolaidis 1979). The glucose sensing ventrolateral preoptic nucleus (VLPO) initiates and maintains slow-wave sleep (SWS), and increasing glucose consumption increases activity in these neurons, thereby promoting sleep (Varin *et al.* 2015). Glucose-induced excitation of sleep-promoting VLPO neurons is also involved in

post meal drowsiness. In addition, orexin, a neuropeptide found in the VLPO, has been shown to promote food consumption and suppress sleep (Chemelli *et al.* 1999). Another neuropeptide, neuropeptide-Y (NPY), which is expressed in the paraventricular nucleus (PVN) of the hypothalamus, also regulates food intake (Allen *et al.* 1983; Billington *et al.* 1991). Further, NPY injection into the PVN induced hyperphagia and reduced energy expenditure (Dryden *et al.* 1995). Leptin, a peptide generated in adipose tissue, is a circulating signaling hormone involved in long-term energy homeostasis. Studies have identified important links between leptin and sleep, circadian rhythms, as well as metabolism. Leptin deficient *ob/ob* mice had significantly increased arousal and increased stage shifts compared to WT mice. These *ob/ob* mice also had increased sleep bout number and shorter bout lengths compared with WT mice. Further, during a recovery period after sleep deprivation, *ob/ob* mice had smaller amounts of NREM and REM sleep. This suggests leptin deficiency disrupts sleep consolidation (Laposky 2005). Although significant work has been done in mammals to understand sleep-metabolism interactions, these models are highly complex, difficult to image, and expensive to maintain. There are physiological differences between mammals and fruit flies; however, the molecular mechanisms underlying the basis of sleep in flies can provide insight for more complex organisms. Therefore, the fruit fly makes an excellent model to dissect the simpler, yet conserved, mechanism to understand sleep.

Drosophila **as a model to study sleep-metabolism interactions:**

Several genes have been identified that regulate sleep-metabolism interactions in *Drosophila*. For example, the *Drosophila foraging* (*for*) gene has naturally occurring

polymorphisms that effect behavioral and neural plasticity that contribute to a number of sleep-related behavioral differences. The gene encodes a cGMP-dependent Protein Kinase (PKG) that regulates feeding behavior and context-dependent regulation of sleep. The first polymorphism, *for*^{rover} (*for*^{R}), causes higher levels of PKG activity than a second polymorphism, *for*^{Sitter} (*for*^{S}). Flies with the *for*^{S} polymorphism do not suppress sleep in response to starvation, suggesting that modulation of PKG activity is critical for the integration of metabolism and sleep (Keene *et al.* 2010a). In addition, *for* appears to also regulate trade-offs between resilience to sleep deprivation and starvation. Enhanced PKG activity increases vulnerability to starvation-induced memory loss but protects against mechanically induced sleep deprivation, suggesting that *for* is involved in a trade-off between sleep and memory loss (Donlea *et al.* 2012). This effect is localized to the mushroom bodies, a region that is not required for starvation-induced sleep suppression (Keene *et al.* 2010a), raising the possibility that *for* acts in different regions of the brain to regulate starvation-induced sleep. Several systems have been developed to validate sleep in fruit flies.

Genetic toolkit of *Drosophila*.

Fruit flies have been well studied for over 100 years since the time of Thomas Morgan to understand basic principles of genetics. Fruit flies make an excellent model organism because of their short life cycle, highly conserved genes to mammals, high-throughput assays, and their brain is composed of 135,000 neurons, which makes it easier to study the genetic basis of their behavior (Banfi *et al.* 1996; Pickeral *et al.* 2000; Adams *et al.* 2000; Reiter *et al.* 2001; Misra *et al.* 2002; Alivisatos *et al.* 2012). Fruit flies also

have extensive genetic tools to manipulate flies at a genetic level; genes can be knocked down, knocked out or overexpressed with spatial and temporal specificity (Brand and Perrimon 1993; Lee and Luo 1999; Suster *et al.* 2004; Dietzl *et al.* 2007; Ni *et al.* 2009; Waddell *et al.* 2015). The GAL4/UAS system is one such tool, consisting of tissue-specific expression of the yeast transcriptional activator GAL4, which binds to an upstream activating sequence (UAS) (Fischer *et al.* 1988; Brand and Perrimon 1993). Flies carrying the GAL4 are mated to flies expressing the UAS. The resulting prodigy will be expressing a transgene of interest in a tissue-specific fashion.

The *Drosophila* Activity Monitoring System (DAMS) is used to record fly activity and correlate it to sleep. Sleep is defined in flies as 5 minutes or more of immobility(Shaw *et al.* 2000a; Greenspan *et al.* 2001). In addition, different food media can be placed in each DAMS tube to monitor how sleep is affected by changes in nutrition(Pfeiffenberger *et al.* 2010a; Cong *et al.* 2015). Another method to study both sleep and metabolism is the sleep and activity metabolic monitoring (SAMM) system, which can be used to measure metabolic rate (MR) in sleep mutants or alterations of diet(Stahl *et al.* 2017). Lastly, sleep depth can be measured in individual fruit flies using the *Drosophila* arousal tracking (DART) system, which as previously been used to measure mechanisms of sleep regulation during starvation(van Alphen *et al.* 2013; Faville *et al.* 2015). Together, the strengths of the fruit fly provide an excellent system to identify and subsequently characterize the genes and neurons regulating behavior.

Sleep in *Drosophila* melanogaster

Sleep homeostasis is the balance between the duration of sleep and wakefulness. The brain uses internal and external cues to assess how much sleep, both in duration and intensity, will be required to compensate for the lack or excess of sleep from the previous day. Sleep homeostasis is essential to many physiological functions, including energy metabolism, neural plasticity and immune functions. Sleep homeostasis is regulated by two independent processes: a circadian and a homeostatic process (Borbély *et al.* 2016). Circadian rhythm genes regulate the propensity to sleep depending on the time of day. In *Drosophila*, the circadian rhythm is regulated by ventral Lateral Neurons (LNvs), pigment dispersion factor (PDF) neurons (Shafer and Taghert 2009), and DN1 clock neurons. These neurons receive inhibitory GABAergic input that function to promote sleep. Homeostatic sleep is regulated by an accumulation of sleep pressure, which continues to build as one remains awake and resets as sleep need is met. In *Drosophila*, sleep has been well characterized through physiological (Nitz *et al.* 2002; Erion and Sehgal 2013) and behavioral correlates (Shaw *et al.* 2000b; Hendricks *et al.* 2000; Greenspan *et al.* 2001). These behavioral correlates are identified as behavioral hallmarks of sleep common to many organisms (Campbell and Tobler 1984). These hallmarks include (Sehgal and Mignot 2011): stereotypical sleep postures(Hendricks *et al.* 2000), sleep deprivation and rebound, elevation of arousal threshold, state of reversibility with stimulation, and maintenance of behavioral quiescence (Hendricks *et al.* 2000). Sleep in flies is primarily defined by behavioral quiescence or period of immobility of 5 mins or greater (Shaw *et al.* 2000a).

These measures of sleep have also been used to examine the functions of sleep and determine how it varies in different environments. For example, sleep regulates many factors such as energy storage/use, synaptic plasticity, and gene expression [19–21]. Sleep can also regulate behavior. For example, changes in sleep in *Drosophila* during early life can cause neuronal alterations that affect courtship behavior well into adulthood (Kayser *et al.* 2014; Murakami and Keene 2014). Moreover, flies collected from different latitudes and regional temperatures of the world display variations in sleep and metabolic function (Rivas *et al.* 2016; Brown *et al.* 2018a). Therefore, sleep can vary among individuals due to both internal and external factors.

Neurobiology of sleep.

Both neuronal and non-neuronal factors regulate sleep in flies. In glial cells, *DmMANF* (downregulation of mesencephalic astrocyte-derived neurotrophic factor) results in neurodegeneration and affects sleep and lifespan in *Drosophila melanogaster* (Walkowicz *et al.* 2017). More recently, *Eaat2* (Excitatory amino acid transporter 2), which is expressed in ensheathing glia, also regulates sleep. Eaat2-deficient flies exhibited an increase in sleep duration while characteristically reducing their metabolic rate during individual sleep bouts (Stahl *et al.* 2018). The neural regulation of sleep has been studied in much greater depth. Substantial research has focused on understanding how specific classes of neurotransmitters, such as dopamine or GABA, as well as specific anatomical brain regions, such as the *Drosophila* fan-shaped body or mushroom body, regulate sleep.

Neurotransmitter systems are highly conserved from flies to mammals and genetic approaches in the fly have made significant headway into understanding how these systems

regulate sleep. Neurotransmitters serotonin and GABA have been identified as sleep-promoting. Serotonin is involved in many behaviors, including sleep. Wild-type flies treated with 5-hydroxytryptophan (5-HTP) significantly increase their sleep. Conversely, mutations in the serotonin receptors d5-HT1A, d5-HT1B, and d5-HT2 resulted in receptor-specific changes in sleep and circadian rhythms. The d5-HT1A receptor mutant flies had short and fragmented sleep, while mutants of the d5-HT2 and d5-HT1B receptors had significantly altered circadian regulation, but didn't have any effect on baseline sleep. Therefore, serotonin affects homeostatic sleep and circadian rhythms through distinct receptors (Yuan *et al.* 2006). In *Drosophila*, the inhibitory neurotransmitter, γ-Aminobutyric acid (GABA) is produced in a small cluster of cells and innervates large portions of the brain (Okada *et al.* 2009). GABA is implicated in learning and memory (Haynes *et al.* 2015), circadian regulation (Parisky *et al.* 2008), and sleep. The GABA-A agonist 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP) significantly increases sleep in flies and restores sleep and memory in *rut* and *dnc* mutants (Dissel *et al.* 2015). The GABA receptors have also been implicated in sleep. The ionotropic $GABA_A$ receptor is expressed in the *l-LNvs* and is encoded by *Resistant to dieldrin (Rdl).* Knockdown of *Rdl* in PDF-expressing clock cells decreases sleep, while overexpression increases sleep (Parisky *et al.* 2008; Chung *et al.* 2009). Moreover, inhibition of GABA release from the DPM neurons decreases sleep duration (Haynes *et al.* 2015). This suggests GABAergic inhibition of arousal-promoting PDF neurons is important in sleep-wake regulation. Overall, serotonin and GABA are two neurotransmitters that promote sleep.

Wake promoting neurotransmitters are dopamine, histamine and octopamine. Dopaminergic cells appear in clusters throughout the *Drosophila* protocerebrum and

innervates neuropils in the central nervous system projecting to the mushroom bodies and central complex (Friggi-Grelin *et al.* 2003; Mao and Davis 2009). PPL1 and PPM3 wake-promoting dopaminergic cells in the posterior protocerebrum of the *Drosophila* brain synapse onto DopR (dopamine receptor)-expressing cells in the dFSB (*Drosophila* fan-shaped body), which is a sleep-promoting brain center (Ueno *et al.* 2012; Liu *et al.* 2012). One of the earliest studies done in *Drosophila* identified mutations in dopamine signaling which altered sleep (Kume *et al.* 2005). A defective dopamine transporter (DAT) mutant, *fumin*, sleeps less than wild-type flies, despite having normal waking activity and circadian rhythms (Kume *et al.* 2005). Further, several drug studies have shown that altering dopamine levels reduces sleep. Methamphetamine promotes dopamine release from presynaptic neurons by competitively inhibiting DA uptake and facilitates the movement of DA out of vesicles and into the cytoplasm (Calipari and Ferris 2013). Cocaine increases dopamine by blocking the DAT, which prevents its clearance from the synaptic cleft (Lebestky *et al.* 2009). Additionally, drugs such as the tyrosine hydroxylase [TH] inhibitor 3IY decreases dopamine levels, thereby increasing sleep (Andretic *et al.* 2005; Lebestky *et al.* 2009). Taken as a whole, dopamine neurotransmitters are wake-promoting (Jiang *et al.* 2016).

Although histamine has various functions that include control photoreception(Hardie 1989; Pantazis *et al.* 2008) and temperature sensing (Hong *et al.* 2006; Lundius *et al.* 2010), it has also been implicated in sleep. Measurements of sleep in hypomorphs of histidine decarboxylase (HDC) and the HisCl1 receptor suggests that histidine is wake-promoting. Knock-down of histamine increases sleep (Yu *et al.* 2015).

Further, when tested for sleep, mutants of *hdc*^{P211} and *hdc*^{P218} reported daytime sleep durations that were significantly longer than those of wild-type flies (Oh *et al.* 2013).

Octopamine, a norepinephrine analog, is involved in memory formation and aggression (Crocker *et al.* 2010). Octopamine-fed flies lowers protein kinase A (PKA), which decreases sleep. Conversely, increasing octopamine leads to increases in sleep (Crocker and Sehgal 2008). Two mutants for the octopamine biosynthesis pathway, *Tdc2*^{RO54} and *T*β*H*^{nm18}, increase their sleep, and sleep can be restored to control sleep levels when octopamine is administered. Further, electrical excitation of octopamine-producing cells decreases total sleep, whereas electrical silencing of these cells increases sleep (Crocker and Sehgal 2008). As dopamine, histamine and octopamine promote wakefulness, other neurotransmitters promote both waking and sleep.

The neurotransmitters acetylcholine and glutamate are both sleep and wake promoting. Acetylcholine (ACh) is the largest class of neurotransmitter in the *Drosophila* brain. Depending on the neuronal population, acetylcholine acts to promote both sleep and wakefulness, and Ach output is also required for sleep regulation. Release and transport of ACh requires vesicular acetylcholine transporter (vAChT), and knockdown of vAChT in a subset of mushroom body neurons significantly decreases sleep, suggesting that acetylcholine promotes sleep by acetylcholine transmission (Yi *et al.* 2013). Acetylcholine receptors are either muscarinic (*mAChR*) or nicotinic (nAChR), both of which play a role in sleep. In mice, the role of sleep for *mAChR*s reveal that cholinergic receptor, muscarinic 1 and 3 (*Chrm1* and *Chrm3)* are important for sleep regulation. Inhibition of *mAChRs* reduced calcium activity during REM sleep, whereas a reduction in calcium was observed during slow wave sleep. Therefore, this result suggests that calcium activity is dependent

on endogenous mAChR activation (Zhou *et al.* 2019). However, the role of *mAChR* in *Drosophila* sleep has not been reported (Niwa *et al.* 2018). The *nAChR* delivers excitatory input to the wake-promoting large ventrolateral neurons (l-LNvs) and modulates their firing in circadian pacemaker cells. This synchronized rhythm and activation of nAChRs increases the excitation and firing of the l-LNvs (McCarthy *et al.* 2011). The nAChRs are also responsible for most of the spontaneous excitatory drive in this circuit in the absence of normal sensory input (Gu 2006). In summary, the neurotransmitter system plays in important role in regulating sleep.

Anatomical regions that regulate *Drosophila* sleep

As we continue to understand how sleep is regulated by the neurotransmitter system, multiple brain regions that regulate sleep in *Drosophila* have been identified. Primary circuits regulating sleep appear to be connections between the ellipsoid body and fan-shaped body of the central complex, interneurons connects the superior arch of fan-shaped body to the ellipsoid body (EB) of the central complex (CX) (Donlea *et al.* 2018). The EB is formed by four ring (R1-4) neurons (Xie *et al.* 2017). In the ellipsoid body, the mechanism for sleep drive is determined by neuroplasticity (Liu *et al.* 2016; Guo *et al.* 2018). The increase in sleep need causes EB to increase Ca^{2+} levels, which strengthens the synapses of R2 cells through the proliferation of NMDA receptors (Liu *et al.* 2016). The dorsal fan-shaped body promotes sleep (Liu *et al.* 2012). Sleep-promoting dFB neurons induce sleep by expression of inhibitory neurotransmitters and the neuropeptide Allatostatin-A (AstA), which are thought to inhibit helicon cells of the central complex. Helicon cells sends signals to R2 ring neurons that regulate locomotion. During

wakefulness, dFB are inactive which allows helicon cells to receive visual input, causing locomotor activity in flies. As sleep pressure builds due to the activity of R2 ring neurons of ellipsoid body, dorsal fan-shaped body becomes active, releasing alliostatinA (AstA) to promote sleep (Donlea *et al.* 2018). Balancing sleep-need and sleep forms an autoregulatory loop between cells that modulate sleep control centers and those that integrate sensory information. Thus the homeostatic regulation of sleep functions within the central complex circuitry.

The mushroom bodies (MBs) and Insulin Producing Cells (IPCs) are sleep promoting brain regions (Yurgel *et al.* 2015a). The mushroom body (MB) is the center for memory and learning and is composed of the alpha (α) and beta (β) lobes, the alpha' (α')and beta' (β') lobes, and the gamma (γ) lobe (Crittenden *et al.* 1998). The MB has been vastly studied for memory consolidation and retrieval involving olfaction (de Belle and Heisenberg 1994; Dubnau *et al.* 2001), courtship conditioning (McBride *et al.* 1999; Joiner and Griffith 2000) and context-dependent visual cues (Liu *et al.* 1999). However, previous studies found MB's are the primary olfactory center of *Drosophila* and MB mutants are deficient in olfactory learning (Heisenberg *et al.* 1985; Pascual 2001). One early experiment assessed the influence of the mushroom bodies on walking activity by using different methods to interfere with mushroom body development and function. The flies with mushroom body defects had increased locomotor activity, suggesting that these flies slept less (Martin *et al.* 1998). However, fewer studies have been published involving the mushroom body in the context of sleep (Joiner *et al.* 2006a; Guo *et al.* 2011; Tomita *et al.* 2017). One neuropeptide critical for sleep regulation both during development and as an adult is short-neuropeptide F (sNPF) (Chen *et al.* 2013; Shang *et al.* 2013). More recently,

optogenetic activation of sNPF neurons induced sleep (Juneau *et al.* 2019). Several studies suggest the MBs have roles in promoting both sleep and wakefulness, suggesting subsets within the MBs have differential function.

The temporal and spatial manipulations were used to study sleep in mushroom bodies of adult *Drosophila* (Joiner *et al.* 2006b). Neurons of the MB can be inactivated by driving the inward rectifying potassium channel (Kir2.1) to significantly increase in sleep. Inversely, the same MB neurons can be activated by driving the bacterial sodium channel (NaChBac) and result in a significant decrease in sleep (Joiner *et al.* 2006b). Further, silencing different populations of MB neurons using the temperature-sensitive silencer (Shi^{TS}) also significantly decreases sleep (Joiner *et al.* 2006a; Pitman *et al.* 2006a; Yi *et al.* 2013; Sitaraman *et al.* 2015c), suggesting that broadly activating or silencing neurons of the MB regulates sleep. Spatial manipulations in specific lobes of the MB can also cause changes in sleep. Activation of 201Y, which is expressed in the γ lobes and the core α/β lobes of the MBs, increases sleep; while, activation of c309, which is expressed in the γ lobes and surface α/β lobes, decreases sleep. Thus, changes in sleep are determined by a small cluster of neurons (Joiner *et al.* 2006b). More recently, two parallel segregated compartment-specific microcircuits that regulate sleep were identified: a GABAergic wake-promoting micro-circuit that originates in α'/β' and a cholinergic sleep-promoting microcircuit that originates in γd Kenyon Cells (Sitaraman *et al.* 2015a). Overall, the function of the MBs in sleep has mainly been studied in isolation. The only evidence of connectivity to other circuits is that the axons of output neurons from the MBs converge in the superior medial protocerebrum (SMP) and crepine (CRE) neuropils, adjacent to the dendrites of the dFB neurons.

Neuroendocrine signaling involved in sleep

Drosophila has emerged as an excellent model in neuropeptide research. Neuropeptides regulate physiology and a broad set of behaviors (Nässel and Zandawala 2019). Neuropeptide signaling is integral to many aspects of neural communication, particularly modulation of membrane excitability and synaptic transmission. Insulin-like peptides and AKH have diverse functions in regulating metabolism and behavior. *Drosophila* has 8 insulin-like peptides (*ilp1-8*) that are homologous to mammalian insulin. Expression of these genes vary in spatial/temporal function and regulates metabolism, behavior, and developmental growth (Brogiolo *et al.* 2001; Britton *et al.* 2002; Rulifson *et al.* 2002; Wu *et al.* 2005; DiAngelo and Birnbaum 2009). The IPCs secrete insulin-like peptides *ilp2*, *ilp3* and *ilp5* expressing in medial neurosecretory cells that regulate many behaviors such as sleep and feeding (Broughton *et al.* 2005). A causal link exists between nutrient sensing and insulin-dependent growth. E.g. overexpression of *ilp2* results in a 51% weight gain in males (Ikeya *et al.* 2002). Interestingly, overexpression of *ilp2*, as well as ectopic activation of insulin signaling in the fat bodies or brain, does not alter sleep, suggesting that insulin-like signaling is not directly responsible for the IPC-dependent regulation of sleep (Erion *et al.* 2012). During development, both *ilp3* and *ilp5* are transcriptionally downregulated in response to starvation, suggesting a role in nutritional state-dependent regulation of behavior and metabolism (Ikeya *et al.* 2002). Deletion of all three *ilps* expressed in the IPCs protects against age-related disruption in sleep, suggesting the *ilp* release from IPCs regulates age- or stress-dependent changes in sleep (Metaxakis *et al.* 2014). Unlike all other *ilps*, *ilp6* is predominantly expressed in the fat body, suggesting

that insulin signaling in the fat body is autoregulated to activate the promotion of fat storage (Saltiel and Kahn 2001; DiAngelo and Birnbaum 2009).

Flies display evidence of neuroendocrine involvement in sleep regulation. The steroid hormone *ecdysone*, which is critical for insect development, also promotes sleep in *Drosophila*, supporting the notion that developmental genes can regulate behavior in the adult animal.(Ishimoto and Kitamoto 2010) . Adipokinetic hormone (AKH), like mammalian glucagon, is a polypeptide that acts as an energy reserve to sustain energy-consuming activities, such as locomotion (Van Der Horst 2003; Kim and Rulifson 2004a). AKH is expressed in peptidergic secretory cells of the corpora cardiaca (CC) (Kim and Rulifson 2004b; Lee and Park 2004; Park *et al.* 2008). The CC receives input from insulin producing cells (IPCs) and secretes AKH into the hemolymph (Rulifson *et al.* 2002; Kim and Rulifson 2004b). AKH binds to the *Adipokinetic Hormone Receptor* (AKHR), a G-protein coupled receptor that is expressed in the brain and fat body (Yin *et al.* 2012). The metabolism of glycogen in muscles and lipids in fat bodies are used for energy (Canavoso *et al.* 2001a; Van Der Horst 2003). Ablation experiments of the corpora cardiaca result in hypoglycemia; suggesting the corpora cardiaca is required in glucose sensing and overall metabolic regulation (Kim and Rulifson 2004b). Disruptions in AKH signaling promote glycogen and triglyceride storage, suggesting this pathway controls carbohydrate and fat metabolism (Kim and Rulifson 2004b; Lee and Park 2004; Isabel *et al.* 2005). Ablation of AKH-producing cells reduces the locomotor and feeding responses to starvation and increases starvation resistance (Lee and Park 2004; Bharucha *et al.* 2008). In summary, ILPs and AKH have diverse functions in regulating metabolism and sleep (Metaxakis *et al.* 2014).

Effect of dietary changes on sleep and metabolism

In nature, the *Drosophila* diet consists of complex sugars and protein obtained by feeding on yeast from rotting fruit (Atkinson and Shorrocks 1977). The diet of *Drosophila* in the laboratory also consists of carbohydrates, protein and fat. A proper dietary balance of sugar and yeast is important for the maintenance of homeostasis and fitness. Flies fed a diet of 5% sucrose-alone have a similar sleep duration to flies fed normal food suggesting that dietary protein is not required for this behavior (Keene *et al.* 2010b). An alternative study examining the contributions of dietary sugar and yeast to sleep architecture reported no difference in total sleep duration between flies fed a high or low calorie diet of sucrose and yeast (Linford *et al.* 2012). Interestingly, increasing the dietary sucrose concentration from 5% to 35% does not alter the total sucrose consumed, but suppresses sleep (Catterson *et al.* 2010). Therefore, these data indicate that flies sleep normally when fed moderate concentrations of sucrose, but suppress sleep on high concentrations of dietary sucrose through a mechanism that is independent of total caloric intake. Flies starved on a diet of agar alone become hyperactive and reduce sleep. By changing the dietary component of food, changes in behavior, physiology, and longevity is clearly observed (Mair *et al.* 2005; Lee and Micchelli 2013). For example, raising dietary sugar concentration increases triglyceride levels in *Drosophila*, which can be suppressed by simultaneously increasing the yeast concentration (Skorupa *et al.* 2008). The protein component of yeast is required for proper growth and development in flies. Larvae fed a sugar diet are severely undersized (Britton and Edgar 1998; Britton *et al.* 2002). Additionally, restricting caloric intake has been implicated in increasing lifespan and reducing reproductive output, reveals diet-related trade-offs between longevity and behavior (Chapman and Partridge 1996; Good

and Tatar 2001; Masek *et al.* 2014b; Piper *et al.* 2014). Interestingly, low amounts of specific amino acids are also responsible for increased lifespan and decreased fecundity observed under caloric restriction, while other nutrients do not contribute to these phenotypes (Harbison *et al.* 2004; Grandison *et al.* 2009). The amino acid methionine appears to be critical for fecundity and lifespan, raising the possibility that methionine may modulate sleep in aging animals (Grandison *et al.* 2009). Emerging evidence indicates there is a role for amino acid metabolism in sleep regulation. Dietary threonine (SPET) links amino acid metabolism in *Drosophila* neurons to GABAergic control of sleep drive in *Drosophila*. SPET increased daily sleep amount and decreased the latency to sleep onset in a dose-dependent manner (Ki and Lim 2019). More recently, nutritional environment influences the impact of microbes on life span. Probiotic gut microbes proliferate in the fly environment during nutrient deprived conditions. The same microbes that extend fly life span on malnourishing diets can shorten lifespan on rich diets (Keebaugh *et al.* 2019). Taken together, diet alone can elicit both acute and chronic behavioral changes.

Nutrient sensors in the *Drosophila* brain

Metabolism is never static. Peripheral organs, such as the fat body and gut control nutritional homeostasis and communicate information to the brain to regulate feeding behavior. In addition, nutrients are also sensed by directly by neurons in the brain providing a direct mechanism to regulating energy balance. The PI is an essential region in the *Drosophila* brain involved in direct nutrient sensing. DH44 neurons express the gene *diuretic neuropeptide 44* (*DH44*), which is needed for the selection of nutritive sugars over a nonnutritive sugars after a period of starvation (Dus *et al.* 2015). Stimulation of *DH44*

neurons targeting the *DH44* receptor promote food intake. Additionally, these neurons target a second *DH44* receptor expressed in the gut to promote gut motility, suggesting that *DH44* neurons have a wide role in sensing macronutrients (Yang *et al.* 2018). The IPCs sense and respond to glucose from hemolymph through the activation of ATP-sensitive potassium channels (Fridell *et al.* 2009; Kréneisz *et al.* 2010). However, a faster mechanism for nutrient sensing was later discovered; *minidisc* demonstrated that the essential amino acid leucine induces insulin-like peptide secretion through the L-type amino acid transporter, (Manière *et al.* 2016). Neurons in the superior protocerebrum of the *Drosophila* brain express a fructose receptor, *Gr43a* (Miyamoto *et al.* 2013). High levels of fructose in the hemolymph are sensed directly through *Gr43a* receptors, regulating food intake in a state-dependent manner. During the state of satiety, *Gr43a* neurons suppress feeding, while during hunger, this receptor functions to promote feeding (Miyamoto *et al.* 2012, 2013). Taken together, there are many neuronal nutrient sensing mechanisms in the brain.

Role of Fat Bodies in *Drosophila*

The fat body (FB) regulates energy stores and brain function, and are therefore critical regulators of sleep and feeding. The *Drosophila* fat body has been previously implicated in regulation of numerous behaviors including courtship, feeding and egg-laying (Lazareva *et al.* 2007; Xu *et al.* 2008, 2011; Sassu *et al.* 2012). The *Drosophila* FB is the main tissue for energy storage, fulfilling functions similar to mammalian adipose tissue and liver (Canavoso *et al.* 2001b; Arrese and Soulages 2010). Energy intake greater than energy expenditure equals increased energy storage; this is a highly conserved method

to maintain energy homeostasis. Adipose tissue senses overall nutrient levels in the animal and modulates behaviors through metabolic control of energy stores and secreted factors that regulate neural function (Ahima and Lazar 2008; Morton and Schwartz 2011). In *Drosophila*, the fat body is the center for energy homeostasis, the primary site of glycogen and triglyceride storage, and the main location for detoxification and immunity. AKH binds a G-protein-coupled transmembrane receptor on fat body cells to increase glycogenolysis and lipolysis, releasing nutrients into hemolymph comprised of circulating glucose, trehalose (disaccharide), and free-glucose (monomeric). Thus, the FB is essential in energy storage.

Several sleep-regulating genes are expressed in the fat body. Manipulations of Angiotensin-converting enzyme peptidase (ACER) have disrupted nightime sleep, suggesting that the fat body functions to promote sleep (Carhan *et al.* 2011). Fat body function also appears to be important in regulating homeostatic sleep changes in response to stressors including, starvation and sleep-deprivation. *Drosophila* mutants for the adipose triglyceride lipase *brummer*, a gene highly expressed in the fat body, have elevated triglyceride stores and have a changed homeostatic response to sleep deprivation (Grönke *et al.* 2005; Thimgan *et al.* 2010a). Conversely, flies mutant for *lipid storage droplet 2* (*lsd2*) have reduced triglyceride levels and do not display a homeostatic rebound in response to sleep deprivation, suggesting that triglyceride stores in the fat body enhance the homeostatic response to sleep deprivation (Thimgan *et al.* 2010a).

