HHS Public Access

Author manuscript

Brain Behav Immun. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Brain Behav Immun. 2015 July ; 47: 75–85. doi:10.1016/j.bbi.2014.09.019.

Differential activation of immune factors in neurons and glia contribute to individual differences in resilience/vulnerability to sleep disruption

Stephane Dissel[1], **Laurent Seugnet**[1,2], **Matthew S. Thimgan**[1,3], **Neal Silverman**[4], **Veena Angadi**[1], **Pamela V. Thacher**[5], **Melissa M. Burnham**[6], and **Paul J. Shaw**[1]

[1]Department of Anatomy & Neurobiology, Washington University in St. Louis, 660 S. Euclid Ave, St. Louis, Missouri, USA

[4]Division of Infectious Diseases, Department of Medicine, University of Massachusetts Medical School, 364 Plantation St., Worcester, MA 01605

[5]Department of Psychology, St Lawrence University, 23 Romoda Drive, Canton, NY 13617

[6]College of Education, University of Nevada, Reno, Reno, NV 89557

Abstract

Individuals frequently find themselves confronted with a variety of challenges that threaten their wellbeing. While some individuals face these challenges efficiently and thrive (resilient) others are unable to cope and may suffer persistent consequences (vulnerable). Resilience/vulnerability to sleep disruption may contribute to the vulnerability to individuals exposed to challenging conditions. With that in mind we exploited individual differences in a fly's ability to form short-term memory (STM) following 3 different types of sleep disruption to identify the underlying genes. Our analysis showed that in each category of flies examined, there are individuals that form STM in the face of sleep loss (resilient) while other individuals show dramatic declines in cognitive behavior (vulnerable). Molecular genetic studies revealed that Antimicrobial Peptides, factors important for innate immunity, were candidates for conferring resilience/vulnerability to sleep deprivation. Specifically, *Metchnikowin* (*Mtk*), *drosocin* (*dro)* and *Attacin* (*Att*) transcript levels seemed to be differentially increased by sleep deprivation in glia (*Mtk*), neurons (*dro*) or primarily in the head fat body (*Att*). Follow-up genetic studies confirmed that expressing *Mtk* in glia but not neurons, and expressing *dro* in neurons but not glia, disrupted memory while

Correspondence should be addressed to P.J.S. (shawp@pcg.wustl.edu).

[2]Current address: Centre for Research in Neurosciences de Lyon, U1028/UMR 5292 - Team WAKING, Université Claude Bernard, 8 avenue Rockefeller, 69373 Lyon Cedex 08.

[3]Current address: Missouri University of Science & Technology, Department of Biological Sciences, Rolla, MO USA.

Author Contributions

S.D., L.S., P.V.T, M.M.B, N.S., and P.J.S. designed the experiments. S.D., L.S., M.S.T. V.A. and P.J.S., completed the behavioral experiments and performed molecular and qPCR experiments. S.D., and P.J.S. analyzed data, and S.D., P.V.T. and P.J.S. wrote this manuscript.

modulating sleep in opposite directions. These data indicate that various factors within glia or neurons can contribute to individual differences in resilience/vulnerability to sleep deprivation.

Keywords

Resilience/vulnerability; Individual differences; Sleep disruption; Short term memory; Immunity; Glia

1. Introduction

In a complex world, individuals frequently find themselves confronted with a variety of challenges that threaten their physical, social, economic and mental wellbeing [1–3]. Some individuals face these challenges efficiently and thrive (resilient) while others are unable to cope and may suffer persistent negative health and psychiatric consequences (vulnerable)[4]. Indeed, vulnerable individuals may be at greater risk for posttraumatic stress disorder, anxiety, major depressive disorder, etc…[1, 2]. Thus, individual differences in resilience/vulnerability have dramatic clinical, social and economic consequences.