The *Drosophia* cytokine *upaired 2* (*upd2*) was identified as an ortholog of mammalian leptin (Rajan and Perrimon 2012). *Upd2* expression is dependent on nutritional status and is produced by the fat body in the fed state. When *upd2* function is perturbed

specifically in the fat body, it results in a systemic reduction in growth and alters energy metabolism (Rajan and Perrimon 2012). Secretion of *upd2* from the fat bodies regulates insulin accumulation and release from the IPCs (Laposky *et al.* 2006). Upd2 secretion from FBs regulates feeding and sleep quality. These findings suggest that *upd2* may function through the IPCs to regulate sleep in response to metabolic changes. Recently, knockdown of *dome*, the upd2 receptor in *Ilp2*-expressing neurons, reduced sleep, suggesting satiety requires signals from peripheral tissues to stop food intake (Ertekin *et al.* 2020) . During starvation, energy stores are metabolized to provide nutrients. The fat bodies regulate energy stores and provide nutrients to the brain; therefore, the FBs are critical regulators of sleep and feeding.

Evolutionary selection of starvation resistance (SR) flies.

While most studies of sleep and metabolism in flies have relied upon inbred lines, the use of experimental evolution also provides the opportunity to investigate these traits (Masek *et al.* 2014b; Slocumb *et al.* 2015a; Brown *et al.* 2019a). Food shortage is particularly challenging to animal survival, and they must adapt developmentally, physiologically and behaviorally to the surrounding environment. Starvation resistance is the ability to survive in response to starvation stress, therefore, starvation-induced sleep suppression plays a key role in survival (Keene *et al.* 2010b). However, another physiological change that might evolve in response to starvation stress is an increase fat storage levels. Three populations of starvation-resistant flies via experimental evolution were generated from wild-caught flies in a Pennsylvania orchard. Each fly population was separated into Fed and Selected A, B and C groups. The fed controls (F) were raised on

standard fly food to propagate normally. The selected groups (S) were placed on nutrient-deprived agar until 15% of the population survived, then placed on food to propagate. This process was repeated in the selected flies for 65+ generations. Each group of S and F flies were placed in DAMS, and then sleep was monitored. (Masek *et al.* 2014a). The S group flies slept significantly longer than their F group control; these starvation-selected lines developed an obese condition, storing nearly twice the level of total lipids than their unselected controls. Although these flies evolved a 3-fold increase in starvation resistance, obesity-associated pathologies in these flies also co-evolved, including: metabolic depression, low activity levels, dilated cardiomyopathy, and disrupted sleeping patterns. A wide range of genetic heterogeneity between the replicates of the selected lines, suggests multiple mechanisms of adaptation (Hardy *et al.* 2018). Therefore, studying starvation-resistance in experimentally evolved populations provide insight into the co-evolution of sleep-metabolism interactions.

Conclusion

As stated earlier, metabolic syndrome encompasses a suite of conditions, most commonly including obesity, heart disease, stroke, and type 2 diabetes. Dysregulation of sleep has been implicated in metabolic syndrome (Zhao *et al.* 2020). However, the genetic mechanisms that regulate sleep-metabolism interactions remain poorly understood. This dissertation utilizes genetic screens to identify novel regulators of sleep and metabolism in *Drosophila melanogaster*. In Chapter 2, a screen identified *translin* as one of the first genes to regulate sleep-metabolism interactions. In Chapter 3, a second screen identifies and subsequently investigates the functional role of *unc79* in the regulation of sleep and

starvation resistance. Together, these works further our understanding of the genetic basis of sleep-metabolism interactions.

CHAPTER 2. TRANSLIN IS NECESSARY FOR THE METABOLIC REGULATION OF SLEEP

Abstract

Dysregulation of sleep or feeding has enormous health consequences. In humans, acute sleep loss is associated with increased appetite and insulin insensitivity, while chronically sleep-deprived individuals are more likely to develop obesity, metabolic syndrome, type II diabetes, and cardiovascular disease. Conversely, metabolic state potently modulates sleep and circadian behavior; yet, the molecular basis for sleep-metabolism interactions remains poorly understood. Here, we describe the identification of *trsn*, a highly conserved RNA/DNA binding protein, as essential for starvation-induced sleep suppression. Strikingly, *trsn* does not appear to regulate energy stores, free glucose levels, or feeding behavior suggesting the sleep phenotype of *trsn* mutant flies is not a consequence of general metabolic dysfunction or blunted response to starvation. While broadly expressed in all neurons, *trsn* is transcriptionally upregulated in the heads of flies in response to starvation. Spatially restricted rescue or targeted knockdown localizes *trsn* function to neurons that produce the tachykinin family neuropeptide Leucokinin. Manipulation of neural activity in Lk neurons revealed these neurons to be required for starvation-induced sleep suppression. Taken together, these findings establish *trsn* as an essential integrator of sleep and metabolic state, with implications for understanding the neural mechanism underlying sleep disruption in response to environmental perturbation.

Introduction

In humans, sleep and feeding are tightly interconnected, and pathological disturbances of either process are associated with metabolism-related disorders. Acute sleep loss correlates with increased appetite and insulin insensitivity, while chronically sleep-deprived individuals are more likely to develop obesity, metabolic syndrome, type II diabetes, and cardiovascular disease (Peppard *et al.* 2000; Taheri *et al.* 2004; Knutson and Van Cauter 2008). Conversely, in humans and rodents, internal metabolic state potently modulates sleep and circadian behavior (MacFadyen *et al.* 1973; Danguir and Nicolaidis 1979; Green *et al.* 2008). Despite the widespread evidence for interactions between sleep loss and metabolic dysfunction, little is known about how these processes integrate within the brain.

Drosophila provides a powerful model system to investigate the integration of sleep and metabolic state. *Drosophila* is amenable to genetic analysis, and most molecular processes regulating sleep and metabolism are conserved from flies to mammals (Sehgal and Mignot 2011; Padmanabha and Baker 2014). Further, flies display all the hallmarks of sleep including extended periods of behavioral quiescence, rebound following deprivation, increased arousal threshold and changes in electrophysiological readouts of brain wave activity (Hendricks *et al.* 2000; Shaw *et al.* 2000a) The value of *Drosophila* as a model for sleep has been highlighted using forward and reverse genetic screens, which have identified novel genetic regulators of sleep including *sleepless*, *Cyclin A*, and the K^+ channel *shaker* (Cirelli *et al.* 2005; Koh *et al.* 2008a; Rogulja and Young 2012). While these findings have provided a framework to understand how sleep is regulated under standard conditions,

much less is known about how sleep is modulated in response to acute environmental changes.

Changes in food availability present a common environmental challenge and potently affect metabolism and sleeping behavior. Interactions between sleep and metabolic state appear to be behaviorally conserved from flies to mammals. Both rodents and insects suppress sleep in response to prolonged food deprivation, presumably to forage for food, and sleep is disrupted in humans during prolonged fasting (MacFadyen *et al.* 1973; Danguir and Nicolaidis 1979; Thimgan *et al.* 2010a; Keene *et al.* 2010a) . Sleep suppression during starvation can occur independently of sensory inputs, or be modified by taste neurons suggesting contributions from both internal metabolic processes and sensory systems to regulate this change in behavior (Keene *et al.* 2010a; Dus *et al.* 2011; Linford *et al.* 2012). A number of genes have been implicated in the metabolic regulation of sleep including the circadian genes *Clock* and *cycle*, the adipose gene *brummer lipase* and the glucagon-like *Adipokinetic Hormone* (AKH) (Lee and Park 2004; Thimgan *et al.* 2010a; Keene *et al.* 2010a). While these genes are modulators of sleep, they are also involved in broader metabolic processes such as regulation of energy stores and feeding behavior, and therefore, may represent more general regulators of physiological or behavioral homeostasis (Bharucha *et al.* 2008; Thimgan *et al.* 2010a; DiAngelo *et al.* 2011).

To further understand the relationship between sleep and feeding state, we combined a powerful behavioral assay with a high-throughput RNAi based screen, which selectively disrupted genetic function in the central nervous system. We identify an essential role for *translin* (*trsn*) in the metabolic regulation of sleep. Knockdown or genetic

mutation of *trsn* impairs starvation-induced sleep suppression. Structurally, *trsn* is evolutionarily conserved from flies to humans (Claußen *et al.* 2006) and has been implicated in regulation of RNA localization, endonuclease function, and monoamine synthesis (Claußen *et al.* 2006; Stein *et al.* 2006; Jaendling and McFarlane 2010). We localize *trsn*-dependent modulation of sleep to Leucokinin (Lk) neurons, which have previously been shown to regulate food intake, water homeostasis and locomotor behavior (De Haro *et al.* 2010; Al-Anzi *et al.* 2010; Liu *et al.* 2015). We find that *trsn* functions in LK neurons to promote wakefulness during starvation. Therefore, these findings indicate *trsn* is a novel regulator of insulin transcription that serves as a selective integrator of sleep and metabolic state.

Methods

Drosophila maintenance and Fly Stocks

The *trsn*-RNAi lines are from the Vienna *Drosophila* Resource Center (Dietzl *et al.* 2007). The RNAi lines have been renamed from original transformant identifiers as follows: *trsn*-IR#1 (GD9963), *trsn*-IR#2 (GD9964) and *trsn*-IR#3 (108456). The *trsn*EP line is the EPgy2 insertion *trsn*EY06981 and has previously been characterized (Bellen *et al.* 2004; Claussen *et al.* 2006; Suseendranathan *et al.* 2007). The $trsn^{null}$ allele is an excision of the *trsn*EY06981 locus derived from mobilizing the EPgy2 insertion in the w1118 background that has been previously described(Claussen *et al.* 2006). This allele removes the entire coding region of the gene and likely represents a null mutation. It has previously been described as Δ*trsn* (Claussen *et al.* 2006). The LK-GAL4 line is a promoter fusion of 3.6 kb upstream of LK, cloned in the laboratory of YJK with a similar expression

pattern to a previously described line (Al-Anzi *et al.* 2010). The lines UAS-TNT and UAS-ShiTS1 have previously been described (Sweeney *et al.* 1995; Kitamoto 2001). The UAS-mCD8::GFP (32184;(Pfeiffer *et al.* 2008)) and UAS-GFP.nls (32184; (Yasunaga *et al.* 2006)) transgenes have previously been described and were obtained from Bloomington. The UAS-*trsn* transgene was generated by amplifying from GM27569 clone into a PhiC31 vector at the attP86Fb docking site on the 3rd chromosome by Zoltan Astolos (Aktogen, Cambridge, UK). Three to five day old mated female flies were used for all experiments in this study, except when noted.

Behavioral Analysis

The DAM system detects activity by monitoring infrared beam crossings for each animal. These data were used to calculate sleep information by extracting immobility bouts of 5 minutes using the *Drosophila* Sleep Counting Macro (Pfeiffenberger *et al.* 2010b). For experiments examining the effects of starvation on sleep, activity was recorded for one day on food, prior to transferring flies into tubes containing 1% agar (Fisher Scientific) at ZT0 and activity was monitored for an additional 24 hours. Change in sleep during starvation or dietary manipulation was calculated as ((sleep duration (mins) experimental-sleep duration (mins) baseline)/(sleep duration (mins) baseline))*100 as previously described(Keene *et al.* 2010a) .For experiments employing thermogenetic manipulation of LK neurons, only nighttime sleep was analyzed because flies were unable to survive 24 hours of starvation at elevated temperatures. Following 24 hours of acclimation, baseline sleep was measured on food at 22°C from ZT12-ZT24. On the following day at ZT8 flies were transferred to new tubes containing either standard fly food (control) or 1% agar. The temperature was increased to 31°C at ZT12 and activity was recorded through ZT24.

For tracking analysis, fly activity was recorded using a custom video acquisition system (Garbe *et al.* 2015). Flies were anesthetized using cold-shock and loaded into standard 24-well tissue culture plates (BD Biosciences 351147), with each well containing either 5% sucrose dissolved in 1% agar (fed group) or 1% agar alone (starved group). The sucrose diet was required as standard fly food is opaque and prevents efficient tracking. The plates were placed in a chamber illuminated with white (6500K) LED lights (Environmental Lights Inc. product no. dlrf3528-120-8-kit) on a 12:12 LD cycle, and with constant illumination from 850-880nm infra-red (IR) lights (Environmental Lights Inc., product no. irrf850-390). Video was recorded using an ICD-49 camera (Ikegami Tsushinki Co., Japan) fitted with an IR- transmitting lens (Computar Inc., Vari Focal H3Z4512 CS-IR 4.5-12.5 mm F 1.2 TV lens). An IR high-pass filter (Edmund Optics Worldwide, filter optcast IR 5x7 in. part no. 46,620) was placed between the camera and the lens to block visible light. Video was recorded at a resolution of 525 lines at 59.94 Hz, 2:1 interlace. Fly activity was analyzed using Ethovision XT 9.0 video tracking software (Noldus Inc.). Sleep was calculated by measuring bouts of inactivity >5 minutes using a previously described Microsoft Excel macro (Garbe *et al.* 2015).

For sleep deprivation experiments, flies were shaken in DAM2 monitors every 3-4 minutes for 12 hours from ZT12 (onset of darkness) through ZT0 (onset of light) as previously described (Masek *et al.* 2014b). Stimulus was applied using a vortexer (Fisher Scientific, MultiTube Vortexer) with a custom milled plate to hold DAM2 monitors and a repeat cycle relay switch (Macromatic, TR63122). Sleep rebound was measured the following day from ZT0-ZT12.

Pharmacological manipulation

For pharmacological manipulation of glucose and fatty acid utilization, flies were loaded into tubes containing standard fly food. Following a 24 hour acclimation period, flies were transferred at ZT0 into tubes containing standard fly food (control), food laced with either 400mM 2-DG, 25μM etomoxir, or 400mM 2-DG and 25μM etomoxir and sleep was measured for an additional 24 hours.

For gene-switch experiments, a 100mM stock solution of RU486 (Sigma, St. Louis) was made in ethanol and stored in -20 °C. The stock solution was added to fly food or 1% agar solution to a final concentration of 0.25mM RU486. Crosses were raised at room temperature in normal fly food vials then transferred to individual DAM tubes containing 0.25mM RU486; the flies were acclimated in the DAM monitor for 24 hrs. On experimental day 1, sleep was recorded. On day 2, flies were flipped to DAM tubes containing 1% agar and 0.25mM RU486; % sleep was recorded. RU486 effects during experiment were calculated by comparing the amount of sleep during the baseline night (without drug) with that during the treatment night.

Paraquat treatment

Paraquat dichloride (Sigma, St. Louis) was dissolved directly into 1% agar with 5% sucrose and poured into plates to obtain a concentration 1mM of Paraquat; DAM tubes were made similarly. Both w^{1118} controls and *trsn* mutant flies were raised at room temperature in normal fly food vials then transferred to individual DAM tubes containing 1mM Paraquat dichloride. The flies were acclimated in the DAM monitor for 24 hrs. Sleep was measured for 5 days under standard light/dark cycles and percent sleep was monitored.

Caffeine treatment

Caffeine (Sigma, St. Louis) was dissolved in melted fly food and poured into plates to a concentration of 4mg/mL. Both w^{1118} and *trsn* mutant flies were raised at room temperature in normal fly food vials then transferred to individual DAM tubes containing standard food. The flies were acclimated in the DAM monitor for 24 hrs. On experimental day 1, sleep was recorded. On day 2, flies were flipped to DAM tubes containing 4mg/mL caffeine and percent sleep was recorded. Caffeine effects during each experiment were calculated by comparing the amount of sleep during the baseline night (without drug) with that during the treatment night.

Protein, glycogen, and triglyceride measurements

Assays for quantifying triglyceride, glycogen and protein content of flies were performed as previously described (Gingras *et al.* 2014). Two female flies aged 3-5 days were homogenized in Tris-HCl containing 140mM NaCl, pH 7.4, 0.1% Triton-X, 1X protease inhibitor cocktail (Sigma Aldrich, P8340). Triglyceride concentration was measured using the Stanbio Liquicolor Kit (Boerne, TX), and protein concentrations were measuring using a BCA Protein Assay Kit (Pierce Scientific). Total glucose levels were determined using the Glucose Oxidase Reagent (Pointe Scientific) in samples previously treated with 8mg/mL amyloglucosidase in 0.2M Sodium Citrate buffer, pH 5.0 (Boston BioProducts). Free glucose was measured in samples not treated with amyloglucosidase and then glycogen concentrations were determined by subtracting the free glucose from total glucose concentration. Both glycogen and triglyceride concentrations were standardized to the total protein content of each sample containing two flies.

Proboscis Extension Reflex (PER)

Three to five day old flies were collected and placed on fresh food for 24 hours, then starved for the designated period of time in vials containing wet Kimwipe paper (Kimberly-Clark Corporation). Flies were then anaesthetized under CO2, and their thorax and wings were glued with nail polish (Electron Microscopy Science) to a microscopy slide, leaving heads and legs unconstrained. Following 3-6 hours recovery in a humidified chamber, the slide was mounted vertically under the dissecting microscope (Leica, S6E) and PER was observed. PER induction was performed as described previously (Masek and Scott 2010). Briefly, flies were satiated with water before and during experiments. Flies that did not water satiate within 5 minutes were excluded from the experiment. A 1 ml syringe (Tuberculin, BD&C) with an attached pipette tip (TipOne) was used for tastant presentation. Tastant was manually applied to tarsi for 2-3 seconds 3 times with 10 second inter-trial intervals, and the number of full proboscis extensions was recorded. Tarsi were then washed with distilled water between applications of different tastants and flies were allowed to drink water during the experiment ad libitum. Each fly was assayed for response to multiple tastants. PER response was calculated as a percentage of proboscis extensions to total number of tastant stimulations to tarsi.

Blue dye assay

Short-term food intake was measured as previously described (Wong *et al.* 2009). Briefly, flies were starved for 24 or 48 hours on wet Kimwipes or maintained on standard fly food. At ZT0 flies were then transferred to food vials containing 1% agar, 5% sucrose, and 2.5% blue dye (FD&C Blue Dye No. 1). Following 30 minutes of feeding flies were flash frozen on dry ice and individually homogenized in 400 µL PBS (pH 7.4, Ambion).

Color spectrophotometry was then used to measure absorbance at 655 nm in a 96-well plate reader (Millipore, iMark). Baseline absorbance was determined by subtracting the absorbance measured in non-dye fed flies from each experimental sample.

Two-choice capillary feeding assay (CAFÉ)

A modified volumetric drinking assay was used to test food consumption (Ja *et al.* 2007) as previously described (Masek *et al.* 2014b). Female flies were allowed to feed on a tube containing 100mM sucrose or 5% yeast extract in water, while a second capillary tube provided access to water alone (WPI, #1B150F-4 ID 1mm, OD 1.5mm, with filament). The capillary tubes were inserted into an empty food vial at a 90° angle and vials were placed at a 45° angle. The openings of the capillaries were aligned with the ceiling of the vial. Following 24 hours of fasting, 30-60 female flies were placed into a vial and food consumption was measured. The volume consumed was calculated as the length of liquid missing from the capillary multiplied by the cross-section of the inner diameter of the capillary. All measurements were adjusted for missing liquid due to evaporation using control capillary tubes without flies. Consumption was measured every hour following the introduction of flies into the assay. Taste compounds were mixed with Allura red food dye (FD&C red #40) to a concentration of 3µl per 1ml dilution for better visibility in the capillary tube. Following the conclusion of the assay flies were anaesthetized and the number of flies in each vial was counted. Total consumption per fly was measured as volume consumed in each capillary divided by number of live flies in the vial.

Quantitative RT-PCR

Flies were collected 5–7 days after eclosion. Ten or more flies were separated into fed and starved groups and were flash frozen. Total RNA was extracted from fly heads

using the QIAGEN RNeasy Tissue Mini kit according to the manufacturer's protocol. RNA samples were reverse transcribed using iScript (Biorad), and the generated cDNA was used for real-time PCR (Biorad CFX96, SsoAdvanced Universal SYBR Green Supermix qPCR Mastermix Plus for SYBRGreen I) using 1.7 ng of cDNA template per well and a primer concentra-tion of approximately 300 nM.

The primers used were: *trsn* (F-5'GCTCCGCCTTCTCCAGATACT3' and (R 5'CCGCCTCCAGGTAAATAACCA3'), actin 5C (F5'AGCGCGGTTACTCTTTCACCAC3') and R 5'GTGGCCATCTCCTGCTCAAAGT3'), and β-tubulin (F-5'GCAGTTCACCGCTATGTTCA3' and R-5'CGGACACCAGATCGTTCAT3'). Triplicate measurements were conducted for each sample. Primers were purchased from IDT technologies.

Immunohistochemistry

Fly brains were dissected in ice-cold PBS and fixed in 4% formaldehyde, PBS, 0.2% Triton-X 100 for 30 minutes. Brains were rinsed 3X with PBS, Triton-X for 10 minutes and incubated overnight at 4°C in 1:4 anti-ELAV, 1:20 NC82 ((Wagh *et al.* 2006) Iowa Hybridoma Bank) and 1:1000 anti-*TRSN* (Claussen *et al.* 2006). The brains were rinsed again in PBS-Triton X, 3X for 10 minutes and placed in secondary antibodies (Goat anti-Mouse 555, and Goat anti-rabbit 488; Life Technologies) for 90 minutes at room temperature. The brains were mounted in Vectashield (VectorLabs) and imaged on a Leica SP8 confocal microscope. Brains were imaged in 2µm sections and are presented as the Z-stack projection through the entire brain. For quantification of whole-brain *TRSN* levels, the entire brain was imaged in 2µm sections, merged into a single Z-stack as maximum

fluorescence, and the total brain fluorescence was determined. For experiments examining co-localization, each channel was imaged separately, and the absence of bleed through was validated.

Statistical Analysis

Statistical analyses were performed using InStat software (GraphPad Software 5.0) or IBM SPSS 22.0 software (IBM). For analysis of sleep, we employed a one- or two-way ANOVA followed by a Tukey's post hoc test. For PER experiments, each fly was sampled three times with the same stimulus. The response was binary (PER yes/no), and these three responses were pooled for values ranging from 0 to 3. The Kruskal-Wallis test (non-parametric ANOVA) was performed on the raw data from single flies, and Dunn's multiple comparisons test was used to compare different groups. For the capillary feeding assay, 30–60 flies were used per tube, and 4–20 tubes per group were tested. The Wilcoxon signed rank test (non-parametric) with two-tailed p value was used to test significance on single groups.

Results

To address how sleep and metabolism are integrated, we sought to sought to identify integrators of these processes in the fruit fly, *Drosophila melanogaster*. Knockdown of genes from randomly selected RNAi lines was achieved by expression of UAS-RNAi under the control of the neuron-specific GAL4 driver, n-Synaptobrevin-GAL4 (nSyb-GAL4) (Dietzl *et al.* 2007; Bushey *et al.* 2009). Following 24 hr of baseline sleep measurements on food, sleep was measured during 24-hr starvation on agar, and the change in sleep was calculated as previously described (Keene *et al.* 2010a). Starvation-induced

sleep suppression was reduced in flies with neuron-specific knockdown of the RNA/DNA binding protein *translin* (*trsn*) **(Fig. 1A-C)**. To confirm the effect of *trsn*-RNAi on sleep, we tested two additional RNAi transgenes. All three RNAi lines showed similar phenotypes; *trsn* knockdown flies slept similarly to control flies on food, while sleep loss resulting from starvation was reduced or absent **(Fig. 1D-E)**. Targeted knockdown of *trsn* in the fat body (yolk-GAL4) or muscle (24b-GAL4), two tissues involved in energy storage, showed normal sleep suppression in response to starvation (**Fig. 2A**), supporting the notion that *trsn* functions primarily in neurons to regulate sleep.

In *Drosophila*, starvation induces hyperactivity in addition to sleep loss (Lee and Park 2004; Mattaliano *et al.* 2007; Keene *et al.* 2010a). To determine whether *trsn* also regulates the hyperactivity response to starvation, we analyzed waking activity in fed and starved *trsn* knockdown flies. Neuronal knockdown of *trsn* had
no effect on waking activity in fed flies but reduced starvation-induced hyperactivity **(Fig. 2B)**. These findings are consistent with the notion that *trsn* does not modulate sleep or activity in the fed state but is required for both sleep and locomotor changes that result from starvation.

To validate that the sleep phenotype in *trsn* knockdown flies was not due to off-target effects of RNAi, we measured sleep in flies with a mutation in the *trsn* locus. Both male and female flies with a P element insertion in the *trsn* locus (*trsn*EP) or the excision allele ($trsn^{null}$) are viable (Claussen *et al.* 2006) and exhibit reduced sleep suppression during starvation (**Fig. 1F-H**, **Fig. 2C,D**), phenocopying flies with neuron-specific RNAi knockdown. The waking activity of $trsn^{null}$ flies phenocopies RNAi knockdown flies under

fed conditions, while starvation-induced hyperactivity is blunted or absent in *trsn* mutants **(Fig. 2E).**

A number of systems have been developed for high-resolution video tracking that may provide a more accurate measure of sleep compared to infrared-based monitoring systems (Zimmerman *et al.* 2008; Donelson *et al.* 2012; Gilestro 2012; Garbe *et al.* 2015). Tracking analysis revealed that w^{1118} control, but not $trsn^{null}$ flies, suppress sleep during starvation, confirming that the results obtained using infrared tracking are not an artifact of the sleep acquisition system **(Fig. 2F)**. Taken together, these findings indicate starvation-induced sleep suppression and locomotor activity are reduced in *trsn* mutant flies.

Starved flies utilize glucose and fatty acids to maintain metabolic homeostasis, and the availability of these energy sources may regulate sleep. To determine the energy source required for normal sleep, we fed flies the glycolysis inhibitor 2-Deoxyglucose (2-DG) (Puschner and Schacht 1997) or the carnitine palmitoyltransferase antagonist, etomoxir, an inhibitor of fatty acid β-oxidation (Lopaschuk *et al.* 1988). Treatment with both of these drugs has been used extensively in mammals, and these inhibitors have similar effects on fly metabolism (Thimgan *et al.* 2010b; Dus *et al.* 2011). Flies were fed standard food laced with 400 mM 2-DG or 25 mM etomoxir and monitored for sleep to determine whether the breakdown products of glucose or triglyceride stores (or both) contribute to reduced sleep during starvation. Flies fed 2-DG, but not etomoxir, significantly reduced sleep, suggesting that reduced glucose availability or the energy derived from its metabolism, rather than fatty acids, contribute to sleep suppression (**Fig. 1I** and data not shown).

When *trsn* mutant flies were subjected to the same protocol, no changes in sleep were observed with 2-DG feeding **(Fig. 1I)**. The finding that *trsn* mutant flies are

insensitive to sleep regulation in response to both acute food deprivation and pharmacological perturbation of energy utilization suggests *trsn* is critical for the integration of sleep and metabolic state. It is possible that the reduced ability of *trsn* mutants to suppress sleep during starvation stems from a general inability to modulate sleep in response to environmental or pharmacological disruption.

To test this, sleep rebound was determined by mechanically shaking flies at 3–4 min intervals for 12 hr during the night (zeitgeber time [ZT]12–ZT24) and measuring sleep for 12 hr (ZT0–ZT12) the following day. Sleep-deprived *trsn* ^{null} flies showed a significant increase in daytime sleep that was not present in undisturbed controls **(Fig. 2G)**. The sleep rebound in *trsn*^{null} flies was comparable to w^{1118} control flies, indicating that *trsn* is dispensable for the homeostatic response to mechanical sleep deprivation **(Fig. 2G)**. In addition to mechanical deprivation, numerous pharmacological agents including the stimulant caffeine and free-radical-inducing agent paraquat disrupt sleep in flies (Koh *et al.* 2006; Wu *et al.* 2009). Both w^{1118} control and *trsn*^{null} flies significantly reduced sleep when fed food laced with caffeine **(Fig. 2H)** or paraquat (**Fig. 2I**), supporting the notion that the loss of starvation-induced sleep suppression in *trsn* mutant flies does not result from a generalized inability to suppress sleep.

Flies with enhanced energy stores do not suppress sleep or increase activity in response to starvation (Lee and Park 2004; Thimgan *et al.* 2010b). *Drosophila* primarily stores energy as triglycerides and glycogen, and prolonged food-deprivation results in depletion of both stores. To test the possibility that *trsn* mutant flies do not suppress sleep when fasted due to increased energy stores, we measured triglyceride and glycogen levels using colorimetric assays standardized to total protein level (Sassu *et al.* 2012; Gingras *et*

al. 2014). No differences in glycogen, triglyceride, or free glucose levels were observed be-tween fed or 24 hr starved *trsn*^{null} flies and w^{1118} controls (**Fig. 4A-C**), indicating that the loss of starvation-induced sleep suppression in *trsn* mutant flies is not due to an increase in energy stores. Many metabolism-related genes regulate both sleep and feeding (Yurgel *et al.* 2015b), raising the possibility that *trsn* is generally required for hunger-dependent behaviors. To determine whether *trsn* modulates reflexive food acceptance response, we measured the proboscis extension reflex (PER) of flies starved for 24 hr prior to behavioral testing (**Fig. 3A**)(Dethier 1977; Masek and Scott 2010). Total PER response did not differ between starved *trsn*^{null} and w^{1118} flies to sucrose concentrations of ranging from 1 to 1,000 mM (**Fig. 3B**), or 5% yeast extract (**Fig. 3C**), indicating that *trsn* is dispensable for reflexive feeding. To measure food consumption, we provided flies with 100 mM sucrose or 5% yeast extract in the capillary tube feeding (CAFE) assay (**Fig. 3D**) (Ja *et al.* 2007). Flies were starved for 24 hr prior to the start of the assay, and consumption was measured over 12 hr. No differences in total consumption of 100 mM sucrose or 5% yeast extract was detected between control and *trsn*^{null} flies (**Fig. 3E**). To quantify feeding over a shorter timeframe, the blue dye assay was used to determine the quantity of food consumed in fed and 24 hr starved flies over a 30 min period (Wong *et al.* 2009). No differences between control and *trsn*^{null} flies were detected in overall consumption in the fed or starved state, indicating that *trsn* does not regulate acute food consumption (**Fig. 3F-G**). Taken together, three independent feeding assays indicate that *trsn* does not regulate feeding behavior during the starved state. In *Drosophila*, *trsn* is expressed in the brain throughout development [30]. To determine whether *trsn* is acutely regulated in response to sleep or feeding state, we measured *trsn* transcript levels by qPCR in flies that were previously

starved or sleep deprived. *trsn* was expressed at low levels in the heads and bodies of fed flies and was specifically upregulated in the head following 24 hr of starvation (**Fig. 5A**). No changes in *trsn* transcript were detected after 12 hr of mechanical sleep deprivation, suggesting the upregulation of *trsn* expression is not a generalized response to stress or environmental perturbation (**Fig. 5B**). To confirm that *TRSN* protein is increased in response to starvation, we performed immunohistochemistry on brains immunostained with anti-*TRSN*. Quantification of whole-brain fluorescence confirmed that *TRSN* protein is increased in response to starvation (**Fig. 5C-D**). In agreement with previous findings, *TRSN* signal is below detection in $trsn^{null}$ mutants and dramatically reduced in nSyb-GAL4>*trsn*-IR flies, confirming the antibody specifically labels *TRSN* (data not shown and (Claussen *et al.* 2006)). Counterstaining with the neuronal marker embryonic lethal abnormal vision (ELAV) revealed that *TRSN* and ELAV are expressed in all neurons during the fed and starved states (**Fig. 5E**), suggesting the observed changes in protein levels are not due to altered protein localization. Together, these data suggest that at the RNA and protein levels, *trsn* is increased in response to starvation. The finding that *trsn* is upregulated in response to starvation raises the possibility that it functions acutely to modulate sleep. RNAi targeted to *trsn* was acutely induced in 3-day-old animals using the GeneSwitch system. Flies were fed food laced with 0.25 mM RU486, and sleep was measured on food and agar. Adult specific pan-neuronal knockdown with all three RNAi lines under regulation of elav-Switch impaired sleep suppression compared to genotype-matched controls not fed RU486 or genetic controls lacking the *trsn*IR transgene ((Osterwalder *et al.* 2001; Roman *et al.* 2001); **Fig. 5F** and **Fig. 6**). These findings, coupled

with the upregulation of *trsn* in response to starvation, provide evidence that *trsn* is required during adult-hood for the integration of sleep and metabolic state.

We next sought to identify neurons where *trsn* functions to modulate sleep. Peptidergic neurons are critical regulators of many behaviors, including sleep and feeding (Nässel and Winther 2010; Taghert and Nitabach 2012; Griffith 2013); therefore, we screened GAL4 lines labeling defined populations of peptidergic neurons or neurons previously shown to regulate sleep. We identified the Leucokinin (Lk) neurons, where knockdown of *trsn* reduced sleep modulation in response to starvation. Lk has been implicated in a host of fly behaviors including feeding and water homeostasis, locomotion, and olfactory behavior (De Haro *et al.* 2010; Al-Anzi *et al.* 2010). Driving membrane tethered CD8::GFP with Lk-GAL4 labeled a single large neuron in the lateral horn and three pairs of neurons in the subesophageal zone ((Al-Anzi *et al.* 2010); **Fig. 7A**). Immunostaining brains of Lk-GAL4 flies driving nuclear GFP (UAS-GFP.nls) revealed that the Lk-GAL4 neurons that are co labeled by *TRSN* antibody (**Fig. 7B**). In addition, all three *trsn*-IR lines impaired starvation-induced sleep suppression when expressed under the control of Lk-GAL4 (**Fig. 7C**), whereas restoration of *trsn* specifically in Lk-GAL4 neurons, or in all neurons with nSyb-GAL4, rescued starvation-induced sleep suppression to control levels (**Fig. 7D**). Therefore, *trsn* function in Lk neurons is essential for starvation-induced sleep loss.

To further examine the role of Lk neurons in sleep regulation, we blocked synaptic release from Lk neurons and measured sleep in fed and starved flies (Al-Anzi *et al.* 2010; Donlea *et al.* 2011). Chronic blockade of synaptic release in Lk neurons with tetanus toxin (TNT) impaired starvation-induced sleep suppression compared to control flies expressing

an inactive form of TNT (UAS-IMP-TNT) or genetic controls harboring only a single transgene (Sweeney *et al.* 1995) (**Fig. 7E and 8B**). In fed conditions, silencing of Lk neurons increased sleep compared to controls that approached significance, raising the possibility that these neurons are wake promoting (**Fig. 8B**). To examine the effects of acutely silencing Lk-GAL4 neurons, the dominant-negative form of the GTPase Shibire (Shi^{TS1}) was expressed in Lk neurons, and sleep was measured in both fed and starved flies during the night period (Kitamoto 2001). Flies expressing Shi^{TS1} in Lk-labeled neurons failed to suppress sleep at the non-permissive temperature of 31°C (**Fig. 7F and Fig. 8 C-E**). Control and experimental groups did not suppress sleep at 22°C due to the lower temperature and shortened duration of the assay (**Fig. 8C**). Therefore, Lk neurons are acutely required for modulation of sleep in response to starvation, supporting the notion that *trsn* function in Lk neurons is essential for the integration of sleep and metabolic state.

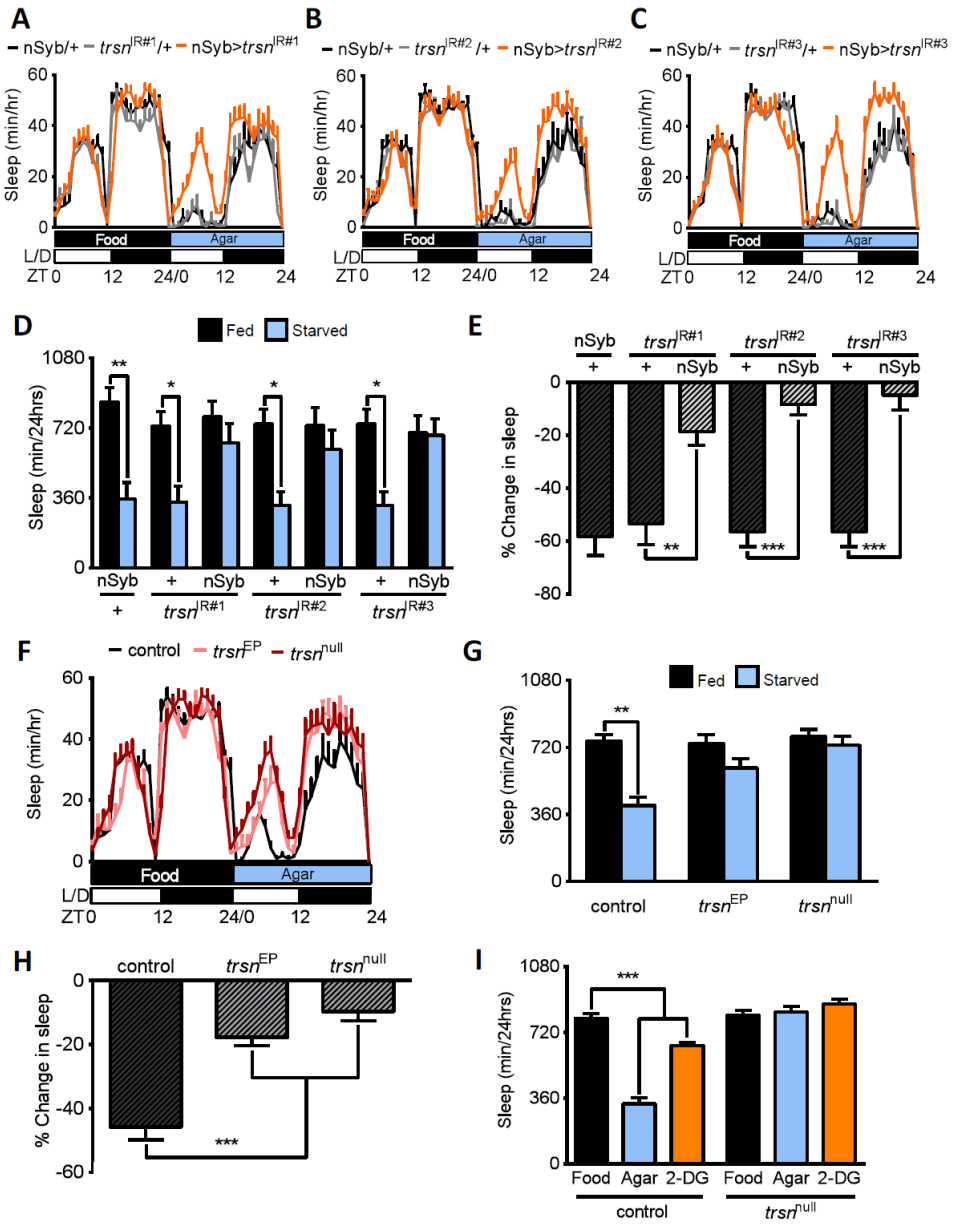

Figure 1: *Trsn* is required for metabolic regulation of sleep.

A-C. Sleep profile for hourly sleep averages over a 48 hour experiment. Flies are on food for day 1, then transferred to agar for day 2. Sleep does not differ between any of the groups for day 1. The *trsn* knockdown groups (*nSyb>trsn*; orange) sleep more than *nSyb*-GAL4/+ (black) and $trsn^{IR}$/+ controls (grey) during day 2 (starved). **D** Control flies (*nSyb*-GAL4/+ and $trsn^{IR}$/+) sleep significantly more on food (black) than when starved (blue, N≥36; P<0.001) while no signficant differences in sleep duration are observed in flies where *nSyb*-GAL4 drives expression of $trsn^{IR\#1}$ (N=45; P>0.98), $trsn^{IR\#2}$ (N=45; P>0.99), or $trsn^{IR\#3}$ (N=36; P>0.98). **E.** Quantifying the percentage change in sleep between fed (day 1) and starved (day 2) states reveals sigificantly greater sleep loss in *nSyb*-GAL4/+ controls

(nSyb-Gal4/+ vs *trsn*$^{IR\#1}$/+, N≥38; P>0.95; nSyb-Gal4/+ vs *trsn*$^{IR\#2}$/+, N≥39; P>0.99; nSyb-Gal4/+ vs *trsn*$^{IR\#3}$/+, N≥37; P>0.99) compared to all three lines with neuronal expression of *trsn*$^{IR\#1}$(N≥38; P<0.01), *trsn*$^{IR\#2}$ (N≥39; P<0.001) and *trsn*$^{IR\#3}$ (N≥36; P<0.01)**. F.** Sleep profile over 48 hours reveals that sleep in *trsn*^{EP} and *trsn*^{null} does not differ from *w*^{1118} control flies on food. Both *trsn*^{EP} and *trsn*^{null} mutant flies sleep more than control flies on agar. **G.** Sleep is significantly reduced in starved control flies (N≥54; P<0.001), while sleep differences are not significant in *trsn*^{EP} (N=69; P>0.23) or *trsn*^{null} flies (N=58; P>0.98). **H.** Percentage sleep loss is also significantly reduced in *trsn*^{EP} and *trsn*^{null} mutants compared to controls (N≥54; P<0.001). **I)** In control flies, sleep is significantly reduced in flies on agar (blue; N=44; P<0.001) or food laced with 2-deoxyglucose (2-DG; orange) (N≥64; P<0.001), compared to flies fed standard food (black). No differences are detected between flies fed standard food compared to agar or 2-DG in *trsn*^{null} mutants (N≥38; P>0.70). Bars for % change in sleep are mean ± SEM by one-way ANOVA. All other bars are mean ± SEM; P<0.01,**; P<0.001,*** by 2-way ANOVA.

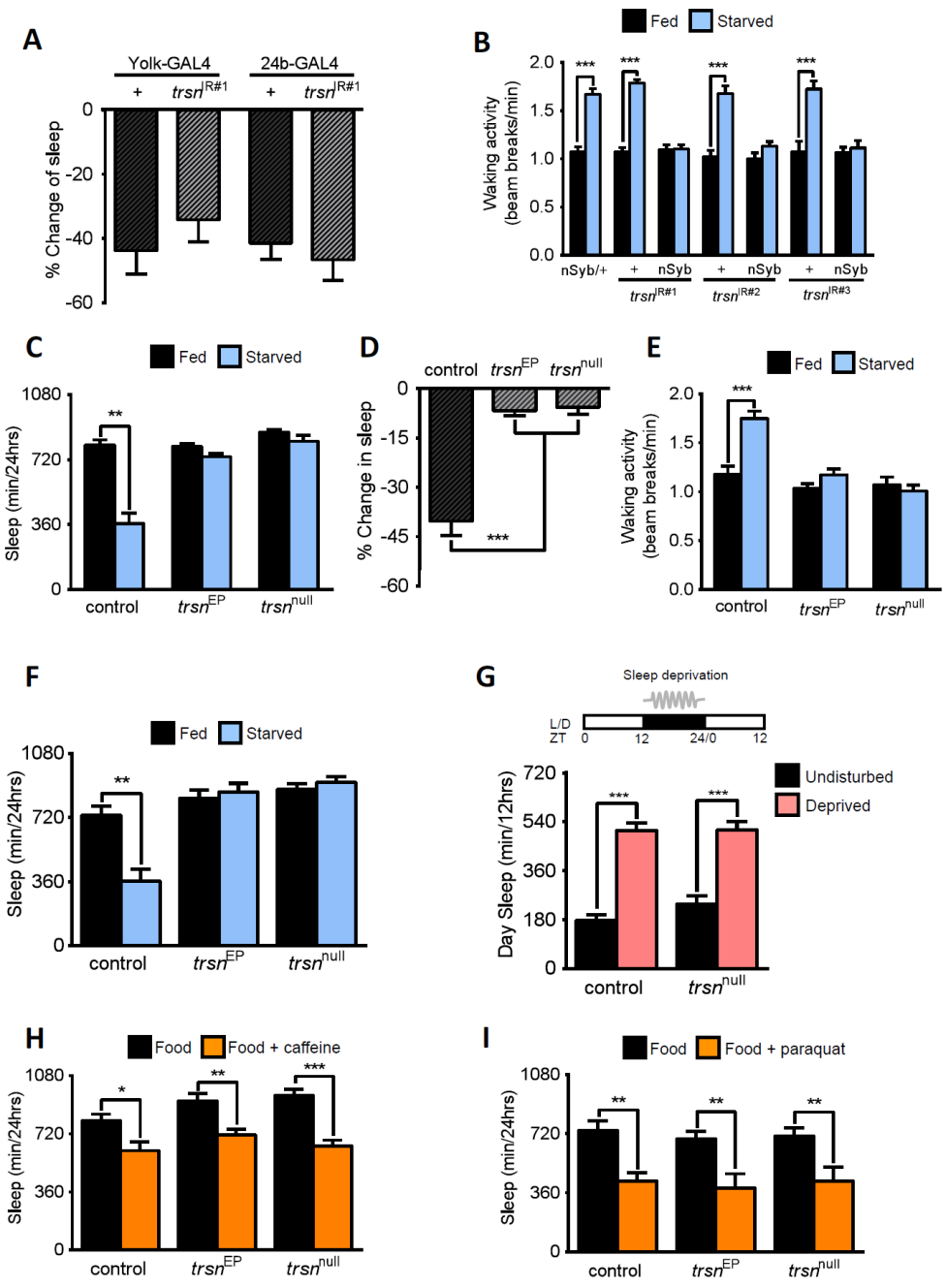

Figure 2: Characterization of sleep in *trsn* deficient flies.

A. Sleep loss (%) in flies expressing *trsn*IR in the fat body (yolk-GAL4) or muscle (24b-GAL4). No significant differences are observed between *trsn* knockdown and control flies harboring GAL4 alone (N≥11; P>0.34 for both groups) **B.** Average waking activity in fed (black) and starved (blue) flies over 24 hrs. Waking activity in fed flies does not differ between any genotypes (N≥36; P<0.99). Under starved conditions, waking activity is increased in *nSyb*-GAL4/+ and $trsn^{IR}$/+ control flies (N≥36; P<0.001), while no change in

waking activity is detected in each of the three *nSyb*-GAL4>*trsn*^{IR} knock down lines. **C.** In male flies, sleep is significantly reduced in starved w^{1118} controls (N=39; P<0.01), while sleep duration of *trsn*^{EP} (N=45; P<0.76) and *trsn*^{null} flies (N=48; P>0.82) does not significantly differ on food and agar. **D.** Change in sleep (%) from fed to starved conditions in male flies show sleep loss is significantly greater in control flies compared to *trsn*^{EP} and *trsn*^{null} flies (N≥39, P<0.001). **E.** Average waking activity in fed (black) and starved (blue) flies over 24 hours. Waking activity in fed flies does not differ between any genotypes (N≥54; P<0.66). Waking activity during starvation is increased in control (N=54; P<0.001) and *trsn*^{EP} flies (N=69; P>0.66), while there is no effect of starvation on waking activity in *trsn*^{null} flies (N=68; P>0.99). Waking activity of starved *trsn*^{EP} flies is reduced compared to controls. **F.** Video tracking analysis of sleep in fed and starved flies. In control flies, sleep is significantly reduced in fed control (black) compared to starved control (blue, N≥37; P<0.001), while no significant differences are observed in fed *trsn*^{EP} or *trsn*^{null} mutant flies (N≥39; P>0.99). **G**. Daytime sleep from ZT0-ZT12 is significantly greater following mechanical sleep deprivation for 12 hours from ZT12-ZT24 (pink) compared to undisturbed flies (black) for w^{1118} control (N=32; P<0.001) and *trsn*^{null} genotypes (N=32; P<0.001). Total sleep does not differ between sleep-deprived control and *trsn*^{null} (N=32; P>0.43) flies or undisrupted control and *trsn*^{null} (N=32, P>0.23) flies from ZT0-ZT12. **H.** Sleep is reduced in control (N=32; P<0.05), *trsn*^{EP} (N=32; P<0.01) and *trsn*^{null} (N=32; P<0.001) flies fed food containing caffeine (orange) compared to flies fed standard fly food (black). I**.** Sleep is reduced in control (N=32; P<0.01), *trsn*^{EP} (N=32; P<0.01), and *trsn*^{null} (N=32; P<0.01) fed paraquat (orange) compared to flies fed standard fly food (black). All error bars are mean ± SEM. ; P<0.01,**; P<0.001,*** by 2-way ANOVA.

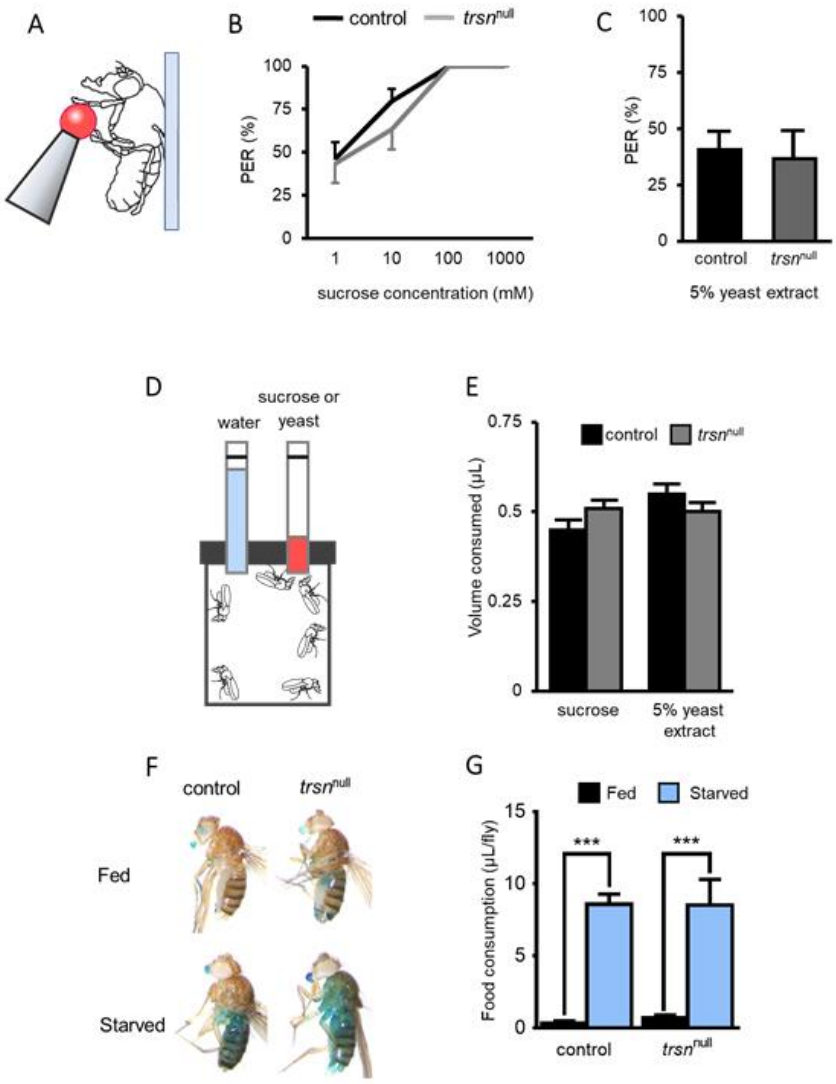

Figure 3: Starvation-induced feeding is normal in *trsn* mutant flies.

A. Diagram of the proboscis extension reflex (PER) assay. Tastant is supplied to the tarsi of a tethered female fly. **B, C.** No significant differences in PER are detected between control (black) and *trsn*^{null} mutants (grey) to increasing concentrations of sucrose (N≥10; 1mM, P>0.84; 10mM, P>0.21; 100mM and 1000mM P>0.95) (B) or 5% yeast extract (N=18; P>0.98) (C). **D.** Diagram of the Capillary Feeder Assay (CAFÉ) assay. **E.** No significant differences in sucrose (left bars, N=4; P>0.34) or yeast (right bars, N>4; P>0.18) were detected between control and *trsn*^{null} flies when presented with each tastant. **F.** Starved or fed flies are placed on food containing blue dye for 30 minutes and consumption is measured. **G.** Quantification of food intake reveals a significant increase in starved controls and *trsn*^{null} flies compared to fed flies from each genotype (N≥26; P<0.001). No differences were observed between genotypes in the fed (N≥29;P>0.99) or starved (N≥26;P>0.99) states All bars are mean ± SEM; P<0.001,*** by 2-way ANOVA.

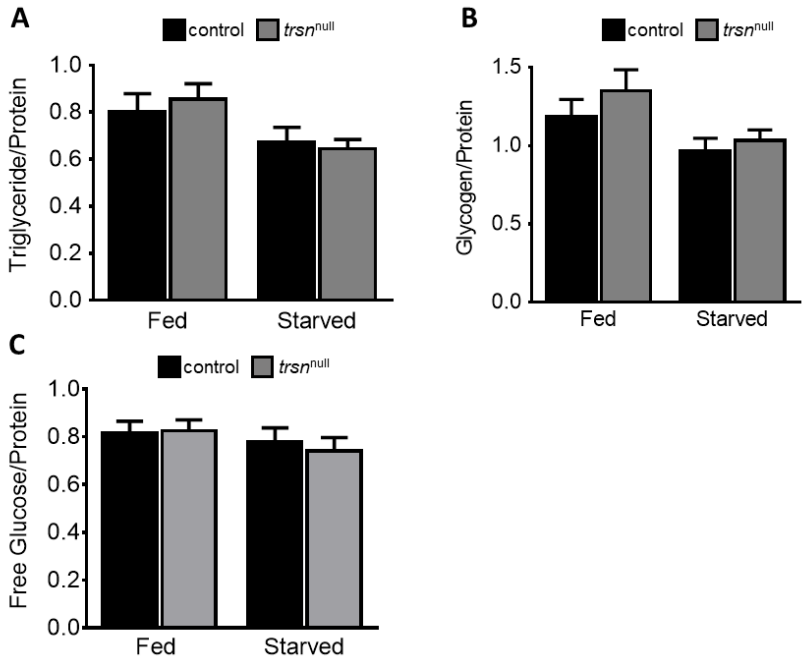

Figure 4: Energy stores and free glucose are normal in *trsn* mutant flies.

A. Triglyceride levels did not differ between control (black) and *trsn*^{null} (grey) in the fed (N=20; P>0.92) or starved state (N=20; P>0.99). **B.** Glycogen levels did not differ between control (black) and *trsn*^{null} (grey) in the fed (N=16; P>0.67) or starved state (N=16; P<0.96). **C.** Free glucose did not differ between control (black) and *trsn*^{null} (grey) in the fed (N≥16; P>0.75) nor starved state (N≥13; P>0.81). All bars are mean ± SEM by two-way ANOVA.

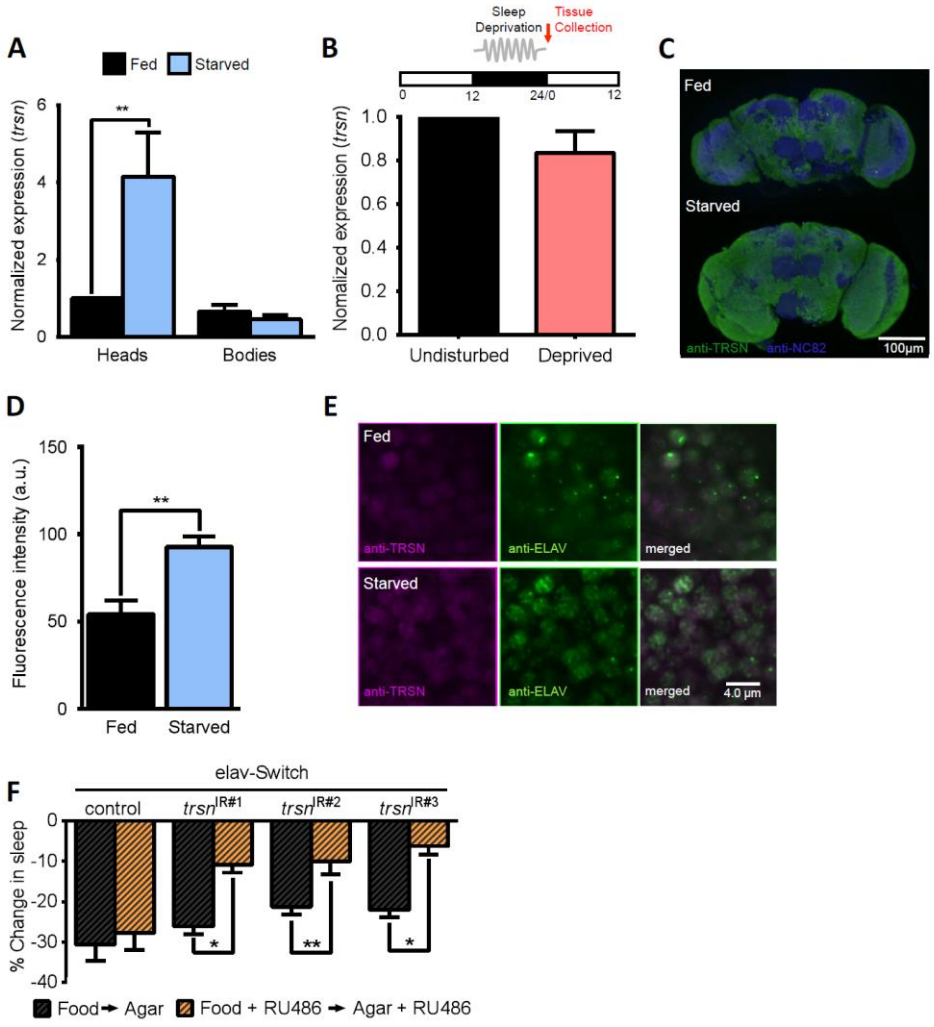

Figure 5: Spatial and temporal localization of *trsn* function.

A. Expression of *trsn* is upregulated in the heads (N≥14; P<0.01) but not bodies of w^{1118} control flies (N≥14;P>0.99) following 24 hours of starvation. **B.** *trsn* transcript does not differ in heads between flies sleep-deprived for 12 hours from ZT12-ZT24 and undisturbed controls (N=3; P>0.17, NS). All bars are mean ± SEM; by t-test. **C,D.** Immunohistochemistry for whole-brain *TRSN* protein (B). Neuropils are labeled by NC82 for reference (magenta) and anti-*TRSN* (green) is observed throughout the brain. Whole-brain *TRSN* protein quantification of fluorescence intesity revealed *TRSN* is increased in starved flies compared to fed control (N≥6; P<0.002) by paired t-test. **E.** Immunostaining for anti-*TRSN* (green) and the neuronal marker anti-ELAV (red) reveals colocalization (yellow) between *TRSN* (green) and ELAV (red) protein levels in brains of fed (upper) and starved (lower) flies. Depicted is a representative section from the dorsomedial central brain, near the lateral horn region. Scale bar denotes 4µm. **F.** Percentage sleep loss in experimental flies treated with RU486 (orange bars) or controls without drug treatment (black bars). Sleep suppression is significantly reduced in elav-

Switch>*trsn*$^{IR\#1}$ flies (N≥36; P>0.031), elav-Switch>*trsn*$^{IR\#2}$ (N≥68; P<0.011) and elav-Switch>*trsn*$^{IR\#3}$ (N≥34; P<0.041) flies fed RU486 compared to non-RU486-fed controls. There is no effect of RU486 feeding in flies harboring the elav-Switch transgene alone (N≥39; P>0.99). All other bars are mean ± SEM; P<0.05,*; P<0.01,**; by 2-way ANOVA

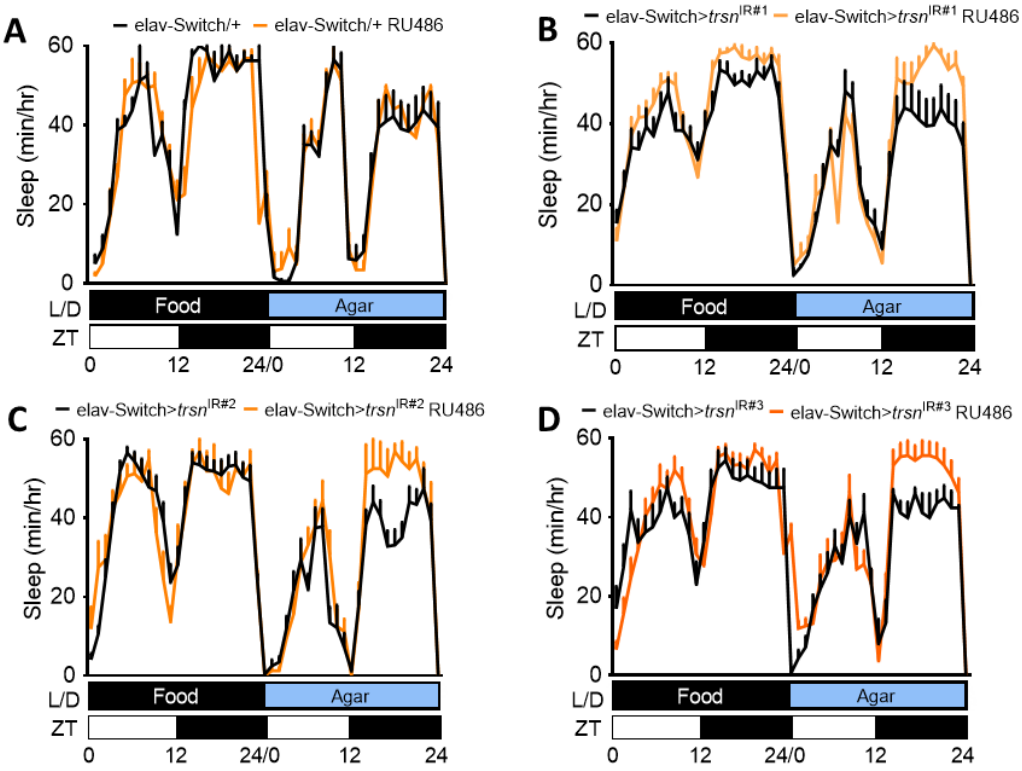

Figure 6: Adult-specific knock down of *trsn* disrupts sleep suppression.