While the mechanisms underlying individual differences in resilience/vulnerability are believed to depend on complex interactions between genetics and the environment, the precise mechanism are not fully understood. Interestingly, humans and animals face a variety of challenging environmental conditions that can dramatically impact sleep and sleep quality. Sleep disruption, by itself, can result in cognitive impairment [5–7], increased emotional reactivity [8], increased risk-taking [9] and may be a contributing factor for developing depression and other psychiatric illnesses [10]. Given the well documented observation that individuals vary greatly in their resilience/vulnerability to sleep loss [11], it seems likely that sleep disruption may enhance the vulnerability to individuals exposed to threatening or challenging conditions.

Indeed, recent studies suggest that the variability observed in individual responses to sleep disruption can be explained, in part, by genetic factors. For example, in humans, polymorphisms in *Period3* (*Per3*), a key circadian gene, are associated with differences in cognitive impairments and sleep homeostasis observed after a night of sleep deprivation [12]. In addition, a polymorphism in *adenosine deaminase* (*ADA*) modulates sleep structure and intensity and contributes to individual differences in cognitive performances [13]. The impact of polymorphisms on the vulnerability to sleep loss extends beyond humans and can even be found in *Drosophila*. For example, polymorphisms in the *foraging* (*for*) gene, which codes for Protein Kinase G (PKG), are associated with resilience/vulnerability to the negative effects of sleep loss on cognition [14]. Unfortunately, while genomic and association studies have begun to provide some clues [15, 16], the mechanisms underlying resilience/vulnerability to sleep disruption remain largely unknown.

Given our interest in understanding how individual differences in resilience/vulnerability to sleep loss impact cognitive behavior [14], we were intrigued by a report suggesting that increased markers of inflammation, may discriminate between intact and cognitively impaired individuals during sleep disruption [17]. That is, cognitive impairments in children

with obstructive sleep apnea (OSA) were associated with increased levels of high-sensitivity C-reactive protein, an important circulating marker of inflammation [17]. Indeed, the relationship between sleep and immune function is well established in humans and animal models [18–23]. Moreover, studies are also beginning to associate immune factors with cognitive impairments [24]. Thus, the immune system can influence both sleep and cognitive functioning either separately or synergistically. In this study, we evaluate the hypothesis that the molecular mechanisms underlying individual differences in resilience/vulnerability to sleep loss are mediated by the immune system.

2. Material and methods

2.1. Flies

Flies were cultured at 25°C with 50–60% relative humidity and kept on a diet of yeast, dark corn syrup and agar under a 12-hour light:12-hour dark cycle. *Cs* flies were obtained from Troy Zars (University of Missouri, Columbia). *UAS-Metchnikowin*, *UAS-drosocin*, *UAS-defensin*, *UAS-drosomycin* and *UAS-Attacin* were obtained from David Wassarman (University of Wisconsin, Madison). *DaGsw-GAL4* were obtained from Marc Tatar (Brown University). *MJ85b-GAL4* flies were obtained from Ralph Greenspan (University of California, San Diego). *UAS-GFP::RpL10A* flies were obtained from Herman Dierick (Baylor College of Medicine). *elav-GAL4* and *repo-GAL4* flies were obtained from the Bloomington Stock Center (Bloomington, Indiana).

2.2. Sleep

Sleep was assessed as previously described [25]. Briefly, female flies were placed into individual 65 mm tubes and all activity was continuously measured through the Trikinetics Drosophila Activity Monitoring System (www.Trikinetics.com, Waltham, Ma). Locomotor activity was measured in 1-minute bins and sleep was defined as periods of quiescence lasting at least 5 minutes. For GeneSwitch experiments, female flies were maintained on RU486 or Vehicle for 2 days before being evaluated [26].

2.3. Sleep Deprivation

Sleep deprivation was performed as previously described [25, 26]. Briefly, female flies were placed into individual 65 mm tubes and the sleep-nullifying apparatus (SNAP) was used to sleep deprive these flies for 12 hours during the dark phase (lights out to lights on).