A. Sleep profiles depicting hourly sleep averages over a 48 hour experiment. Flies are placed on food for day 1, then transferred to agar for day 2. Flies harboring elav-Switch alone with RU486 treatment (orange) or elav-Switch alone without treatment (black). **B-D.** Experimental flies (elav-Switch>*trsn*^{IR}; orange) fail to suppress sleep compared to genotype-matched controls without drug treatment (black).

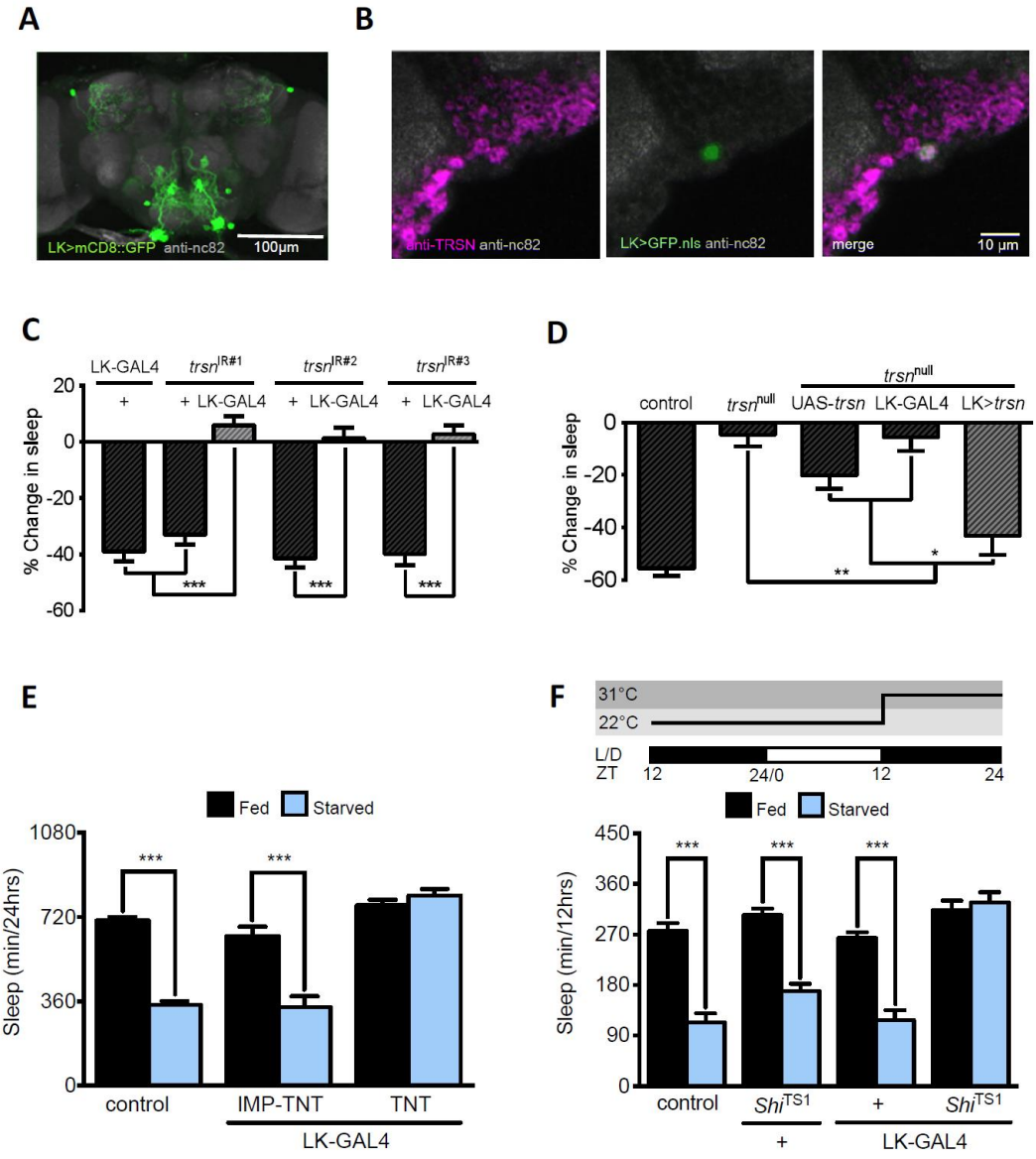

Figure 7: *Trsn* functions in leucokinin neurons to regulate sleep.

A. Whole-brain confocal reconstruction of Lk-GAL4>mCD8::GFP. GFP-expressing neurons (green) labeled the subeosphogeal zone and dorsal protocerebrum. The brain was counterstained with the neuropil marker nc82 (grey). Scale bar denotes 100µm.**B.** Immunostaining for anti-*TRSN* (red) in the brain of Lk-GAL4>UAS-GFP.nls reveals *trsn* is expressed in neurons labeled by Lk-GAL4. Depicted is a representative 2µm section from the lateral horn region. Scale bar denotes 10µm. The neuropil marker anti-nc82 (grey) is used as background. **C.** Knock down of *trsn* in Lk-GAL4 neurons

aloneresulted in significantly reduced starvation-induced sleep loss in all three *trsn*^{IR} lines compared to control flies harboring a UAS-*trsn*^{IR} transgene alone (N≥52; P<.001) or Lk-GAL4 transgenes alone (N≥64; P<0.001). **D.** Expression of UAS-*trsn* under Lk-GAL4 control in the background of a *trsn*^{null} mutation restores starvation-induced sleep suppression compared to flies harboring either UAS-*trsn* (N=87; P<0.05); or the GAL4 lines alone (N=79; P<0.01). No significant differences were detected between Lk rescue and w^{1118} control flies (N≥38; P>0.10). **E.** Starvation-induced sleep suppression is abolished in flies expressing TNT in Lk-GAL4 neurons (N=39; P=0.96) compared to controls expressing inactive UAS-IMP-TNT (N=33, P<0.0001). Sleep duration on food does not differ significantly in Lk-GAL4>UAS-TNT (N≥34, P=0.50) compared to control flies, and flies expressing UAS-IMP-TNT (P=0.58). **F.** Flies were transferred to agar at ZT9 then sleep was measured at 31°C on food (black) or agar (blue) over the 12hr night (ZT12-ZT24). Genetic silencing of Lk-GAL4 abolished starvation-induced sleep suppression (N≥40, P=0.99) while control flies robustly suppressed sleep (N≥79, P<0.0001; UAS- *Shi*^{TS} /+, N≥42, P<0.0001; Lk-GAL4/+, N≥51, P<0.0002). No differences were observed between genotypes in the fed state (UAS-*Shi*^{TS}/+, P=0.84; Lk-GAL4/+, P= 0.76; Lk-GAL4>UAS-*Shi*^{TS} , P=0.73; All columns are mean ± SEM; P<0.01,**; P<0.001,*** by 2-way ANOVA.

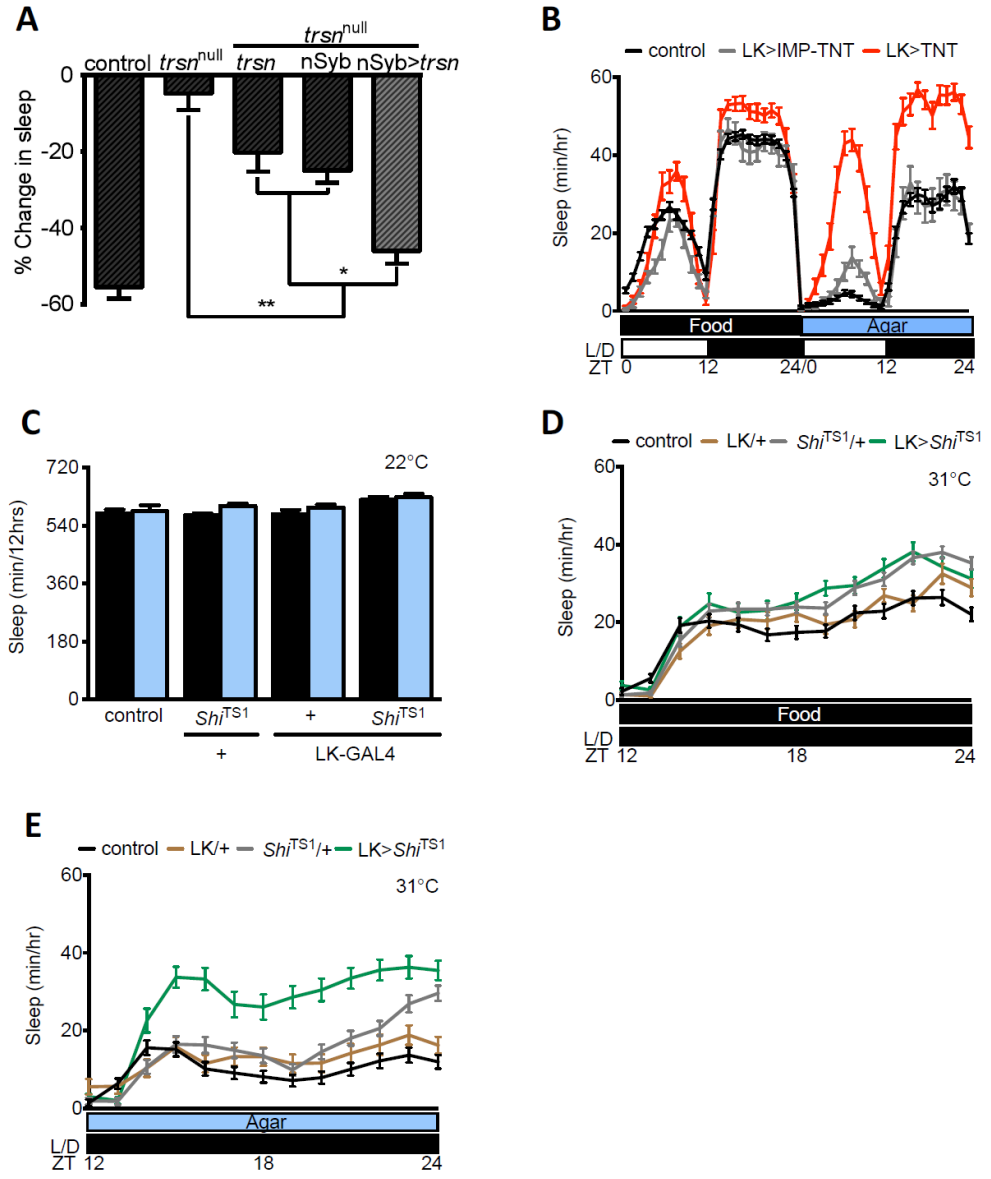

Figure 8: Lk neurons are acutely required for starvation-induced sleep suppression.

A. Expression of UAS-*trsn* under control *nSyb*-GAL4 in the background of a *trsn*^{null} mutation restores starvation-induced sleep suppression compared to flies harboring either UAS-*trsn* (N≥37; P<0.05) or the GAL4 line alone (N≥82;P<0.01). No significant difference was detected between *nSyb* rescue and control flies (N≥37; P>0.66) .**B.** Sleep profile over 48 hours reveals that sleep in Lk-GAL4>UAS-TNT (red) flies is moderately increased compared to w^{1118} control flies (black) or flies expressing inactive IMP-TNT (grey) for day one on food. Sleep in Lk-GAL4>UAS-TNT is significantly greater for day two on agar compared to control and IMP-TNT-expressing flies. **C.** No significant differences for sleep duration on food (black) or agar (blue) were detected for any of the

genotypes tested when flies were housed at 22°C (Fed vs Starved: control, N≥38, P=0.99; UAS- *Shi*^{TS}/+; N≥87, P=0.83, Lk-GAL4/+, N=26, P=0.97, Lk-GAL4>UAS- *Shi*^{TS}; N≥23, P=0.99). **D.** Sleep profile over 12 hours on food at 31°C reveals that sleep in Lk-GAL4>UAS-*Shi*^{TS} (blue) flies does not differ from controls (black) or respective heterozygote controls (grey, brown). **E.** Sleep profile over 12 hours on agar at 31°C reveals that sleep suppression in Lk-GAL4>UAS-*Shi*^{TS} (blue) sleep significantly more than *w*^{1118} controls (black) and heterozygote controls (grey, brown). All columns are mean ± SEM; P<0.01,**; P<0.001,*** by 2-way ANOVA.

Discussion

Here, we have identified *trsn* as an essential regulator of sleep-metabolism interactions. While many genes have been identified as genetic regulators of sleep or metabolic state, multiple lines of evidence indicate that *trsn* functions as a unique integrator of these processes. *trsn* is not required for the homeostatic increase in sleep following mechanical deprivation or response to stimulants, suggesting *trsn* is not generally required for acute modulation of sleep. Further, *trsn*-deficient flies display normal feeding behavior, indicating that it is not required for modulation of behavior in response to food deprivation. Finally, energy stores in *trsn* mutant flies are normal, indicating that the starvation-induced sleep suppression phenotype is not due to increased nutrient storage. These results provide evidence that *trsn* is not required for the perception of starvation or the general induction of hunger-related behaviors but is required for the induction of wakefulness in the absence of food. While *trsn* is broadly expressed in the *Drosophila* nervous system, we localize the function of *trsn* in metabolic regulation of sleep to LK-expressing neurons. Targeted knockdown of *trsn* in Lk neurons disrupts metabolic control of sleep, while restoring *trsn* to Lk neurons rescues sleep regulation in *trsn* mutants.

In addition to regulating sleep, ablation of Lk neurons reduces meal number, while increasing consumption during individual feeding bouts, suggesting a role in feeding behavior (Al-Anzi *et al.* 2010). Lk is expressed in the subesophageal zone, the insect taste center, and in modulatory neurons within the lateral horn, raising the possibility that the sleep and feeding phenotypes associated with Lk mutations or manipulation of Lk neurons may localize to distinct brain regions (Al-Anzi *et al.* 2010). It is possible that the same populations of Lk neurons regulate meal frequency and sleep or distinct neurons modulate

each process. Combinatorial genetic approaches to manipulate subsets of GAL4-labeled neurons in combination with recent advances in behavioral analysis of meal frequency may allow for the localization of LK neurons involved in each behavioral process (Luan *et al.* 2006; Itskov and Ribeiro 2013; Ro *et al.* 2014).

In addition to its known role in the synthesis of non-coding RNA, *TRSN* physically associates with Translin- Associated Protein X (TRAX) (Aoki *et al.* 1999; Wu *et al.* 2003). *TRSN* and TRAX are essential components for the RNA-induced silencing complex (RISC), suggesting a role in post-transcriptional gene silencing through the generation of small RNAs. *trsn* knockout mice have diminished forebrain monoamine levels, indicating that *trsn* may serve to regulate neurotransmitter synthesis (Stein *et al.* 2006). Further investigation of the mechanistic relationship between *trsn* and neural regulation of sleep will provide a framework to study the molecular properties and neural networks that are associated with interactions between sleep and metabolic state.

CHAPTER 3. A SCREEN FOR SLEEP AND STARVATION RESISTANCE IDENTIFIES A WAKE-PROMOTING ROLE FOR THE AUXILIARY CHANNEL *UNC79*

Abstract

The regulation of sleep and metabolism are highly interconnected, and dysregulation of sleep is linked to metabolic diseases that include obesity, diabetes, and heart disease. Further, both acute and long-term changes in diet potently impact sleep duration and quality. To identify novel factors that modulate interactions between sleep and metabolic state, we performed a genetic screen for their roles in regulating sleep duration, starvation resistance, and starvation-dependent modulation of sleep. This screen identified a number of genes with potential roles in regulating sleep, metabolism or both processes. One such gene encodes the auxiliary ion channel *UNC79*, which was implicated in both the regulation of sleep and starvation resistance. Genetic knockdown or mutation of *unc79* results in flies with increased sleep duration, as well as increased starvation resistance. Previous findings have shown that *unc79* is required in pacemaker for 24-hour circadian rhythms. Here, we find that *unc79* functions in the mushroom body, but not pacemaker neurons, to regulate sleep duration and starvation resistance. Together, these findings reveal spatially localized separable functions of *unc79* in the regulation of circadian behavior, sleep, and metabolic function.

Introduction

Sleep acutely regulates metabolic function, and growing evidence suggests that these processes interact to regulate many biological functions including cognition, physiology, and longevity (Hartmann 1974; Siegel 2005; Joiner 2016; Beckwith and French 2019). At the clinical level, many diseases related to metabolic dysfunction including diabetes, heart disease and obesity are associated with chronic sleep loss (Taheri *et al.* 2004; Arble *et al.* 2015; Reutrakul and Van Cauter 2018). In addition, diet potently influences sleep duration and quality, indicating that neural systems regulating sleep are sensitive to internal nutrient stores and food availability (Grandner *et al.* 2010, 2014b; Catterson *et al.* 2010; Linford *et al.* 2012). Identifying how sleep, diet, and metabolic regulation are interconnected is critical to understanding the fundamental functions of sleep.

Interactions between sleep, feeding, and metabolic regulation are highly conserved between the fruit fly and mammals (Griffith 2013; Yurgel *et al.* 2015b; Beckwith and French 2019). Experimental evolution and artificial selection approaches have revealed a relationship between sleep, feeding, and starvation resistance (Masek *et al.* 2014a; Slocumb *et al.* 2015b; Brown *et al.* 2019b). For example, selection for short-sleeping flies results in reduced energy stores and sensitivity to starvation, while selecting for starvation resistance increases sleep duration (Seugnet *et al.* 2009; Masek *et al.* 2014a; Slocumb *et al.* 2015b). Further, examining naturally occurring genetic variation in sleep and starvation resistance in *Drosophila melanogaster* from different geographic localities suggests sleep and starvation resistance are inversely related (Brown *et al.* 2018b; Sarikaya *et al.* 2020).

The interactions between these traits under conditions of experimental evolution raise the possibility that shared genetic factors underlie sleep and starvation resistance.

Energy conservation has long been proposed to be primary function of sleep (Hartmann 1973; Berger and Phillips 1995). *Drosophila* live for only a few days in the absence of food, providing an excellent model to examine the effects of sleep on metabolic regulation and energy conservation (Yurgel *et al.* 2015b; Ly *et al.* 2018). Quantifying longevity under starvation conditions provides a readout of overall energy stores and metabolic rate (Baldal *et al.* 2006; Schwasinger-Schmidt *et al.* 2012). In addition, flies acutely suppress sleep and increase activity in response to starvation, providing a system to investigate acute modulation of sleep and metabolic function (Lee and Park 2004; Keene *et al.* 2010a). Genetic screens and genomic analyses have identified many regulators of sleep, metabolic regulation, and starvation resistance, establishing flies as a model for studying the interactions between these processes (Harbison *et al.* 2005; Jumbo-Lucioni *et al.* 2010; Murakami *et al.* 2016; Sonn *et al.* 2018). Many of the genes initially identified through screening for short-sleeping mutants have reduced life spans or increased sensitivity to stressors, though the relationship with starvation resistance is less clear (Koh *et al.* 2008b; Bushey *et al.* 2010; Hill *et al.* 2018). A complete understanding of how these processes are integrated requires the localization of genes and neurons that regulate sleep and metabolic processes.

The study of sleep in flies has predominantly focused on the role of genes and neurons under fed conditions, leading to the identification of many distinct circuits that promote sleep and wakefulness (Allada and Siegel 2008; Sehgal and Mignot 2011; Ly *et al.* 2018). There is growing evidence that additional cell types are critical regulators of

sleep including multiple classes of glia, endocrine cells, and the fat body (A rtiushin *et al.* 2018; Stahl *et al.* 2018; Vanderheyden *et al.* 2018; Yurgel *et al.* 2018; Ertekin *et al.* 2020). Further, the genes and neurons regulating sleep can differ based on environmental context (Griffith 2013; Beckwith and French 2019; Shafer and Keene 2021). These studies highlight brain-periphery interactions that are change in response numerous environmental contexts including food availability. Therefore, identifying genetic regulators that impact both sleep and metabolic function requires investigating both neuronal and non-neuronal cell-types.

Here, we have performed a genetic screen to identify genetic regulators of sleep and metabolic function, targeting genes ubiquitously to identify factors that function both within the brain and the periphery. Flies were tested in a pipeline that measured sleep parameters under fed and starved conditions, followed by assessment of starvation resistance. This screen identified several candidate genes regulating sleep and metabolic function including the sodium leak channel *NALCN* accessory subunit *unc79*. The phenotype of *unc79* is unique because it contributes to all three of these processes, suggesting *unc79* modulates both sleep and starvation resistance and potentially has a dual role in the regulation of sleep and metabolic function.

Methods

Fly husbandry:

Flies for behavioral experiments were maintained and tested in humidified incubators at 25°C and under 65% humidity (Powers Scientific). Flies were reared on a 12 h:12 h light–dark cycle for experiments prior to behavioral analysis. All flies were

maintained on Nutri-fly Drosophila food (Genesee Scientific). All RNAi lines tested were obtained from the Bloomington *Drosophila* Stock Center (Bloomington, IN, USA) (Ni *et al.* 2008; Perkins *et al.* 2015) and the (# 45780) Vienna *Drosophila* Resource Center (Vienna, Austria) (Dietzl *et al.* 2007) (See table 1). Bridget Lear (Northwestern) generously provided *unc79* ^{F01615} and *unc79* ^{F01615} lines (Lear *et al.* 2013).

P{TRiP.HMC03213}attP2, BDSC, 51471; P{CaryP }attP2, BDSC, 36303; P{GMR57C10-GAL4}attP2, BDSC, 39171; P{GawB}OK107, BDSC, 854; P{GawB}Hr39[c739], BDSC, 7362; P{GawB}Cka[c305a], BDSC, 30829; P{GawB}1471, BDSC, 9465; P{GAL4-tim.E}62, BDSC, 7126; P{PDF-GAL4/cyo}, (Tomchik lab); P{GawB}dimm[929], BDSC, 25373; P{GAL4}repo, BDSC, 7415; P{GMR69F08-GAL4}attP2, BDSC, 39499; P{GMR23E10-GAL4}attP2, BDSC, 49032; P{mb247-GAL80.T}2, BDSC, 68265; w1118; P{GD11587}, VDRC, v45780; w1118; P{GD330}v1309, VDRC, v1309; and w1118; P{GD1172}, v3306, VDRCv3306.

Behavioral Analysis:

Drosophila Activity Monitors (DAM; Trikinetics, Waltham, MA) were used for all behavioral analyses. The DAM system detects activity by monitoring infrared beam crossings for each animal (Pfeiffenberger *et al.* 2010a). These data were used to calculate sleep information by extracting immobility bouts of 5 minutes using the Drosophila Sleep Counting Macro (Pfeiffenberger *et al.* 2010b). All behavioral experiments used 5-7 days old mated female flies unless otherwise noted. For experiments examining the effects of starvation on sleep, activity was then measured for 24 hours on food, prior to transferring flies into tubes containing 1% agar (Fisher Scientific) at ZT0 and activity was recorded for an additional 48 hours.

To measure starvation resistance, flies were starved on experimental day 2 by transferring them from food 1% agar (Fisher Scientific) individual DAM tubes containing 1% agar. Activity across the first 48 hours on agar was used to measure starvation-induced sleep suppression as previously described (Masek *et al.* 2014a). While previous studies measured starvation-induced sleep suppression on day 1 of starvation, we measured this process for multiple days (Keene *et al.* 2010a). To measure starvation resistance, activity was then recorded until death. Death was manually determined as the last activity time point from the final recorded activity bout for each individual fly.

For experiments quantifying circadian rhythm analysis, locomotor activity under free-running conditions was measured using the DAM system as previously described (Chiu *et al.* 2010). Individual flies were housed in 10% sucrose DAM tubes instead of standard fly food to prevent larval development that interferes that the circadian assay. Five day old adult flies were entrained to light-dark (LD) 12 hour: 12 hour (12:12) cycles for three days, then transferred to constant darkness (DD) for 7-8 days. Locomotor activity data were analyzed using Clocklab software (ActiMetrics, Version 2.72). Individual periods were calculated from 7-8 days activity data during DD using chi-square periodogram. Rhythm strength was determined by Fast Fourier Transform (FFT) analysis as previously described (Chiu *et al.* 2010).

Statistical Analysis

Statistical analyses were performed using InStat software (GraphPad Software 6.0). For analysis of sleep, we employed a one- or two-way ANOVA followed by a Tukey's post hoc test. For starvation resistance, we applied Kaplan–Meier analysis by grouping each genotype.

Results

To screen for novel regulators of sleep and starvation resistance we ubiquitously knocked down genes by expressing RNAi transgenes from the TRiP Collection under control of the *Actin5C*-GAL4 driver (Perkins *et al.* 2015). To enrich for genes that may be involved in sleep or metabolic function, candidate genes were selected from a genome-wide analysis of polymorphisms and genomic markers of selection in flies selectively bred for starvation resistance (Hardy *et al.* 2018). Sleep was measured for 24 hours on standard food, after which, flies were transferred to agar where they were maintained until death to measure starvation-induced sleep suppression and starvation resistance **(Fig. 9A)**. In control flies harboring *Actin5C*-GAL4 driving UAS-*luciferase*-RNAi (*Act5c>Luc*^{RNAi}), a control with no endogenous targets, flies suppressed sleep during the first day of starvation, and an even greater suppression was observed on day two of starvation. Flies survived approximately three days on agar, providing a robust readout of sleep and starvation resistance.

To identify novel regulators of these traits we sought to screen genes that were previously identified in a *Drosophila* genome-wide association study as regulating starvation resistance. Of the 1429 significant genes from this analysis, we identified 914 genes that TRiP RNAi stocks available (Perkins *et al.* 2015; Hardy *et al.* 2018). Of these, 299 lines (32.7%) were lethal with ubiquitous knockdown and, therefore, were not screened. In total, we screened 616 lines for sleep, starvation-induced sleep suppression (**Fig. 9B**).Ubiquitous knockdown of the previously identified Ly-6 transmembrane protein, *qvr/sleepless,* resulted in the shortest sleep duration, confirming the ability of the screening procedure to effectively identify genetic regulators of sleep (Koh *et al.* 2008b). To examine

the relationship between sleep and starvation resistance we plotted the average for each trait. There was no association between the traits (r^2<0.001), suggesting these two traits are largely independently regulated (**Fig. 9C**). However, we identified a number of genes where ubiquitous knockdown resulted in increased sleep on food and greater starvation resistance (**Fig. 9C**)**.** We also examined the correlation between genes screened for different sleep parameters. For example, daytime sleep duration is correlated with nighttime sleep duration, suggesting shared genes regulate both processes (**Fig 10A)**. We found average bout length was inversely correlated with sleep bout number, suggesting these traits are functionally related (**Fig. 10B**). However, no correlation was observed between waking activity and total sleep (**Fig. 10C**) suggesting independent regulation of these traits. We chose to focus on the gene encoding for the NALCN auxiliary protein, *uncoordinated 79* (*unc79*), because of the robustness of each phenotype and its role as an essential regulator of circadian rhythms sleep regulation (Lear *et al.* 2005; Joiner *et al.* 2013).

To validate the sleep and starvation phenotypes associated with *unc79* we repeated experiments and examined the sleep profile. Flies with ubiquitous knockdown of *unc79* (*Act5c>unc79*^{RNAi}) slept significantly more than control flies (**Fig. 11A, B**). Further, while both control groups suppressed sleep during day 1 and 2 of starvation, *Act5c>unc79*^{RNAi} flies did not suppress sleep, suggesting that *unc79* is required for metabolic regulation of sleep (**Fig. 11A, B**). In addition, starvation resistance was significantly increased in *Act5c>unc79*^{RNAi} flies compared to *Act5c>Luc* ^{RNAi} controls (**Fig. 11C**). In agreement with our previous findings, waking activity in female control flies increased starvation, but was unchanged in *Act5C>Unc79*^{RNAi} flies (Fig 12A) (Keene *et al.* 2010a). Further, the overall

waking activity was elevated in *Act5c>Unc79*^{RNAi} flies compared to *Act5c>Luc* ^{RNAi} controls under fed conditions suggestions that the increased sleep in flies deficient for *unc79* is not due to general lethargy (**Fig. 12A**).

To confirm that the observed phenotypes are not due to RNAi off targets we tested flies with a genetic mutation in the *unc79* locus. The independent Pbac element insertions in the *unc79* locus slept longer on food and failed to suppress sleep when starved, phenocopying RNAi knockdown **(Fig. 11D,E)** (Lear *et al.* 2013)**.** Further, *unc79* mutants (*unc79*^{F03453} and *unc79*^{F01615}) survived significantly longer on agar than respective controls (**Fig. 11F**). Waking activity for control increased, but waking activity for *unc79* hypomorphs remains unchanged (Fig. 12B). We also assessed male flies to determine whether these phenotypes generalize across sexes. Male *unc79* mutants (*unc79*^{F03453} and *unc79*^{F01615}) flies slept longer on food and failed to suppress sleep when starved (**Fig.12C**) and survived longer on agar than respective controls **(Fig. 12D**), but the response was attenuated compared to female flies. Waking activity in male flies did not differ between fed and starved groups of *unc79* mutants, while controls increase waking activity (**Fig. 12E**). Therefore, ubiquitous RNAi knockdown or genetic mutation of *unc79* results in increased sleep and starvation resistance, and impaired metabolic regulation of sleep.

To localize *unc79* function in metabolism and sleep we first targeted *unc79* ^{RNAi} to all neurons using the driver n-synaptobrevin-GAL4 (*nsyb*-GAL4) (Riabinina *et al.* 2015). Knockdown in neurons led to flies that slept significantly more than background controls harboring expressing RNAi to luciferase under fed conditions (**Fig. 13A**). Further, flies with pan-neuronal knockdown of *unc79* (*nSyb*-GAL4>*unc79*^{RNAi}) also did not significantly reduce sleep during starvation and survived significant longer, suggesting *unc79* functions

in neurons to regulate sleep and starvation resistance (**Fig. 13 A,B**). To further localize the function of *unc79* we targeted RNAi to six types of neurons known to modulate sleep. Knockdown in the circadian neurons using *Pdf*-GAL4 or *Tim*-GAL4 did not affect sleep or starvation resistance, suggesting the effects on sleep and metabolic function are independent of its role in circadian activity (**Fig. 14AB**). Further no effect was observed knocking down *unc79* in the sleep promoting central complex (*23E10*-GAL4) broad classes of peptidergic cells (*C929*-GAL4) (**Fig. 14AB**).

The driver *OK107*-GAL4 expresses in some neurons that are extrinsic to the mushroom bodies including the Pars Intercerebralis (Aso *et al.* 2009). These flies also slept longer than controls, failed to suppress sleep and survived longer under starvation conditions (**Fig. 13C**). Knockdown of *unc79* selectively in the mushroom bodies (*OK107*-GAL4) increased total sleep, specifically during the day, and resulted in increased starvation resistance (**Fig. 13D,E**). Therefore, selective knockdown with *OK107*-GAL4 largely phenocopies ubiquitous knockdown, raising the possibility that *unc79* functions in the mushroom body to regulate sleep and starvation resistance (**Fig. 13F**). Therefore, these findings confirm that *unc79* functions in the mushroom bodies to regulate sleep and starvation resistance.

To determine whether the phenotypes observed are specific to lobes of the mushroom body, we tested the effects of *unc79* knockdown in the α/β lobes (*c739*-GAL4), α′/β′ lobes (*c305a*-GAL4), and the γ lobes (*1471* GAL4 drivers) (Krashes *et al.* 2007; Aso *et al.* 2009). Flies with *unc79* knockdown in α/β lobes and α′/β′ lobes fail to suppress sleep compared to *luc* control, while knockdown in the γ lobes increased total sleep duration compared to *luc* control and fail to suppress sleep when starved (**Fig. 15A**). Starvation

resistance is increased in *unc79* knockdown in each mushroom body subtype compared to their respective controls expressing *luciferase*-RNAi (**Fig. 15B**). Therefore, loss of *unc79* function in each subset of mushroom body neurons impacts sleep and metabolic regulation, while selective loss in the γ lobes largely recapitulates the full extent of ubiquitous knockdown. These findings reveal that *unc79* is required in all lobes of the mushroom body for proper sleep and metabolic regulation.

To verify that the increase in sleep and starvation resistance is specific to the mushroom bodies, we examined whether including of the *MB*-GAL80 transgene reverses the effects of *unc79* knockdown. Expression of GAL80 in the mushroom bodies restores sleep and starvation-induced sleep suppression to control levels (**Fig. 15CD**). Starvation resistance increased in flies with *unc79* selectively knocked down in the γ lobes (*1471*-GAL4>*unc79*^{RNAi}). Blocking expression of GAL4 within the mushroom body restored starvation resistance, and partially restored sleep duration on food, confirming that loss of *unc79* in the mushroom body leads to dysregulated sleep (**Fig. 15C**). In addition, the expression of *MB*-GAL80 restored normal starvation resistance to *1471*-GAL4>*unc79*^{RNAi} flies (**Fig. 15D**). Therefore, loss of *unc79* function within the γ-lobe of mushroom bodies increases both sleep and starvation resistance.

Previous work has revealed that *unc79* functions within the circadian neurons in association with the *unc80* accessory protein and the ion channel *narrow abdomen* (*na*) to maintain locomotor rhythms during constant darkness (Lear *et al.* 2005, 2013; Moose *et al.* 2017). To further investigate whether the sleep and circadian phenotypes are controlled by shared or distinct neural circuits, we knocked down additional components of the *unc79* complex in the mushroom bodies and measured the effects on sleep and starvation

resistance. Knockdown of *na* or *unc80* throughout the mushroom bodies did not increase sleep duration on food or disrupt starvation-induced sleep suppression **(Fig 16A)**. In addition, knockdown of *unc*80 and *na* in the mushroom body had little impact on starvation resistance (**Fig 16B**). These findings suggest *unc79* functions independently of its canonical complex with *unc80* and *na* to regulate sleep and starvation resistances.

Expression of *unc79* is required within pacemaker neurons to circadian rhythms. Therefore, it is possible that *unc79* functions in distinct populations of neurons to regulate circadian rhythms, from those regulating sleep and metabolic phenotypes. To directly test this possibility, we measured the effects of mushroom body-specific knockdown of *unc79* on free-running activity in entrained animals. As expected, flies with pan-neuronal knockdown are arrhythmic under conditions of constant darkness, while control flies show robust rhythms **(Fig 16C-F).** Conversely, knockdown in OK107-expressing cells does not impact circadian activity (Fig 5G-H). Circadian analysis for power and periodicity reveals that pan-neuronal knockdown of *unc79* (nSyb GAL4>*unc79* RNAi, power equals 45.4±7.049, periodicity equals 26.32±0.9583, N=31) compared mushroom body knockdown of *unc79* (OK107GAL4>*unc79* RNAi, power equals 129±13.16, periodicity equals 23.58±0.1117, n =30) or other exp controls (W1118 (+), power equals 145.5±18.38, Periodicity 24.78±0.1326, N=32; luc RNAi /+, power equals 418±38.99, periodicity equals 24.24±0.09269, n=32; *unc79* RNAi/+ power equals 207.7±22.14, periodicity equals 23.84±0.04861, n=32; nSybGAL4>luc RNAi, power equals 420.4±56.79, periodicity equals 25.58±0.02795, n=32 and OK107GAL4>luc RNAi, power equals 309.8±38.95, periodicity equals 23.54±0.09379, n=32). Therefore, *unc79* function to regulate circadian rhythms through distinct neural mechanisms that regulate sleep and starvation resistance.

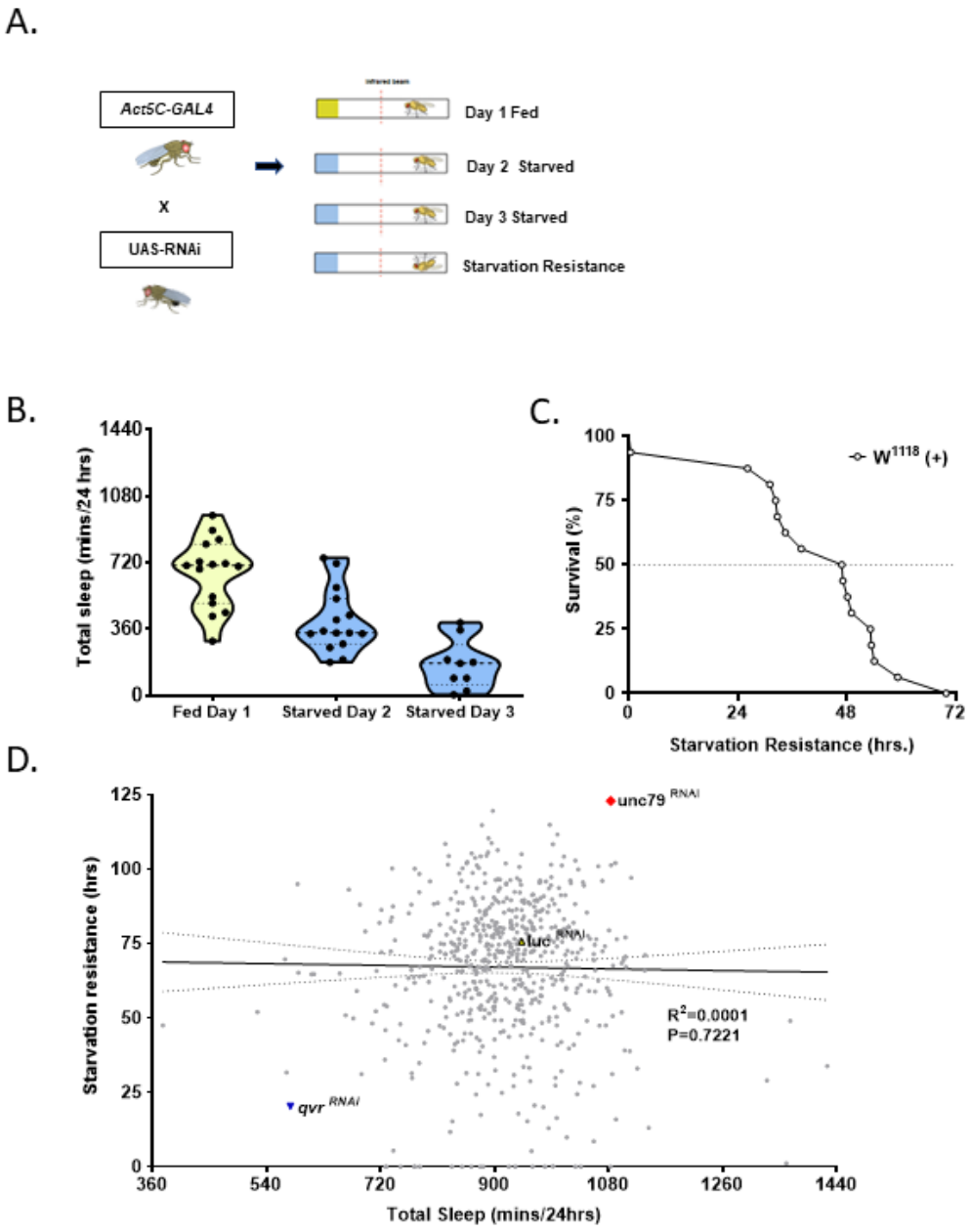

Figure 9: Screening for sleep and starvation resistance.

A. Schematic of TRiP Screen. Ubiquitous Act5c GAL4 driver is crossed to TRiP lines, F1 flies were transferred to Drosophila Activity Monitor tubes. Sleep was measured for 24 hours on standard food, after which, flies were transferred to agar where they remained until death to quantify starvation-induced sleep suppression and starvation resistance. B. Flies suppressed sleep during the first day of starvation, and an even greater suppression was observed on day two of starvation. C. Flies survived approximately three days on agar, providing a robust readout sleep and starvation resistance. Total sleep (mins) is measured over 24 hour period and starvation resistance is measured in hours. D. Scatter plot for fed

total sleep on x-axis plotted (mins) to starvation resistance on y-axis (hrs) (Simple Linear Regression: F (1, 609) equals 0.1265, R2 value equal 0.0001, P-value > 0.7223). Control flies with no endogenous targets Act5c GAL4 drive luc RNAi (yellow), lines tested in (grey), qvr has lowest sleep and SR (blue), and *unc79* highest sleep and SR (red).

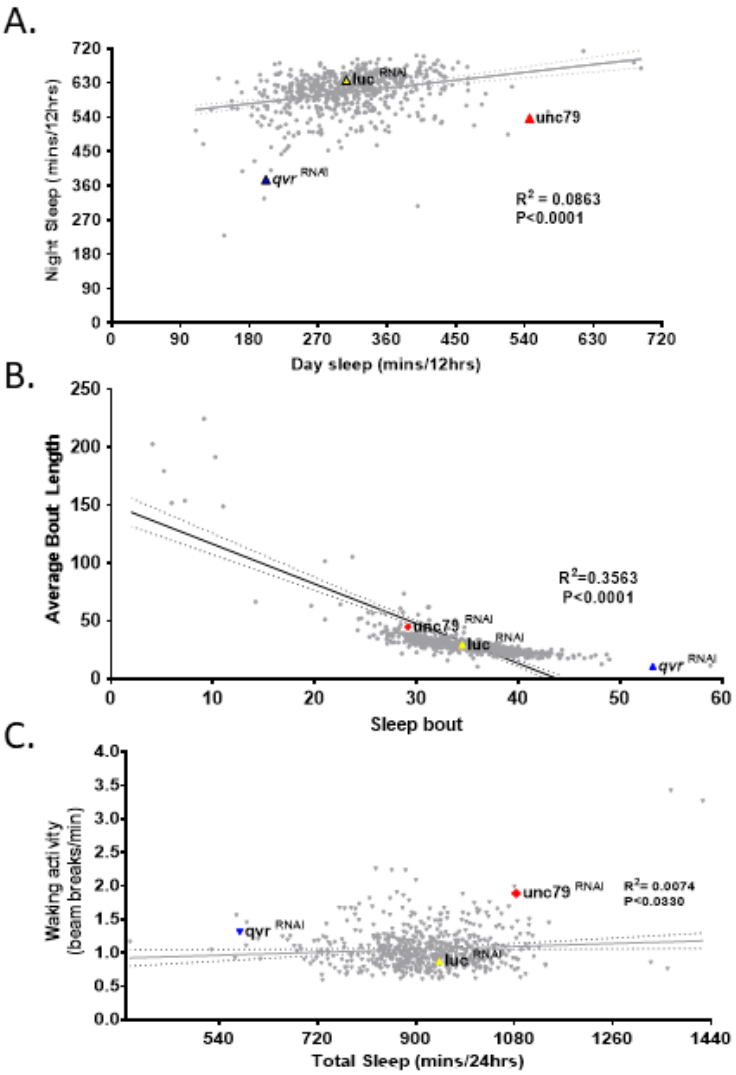

Figure 10: Scatter plots depicting different characteristics of *unc79*.

A. Scatter plot depicting day time and nighttime sleep scatter plot. Fed day sleep on x-axis plotted (mins/12 hrs) to Fed night sleep on y-axis (mins/12 hrs) (Simple Linear Regression: F (1, 609) equals 57.65, R2 equals 0.0863, P-value equals<0.0001). **B.** Scatter plot depicting the average number of sleep bouts compared to the average bout length. Sleep bout on x-axis to average bout length on y-axis Simple Linear Regression: F (1, 611) equals 338.2, R squared 0.3563, P value <0.0001). **C.** Total sleep/waking activity scatter plot. Fed total sleep on x-axis plotted (mins/24 hrs) to waking activity on y-axis (beam breaks/min (Simple Linear Regression: F (1, 612) equals 4.568, R2 equals 0.0074, P-value equals<0.0330). Control flies with no endogenous targets Act5c GAL4 drive luc RNAi (yellow), lines tested in (grey), and *unc79* highest sleep and SR (red).

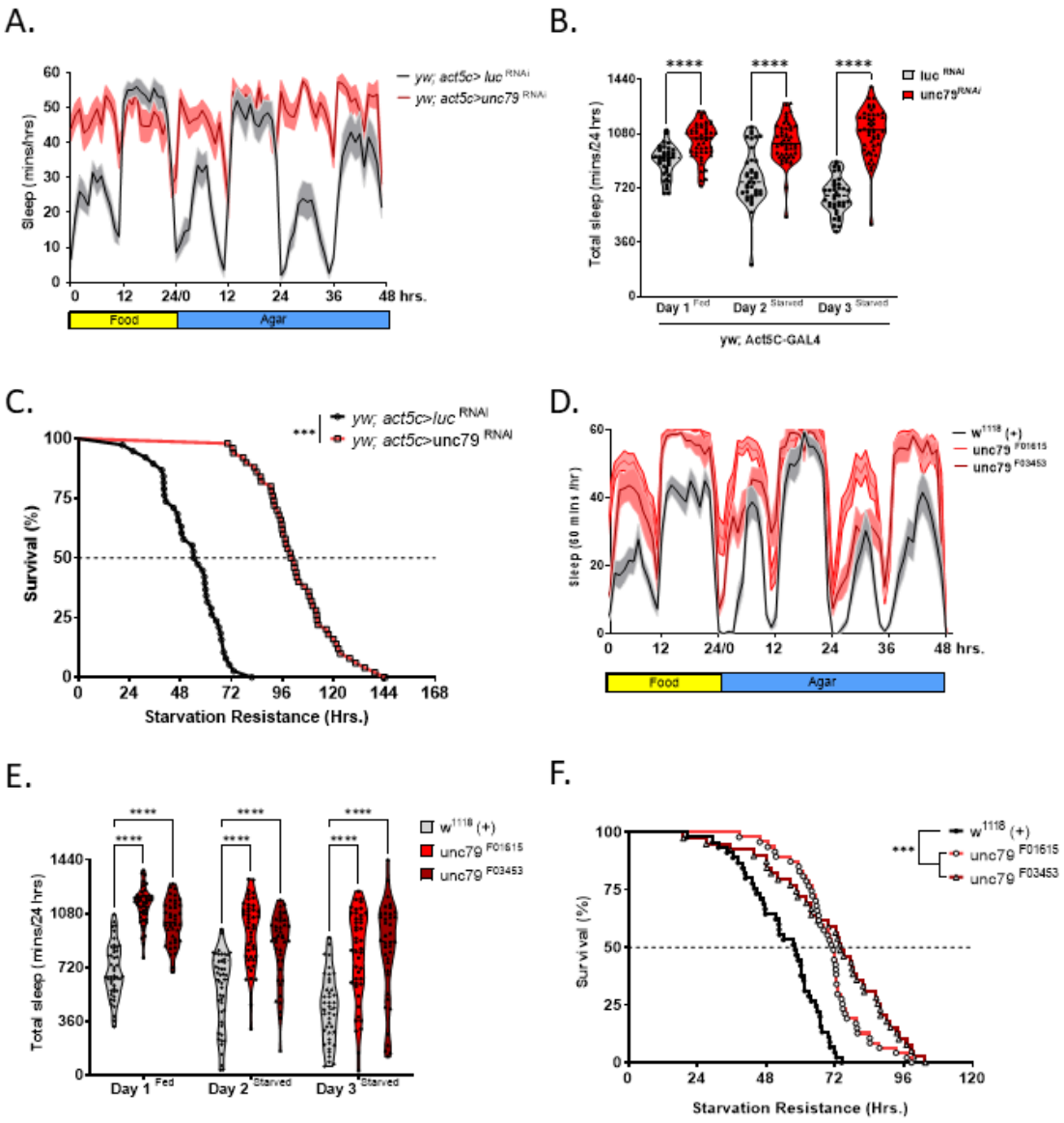

Figure 11: *unc79* RNAI and mutants have increased sleep and sr

A. Sleep profiles depicting the average sleep each hour over a 72 hour experiment for Act5c>luc^{RNAi} (grey) and Act5c>*unc79*^{RNAi} (red). Flies were on food for day 1, then transferred to agar for days 2 and 3. **B.** Act5c>*unc79* ^{RNAi} (red) during fed (Two-way ANOVA: $F_{(2, 261)} = 8.551$, $P<0.0001$ $N>39$), starved day 1 (Two-way ANOVA: $F(2, 261) = 8.551$, $P<0.0001$, $N>39$) and starved day 2 (Two-way ANOVA: $F_{(2, 261)} = 8.551$, $P<0.0001$ $N>39$) flies slept significatly longer compared to Act5c>luc ^{RNAi} (grey) controls. **C.** Starvation resistance of Act5c>*unc79*^{RNAi} (red) is significatly higher than Act5c>luc^{RNAi} (black) control (Gehan-Breslow-Wilcoxon test: Chi $^2 = 94.42$, df = 1, P-value < 0.0001). **D.** Sleep profile for hourly sleep averages over a 72 hour experiment for w^{1118}, *unc79* ^{F01615} (red) and *unc79* ^{F03453} (maroon) flies are on food for day 1, then transferred to agar for day

2 and 3. **E.** Total sleep is greater in *unc79* ^{F03453} mutant under fed (maroon, $F_{(2, 651)} = 71.46$, $P<0.0001$, $N\geq39$), starved day 1 ($P<0.001$; $N\geq39$) and starved day 2 ($P<0.001$; $N\geq39$) conditions compared to control (grey). Total sleep is greater in *unc79* ^{F01615} mutant (red) under fed ($F(2, 651) = 71.46$, $P<0.0001$, $N\geq39$), starved day 1 ($P<0.001$; $N\geq39$) and starved day 2 ($P<0.001$; $N\geq39$) conditions compared to w^{1118} control (grey). **F.** Starvation resistance is greater in *unc79*^{F03453} (maroon, Gehan-Breslow-Wilcoxon test: Chi 2 = 29.1, df = 1, P-value < 0.0001) and *unc79*^{F01615} (red, (Gehan-Breslow-Wilcoxon test: Chi ^{2} = 18.6, df = 1, P-value < 0.0001) flied compared to w^{1118} control (grey). All sleep data are violin plots and SR data are survival curves. ****p < 0.0001.

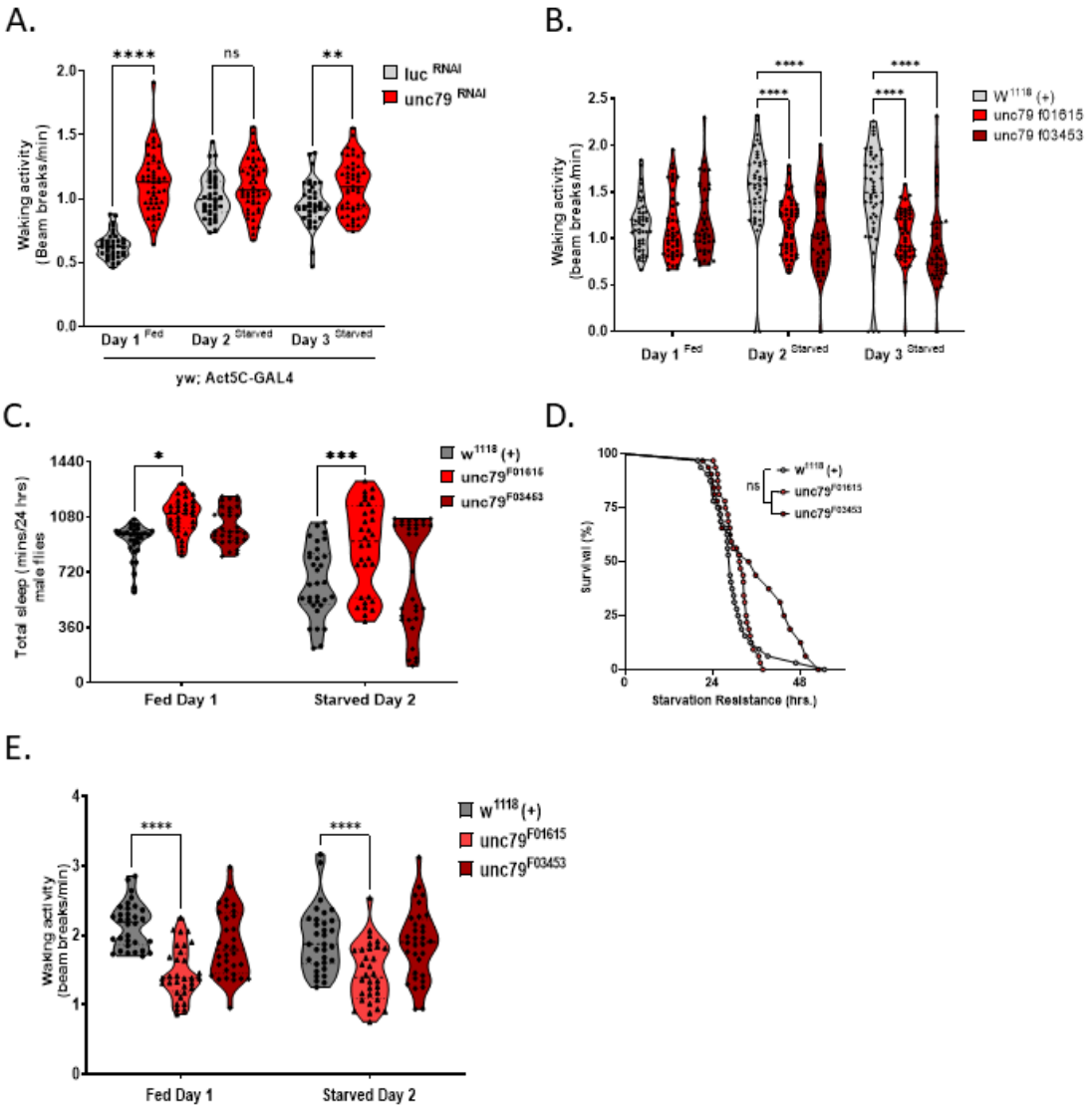

Figure 12: Waking activity for *unc79* knockdown and hypomorphs.

A. Waking activity violin plots over a 72-hour experiment for female Act5c>lucRNAi (grey) and Act5c>*unc79*RNAi (red). Waking activity is higher during Day 1 fed (N≥37; P<0.0001) and Day 3 starved (N≥37; P<0.01), while not different during Day 2 (N≥32; P<.1199). **B.** Waking activity violin plots over a 72-hour experiment for female *unc79* mutants. Waking activity differ during starved state of *unc79*F03453 (maroon) *unc79*F01615 (red) more than w1118 (grey) and *unc79*/+ controls (pink) (N≥39; P<0.0001), but not during fed state (N≥39; P<0.856). **C.** Total sleep violin plot over a 48-hour experiment for male *unc79* mutants. Waking activity differ during fed and starved state of *unc79*F01615 (red) more than w1118 (grey) and *unc79*/+ controls (pink) (N≥39; P<0.0001), but not during fed or starved state for *unc79* F03453 (maroon) (N≥39; P<0.8559). **D.** Starvation resistance of male flies did not differ between *unc79*F03453 (maroon) *unc79*F01615 flies (red), and both were greater than w1118 (grey) and *unc79*/+ controls (pink). E. Waking activity for male flies for 48-hour period. Mutant *unc79*F01615

(red) is less active than w1118 (grey) (N≥32; P<0.0001), while *unc79*F03453 (maroon) (N=32, P>0.0648), and heterozygous controls (pink) (N=32; P>0.0733). All sleep data are violin plots and SR data are survival curves. ***p < 0.001; ****p < 0.0001.