2.4. Short-term memory

Short-term memory (STM) was assessed by Aversive Phototaxic Suppression (APS) as previously described [26, 27]. The experimenters were blinded to condition. In the APS, flies are individually placed in a T-maze and allowed to choose between a lightened and darkened chamber over 16 trials. Flies that do not display phototaxis during the first block of 4 trials are excluded from further analysis [27, 28]. During 16 trials, flies learn to avoid the lighted chamber that is paired with an aversive stimulus (quinine/humidity). The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials of the 16 trial test. In the absence of quinine, where no learning is possible, it is common to observe flies choosing the dark vial once during the last 4 trials in

Block 4 [27]. In contrast, flies never choose the dark vial 2 or more times during block 4 in the absence of quinine [27]. Thus, STM is defined as two or more photonegative choices in Block 4. For STM experiments following a 12 h sleep deprivation, the deprivation continued until evaluation in the APS. All flies were tested in the morning. Power analysis using G*Power calculates a Cohen's d of 1.8 and indicates that eight flies/group are needed to obtain statistical differences [27].

2.5. Photosensitivity

Photosensitivity was evaluated as previously described [27]. Briefly, flies were put in the T-maze over 10 trials in the absence of filter paper. The lightened and darkened chambers appeared equally on both the left and right. The percentage of times the flies choose the lighted chamber for the 10-trial test is tabulated. The photosensitivity index (PI) is the average of the percent photopositive scores obtained for 5–6 flies ± s.e.m..

2.6. Quinine sensitivity

Quinine sensitivity index (QSI) was evaluated as previously described [26, 27]. Briefly, flies were individually placed at the bottom of a 14 cm transparent cylindrical tube which was uniformly lighted and maintained horizontal after the introduction of the animal. Each half of the apparatus contained separate pieces of filter paper which could be wetted with quinine or kept dry. The QSI was determined by calculating the time in seconds that the fly spent on the dry side of the tube when the other side had been wetted with quinine, during a 5 min period.

2.7. QPCR

We performed QPCR on mRNAs obtained from whole heads (cuticle, eyes, fat body, neurons and glia), brains (neurons and glia) or neurons only (using the TRAP system).

Isolation of mRNAs from neurons only: approximately 100 heads from adult *MJ85b-GAL4/+>UAS-GFP::RpL10A/+* flies were collected and homogenized in extraction buffer (20mM HEPES pH 7.5, 150mM KCl, 5mM MgCl2, 1% Triton-x, 0.5mM DTT, 100ug/mL cyclohexamide, 1x Complete protease inhibitors, 100U/mL Rnase OUT). The lysate was centrifuged to separate insoluble material and the protein extract was added to Protein A Sepharose beads conjugated to Rabbit anti-GFP (NeuroMab αGFP, clone N86/38). Lysate-bead slurry was then incubated overnight at 4°C followed by washing in Wash Buffer (150mM NaCl, 0.05% Triton X-100, 50mM Tris, 5mM $MgCl_2$, and 40U/mL RNase OUT) at 4°C. RNA was extracted using standard Trizol extraction methods for downstream analysis (see below). For mRNAs isolated from brains, ~30 fly heads were collected and brains were dissected on dry ice before using standard Trizol RNA extraction methods for downstream analysis. For mRNAs isolated from whole heads, total RNA was isolated from ~20 fly heads with Trizol (Invitrogen, Carlsbad, CA). QPCR were performed as previously described [25, 26]. Briefly, total RNA was digested with DNAse. cDNA synthesis was performed in triplicate using Superscript III (Invitrogen, Carlsbad, CA), according to manufacturer protocol. In order to evaluate the efficiency of each reverse transcription, equal amounts of cDNA were used as a starting material to amplify RP49 as previously described. cDNA from comparable reverse transcription reactions were pooled and used as a

starting material to run three QPCR replicates. Expression values for RP49 were used to normalize results between groups.

2.8. Statistics

All comparison were done using a Student's t-test or, if appropriate, ANOVA and subsequent planned comparisons using modified Bonferroni test unless otherwise stated. All statistically different groups are defined as *P < 0.05.