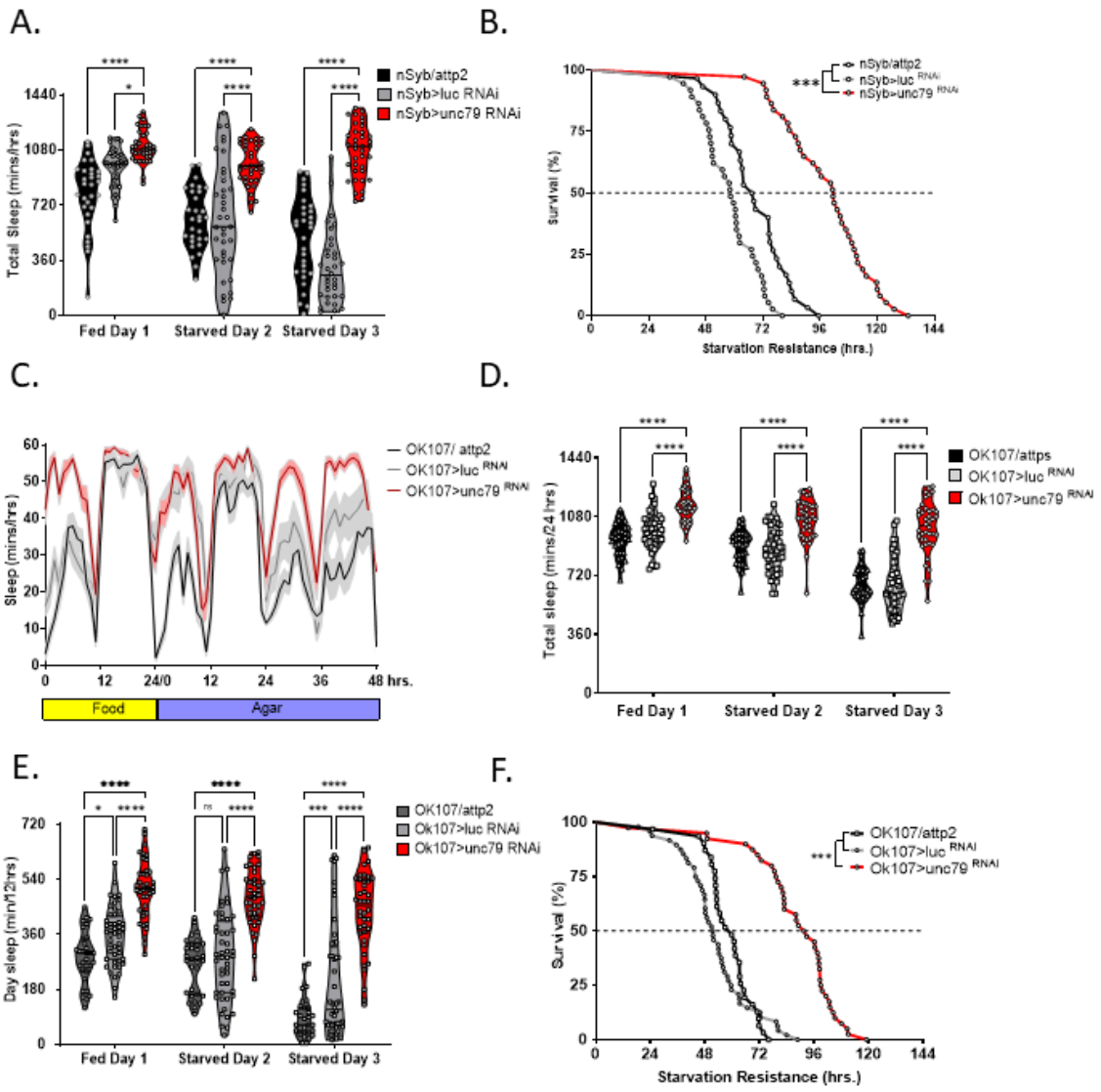

Figure 13: Localization of sleep and sr phenotype to mushroom body

A. Pan-neuronal knockdown of *unc79* (nSyb>*unc79* ^{RNAi} ,red) is significantly increased in sleep during fed (Two-Way ANOVA: $F_{(2, 300)} = 57.82$, $P<0.0001$, $N>30$), starved day 1 ($P<0.0001$, $N>30$), and starved day 2 ($P<0.0001$, $N>30$); while, nSyb>attp2 (light grey) and nSyb>luc RNAi controls shows starvation-induced sleep suppression. **B.** Starvation resistance for pan-neuronal knockdown of *unc79* nSyb>*unc79*^{RNAi} (red) is significantly increased compared to nSyb>attp2 (grey, Gehan-Breslow-Wilcoxon test: Chi ^{2} equal 42, df equals 1, P-value<0.0001, N>30) and nSyb>luc RNAi (light grey, Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 64.6, df equals 1, P-value <0.0001, N>37) control flies. **C.** Sleep profile hourly sleep averages over a 72-hour experiment for mushroom body knockdown of *unc79*. Flies are on food for day 1, then transferred to agar for day 2 and 3. **D.** Mushroom body knockdown of *unc79* (OK107>*unc79* ^{RNAi}, red) is significantly increased in sleep during fed (Two-Way ANOVA: $F_{(2, 300)} = 57.82$, $P<0.0001$, $N>30$)

starved day 1 (P<0.0001, N>31), and starved day 2 (P<0.0001, N>31); while, Ok107>attp2 (light grey) and Ok107>luc RNAi controls shows starvation-induced sleep suppression. **E.** Daytime sleep in flies with mushroom body knockdown of *unc79* (OK107>*unc79* RNAi, red) is significantly increased under fed conditions (Two-Way ANOVA: $F_{(2, 348)} = 43.42$, P<0.0001, N>30), starved day 1 (P<0.0001, N>31), and starved day 2 (P<0.0001, N>31); while, nSyb>attp2 (grey) and nSyb>luc ^{RNAi} (light grey) controls maintain normal daytime sleep. **F.** Starvation resistance is increased in OK107>*unc79*^{RNAi} flies compared to Ok107>attp2 (grey, Gehan-Breslow-Wilcoxon test: Chi 2 equals 47.6, df equals 1, P-value <0.0001, N>31) and OK107>*luc* ^{RNAi} (light grey, Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 50.5, df equals 1, P-value <0.0001, N>31) control flies. All sleep data represent violin plots and SR data are survival curves. ****p < 0.0001.

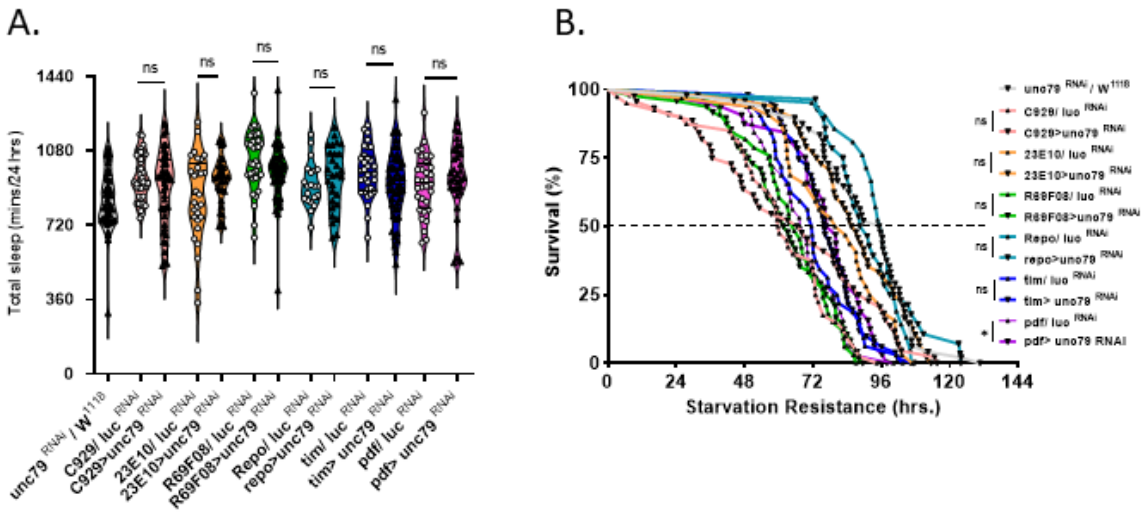

Figure 14: Knockdown *unc79* in different brain regions has no change sleep or sr.

A. Knockdown of *unc79* in different brain regions show no differences to respective flies expressing luc-RNAi (One-way ANOVA : $F_{(12, 454)}$ equal 2.247). Peptidergic knockdown of *unc79* (C929>*unc79* RNAi) does not differ in control (C929/ luc RNAi) (pink, P-value equals 0.9665, N>40). Fan-shaped body knockdown of *unc79* (23E10>*unc79* RNAi) does not differ in control (23E10/ luc RNAi) (orange, P-value equals 0.698, N>29).
Central complex knockdown of *unc79* (R69F08>*unc79* RNAi) does on differ in control (R69F08/ luc RNAi) (green, P-value equals 0.8795, N>33). Glial knockdown of *unc79* (repo>*unc79* RNAi) (teal, P-value equals 0.9999, N>21) does on differ in control (Repo/ luc RNAi). Circadian genes knockdown of *unc79* timeless (tim> *unc79* RNAi) (blue, P-value equals 0.8226, N>32) does on differ in control (tim/ luc RNAi) and pigment dispersion factor (pdf> *unc79* RNAi) (purple, P-value equals 0.9997, N>32) does on differ in control (pdf/ luc RNAi). **B**. Starvation resistance for knockdown of *unc79* in brain region drivers compared to luc control (Gehan-Breslow-Wilcoxon test). Peptidergic knockdown of *unc79* (C929>*unc79* RNAi) does not differ in control (C929/ luc RNAi) (pink, df equal 1, P-value equals 0.668, N>40). Fan-shaped body knockdown of *unc79* (23E10>*unc79* RNAi) does not differ in control (23E10/ luc RNAi) (orange, df equals 1, P-value equals 0.0879, N>32). Central complex knockdown of *unc79* (R69F08>*unc79* RNAi) does on differ in control (R69F08/ luc RNAi) (green, df equals 1, P-value equals 0.6268, N>33). Glial knockdown of *unc79* (repo>*unc79* RNAi) (teal, df equals 1, P-value equals 0.3464, N>21) does on differ in control (Repo/ luc RNAi). Circadian genes knockdown of *unc79* timeless (tim> *unc79* RNAi) (blue, df equals 1, P-value equals 0.072, N>32) does on differ in control (tim/ luc RNAi). Pigment dispersion factor (pdf> *unc79* RNAi) (purple, df equals 1, P-value equals 0.0191, N>32) is significantly different than control (pdf/ luc RNAi). All sleep data are violin plots and SR data are survival curves. ***p < 0.001; ****p < 0.0001.

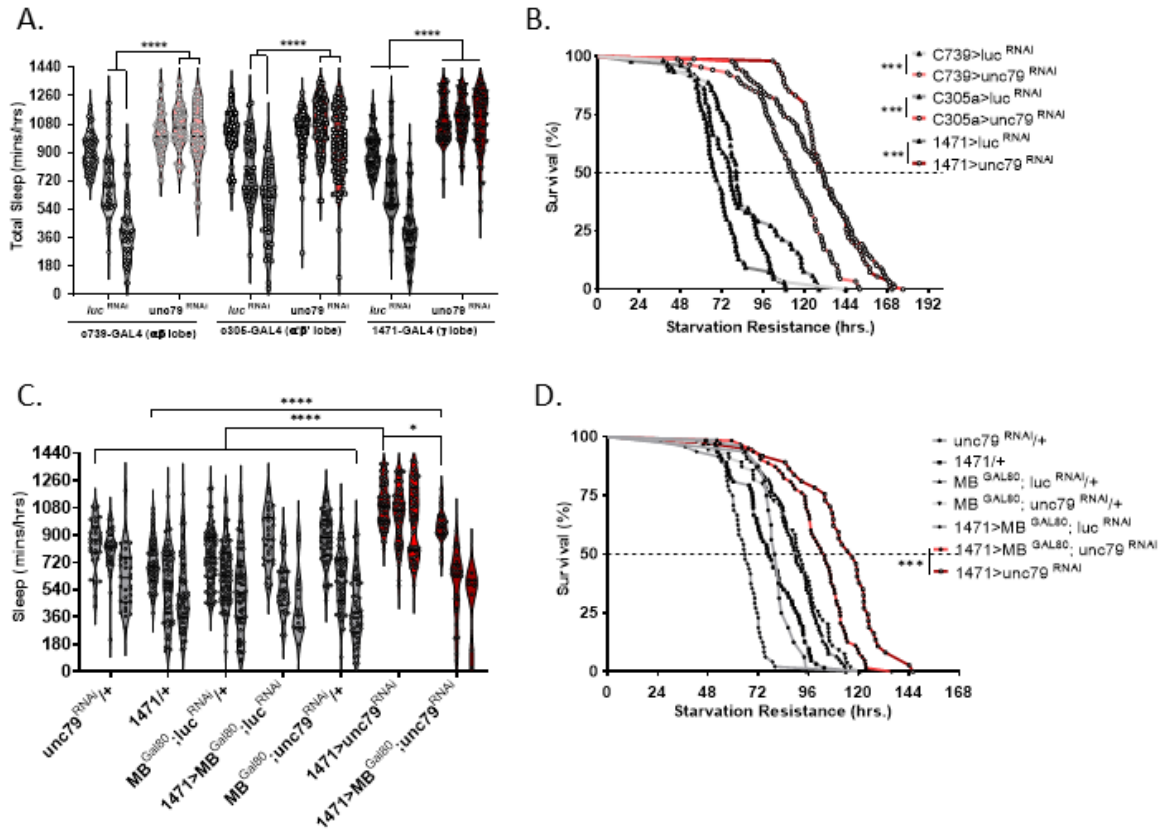

Figure 15: *Unc79* function localized to mb γ-lobe to regulate sleep and sr.

A. Flies with *unc79* knocked down in the αβ lobes (c739>*unc79* RNAi, pink) fail to suppress starvation during starved day 1 (Two-way ANOVA $F_{(10, 1075)} = 28.56$, P<0.0001, N>53) and starved day 2 (P<0,0001) compared to control (c739>luc ^{RNAi}, light grey); while fed day 1 did not differ (P>0.1587). Mushroom body α'β' knockdown of *unc79* (c305a> *unc79* RNAi, red) fails to suppress starvation during starved day 1 (Two-way ANOVA $F_{(10, 1075)} = 28.56$, P<0.0001, N>51) and starved day 2 (P<0.0001) compared to control (c305a>luc RNAi, light grey); while fed day 1 did not differ (P>0.999). Mushroom body γ knockdown of *unc79* (1471 >*unc79* ^{RNAi}, maroon) significantly increase total sleep during fed day (Two-way ANOVA $F_{(10, 1075)} = 28.56$, P<0.0001, N>53) and fails to suppress sleep on starved day 1(P< 0.0001), and starved day 2 (P<0.0001) compared to control (1471>luc ^{RNAi}, dark grey). **B.** Starvation resistance increased when mushroom body αβ knockdown of *unc79* (c739 >*unc79* ^{RNAi}, pink) is significant (Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 71.36, df equals 1, P-value<0.0001, N>63) compared to control (c739>luc ^{RNAi}, light grey). Starvation resistance increased when mushroom body α'β' knockdown of *unc79* (c305a >*unc79* ^{RNAi}, red) is significant (Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 90.45, Df equals 1, P-value <0.0001, N>51) compared to control (c305a>luc ^{RNAi}, grey). Mushroom body γ knockdown of *unc79* (1471 >*unc79* ^{RNAi}, maroon) is significant (Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 124.6, df equals 1, P-value<0.0001, N>53) compared to control (1471>luc RNAi, dark grey). **C.** Mushroom body GAL80 rescue γ knockdown of *unc79* ^{RNAi} (1471>MB^{Gal80}; *unc79* ^{RNAi}) significantly rescues total sleep compared to γ

knockdown of *unc79* RNAi (1471>*unc79*RNAi, P-value <0.0432); while, total sleep for γ knockdown of *unc79* RNAi (1471>*unc79*RNAi) is high compares to other control groups (MBGal80;lucRNAi/+, P-value <0.0001; *unc79*RNAi/+, P-value <0.0001; 1471/+, P-value <0.0001; MBGal80;lucRNAi/+, P-value <0.0001; 1471>MBGal80;lucRNAi, P-value <0.0001; and, MBGal80;*unc79*RNAi/+, P-value <0.0001). Mushroom body GAL80 rescue γ knockdown of *unc79* RNAi (1471>MBGal80; *unc79* RNAi) restored total sleep to controls (*unc79*RNAi/+, P-value equal 0.3715; MBGal80;*unc79*RNAi/+, P-value equals 0.7292; and, 1471>MBGal80;lucRNAi, P-value quals 0.8177). However, 1471>MBGal80;*unc79*RNAi vs.1471/+ remained significant (P-value<0.0001). **D.** Starvation resistance of mushroom body GAL80 rescue γ knockdown of *unc79* RNAi (1471>MBGal80; *unc79* RNAi) significantly lower (Gehan-Breslow-Wilcoxon test: Chi ^{2} equals 11.13, Df equals 1, P-value<0.0009, N>37) compared to mushroom body γ knockdown of *unc79* (1471 >*unc79* RNAi, maroon). All sleep data are violin plots and SR data are survival curves. ***p < 0.001; ****p < 0.0001.

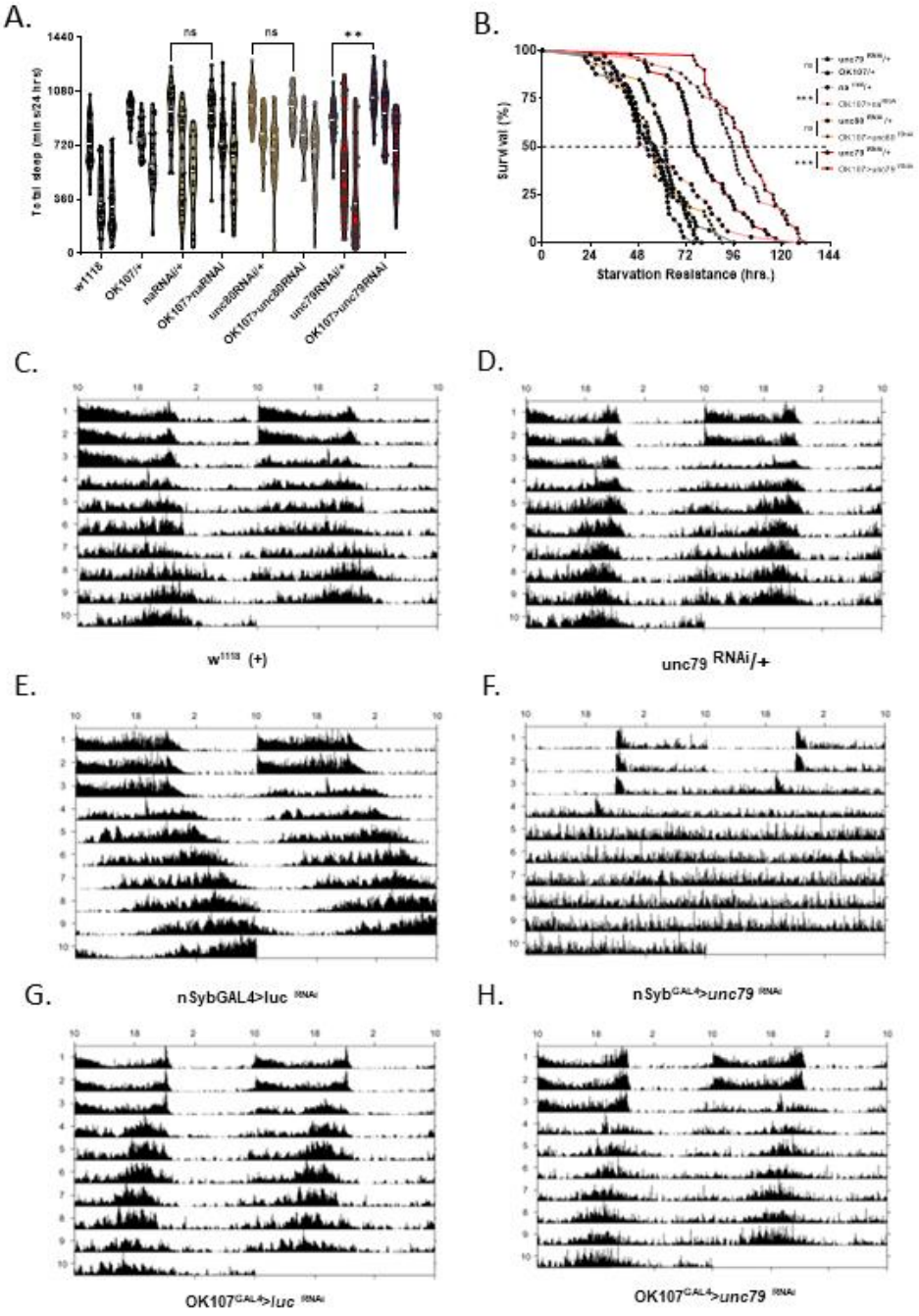

Figure 16: mb knockdown of *unc79* doesn't alter circadian rhythm.

A. Mushroom body knockdown of *unc79* (OK107>*unc79*-RNAi) significantly increased sleep (Two-way ANOVA: $F_{(2, 711)} = 169$, $P<0.0029$, $N>40$) compared to control (*unc79*RNAi/+). Mushroom body knockdown of narrow abdomen (OK107>na-RNAi) did not differ ($P>0.9999$, $N>35$) compared to control (*na*-RNAi/+). Mushroom body knockdown of unc80 (OK107>unc80RNAi) did not differ ($P>0.9964$, $N>42$) compared to control (unc80 RNAi/+). **B**. Starvation resistance mushroom body knockdown of *unc79* (OK107>*unc79* RNAi) is increased (Gehan-Breslow-Wilcoxon test: Chi ^{2} equal 25.95, df

equal 1, P-value <0.0001, N>40) compared to control (*unc79*-RNAi/+). Starvation resistance mushroom body knockdown of narrow abdomen (OK107>na RNAi) is increased (Gehan-Breslow-Wilcoxon test: Chi ^{2} equal 33.54, df equal 1, P-value <0.0001, N>42) compared to control (na RNAi/+). Starvation resistance mushroom body knockdown of narrow abdomen (OK107>unc80 RNAi) is no different (Gehan-Breslow-Wilcoxon test: Chi ^{2} equal 2.486, df equal 1, P-value <0.1148, N>43) compared to control (unc80 RNAi/+). **C-H.** Actogram double plot. Female flies were entrained in light-dark cycle for days 1-3 and dark-dark cycles 4-10 days. A, B, C, E and F have normal rhythm. Circadian rhythm is disrupted when *unc79* is knocked down pan-neuronally, while *unc79* knockdown in mushroom body has restored rhythm. All sleep data are violin plots and SR data are survival curves. ***p < 0.001; ****p < 0.0001.

Discussion

Here, we screened by targeting gene function ubiquitously to identify regulators of sleep and metabolic function. Growing evidence suggests sleep is regulated by complex interactions between the brain and periphery, including the findings that mutants impacting fat storage, and communication from the fat body to the brain significantly impact sleep (Thimgan *et al.* 2010a; Slocumb *et al.* 2015b; Ertekin *et al.* 2020). We have identified numerous candidate regulators of sleep, including a novel role for *unc79* in the regulation of sleep and metabolic function. *Unc79* and *unc80* are auxiliary subunits of the sodium leak channel *na*, an ortholog of mammalian *NALCN* family of ion channels (Swayne *et al.* 2009). A number of functions have been identified for this complex including a role in the regulation of circadian rhythms, and anesthesia sensitivity (Lear *et al.* 2005; Humphrey *et al.* 2007). Previous work found that loss of narrow abdomen or *unc79* increased sensitivity to the anesthetics, halothane and isofluorane, and increases sleep (Humphrey *et al.* 2007; Joiner *et al.* 2013) consistent with our findings of increased quiescence in *unc79* mutants. Mutation of *unc79* also facilitates the emergence from anesthesia, raising the possibility that loss of *unc79* promotes state transitions, rather than directly impacting isofluorane sensitivity (Joiner *et al.* 2013). Therefore, suppression of arousal may be involved in anesthesia and sleep (Joiner *et al.* 2013). Mutation of *na* also impacts a number of complex behaviors including social clustering (the distance maintained between individual flies)

(Burg *et al.* 2013), and light-mediated locomotor activity (Nash *et al.* 2002).These findings suggest a complex role for *na* and associated *unc79* genes in regulating brain function.

Multiple lines of evidence suggest the role of *unc79* in the regulation of sleep, metabolic regulation of sleep, and starvation resistance is separate from its essential role in regulating circadian rhythms. First, we localize function to the mushroom body, a region that is critical for regulation of sleep and modulation of behavior in accordance with feeding state (Joiner *et al.* 2006a; Pitman *et al.* 2006b; Sitaraman *et al.* 2015a; Tsao *et al.* 2018). We previously reported that the mushroom bodies are dispensable for starvation-induced sleep suppression, however the manipulations that led to this conclusion involved pharmacological ablation or acute genetic silencing of the mushroom bodies (Keene *et al.* 2010a). Therefore, it is possible that loss of *unc79* function impacts sleep circuitry through a mechanism that would not be detected in flies with the previously applied genetic manipulations. Second, *na* and *unc80*, two components of a complex that interacts with *unc79* to regulate circadian rhythms, are dispensable for regulation of sleep and starvation resistance in the mushroom bodies. These findings raise the possibility that *unc79* may function independently of its canonical complex with *unc80* and *na.* A central question is how *unc79* functions to modulate mushroom body physiology and sleep circuitry. Studies examining the role of *unc79* in circadian function and anesthesia sensitivity suggests it functions by regulating *na* activity to modulate neural activity (Moose *et al.* 2017), and it is possible that *unc79* modulates the function of a different ion channel within the mushroom bodies.

We identify three independent phenotypes to the mushroom bodies. First, we find knockdown of *unc79* in the mushroom bodies promotes sleep suggesting a wake-promoting role for mushroom bodies. The mushroom bodies contain both wake and sleep-promoting neurons, and genetic ablation or silencing of the mushroom body increases

wakefulness (Joiner *et al.* 2006a; Pitman *et al.* 2006b; Sitaraman *et al.* 2015a). It is possible that loss of *unc79* is functioning in either sleep promoting or wake-promoting neurons to elicit this phenotype. We also identify two independent metabolic phenotypes to the mushroom bodies. In *Drosophila*, the mushroom circuits have been well-defined including the identification of modulatory neurons, and output neurons that modulate sleep (Aso *et al.* 2014b; Haynes *et al.* 2015; Sitaraman *et al.* 2015d). Therefore, the identification of *unc79* as a regulator of sleep provides the opportunity to examine how gamma lobe output neurons are regulated by input neurons and impact the physiology of output neurons.

In addition to the sleep phenotypes, we find *unc79* mutants are resistant to starvation. This finding is particularly interesting because the list of genes chosen for the screen derived from those identified in a Genome Wide Analysis Study for factors associated with starvation resistance (Harbison *et al.* 2004; Hardy *et al.* 2018). Many different factors contribute to starvation resistance including energy stores, basal metabolic rate, and changes in metabolic rate upon starvation. Animals selected for starvation resistance have elevated sleep and do not suppress sleep when starved (Masek *et al.* 2014a). Therefore, future work studying starvation selected lines, or other populations of outbred fly lines have potential to identify whether variable expression of *unc79* is associated with naturally occurring differences in sleep and metabolic regulation.

We find that *unc79* most potently impacts sleep and starvation resistance in the gamma lobes, suggesting this population is critical for both sleep metabolic regulation. Output neurons from the gamma lobes have been directly implicated in feeding and fat storage supporting the notion that this region is critical for metabolic regulation (Al-Anzi and Zinn 2018). Future work examining the effects of *unc79* deficiency on the physiology

and function of mushroom body output neurons may help identify the role of *unc79* in regulating mushroom body circuits that ultimately regulate behavior and metabolic function. Taken together, these findings add to growing evidence that sleep and metabolic function are integrated. The identification of additional genetic factors that regulate the relationship between sleep and nutritional state through behavioral studies will improve our understanding of the strong associations between sleep loss and metabolism-related diseases. The ubiquitous screen has identified numerous candidate genes that impact sleep, starvation-induced sleep suppression, and starvation resistance, providing candidates that function within and outside of the nervous system. Future study of these genes, such as *unc79*, has potential to advance our understanding of sleep-metabolism interactions and brain-periphery communication.

CHAPTER 4. FINAL REMARKS

Genetic screens have been useful to identify novel regulators of sleep-metabolism interactions in the fruit fly. This work has identified genes in different brain regions that regulate sleep. In Chapter 2, this work characterized one of the first genes that regulate sleep-metabolism interactions. *trsn* flies failed to suppress sleep during starvation compared to control flies that suppress sleep due to starvation. Through qPCR analysis, *trsn* is transcriptionally upregulated in the heads of wild type flies during starvation. Through spatial localization, *trsn* functions in *Lk*-expressing neurons. Further, silencing *Lk* neurons cause a failure to suppress sleep, thereby strengthening the critical role for both *trsn* and *Lk*-expressing neurons in the metabolic regulation of sleep.

Translin and *Trax* are components of an evolutionarily conserved RNA binding complex. Deletion of *translin* (TB-RBP) -null mice exhibited behavioral abnormalities such as increased docility and exhibited reduced Rota-Rod performance (Chennathukuzhi *et al.* 2003). Analysis of *translin* KO mice also revealed alterations in levels of transcripts encoding synaptic proteins. A confluence of localization, biochemical and RNA trafficking studies supports the view that this complex mediates dendritic trafficking of RNAs, a process thought to play a critical role in synaptic plasticity (Aoki *et al.* 1997; Meng *et al.* 2000). The *translin*/*trax* complex are among the cadre of RNA binding complexes that regulate dendritic trafficking of RNA in neurons (Li *et al.* 2008). Further downstream, the *translin/trax* complex is involved with ribonucleo-protein complexes called RNA-induced

silencing complexes (RISCs), which target nucleic acid sequences for gene-silencing (Pratt and MacRae 2009). Since the microRNA system plays a

prominent role in regulating metabolism, *translin* KO mice induced unusual metabolic profiles characterized by robust adiposity, normal body weight and glucose tolerance (Fu *et al.* 2020). Further experiments ought to be conducted to further understand the role of *trsn* in sleep-metabolism interactions. Since TRAX and RISC are downstream of translin, the knockdown of these genes in LK neurons should phenocopy starvation-induced sleep suppression. Single-cell analysis of Lk neurons to identify metabolic changes in different fed and starved states will also be useful. Single-cell genomics can characterize cell identity and function for the profiling of thousands of individual cells. Drop-seq analyzes mRNA transcripts from thousands of individual cells simultaneously while remembering transcripts' cell of origin (Macosko *et al.* 2015).

In Chapter 3, This work investigated the functional role of *unc79* in the regulation of sleep and starvation resistance. This work started by using a RNAi screen to identify novel genes to regulate sleep. This work found that *unc79* is required for sleep, starvation-induced sleep suppression, and starvation resistance. This work demonstrated through circadian analysis, that pan-neuronal knockdown of *unc79* altered circadian rhythmicity, but retained normal circadian rhythmicity in mushroom body knockdown, suggesting that *unc79* is an essential circadian and homeostatic sleep regulator but is independent of the mushroom body. Further, knockdown of *unc79* in different lobes within the mushroom body revealed different sleep and starvation-induced sleep suppression phenotypes. Lastly, *unc79* seems to regulate sleep, starvation-induced sleep suppression, and starvation

resistance within the gamma (γ) lobe of the mushroom body neurons. *unc79* is highly conserved and forms a larger *Na-unc80-unc79* complex.

In mammals and invertebrates, animal models revealed that this complex is involved in many processes such as locomotor behavior (Pierce-Shimomura *et al.* 2008), sensitivity to volatile anesthetics (Sedensky and Meneely 1987; Humphrey *et al.* 2007), and respiratory rhythms (Cochet-Bissuel *et al.* 2014). Interestingly, heterozygotes for NCA are smaller than wild-type animals and mildly hyperactive when exposed to a novel environment. Lightweight heterozygotes exhibit increased food consumption, yet have a leaner body composition (Speca *et al.* 2010). Since these mice are heterozygous and not full KO, it's difficult to observe translational studies to *Drosophila*. However, further experiments in *Drosophila* can further elucidate its function. Observing changes in metabolic rate and calcium activity of MB dung fed and starved states may give insight into this sleep-metabolism interaction.

unc79 function was localized to MBs, however, the role of *unc79* in mushroom body output neuron function is unknown. Sleep-promoting KCs that innervate g dorsal(g d) preferentially activate sleep-promoting MBONs (MBON-g2a'1), while wake-promoting KCs that innervate a'/b' or g main(g m) preferentially excite wake-promoting MBONs (MBON-g5b'2a/'2mp/b'2mp bilateral) (Aso *et al.* 2014a). More recently, Sitaraman et al. have determined two classes of sleep-regulating KCs (Sitaraman *et al.* 2015b; Barnstedt *et al.* 2016). A cholinergic MBON class promotes sleep and a glutamatergic MBON class promotes wakefulness (Aso *et al.* 2014c). Sleep-promoting and wake-promoting MBONs project to two brain areas, termed the crepine (CRE) and the superior medial protocerebrum (SMP). These results indicate that the sleep-promoting KCs-to-MBONs circuits play an

essential role for propagating homeostatic sleep signals. Further experiments to knockdown *unc79* in MBON will further localize sleep and starvation resistance.

Taken together, experiments performed in Chapters 2 and 3 established that genetic screening is still relevant in the identification of novel genes that regulate sleep and metabolism interactions. The knowledge that these works will bring greater understanding of the mechanisms underlying sleep-metabolism interactions, not only in fruit flies, but in other more complex organisms. A the prevalence of obesity, Type II diabetes, and other metabolic syndromes increase, there is an increasing need to understand the mechanisms underlying the interrelationship between these processes. The present study provides an entry point to investigating the genes and neural circuits regulating sleep-metabolism interactions.

Acknowledgements of contributions to dissertation

In Chapter 2 of this dissertation, the research project identified translin (*trsn*); one of the first genes that regulates the interaction between sleep and metabolism. The research shown has been peer reviewed and published (Murakami *et al.* 2016). Maria E. Yurgel conducted behavioral experiments with silencing experiments, colocalizing *trsn* antibody to the lateral horn neurons. Bethany Stahl (Stanhope) performed the immunohistochemistry, Pavel Masek performed the feeding experiments, Aradhana Mehta and Rebecca Heidker performed behavioral. Wesley Bollinger conducted the video tracking experiments. Finally, Robert M. Gingras and Justin R. DiAngelo contributed energy storage quantification. In Chapter 3 of this dissertation, the research project identified uncoordinated 79 (*unc79*) as a regulator sleep and concurrently increases starvation

resistance in specific neuronal subset of the mushroom bodies. Bethany Stanhope assisted in genetic screening. Justin Palermo conducted biochemical assays. Allen Gibbs is the originator of the starvation resistance flies and GWAS hits which led to the sleep and starvation resistance screen. Currently, this work is not published.

REFERENCES

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of Drosophila melanogaster. Science 287: 2185–95.

Aggarwal, S., R. S. Loomba, R. R. Arora, and J. Molnar, 2013 Associations between sleep duration and prevalence of cardiovascular events. Clin. Cardiol. 36: 671–6.

Ahima, R. S., and M. A. Lazar, 2008 Adipokines and the peripheral and neural control of energy balance. Mol. Endocrinol. 22: 1023–31.

Al-Anzi, B., E. Armand, P. Nagamei, M. Olszewski, V. Sapin *et al.*, 2010 The leucokinin pathway and its neurons regulate meal size in Drosophila. Curr. Biol. 20: 969–78.

Al-Anzi, B., and K. Zinn, 2018 Identification and characterization of mushroom body neurons that regulate fat storage in Drosophila. Neural Dev.

Alivisatos, A. P., M. Chun, G. M. Church, R. J. Greenspan, M. L. Roukes *et al.*, 2012 The Brain Activity Map Project and the Challenge of Functional Connectomics. Neuron 74: 970–974.

Allada, R., and J. M. Siegel, 2008 Unearthing the Phylogenetic Roots of Sleep. Curr. Biol. 18: 14–20.

Allen, Y. S., T. E. Adrian, J. M. Allen, K. Tatemoto, T. J. Crow *et al.*, 1983 Neuropeptide Y distribution in the rat brain. Science (80-.).

van Alphen, B., M. H. W. Yap, L. Kirszenblat, B. Kottler, and B. van Swinderen, 2013 A dynamic deep sleep stage in Drosophila. J. Neurosci. 33: 6917–27.

Anafi, R. C., R. Pellegrino, K. R. Shockley, M. Romer, S. Tufik *et al.*, 2013 Sleep is not just for the brain: transcriptional responses to sleep in peripheral tissues. BMC Genomics 14: 362.

Andretic, R., B. Van Swinderen, and R. J. Greenspan, 2005 Dopaminergic modulation of arousal in Drosophila. Curr. Biol. 15: 1165–1175.

Aoki, K., J. Inazawa, T. Takahashi, K. Nakahara, and M. Kasai, 1997 Genomic structure and chromosomal localization of the gene encoding Translin, a recombination hotspot binding protein. Genomics.

Aoki, K., K. Suzuki, R. Ishida, and M. Kasai, 1999 The DNA binding activity of Translin is mediated by a basic region in the ring-shaped structure conserved in evolution. FEBS Lett. 443: 363–366.

Arble, D. M., J. Bass, C. D. Behn, M. P. Butler, E. Challet *et al.*, 2015 Impact of Sleep and Circadian Disruption on Energy Balance and Diabetes: A Summary of Workshop Discussions. Sleep 38:.

Arrese, E. L., and J. L. Soulages, 2010 Insect fat body: Energy, metabolism, and regulation. Annu. Rev. Entomol.

Artiushin, G., S. L. Zhang, H. Tricoire, and A. Sehgal, 2018 Endocytosis at the Drosophila blood–brain barrier as a function for sleep. Elife e43326.

Aso, Y., K. Grübel, S. Busch, A. B. Friedrich, I. Siwanowicz *et al.*, 2009 The mushroom body of adult Drosophila characterized by GAL4 drivers. J. Neurogenet. 23: 156–172.

Aso, Y., D. Hattori, Y. Yu, R. M. Johnston, N. A. Iyer *et al.*, 2014a The neuronal architecture of the mushroom body provides a logic for associative learning. Elife 3: e04577.

Aso, Y., D. Sitaraman, T. Ichinose, K. R. Kaun, K. Vogt *et al.*, 2014b Mushroom body output neurons encode valence and guide memory-based action selection in Drosophila. Elife 3: e04580.

Aso, Y., D. Sitaraman, T. Ichinose, K. R. Kaun, K. Vogt *et al.*, 2014c Mushroom body output neurons encode valence and guide memory-based action selection in Drosophila. Elife 3: e04580.

Atkinson, W., and B. Shorrocks, 1977 Breeding site specificity in the domestic species of Drosophila. Oecologia 29: 223–232.

Baldal, E. A., P. M. Brakefield, and B. J. Zwaan, 2006 MULTITRAIT EVOLUTION IN LINES OF DROSOPHILA MELANOGASTER SELECTED FOR INCREASED STARVATION RESISTANCE: THE ROLE OF METABOLIC RATE AND IMPLICATIONS FOR THE EVOLUTION OF LONGEVITY . Evolution (N. Y).

Banfi, S., G. Borsani, E. Rossi, L. Bernard, A. Guffanti *et al.*, 1996 Identification and mapping of human cDNAs homologous to Drosophila mutant genes through EST database searching. Nat. Genet. 13: 167–174.

Barnstedt, O., D. Owald, J. Felsenberg, R. Brain, J. P. Moszynski *et al.*, 2016 Memory-Relevant Mushroom Body Output Synapses Are Cholinergic. Neuron 89: 1237–1247.

Beckwith, E. J., and A. S. French, 2019 Sleep in Drosophila and Its Context. Front. Physiol. 10: 1–19.

de Belle, J., and M. Heisenberg, 1994 Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. 263: 692–695.

Bellen, H. J., R. W. Levis, G. Liao, Y. He, J. W. Carlson *et al.*, 2004 The BDGP gene disruption project: Single transposon insertions associated with 40% of Drosophila genes. Genetics 167: 761–781.

Berger, R. J., and N. H. Phillips, 1995 Energy conservation and sleep, pp. 65–73 in *Behavioural Brain Research*,.

Bharucha, K. N., P. Tarr, and S. L. Zipursky, 2008 A glucagon-like endocrine pathway in Drosophila modulates both lipid and carbohydrate homeostasis. J. Exp. Biol. 211: 3103–10.

Billington, C. J., J. E. Briggs, M. Grace, and A. S. Levine, 1991 Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. - Regul. Integr. Comp. Physiol.

Borbely, A. A., 1982 A two process model of sleep regulation. Hum. Neurobiol.

Borbély, A. A., S. Daan, A. Wirz-Justice, and T. Deboer, 2016 The two-process model of sleep regulation: A reappraisal. J. Sleep Res. 25: 131–143.

Brand, A. H., and N. Perrimon, 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118: 401–15.

Britton, J. S., and B. A. Edgar, 1998 Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development 125: 2149–58.

Britton, J. S., W. K. Lockwood, L. Li, S. M. Cohen, and B. A. Edgar, 2002 Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. Dev. Cell 2: 239–49.

Brogiolo, W., H. Stocker, T. Ikeya, F. Rintelen, R. Fernandez *et al.*, 2001 An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr. Biol. 11: 213–21.

Broughton, S. J., M. D. W. Piper, T. Ikeya, T. M. Bass, J. Jacobson *et al.*, 2005 Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. U. S. A. 102: 3105–10.

Brown, E. B., M. E. Slocumb, M. Szuperak, A. Kerbs, A. G. Gibbs *et al.*, 2019a Starvation resistance is associated with developmentally specified changes in sleep, feeding and metabolic rate. J. Exp. Biol. 222: 1–9.

Brown, E. B., M. E. Slocumb, M. Szuperak, A. Kerbs, A. G. Gibbs *et al.*, 2019b Starvation resistance is associated with developmentally specified changes in sleep, feeding and metabolic rate. J. Exp. Biol. 222:.

Brown, E. B., J. Torres, R. A. Bennick, V. Rozzo, A. Kerbs *et al.*, 2018a Variation in sleep and metabolic function is associated with latitude and average temperature in Drosophila melanogaster. Ecol. Evol. 4084– 4097.

Brown, E. B., J. Torres, R. A. Bennick, V. Rozzo, A. Kerbs *et al.*, 2018b Variation in sleep and metabolic function is associated with latitude and average temperature in Drosophila melanogaster. Ecol. Evol. 8: 4084–4097.

Burg, E. D., S. T. Langan, and H. A. Nash, 2013 Drosophila social clustering is disrupted by anesthetics and in narrow abdomen ion channel mutants. Genes, Brain Behav. 12: 338–347.

Bushey, D., K. A. Hughes, G. Tononi, and C. Cirelli, 2010 Sleep, aging, and lifespan in Drosophila. BMC Neurosci. 11: 56.

Bushey, D., G. Tononi, and C. Cirelli, 2009 The Drosophila fragile X mental retardation gene regulates sleep need. J. Neurosci. 29: 1948–1961.

Calipari, E. S., and M. J. Ferris, 2013 Amphetamine mechanisms and actions at the dopamine terminal revisited. J. Neurosci.

Campbell, S. S., and I. Tobler, 1984 Animal sleep: a review of sleep duration across phylogeny. Neurosci. Biobehav. Rev. 8: 269–300.

Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Pennington, and M. A. Wells, 2001a Fat metabolism in insects. Annu. Rev. Nutr. 21: 23–46.

Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Pennington, and M. A. Wells, 2001b Fat metabolism in insects. Annu. Rev. Nutr.

Carhan, A., K. Tang, C. A. Shirras, A. D. Shirras, and R. E. Isaac, 2011 Loss of Angiotensin-converting enzyme-related (ACER) peptidase disrupts night-time sleep in adult Drosophila melanogaster. J. Exp. Biol. 214: 680–6.

Catterson, J. H., S. Knowles-Barley, K. James, M. M. S. Heck, A. J. Harmar *et al.*, 2010 Dietary modulation of Drosophila sleep-wake behaviour. PLoS One 5: e12062.

Chapman, T., and L. Partridge, 1996 Female fitness in Drosophila melanogaster: an interaction between the effect of nutrition and of encounter rate with males. Proc. Biol. Sci. 263: 755–9.

Chemelli, R. M., J. T. Willie, C. M. Sinton, J. K. Elmquist, T. Scammell *et al.*, 1999 Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation. Cell 98: 437–451.

Chen, W., W. Shi, L. Li, Z. Zheng, T. Li *et al.*, 2013 Regulation of sleep by the short neuropeptide F (sNPF) in Drosophila melanogaster. Insect Biochem. Mol. Biol.

Chennathukuzhi, V., J. M. Stein, T. Abel, S. Donlon, S. Yang *et al.*, 2003 Mice Deficient for Testis-Brain RNA-Binding Protein Exhibit a Coordinate Loss of TRAX, Reduced Fertility, Altered Gene Expression in the Brain, and Behavioral Changes. Mol. Cell. Biol.

Chiu, J. C., K. H. Low, D. H. Pike, E. Yildirim, and I. Edery, 2010 Assaying Locomotor Activity to Study Circadian Rhythms and Sleep Parameters in Drosophila J. Vis. Exp.

Chung, B. Y., V. L. Kilman, J. R. Keath, J. L. Pitman, and R. Allada, 2009 The GABAA Receptor RDL Acts in Peptidergic PDF Neurons to Promote Sleep in Drosophila. Curr. Biol. 19: 386–390.

Cirelli, C., D. Bushey, S. Hill, R. Huber, R. Kreber *et al.*, 2005 Reduced sleep in Drosophila Shaker mutants. Nature 434: 1087–1092.

Claussen, M., R. Koch, Z.-Y. Jin, and B. Suter, 2006 Functional characterization of Drosophila Translin and Trax. Genetics 174: 1337–47.

Claußen, M., R. Koch, Z. Y. Jin, and B. Suter, 2006 Functional characterization of Drosophila Translin and Trax. Genetics 174: 1337–1347.

Cochet-Bissuel, M., P. Lory, and A. Monteil, 2014 The sodium leak channel, NALCN, in health and disease. Front. Cell. Neurosci.

Cong, X., H. Wang, Z. Liu, C. He, C. An *et al.*, 2015 Regulation of Sleep by Insulin-like Peptide System in Drosophila melanogaster. Sleep 38: 1075–83.

Crittenden, J. R., E. M. C. Skoulakis, K.-A. Han, D. Kalderon, and R. L. Davis, 1998 Tripartite Mushroom Body Architecture Revealed by Antigenic Markers. Learn. Mem. 5: 38–51.

Crocker, A., and A. Sehgal, 2008 Octopamine regulates sleep in Drosophila through protein kinase A-dependent mechanisms. J. Neurosci. 28: 9377–9385.

Crocker, A., M. Shahidullah, I. B. Levitan, and A. Sehgal, 2010 Identification of a Neural Circuit that Underlies the Effects of Octopamine on Sleep:Wake Behavior. Neuron 65: 670–681.

Danguir, J., and S. Nicolaidis, 1979 Dependence of sleep on nutrient's availability. Physiol. Behav. 22: 735–740.

Dethier, V. G., 1977 The Hungry Fly: A Physiological Study of the Behavior Associated With Feeding. JAMA J. Am. Med. Assoc. 237: 1010.

DiAngelo, J. R., and M. J. Birnbaum, 2009 Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol. Cell. Biol. 29: 6341–52.

DiAngelo, J. R., R. Erion, A. Crocker, and A. Sehgal, 2011 The central clock neurons regulate lipid storage in Drosophila. PLoS One 6:.

Dietzl, G., D. Chen, F. Schnorrer, K.-C. Su, Y. Barinova *et al.*, 2007 A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature 448: 151–6.

Dissel, S., V. Angadi, L. Kirszenblat, Y. Suzuki, J. Donlea *et al.*, 2015 Sleep restores behavioral plasticity to drosophila mutants. Curr. Biol. 25: 1270–1281.

Donelson, N., E. Z. Kim, J. B. Slawson, C. G. Vecsey, R. Huber *et al.*, 2012 High-resolution positional tracking for long-term analysis of Drosophila sleep and locomotion using the “tracker” program. PLoS One 7:.

Donlea, J., A. Leahy, M. S. Thimgan, Y. Suzuki, B. N. Hughson *et al.*, 2012 Foraging alters resilience/vulnerability to sleep disruption and starvation in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 109: 2613–8.

Donlea, J. M., D. Pimentel, C. B. Talbot, A. Kempf, J. J. Omoto *et al.*, 2018 Recurrent Circuitry for Balancing Sleep Need and Sleep. Neuron 97: 378-389.e4.

Donlea, J. M., M. S. Thimgan, Y. Suzuki, L. Gottschalk, and P. J. Shaw, 2011 Inducing sleep by remote control facilitates memory consolidation in Drosophila. Science 332: 1571–1576.

Dryden, S., L. Pickavance, H. M. Frankish, and G. Williams, 1995 Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. Brain Res.

Dubnau, J., L. Grady, T. Kitamoto, and T. Tully, 2001 Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature 411: 476–480.

Dus, M., J. S. Y. Lai, K. M. Gunapala, S. Min, T. D. Tayler *et al.*, 2015 Nutrient Sensor in the Brain Directs the Action of the Brain-Gut Axis in Drosophila. Neuron 87: 139–151.

Dus, M., S. Min, A. C. Keene, G. Y. Lee, and G. S. B. Suh, 2011 Taste-independent detection of the caloric content of sugar in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 108: 11644–11649.

Erion, R., J. R. DiAngelo, A. Crocker, and A. Sehgal, 2012 Interaction between sleep and metabolism in Drosophila with altered octopamine signaling. J. Biol. Chem. 287: 32406–32414.

Erion, R., and A. Sehgal, 2013 Regulation of insect behavior via the insulin-signaling pathway. Front. Physiol. 4 DEC: 1–6.

Ertekin, D., L. Kirszenblat, R. Faville, and B. van Swinderen, 2020 Down-regulation of a cytokine secreted from peripheral fat bodies improves visual attention while reducing sleep in Drosophila. PLOS Biol. 18: e3000548.

Faville, R., B. Kottler, G. J. Goodhill, P. J. Shaw, and B. van Swinderen, 2015 How deeply does your mutant sleep? Probing arousal to better understand sleep defects in Drosophila. Sci. Rep. 5: 8454.

Fischer, J. A., E. Giniger, T. Maniatis, and M. Ptashne, 1988 GAL4 activates transcription in Drosophila. Nature 332: 853–856.

Ford, E. S., W. H. Giles, and W. H. Dietz, 2002 Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287: 356–9.

Fridell, Y.-W. C., M. Hoh, O. Kréneisz, S. Hosier, C. Chang *et al.*, 2009 Increased uncoupling protein (UCP) activity in Drosophila insulin-producing neurons attenuates insulin signaling and extends lifespan. Aging (Albany. NY). 1: 699–713.

Friggi-Grelin, F., H. Coulom, M. Meller, D. Gomez, J. Hirsh *et al.*, 2003 Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. J. Neurobiol. 54: 618–627.

Fu, X., A. P. Shah, Z. Li, M. Li, K. L. Tamashiro *et al.*, 2020 Genetic inactivation of the translin/trax microRNA-degrading enzyme phenocopies the robust adiposity induced by Translin (Tsn) deletion. Mol. Metab.

Garbe, D. S., W. L. Bollinger, A. Vigderman, P. Masek, J. Gertowski *et al.*, 2015 Context-specific comparison of sleep acquisition systems in Drosophila. Biol. Open 4: 1558–1568.

Gilestro, G. F., 2012 Video tracking and analysis of sleep in Drosophila melanogaster. Nat. Protoc. 7: 995–1007.

Gingras, R. M., M. E. Warren, A. A. Nagengast, and J. R. Diangelo, 2014 The control of lipid metabolism by mRNA splicing in Drosophila. Biochem. Biophys. Res. Commun. 443: 672–676.

Good, T. P., and M. Tatar, 2001 Age-specific mortality and reproduction respond to adult dietary restriction in Drosophila melanogaster. J. Insect Physiol. 47: 1467–1473.

Grandison, R. C., M. D. W. Piper, and L. Partridge, 2009 Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. Nature 462: 1061–4.

Grandner, M. A., N. Jackson, J. R. Gerstner, and K. L. Knutson, 2014a Sleep symptoms associated with intake of specific dietary nutrients. J. Sleep Res. 23: 22–34.

Grandner, M. A., N. Jackson, J. R. Gerstner, and K. L. Knutson, 2014b Sleep symptoms associated with intake of specific dietary nutrients. J. Sleep Res. 23: 22–34.

Grandner, M. A., D. F. Kripke, N. Naidoo, and R. D. Langer, 2010 Relationships among dietary nutrients and subjective sleep, objective sleep, and napping in women. Sleep Med. 11: 180–184.

Green, C. B., J. S. Takahashi, and J. Bass, 2008 The Meter of Metabolism. Cell 134: 728–742.

Greenspan, R. J., G. Tononi, C. Cirelli, and P. J. Shaw, 2001 Sleep and the fruit fly. Trends Neurosci. 24: 142–145.

Griffith, L. C., 2013 Neuromodulatory control of sleep in Drosophila melanogaster: Integration of competing and complementary behaviors. Curr. Opin. Neurobiol. 23: 819–823.

Grönke, S., A. Mildner, S. Fellert, N. Tennagels, S. Petry *et al.*, 2005 Brummer lipase is an evolutionary conserved fat storage regulator in Drosophila. Cell Metab. 1: 323–30.

Gu, H., 2006 Cholinergic Synaptic Transmission in Adult Drosophila Kenyon Cells In Situ. J. Neurosci. 26: 265–272.

Guo, F., M. Holla, M. M. Díaz, and M. Rosbash, 2018 A Circadian Output Circuit Controls Sleep-Wake Arousal in Drosophila. Neuron.

Guo, F., W. Yi, M. Zhou, and A. Guo, 2011 Go signaling in mushroom bodies regulates sleep in Drosophila. Sleep 34: 273–81.

Harbison, S. T., S. Chang, K. P. Kamdar, and T. F. C. Mackay, 2005 Quantitative genomics of starvation stress resistance in Drosophila. Genome Biol. 6:.

Harbison, S. T., A. H. Yamamoto, J. J. Fanara, K. K. Norga, and T. F. C. Mackay, 2004 Quantitative trait loci affecting starvation resistance in Drosophila melanogaster. Genetics 166: 1807–1823.

Hardie, R. C., 1989 A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse. Nature.

Hardy, C. M., M. K. Burke, L. J. Everett, M. V. Han, K. M. Lantz *et al.*, 2018 Genome-Wide Analysis of Starvation-Selected Drosophila melanogaster-A Genetic Model of Obesity. Mol. Biol. Evol.

De Haro, M., I. Al-Ramahi, J. Benito-Sipos, B. López-Arias, B. Dorado *et al.*, 2010 Detailed analysis of leucokinin-expressing neurons and their candidate functions in the Drosophila nervous system. Cell Tissue Res. 339: 321–336.

Hartmann, E., 1974 The function of sleep. Annu. Psychoanal. 2: 271–289.

Hartmann, E. L., 1973 *The Functions of Sleep*. Yale University Press.

Haynes, P. R., B. L. Christmann, and L. C. Griffith, 2015 A single pair of neurons links sleep to memory consolidation in Drosophila melanogaster. Elife 4: 1–24.

Healy, K., 2016 A Theory of Human Motivation by Abraham H. Maslow - reflection. Br. J. Psychiatry 208: 313.

Heisenberg, M., A. Borst, S. Wagner, and D. Byers, 1985 drosophila mushroom body mutants are deficient in olfactory learning: Research papers. J. Neurogenet.

Hendricks, J. C., S. M. Finn, K. A. Panckeri, J. Chavkin, J. A. Williams *et al.*, 2000 Rest in Drosophila is a sleep-like state. Neuron 25: 129–38.

Hill, V. M., R. M. O'Connor, G. B. Sissoko, I. S. Irobunda, S. Leong *et al.*, 2018 A bidirectional relationship between sleep and oxidative stress in Drosophila. PLoS Biol. 16: e2005206.

Hong, S. T., S. Bang, D. Paik, J. Kang, S. Hwang *et al.*, 2006 Histamine and its receptors modulate temperature-preference behaviors in Drosophila. J. Neurosci.

Van Der Horst, D. J., 2003 Insect adipokinetic hormones: Release and integration of flight energy metabolism. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 136: 217–226.

Huang, T., and S. Redline, 2019 Cross-sectional and prospective associations of actigraphy-assessed sleep regularity with metabolic abnormalities: The multi-ethnic study of atherosclerosis. Diabetes Care 42: 1422–1429.

Humphrey, J. A., K. S. Hamming, C. M. Thacker, R. L. Scott, M. M. Sedensky *et al.*, 2007 A Putative Cation Channel and Its Novel Regulator: Cross-Species Conservation of Effects on General Anesthesia. Curr. Biol. 17: 624–629.

Ikeya, T., M. Galic, P. Belawat, K. Nairz, and E. Hafen, 2002 Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Curr. Biol. 12: 1293–300.

Isabel, G., J.-R. Martin, S. Chidami, J. A. Veenstra, and P. Rosay, 2005 AKH-producing neuroendocrine cell ablation decreases trehalose and induces behavioral changes in Drosophila. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288: R531-8.

Ishimoto, H., and T. Kitamoto, 2010 The steroid molting hormone ecdysone regulates sleep in adult Drosophila melanogaster. Genetics.

Itskov, P. M., and C. Ribeiro, 2013 The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in Drosophila. Front. Neurosci. 7: 12.

Ja, W. W., G. B. Carvalho, E. M. Mak, N. N. de la Rosa, A. Y. Fang *et al.*, 2007 Prandiology of Drosophila and the CAFE assay. Proc. Natl. Acad. Sci. U. S. A. 104: 8253–8256.

Jaendling, A., and R. J. McFarlane, 2010 Biological roles of translin and translin-associated factor-X: RNA metabolism comes to the fore. Biochem. J. 429: 225–34.

Jiang, Y., E. Pitmon, J. Berry, F. W. Wolf, Z. Mckenzie *et al.*, 2016 A Genetic Screen To Assess Dopamine Receptor (DopR1) Dependent Sleep Regulation in Drosophila. G3 Genes, Genomes, Genet. 6:.

Joiner, W. J., 2016 Unraveling the Evolutionary Determinants of Sleep. Curr. Biol. 26: R1073–R1087.

Joiner, W. J., A. Crocker, B. H. White, and A. Sehgal, 2006a Sleep in Drosophila is regulated by adult mushroom bodies. Nature 441: 757–760.

Joiner, W. J., A. Crocker, B. H. White, and A. Sehgal, 2006b Sleep in Drosophila is regulated by adult mushroom bodies. Nature.

Joiner, W. J., E. B. Friedman, H. T. Hung, K. Koh, M. Sowcik *et al.*, 2013 Genetic and Anatomical Basis of the Barrier Separating Wakefulness and Anesthetic-Induced Unresponsiveness. PLoS Genet. 9: 1–12.

Joiner, M. A., and L. C. Griffith, 2000 Visual Input Regulates Circuit Configuration in Courtship Conditioning of Drosophila melanogaster. Learn. Mem. 7: 32–42.

Jumbo-Lucioni, P., J. F. Ayroles, M. M. Chambers, K. W. Jordan, J. Leips *et al.*, 2010 Systems genetics analysis of body weight and energy metabolism traits in Drosophila melanogaster. BMC Genomics 11: 297.

Juneau, B. A., J. M. Stonemetz, R. F. Toma, D. R. Possidente, R. C. Heins *et al.*, 2019 Optogenetic activation of short neuropeptide F (sNPF) neurons induces sleep in Drosophila melanogaster. Physiol. Behav.

Kayser, M. S., Z. Yue, and A. Sehgal, 2014 A critical period of sleep for development of courtship circuitry and behavior in Drosophila. Science (80-.). 344: 269–74.

Keebaugh, E. S., R. Yamada, and W. W. Ja, 2019 The nutritional environment influences the impact of microbes on Drosophila melanogaster life Span. MBio.

Keene, A. C., E. R. Duboué, D. M. McDonald, M. Dus, G. S. B. Suh *et al.*, 2010a Clock and cycle limit starvation-induced sleep loss in Drosophila. Curr. Biol. 20: 1209–15.

Keene, A. C., E. R. Duboué, D. M. McDonald, M. Dus, G. S. B. Suh *et al.*, 2010b Clock and cycle limit starvation-induced sleep loss in drosophila. Curr. Biol. 20:.

Ki, Y., and C. Lim, 2019 Sleep-promoting effects of threonine link amino acid metabolism in Drosophila neuron to GABAergic control of sleep drive. Elife 8: 1–24.

Kim, S. K., and E. J. Rulifson, 2004a Conserved mechanisms of glucose sensing and regulation by *Drosophila* corpora cardiaca cells. Nature 431: 316–320.

Kim, S. K., and E. J. Rulifson, 2004b Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. 431: 316–320.

Kitamoto, T., 2001 Conditional modification of behavior inDrosophila by targeted expression of a temperature-sensitiveshibire allele in defined neurons. J. Neurobiol. 47: 81–92.

Knutson, K. L., and E. Van Cauter, 2008 Associations between sleep loss and increased risk of obesity and diabetes. Ann. N. Y. Acad. Sci. 1129: 287–304.

Koh, K., J. M. Evans, J. C. Hendricks, and A. Sehgal, 2006 A Drosophila model for age-associated changes in sleep:wake cycles. Proc. Natl. Acad. Sci. U. S. A. 103: 13843–13847.

Koh, K., W. J. Joiner, M. N. Wu, Z. Yue, C. J. Smith *et al.*, 2008a Identification of SLEEPLESS, a sleep-promoting factor. Science 321: 372–376.

Koh, K., W. J. Joiner, M. N. Wu, Z. Yue, C. J. Smith *et al.*, 2008b Identification of SLEEPLESS, a Sleep-Promoting Factor. Science (80-.). 321: 372–376.