3. Results

3.1. The ability to form STM is a stable trait

To investigate individual differences in learning ability in the face of different types of sleep disruptions, we first needed to establish that the ability to form short-term memory (STM) is a stable trait. We chose to evaluate STM using Aversive Phototaxic Suppression (APS). The APS has many advantages over other *Drosophila* memory assays. It is simple, reliable, and while performance scores are extremely sensitive to sleep disruption, STM is not strongly influenced by genetic background [14, 26, 27, 29–31]. In the APS, flies are placed in a T-maze and allowed to choose between a lighted and darkened alley. Flies that do not display phototaxis during the first block of 4 trials are excluded from further analysis [27, 28]. Quinine is then placed into the lighted alley to provide an aversive association [26–28]. The number of photonegative choices is tabulated during 4 Blocks of 4 trials where the light and quinine appear equally on both the right and left side of the apparatus. The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials (Block 4) of the 16 trial test. In the absence of quinine, where no learning is possible, flies never choose the dark alley twice during the last four trials [27]. Thus STM is defined for an individual fly as 2 or more photonegative choices in Block 4.

Individual *Cs* flies were evaluated in the APS on two trials spaced 2 days apart. As seen in Figure 1, on test 1 (blue bars), 28 out of 32 flies formed STM. We observed only 4 flies that were memory impaired on test 1. When the same individuals were retested two days later (test 2, red bars), 27 out of the 28 flies that had a STM on test 1, maintained the ability to form STM on test 2. In addition, 3 out of the 4 flies that did not form STM on test 1 remained learning impaired on test 2. We observed only one individual fly that made two photonegative choices during Block 4 during the first trial but did not show evidence of STM on test 2. Conversely, one fly improved its performance between tests. Thus, in 94% of the flies tested (30/32), the ability to form STM was stable between two independent trials performed days apart. This result demonstrates that memory performance is a stable trait in individual flies and that STM formation (as assayed with APS) is a valid behavior to investigate individual differences.

3.2. Individual differences in the ability to form STM are common

We have thoroughly assessed STM using the APS in a variety of mutants and conditions (i.e., undisturbed sleep, sleep deprivation, sleep fragmentation, starvation, etc…)[14, 26, 27, 29–33]. Our data reveal that individual differences in the ability to form STM are common. Indeed, while the majority of wild-type flies display STM during baseline, when sleep is

undisturbed, it is not uncommon to find individual flies to exhibit cognitive impairments (Figure 1). Similarly, while sleep deprivation substantially impairs STM, it is not uncommon to find a minority of individuals within a population of wild type flies that can form STM after sleep deprivation (i.e., they are resilient to sleep loss).

To further investigate the extent to which flies display individual differences in cognitive behavior, we quantified the proportion of flies that could form STM during baseline and following sleep disruption. As seen in Figure 2A, 88% of "good sleepers" (as defined by consolidated sleep at night with an average sleep bout duration >40 min), form STM. In comparison, only 41% of sleep deprived individuals are able to maintain normal STM while 59% are cognitively impaired, (Figure 2B). Similarly, only 38% of *Cs* flies that spontaneously exhibit fragmented sleep (short nighttime sleep bouts coupled with normal levels of total sleep time) can achieve optimal STM (Figure 2C); note that ~5–10% of *Cs* flies spontaneously exhibit fragmented sleep [26, 34]. Finally, while 55% of immature flies that were sleep deprived on their first full day of adult life show cognitive impairments when tested 5 days later, 45% of their sleep deprived siblings continued to exhibit wild-type STM indicating that they are resilient to the effects of sleep deprivation during a critical stage of development. (Figure 2D). Thus, individuals within a population are either vulnerable or resilient to three different forms of sleep disruption.