Krashes, M. J., A. C. Keene, B. Leung, J. D. Armstrong, and S. Waddell, 2007 Sequential use of mushroom body neuron subsets during drosophila odor memory processing. Neuron 53: 103–15.

Kréneisz, O., X. Chen, Y.-W. C. Fridell, and D. K. Mulkey, 2010 Glucose increases activity and Ca2+ in insulin-producing cells of adult Drosophila. Neuroreport 21: 1116–20.

Kume, K., S. Kume, S. K. Park, J. Hirsh, and F. R. Jackson, 2005 Cellular/Molecular Dopamine Is a Regulator of Arousal in the Fruit Fly. J. Neurosci. 25: 7377–7384.

Laposky, A. D., 2005 Altered sleep regulation in leptin-deficient mice. AJP Regul. Integr. Comp. Physiol. 290: R894–R903.

Laposky, A. D., J. Shelton, J. Bass, C. Dugovic, N. Perrino *et al.*, 2006 Altered sleep regulation in leptin-deficient mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290: R894-903.

Lazareva, A. a, G. Roman, W. Mattox, P. E. Hardin, and B. Dauwalder, 2007 A role for the adult fat body in Drosophila male courtship behavior. PLoS Genet. 3: e16.

Lear, B. C., E. J. Darrah, B. T. Aldrich, S. Gebre, R. L. Scott *et al.*, 2013 UNC79 and UNC80, putative auxiliary subunits of the NARROW ABDOMEN ion channel, are indispensable for robust circadian locomotor rhythms in Drosophila. PLoS One 8:.

Lear, B. C., J. M. Lin, J. R. Keath, J. J. McGill, I. M. Raman *et al.*, 2005 The ion channel narrow abdomen is critical for neural output of the Drosophila circadian pacemaker. Neuron 48: 965–976.

Lebestky, T., J.-S. C. Chang, H. Dankert, L. Zelnik, Y.-C. Kim *et al.*, 2009 Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. Neuron 64: 522–36.

Lee, T., and L. Luo, 1999 Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22: 451–61.

Lee, W.-C., and C. A. Micchelli, 2013 Development and characterization of a chemically defined food for Drosophila. PLoS One 8: e67308.

Lee, G., and J. H. Park, 2004 Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in Drosophila melanogaster. Genetics 167: 311–323.

Leng, Y., F. P. Cappuccio, P. G. Surtees, R. Luben, C. Brayne *et al.*, 2016 Daytime napping, sleep duration and increased 8-year risk of type 2 diabetes in a British population. Nutr. Metab. Cardiovasc. Dis. 26: 996–1003.

Li, Z., Y. Wu, and J. M. Baraban, 2008 The Translin/Trax RNA binding complex: Clues to function in the nervous system. Biochim. Biophys. Acta - Gene Regul. Mech.

Linford, N. J., T. P. Chan, and S. D. Pletcher, 2012 Re-patterning sleep architecture in Drosophila through gustatory perception and nutritional quality. PLoS Genet. 8: e1002668.

Liu, Q., S. Liu, L. Kodama, M. R. Driscoll, and M. N. Wu, 2012 Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. Curr. Biol. 22: 2114–23.

Liu, S., Q. Liu, M. Tabuchi, and M. N. Wu, 2016 Sleep drive is encoded by neural plastic changes in a dedicated circuit. Cell 165:.

Liu, Y., J. Luo, M. Carlsson, and D. Nassel, 2015 Serotonin and insulin-like peptides modulate leucokinin-producing neurons that affect feeding and water homeostasis in Drosophila. J Comp Neurol 12: 1840–63.

Liu, L., R. Wolf, R. Ernst, and M. Heisenberg, 1999 Context generalization in Drosophila visual learning requires the mushroom bodies. Nature 400: 753–756.

Lopaschuk, G. D., S. R. Wall, P. M. Olley, and N. J. Davies, 1988 Etomoxir, a carnitine palmitoyltransferase I inhibitor, protects hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine. Circ. Res. 63: 1036–1043.

Luan, H., N. C. Peabody, C. R. Vinson, and B. H. White, 2006 Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. Neuron 52: 425–36.

Luan, H., N. C. Peabody, C. R. Vinson, B. H. White, L. Li *et al.*, 2012 Hypothalamic regulation of sleep and circadian rhythms. J. Neurosci. 2: pl6.

Lundius, E. G., M. Sanchez-Alavez, Y. Ghochani, J. Klaus, and I. V. Tabarean, 2010 Histamine influences body temperature by acting at H1 and H3 receptors on distinct populations of preoptic neurons. J. Neurosci.

Ly, S., A. I. Pack, and N. Naidoo, 2018 The neurobiological basis of sleep: Insights from Drosophila. Neurosci. Biobehav. Rev. 87: 67–86.

MacFadyen, U. M., I. Oswald, and S. A. Lewis, 1973 Starvation and human slow-wave sleep. J. Appl. Physiol. 35: 391–4.

Macosko, E. Z., A. Basu, R. Satija, J. Nemesh, K. Shekhar *et al.*, 2015 Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell.

Mair, W., M. D. W. Piper, and L. Partridge, 2005 Calories do not explain extension of life span by dietary restriction in Drosophila. PLoS Biol. 3: e223.

Manière, G., A. B. Ziegler, F. Geillon, D. E. Featherstone, and Y. Grosjean, 2016 Direct Sensing of Nutrients via a LAT1-like Transporter in Drosophila Insulin-Producing Cells. Cell Rep. 17: 137–148.

Mao, Z., and R. L. Davis, 2009 Eight different types of dopaminergic neurons innervate the Drosophila mushroom body neuropil: Anatomical and physiological heterogeneity. Front. Neural Circuits 3: 1–17.

Martin, J. R., R. Ernst, and M. Heisenberg, 1998 Mushroom bodies suppress locomotor activity in Drosophila melanogaster. Learn. Mem. 5: 179–191.

Masek, P., L. a Reynolds, W. L. Bollinger, C. Moody, A. Mehta *et al.*, 2014a Altered regulation of sleep and feeding contribute to starvation resistance in Drosophila. J. Exp. Biol. 217: 3122–3132.

Masek, P., L. A. Reynolds, W. L. Bollinger, C. Moody, A. Mehta *et al.*, 2014b Altered regulation of sleep and feeding contributes to starvation resistance in Drosophila melanogaster. J. Exp. Biol. 217:.

Masek, P., and K. Scott, 2010 Limited taste discrimination in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 107: 14833–14838.

Mattaliano, M. D., E. S. Montana, K. M. Parisky, J. T. Littleton, and L. C. Griffith, 2007 The Drosophila ARC homolog regulates behavioral responses to starvation. Mol. Cell. Neurosci. 36: 211–221.

McBride, S. M. ., G. Giuliani, C. Choi, P. Krause, D. Correale *et al.*, 1999 Mushroom body ablation impairs short-term memory and long-term memory of courtship conditioning in Drosophila melanogaster. Neuron 24: 967–77.

McCarthy, E. V., Y. Wu, T. deCarvalho, C. Brandt, G. Cao *et al.*, 2011 Synchronized bilateral synaptic inputs to Drosophila melanogaster neuropeptidergic rest/arousal neurons. J. Neurosci. 31: 8181–8193.

McDonald, D. M., and A. C. Keene, 2010 The sleep-feeding conflict: Understanding behavioral integration through genetic analysis in Drosophila. Aging (Albany. NY). 2: 519–22.

Meng, G., K. Aoki, K. Tokura, K. Nakahara, J. Inazawa *et al.*, 2000 Genomic structure and chromosomal localization of the gene encoding TRAX, a translin-associated factor X. J. Hum. Genet.

Mesarwi, O., J. Polak, J. Jun, and V. Y. Polotsky, 2013 Sleep disorders and the development of insulin resistance and obesity. Endocrinol. Metab. Clin. North Am. 42: 617–34.

Metaxakis, A., L. S. Tain, S. Grönke, O. Hendrich, Y. Hinze *et al.*, 2014 Lowered Insulin Signalling Ameliorates Age-Related Sleep Fragmentation in Drosophila. PLoS Biol. 12:.

Misra, S., M. A. Crosby, C. J. Mungall, B. B. Matthews, K. S. Campbell *et al.*, 2002 Annotation of the Drosophila melanogaster euchromatic genome: a systematic review. Genome Biol. 3: RESEARCH0083.

Miyamoto, T., J. Slone, X. Song, and H. Amrein, 2012 A fructose receptor functions as a nutrient sensor in the drosophila brain. Cell 151: 1113–1125.

Miyamoto, T., G. Wright, and H. Amrein, 2013 Nutrient sensors. Curr. Biol. 23:.

Moose, D. L., S. J. Haase, B. T. Aldrich, and B. C. Lear, 2017 The Narrow Abdomen Ion Channel Complex Is Highly Stable and Persists from Development into Adult Stages to Promote Behavioral Rhythmicity. Front. Cell. Neurosci. 11: 159.

Morton, G. J., and M. W. Schwartz, 2011 Leptin and the central nervous system control of glucose metabolism. Physiol. Rev. 91: 389–411.

Murakami, K., and A. C. Keene, 2014 Development: Better sleep on it, children. Curr. Biol. 24: R569–R571.

Murakami, K., M. E. Yurgel, B. A. Stahl, P. Masek, A. Mehta *et al.*, 2016 Translin Is Required for Metabolic Regulation of Sleep. Curr. Biol. 26: 972–980.

Nash, H. A., R. L. Scott, B. C. Lear, and R. Allada, 2002 An unusual cation channel mediates photic control of locomotion in Drosophila. Curr. Biol. 12: 2152–2158.

Nässel, D. R., and Å. M. E. Winther, 2010 Drosophila neuropeptides in regulation of physiology and behavior. Prog. Neurobiol. 92: 42–104.

Nässel, D. R., and M. Zandawala, 2019 Recent advances in neuropeptide signaling in Drosophila, from genes to physiology and behavior. Prog. Neurobiol.

Ni, J. Q., L. P. Liu, R. Binari, R. Hardy, H. S. Shim *et al.*, 2009 A drosophila resource of transgenic RNAi lines for neurogenetics. Genetics 182: 1089–1100.

Ni, J. Q., M. Markstein, R. Binari, B. Pfeiffer, L. P. Liu *et al.*, 2008 Vector and parameters for targeted transgenic RNA interference in Drosophila melanogaster. Nat. Methods 5: 49–51.

Nitz, D. A., B. Van Swinderen, G. Tononi, and R. J. Greenspan, 2002 Electrophysiological correlates of rest and activity in Drosophila melanogaster. Curr. Biol. 12: 1934–1940.

Niwa, Y., G. N. Kanda, R. G. Yamada, S. Shi, G. A. Sunagawa *et al.*, 2018 Muscarinic Acetylcholine Receptors Chrm1 and Chrm3 Are Essential for REM Sleep. Cell Rep. 24: 2231-2247.e7.

Oh, Y., D. Jang, J. Y. Sonn, and J. Choe, 2013 Histamine-HisCl1 Receptor Axis Regulates Wake-Promoting Signals in Drosophila melanogaster. PLoS One 8:.

Okada, R., T. Awasaki, and K. Ito, 2009 Gamma-aminobutyric acid (GABA)-mediated neural connections in the Drosophila antennal lobe. J. Comp. Neurol. 514: 74–91.

Osterwalder, T., K. S. Yoon, B. H. White, and H. Keshishian, 2001 A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. U. S. A. 98: 12596–12601.

Padmanabha, D., and K. D. Baker, 2014 Drosophila gains traction as a repurposed tool to investigate metabolism. Trends Endocrinol. Metab. 25: 518–527.

Pantazis, A., A. Segaran, C. H. Liu, A. Nikolaev, J. Rister *et al.*, 2008 Distinct roles for two histamine receptors (hclA and hclB) at the Drosophila photoreceptor synapse. J. Neurosci.

Parisky, K. M., J. Agosto, S. R. Pulver, Y. Shang, E. Kuklin *et al.*, 2008 PDF Cells Are a GABA-Responsive Wake-Promoting Component of the Drosophila Sleep Circuit. Neuron 60: 672–682.

Park, D., J. a Veenstra, J. H. Park, and P. H. Taghert, 2008 Mapping peptidergic cells in Drosophila: where DIMM fits in. PLoS One 3: e1896.

Pascual, A., 2001 Localization of Long-Term Memory Within the Drosophila Mushroom Body. Science (80-.). 294: 1115–1117.

Peppard, P. E., T. Young, M. Palta, J. Dempsey, and J. Skatrud, 2000 Longitudinal Study of Moderate Weight Change and Sleep-Disordered Breathing. JAMA 284: 3015.

Perkins, L. A., L. Holderbaum, R. Tao, Y. Hu, R. Sopko *et al.*, 2015 The transgenic RNAi project at Harvard medical school: Resources and validation. Genetics 201: 843–852.

Petit, J. M., S. Burlet-Godinot, P. J. Magistretti, and I. Allaman, 2014 Glycogen metabolism and the homeostatic regulation of sleep. Metab. Brain Dis. 30: 263–279.

Pfeiffenberger, C., B. C. Lear, K. P. Keegan, and R. Allada, 2010a Locomotor activity level monitoring using the Drosophila activity monitoring (DAM) system. Cold Spring Harb. Protoc. 5: 1238–1242.

Pfeiffenberger, C., B. C. Lear, K. P. Keegan, and R. Allada, 2010b Processing sleep data created with the Drosophila activity monitoring (DAM) system. Cold Spring Harb. Protoc. 5:.

Pfeiffer, B. D., A. Jenett, A. S. Hammonds, T.-T. B. Ngo, S. Misra *et al.*, 2008 Tools for neuroanatomy and neurogenetics in Drosophila. Proc. Natl. Acad. Sci. 105: 9715–9720.

Pickeral, O. K., J. Z. Li, I. Barrow, M. S. Boguski, W. Makałowski *et al.*, 2000 Classical oncogenes and tumor suppressor genes: a comparative genomics perspective. Neoplasia 2: 280–6.

Pierce-Shimomura, J. T., B. L. Chen, J. J. Mun, R. Ho, R. Sarkis *et al.*, 2008 Genetic analysis of crawling and swimming locomotory patterns in C. elegans. Proc. Natl. Acad. Sci. U. S. A.

Piper, M. D. W., E. Blanc, R. Leitão-Gonçalves, M. Yang, X. He *et al.*, 2014 A holidic medium for Drosophila melanogaster. Nat. Methods 11: 100–5.

Pitman, J. L., J. J. McGill, K. P. Keegan, and R. Allada, 2006a A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature.

Pitman, J. L., J. J. McGill, K. P. Keegan, and R. Allada, 2006b A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature 441: 753–756.

Pratt, A. J., and I. J. MacRae, 2009 The RNA-induced silencing complex: A versatile gene-silencing machine. J. Biol. Chem.

Puschner, B., and J. Schacht, 1997 Energy metabolism in cochlear outer hair cells in vitro. Hear. Res. 114: 102–106.

Rajan, A., and N. Perrimon, 2012 Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. Cell 151: 123–37.

Reiter, L. T., L. Potocki, S. Chien, M. Gribskov, and E. Bier, 2001 A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. Genome Res. 11: 1114–1125.

Reutrakul, S., and E. Van Cauter, 2018 Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. Metabolism.

Riabinina, O., D. Luginbuhl, E. Marr, S. Liu, M. N. Wu *et al.*, 2015 Improved and expanded Q-system reagents for genetic manipulations. Nat. Methods 12: 219–222.

Rivas, G. B. S., L. G. S. da R. Bauzer, and A. C. A. Meireles-Filho, 2016 "The environment is everything that isn't me": Molecular mechanisms and evolutionary dynamics of insect clocks in variable surroundings. Front. Physiol. 6:.

Ro, J., Z. M. Harvanek, and S. D. Pletcher, 2014 FLIC: high-throughput, continuous analysis of feeding behaviors in Drosophila. PLoS One 9: e101107.

Rogulja, D., and M. W. Young, 2012 Control of sleep by cyclin A and its regulator. Science 335: 1617–21.

Roman, G., K. Endo, L. Zong, and R. L. Davis, 2001 P[Switch], a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. 98: 12602–12607.

Rulifson, E. J., S. K. Kim, and R. Nusse, 2002 Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296: 1118–20.

Saltiel, A. R., and C. R. Kahn, 2001 Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799–806.

Sarikaya, D. P., J. Cridland, A. Tarakji, H. Sheehy, S. Davis *et al.*, 2020 Phenotypic coupling of sleep and starvation resistance evolves in D. melanogaster. BMC Evol. Biol. 20:.

Sassu, E. D., J. E. McDermott, B. J. Keys, M. Esmaeili, A. C. Keene *et al.*, 2012 Mio/dChREBP coordinately increases fat mass by regulating lipid synthesis and feeding behavior in Drosophila. Biochem. Biophys. Res. Commun. 426: 43–48.

Schoenborn, C. A., and P. E. Adams, 2010 Health behaviors of adults: United States, 2005-2007. Vital Health Stat. 10. 1–132.

Schwasinger-Schmidt, T. E., S. D. Kachman, and L. G. Harshman, 2012 Evolution of starvation resistance in Drosophila melanogaster: Measurement of direct and correlated responses to artificial selection. J. Evol. Biol. 25: 378–387.

Sedensky, M. M., and P. M. Meneely, 1987 Genetic analysis of halothane sensitivity in Caenorhabditis elegans. Science (80-.).

Sehgal, A., and E. Mignot, 2011 Genetics of sleep and sleep disorders. Cell 146: 194–207.

Seugnet, L., Y. Suzuki, M. Thimgan, J. Donlea, S. I. Gimbel *et al.*, 2009 Identifying sleep regulatory genes using a Drosophila model of insomnia. J. Neurosci. 29: 7148–7157.

Shafer, O. T., and A. C. Keene, 2021 The Regulation of Drosophila Sleep. Curr. Biol. 31: R38–R49.

Shafer, O. T., and P. H. Taghert, 2009 RNA-interference knockdown of drosophila pigment dispersing factor in neuronal subsets: The anatomical basis of a neuropeptide's circadian functions. PLoS One 4:.

Shang, Y., N. C. Donelson, C. G. Vecsey, F. Guo, M. Rosbash *et al.*, 2013 Short Neuropeptide F Is a Sleep-Promoting Inhibitory Modulator. Neuron.

Shaw, P. J., C. Cirelli, R. J. Greenspan, and G. Tononi, 2000a Correlates of sleep and waking in Drosophila melanogaster. Science 287: 1834–7.

Shaw, P. J., C. Cirelli, R. J. Greenspan, G. Tononi, S. S. Campbell *et al.*, 2000b Correlates of sleep and waking in Drosophila melanogaster. Science 287: 1834–7.

Shaw, P. J., G. Tortoni, R. J. Greenspan, and D. F. Robinson, 2002 Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature 417: 287–291.

Siegel, J. M., 2005 Clues to the functions of mammalian sleep. Nature 437: 1264–1271.

Sitaraman, D., Y. Aso, X. Jin, N. Chen, M. Felix *et al.*, 2015a Propagation of Homeostatic Sleep Signals by Segregated Synaptic Microcircuits of the Drosophila Mushroom Body. Curr. Biol. 25: 2915–2927.

Sitaraman, D., Y. Aso, G. M. Rubin, and M. N. Nitabach, 2015b Control of sleep by dopaminergic inputs to the drosophila mushroom body. Front. Neural Circuits.

Sitaraman, D., Y. Aso, G. M. Rubin, and M. N. Nitabach, 2015c Control of Sleep by Dopaminergic Inputs to the Drosophila Mushroom Body. Front. Neural Circuits 9: 1–8.

Sitaraman, D., Y. Aso, G. M. Rubin, and M. N. Nitabach, 2015d Control of Sleep by Dopaminergic Inputs to the Drosophila Mushroom Body. Front Neural Circuits 9: 73.

Skorupa, D. a, A. Dervisefendic, J. Zwiener, and S. D. Pletcher, 2008 Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. Aging Cell 7: 478–90.

Slocumb, M. E., J. M. Regalado, M. Yoshizawa, G. G. Neely, P. Masek *et al.*, 2015a Enhanced sleep is an evolutionarily adaptive response to starvation stress in Drosophila. PLoS One 10:.

Slocumb, M. E., J. M. Regalado, M. Yoshizawa, G. G. Neely, P. Masek *et al.*, 2015b Enhanced sleep is an evolutionarily adaptive response to starvation stress in Drosophila. PLoS One.

Sonn, J. Y., J. Lee, M. K. Sung, H. Ri, J. K. Choi *et al.*, 2018 Serine metabolism in the brain regulates starvation-induced sleep suppression in Drosophila melanogaster. Proc. Natl. Acad. Sci. U. S. A. 115: 7129–7134.

Speca, D. J., D. Chihara, A. M. Ashique, M. S. Bowers, J. T. Pierce-Shimomura *et al.*, 2010 Conserved role of unc-79 in ethanol responses in lightweight mutant mice. PLoS Genet.

Spiegel, K., R. Leproult, and E. Van Cauter, 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354: 1435–1439.

Stahl, B. A., E. Peco, S. Davla, K. Murakami, D. J. Van Meyel *et al.*, 2018 The Taurine Transporter Eaat2 Functions in Ensheathing Glia to Modulate Sleep and Metabolic Rate. 1–9.

Stahl, B. A., M. Slocumb, H. Chaitin, J. DiAngelo, and A. C. Keene, 2017 Sleep-Dependent Modulation Of Metabolic Rate In Drosophila. Sleep XX:

Stein, J. M., W. Bergman, Y. Fang, L. Davison, C. Brensinger *et al.*, 2006 Behavioral and neurochemical alterations in mice lacking the RNA-binding protein translin. J. Neurosci. 26: 2184–2196.

Suseendranathan, K., K. Sengupta, R. Rikhy, J. S. D'Souza, M. Kokkanti *et al.*, 2007 Expression pattern of Drosophila translin and behavioral analyses of the mutant. Eur. J. Cell Biol. 86: 173–186.

Suster, M. L., L. Seugnet, M. Bate, and M. B. Sokolowski, 2004 Refining GAL4-driven transgene expression in Drosophila with a GAL80 enhancer-trap. Genesis 39: 240–245.

Swayne, L. A., A. Mezghrani, A. Varrault, J. Chemin, G. Bertrand *et al.*, 2009 The NALCN ion channel is activated by M3 muscarinic receptors in a pancreatic β-cell line. EMBO Rep. 10: 873–880.

Sweeney, S. T., K. Broadie, J. Keane, H. Niemann, and C. J. O 'kane, 1995 Targeted Expression of Tetanus Toxin Light Chain in Drosophila Specifically Eliminates Synaptic Transmission and Causes Behavioral Defects. Neuron 14: 341–351.

Taghert, P. H., and M. N. Nitabach, 2012 Peptide Neuromodulation in Invertebrate Model Systems. Neuron 76: 82–97.

Taheri, S., L. Lin, D. Austin, T. Young, and E. Mignot, 2004 Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med. 1: 210–217.

Thimgan, M. S., Y. Suzuki, L. Seugnet, L. Gottschalk, and P. J. Shaw, 2010a The perilipin homologue, lipid storage droplet 2, regulates sleep homeostasis and prevents learning impairments following sleep loss. PLoS Biol. 8:.

Thimgan, M. S., Y. Suzuki, L. Seugnet, L. Gottschalk, and P. J. Shaw, 2010b The Perilipin Homologue, Lipid Storage Droplet 2, Regulates Sleep Homeostasis and Prevents Learning Impairments Following Sleep Loss (P. E. Hardin, Ed.). PLoS Biol. 8: e1000466.

Tomita, J., G. Ban, and K. Kume, 2017 Genes and neural circuits for sleep of the fruit fly. Neurosci. Res. 118: 82–91.

Touitou, Y., A. Reinberg, and D. Touitou, 2017 Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. Life Sci. 173: 94–106.

Tsao, C. H., C. C. Chen, C. H. Lin, H. Y. Yang, and S. Lin, 2018 Drosophila mushroom bodies integrate hunger and satiety signals to control innate food-seeking behavior. Elife 7:.

Ueno, T., J. Tomita, H. Tanimoto, K. Endo, K. Ito *et al.*, 2012 Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. Nat. Neurosci. 15: 1516–1523.

Vanderheyden, W. M., A. G. Goodman, R. H. Taylor, M. G. Frank, H. P. A. Van Dongen *et al.*, 2018 Astrocyte expression of the Drosophila TNF-alpha homologue, Eiger, regulates sleep in flies. PLoS Genet. 14: e1007724.

Varin, C., A. Rancillac, H. Geoffroy, S. Arthaud, P. Fort *et al.*, 2015 Glucose Induces Slow-Wave Sleep by Exciting the Sleep-Promoting Neurons in the Ventrolateral Preoptic Nucleus: A New Link between Sleep and Metabolism. J. Neurosci. 35: 9900–11.

Waddell, S., D. Owald, S. Lin, and S. Waddell, 2015 Light, heat, action: neural control of fruit fly behaviour. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 370: 20140211.

Wagh, D. A., T. M. Rasse, E. Asan, A. Hofbauer, I. Schwenkert *et al.*, 2006 Bruchpilot, a Protein with Homology to ELKS/CAST, Is Required for Structural Integrity and Function of Synaptic Active Zones in Drosophila. Neuron 49: 833–844.

Walkowicz, L., E. Kijak, W. Krzeptowski, J. Górska-Andrzejak, V. Stratoulias *et al.*, 2017 Downregulation of DmMANF in glial cells results in neurodegeneration and affects sleep and lifespan in Drosophila melanogaster. Front. Neurosci. 11: 1–15.

Wong, R., M. D. W. Piper, B. Wertheim, and L. Partridge, 2009 Quantification of food intake in Drosophila. PLoS One 4:.

Wu, M. N., K. Ho, A. Crocker, Z. Yue, K. Koh *et al.*, 2009 The effects of caffeine on sleep in Drosophila require PKA activity, but not the adenosine receptor. J. Neurosci. 29: 11029–11037.

Wu, R. F., K. Osatomi, L. S. Terada, and K. Uyeda, 2003 Identification of Translin/Trax complex as a glucose response element binding protein in liver. Biochim. Biophys. Acta - Gen. Subj. 1624: 29–35.

Wu, Q., Z. Zhao, and P. Shen, 2005 Regulation of aversion to noxious food by Drosophila neuropeptide Y- and insulin-like systems. Nat. Neurosci. 8: 1350–5.

Xie, L., H. Kang, Q. Xu, M. J. Chen, Y. Liao *et al.*, 2013 Sleep drives metabolite clearance from the adult brain. Science 342: 373–7.

Xie, X., M. Tabuchi, M. P. Brown, S. P. Mitchell, M. N. Wu *et al.*, 2017 The laminar organization of the drosophila ellipsoid body is semaphorin-dependent and prevents the formation of ectopic synaptic connections. Elife.

Xu, K., J. R. DiAngelo, M. E. Hughes, J. B. Hogenesch, and A. Sehgal, 2011 The circadian clock interacts with metabolic physiology to influence reproductive fitness. Cell Metab. 13: 639–54.

Xu, K., X. Zheng, and A. Sehgal, 2008 Regulation of feeding and metabolism by neuronal and peripheral clocks in Drosophila. Cell Metab. 8: 289–300.

Yang, Z., R. Huang, X. Fu, G. Wang, W. Qi *et al.*, 2018 A post-ingestive amino acid sensor promotes food consumption in Drosophila. Cell Res. 28: 1013–1025.

Yasunaga, K., K. Saigo, and T. Kojima, 2006 Fate map of the distal portion of Drosophila proboscis as inferred from the expression and mutations of basic patterning genes. Mech. Dev. 123: 893–906.

Yi, W., Y. Zhang, Y. Tian, J. Guo, Y. Li *et al.*, 2013 A subset of cholinergic mushroom body neurons requires Go signaling to regulate sleep in Drosophila. Sleep 36: 1809–21.

Yin, Z., A. Sadok, H. Sailem, A. McCarthy, X. Xia *et al.*, 2012 Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. Nature 2004: pl6.

Yu, X., Z. Ye, C. M. Houston, A. Y. Zecharia, Y. Ma *et al.*, 2015 Wakefulness Is Governed by GABA and Histamine Cotransmission. Neuron 87: 164–178.

Yuan, Q., W. J. Joiner, and A. Sehgal, 2006 A Sleep-Promoting Role for the Drosophila Serotonin Receptor 1A. Curr. Biol. 16: 1051–1062.

Yurgel, M. E., P. Masek, J. DiAngelo, and A. C. Keene, 2015a Genetic dissection of sleep-metabolism interactions in the fruit fly. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 201: 869–77.

Yurgel, M. E., P. Masek, J. DiAngelo, and A. C. Keene, 2015b Genetic dissection of sleep–metabolism interactions in the fruit fly. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 201:.

Yurgel, M. E., K. D. Shah, E. B. Brown, C. Burns, R. A. Bennick *et al.*, 2018 Ade2 Functions in the Drosophila Fat Body To Promote Sleep. G3 (Bethesda). 8: 3385–3395.

Zager, A., and M. Andersen, 2007 Effects of acute and chronic sleep loss on immune modulation of rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293: R504–R509.

Zhao, J. J., T. T. Zhang, X. H. Liu, J. X. Sun, Y. H. Liu *et al.*, 2020 A Meta-analysis on the association between sleep duration and metabolic syndrome in adults. Zhonghua Liu Xing Bing Xue Za Zhi.

Zhou, H., K. R. Neville, N. Goldstein, S. Kabu, N. Kausar *et al.*, 2019 Cholinergic modulation of hippocampal calcium activity across the sleep-wake cycle. Elife.

Zimmerman, J. E., D. M. Raizen, M. H. Maycock, G. Maislin, and A. I. Pack, 2008 A video method to study Drosophila sleep. Sleep 31: 1587–98.

Zimmet, P., K. G. M. M. Alberti, N. Stern, C. Bilu, A. El-Osta *et al.*, 2019 The Circadian Syndrome: is the Metabolic Syndrome and much more! J. Intern. Med. 286: 181–191.