3.3. Immune genes are associated with vulnerability to sleep disruption

To identify genetic factors involved in the resilience/vulnerability to sleep loss we employed a gene profiling approach as detailed in Figure 3. Briefly, we first evaluated sleep in a population of *Cs* flies to identify "good sleepers" with consolidated nighttime sleep (Figure 3A). Next, we assessed STM in individual "good sleepers" using the APS and identified two subgroups: individuals that expressed STM and those that are cognitively impaired (no STM). The "good sleeping" individuals that form STM and their cognitively impaired siblings were pooled to form two groups (Good Sleepers with STM and Good Sleepers without STM) for RNA extraction (Figure 3B). Similarly, flies that were identified as "good sleepers" were subjected to 12 h of sleep deprivation and assessed for STM. Individual flies that exhibited STM (resilient) following sleep deprivation, and their impaired siblings (vulnerable), were placed into separate groups for RNA extraction (Figure 3C). To minimize the immediate influence of the deprivation stimulus on gene expression, we evaluated two additional groups: Flies that spontaneously exhibit fragmented nighttime sleep and flies that were sleep deprived on their first full day of adult life and then allowed to rest unperturbed for 5 days [26, 30]. As before, individual flies were tested for STM and resilient and vulnerable individuals were isolated and placed into separate groups for RNA extraction (Figure 3D, E).

Before evaluating gene profiles we wanted to ensure that the differences in STM that were observed in each of the 4 groups of flies highlighted in Figure 3 were not due to pre-existing differences in sleep. Thus, we assessed sleep for each set of flies. As seen in Figure 4A, B, sleep time and average sleep bout duration at night are not different between "good sleepers" that form STM and their impaired siblings. Similarly, sleep deprived flies with and without STM slept similarly on the day preceding sleep deprivation (Figure 4C, D) and all

flies exhibited similar amounts of waking during the sleep deprivation protocol consistent with previous reports (data not shown) [31, 35]. Moreover, we did not observe any differences in sleep time between sleep fragmented siblings with and without STM (Figure 4E, F). Finally, flies that were sleep deprived on their first full day of adult life exhibited similar sleep metrics regardless of whether they could form STM as adults (Figure 4G, H). Thus, we can conclude that within each of the four categories of *Cs* flies used for RNA extraction, there are no differences in sleep parameters between individuals that form STM and those that are cognitively impaired.

To identify candidate genes that are associated with memory impairments during sleep disruption, transcripts from cognitively impaired flies (no STM) in each category were expressed as a percent change from their resilient siblings (STM). Transcripts that were significantly increased in memory-impaired, good-sleeping flies are likely to represent genes that directly impact STM independently of their effects on cognitive impairment during sleep disruption since these flies do not exhibit any sleep deficits. Thus, we only considered transcripts whose expression pattern differed between good-sleeping memory-impaired flies and sleep-disrupted flies with STM deficits (i.e. genes specifically associated with memory impairment during sleep disruption vs. memory impairment without sleep disruption). We took a candidate gene approach based upon results from our own microarray studies. We evaluated ~100 genes representing different molecular pathways. One of the most promising transcripts was *Metchnikowin* (*Mtk*), an antimicrobial peptide (AMP), which is a component of the immune response in *Drosophila* [36, 37]. As seen in Figure 4I, *Mtk* transcripts were modestly reduced in memory-impaired good-sleepers (blue). However, *Mtk* transcripts were dramatically increased in STM-impaired flies following sleep deprivation (red), sleep fragmentation (green) and developmental sleep deprivation (purple). Given the profiles seen for *Mtk*, we evaluated additional immune related transcripts, including *defensin* (*def*), *drosocin*, (*dro*), *drosomycin* (*drs*) and *AttacinB* (*AttB*). Interestingly *def*, *drs* and *AttB* were not as tightly associated with cognitive impairment following sleep disruption (Figure 4I). However, transcripts for *dro* were significantly increased following sleep deprivation, and sleep fragmentation. Thus, our data provides strong evidence that *Mtk*, and *dro* are candidates for modulating resilience/vulnerability to sleep disruption.

3.4. Adult-specific expression of immune genes results in memory impairment

To further investigate the role of AMPs in modulating sleep and STM, we specifically increased the level of each of the five AMP genes for 2 days in adult flies using the ubiquitous *Daughterless-GeneSwitch* (*DaGs*) driver. We chose to use the GeneSwitch system to activate AMP expression for a brief period of time in young flies to avoid complications in cellular health that arise when AMPs are chronically induced [38]. As seen in Figure 5A, RU486 (RU)-fed *DaGs/+>UAS-Mtk/+* flies increase sleep compared with their vehicle (veh)-fed siblings. The increase in sleep is accompanied by an increase in sleep consolidation at night (Figure 5B). Importantly, waking-activity in RU-fed *DaGs/+>UAS-Mtk/+* flies does not differ from vehicle fed controls indicating that the increase in sleep is not due to a lethargic or sick fly (data not shown, t-test; p=0.32)[39]. Although our evaluation of transcripts in sleep-disrupted, memory-impaired flies is correlative, the expression pattern would predict that the expression of *Mtk* would result in cognitive

impairments. Indeed, RU-fed *DaGs/+>UAS-Mtk/+* flies exhibit significant disruption in STM compared to vehicle fed siblings (Figure 5C). To rule out the possibility that the expression of *Mtk* would alter sensory modalities that might influence performance in the APS, we evaluated photosensitivity and quinine sensitivity in RU and Vehicle fed *DaGs/+>UAS-Mtk/+* siblings. As seen in Table S1, the expression of *Mtk* does not alter either sensory modality. Thus, the ubiquitous expression of *Mtk* results in cognitive impairment.

In contrast with *Mtk*, the adult specific expression of *def* (Figure 5D, E), *dro* (Figure 5G, H), *drs* (Figure 5J, K) *and Att* (Figure 5M, N) with the *DaGs* driver does not alter sleep parameters. However, with the exception of *DaGs/+>UAS-Att/+*, STM formation is significantly impaired in RU-fed *DaGs/+>UAS-def/+*, *DaGs/+>UAS-dro/+* and *DaGs/+>UAS-drs/+* flies compared with their veh-fed siblings (Figure 5F, I, L, O). As above, photosensitivity or quinine sensitivity are not altered in RU-fed flies indicating that the impaired performance in the APS is not due to changes in sensory thresholds (Table S1).

3.5. Neurons and glia contribute to sleep loss induced memory impairment

The results we obtained with ubiquitous upregulation of individual AMPs in adult flies prompted us to further investigate the cellular origin of AMP gene expression during learning impairment. Firstly, we wanted to obtain a better understanding of the contribution of the different tissues found within a fly head to the increased immune gene expression seen after sleep deprivation. To do so, we extracted mRNAs from whole heads (which contain eyes, cuticle, fat body, neurons and glia) and from brains (neurons and glia) of female *Cs* flies under baseline (undisturbed sleep) and after 12 h of sleep deprivation. In order to obtain mRNAs from neurons only, we took advantage of the recently developed translating ribosome affinity purification (TRAP) method that can be used to profile actively translated mRNAs [40]. GFP-tagged *UAS-RpL10A* incorporates into assembled ribosomes and polysomes such that mRNA from the immunoprecipitated polysome can be evaluated. We targeted the expression of *UAS-GFP::RpL10A* pan-neuronally using the *MJ85b-GAL4* driver. Unfortunately, expressing *UAS-GFP::RpL10A/+* using glial-*GAL4* drivers disrupted behavior, preventing us from using the TRAP system with glia. We collected mRNAs from *MJ85b-GAL4/+>UAS-GFP::RpL10A/+* flies under baseline and after 12 h of sleep deprivation. Given the low yield of mRNA obtained using the TRAP system, it was not practical to extract RNA from flies with and without STM. We compared expression levels of genes after sleep deprivation relative to baseline. As proof of principle that transcripts extracted from heads, brains and neurons (e.g. TRAP system) can be used to evaluate how transcripts change in different cellular compartments, we examined levels of a transcript, the D1 dopamine receptor (*dDA1*), which is strongly modulated by sleep loss [26]. Previous studies have found that *dDA1* transcripts extracted from whole head are reduced following 12 h of sleep deprivation [26]. As seen in Figure 6A, blue, *dDA1* levels are down-regulated in whole heads following sleep deprivation compared to their untreated siblings as previously reported. Interestingly, the magnitude of the decline in *dDA1* transcripts is similar in mRNA extracted from brains, suggesting that the eye, the cuticle, and the fat body may not contribute substantially to reduced *dDA1* transcripts following sleep loss (Figure 6A, red). A similar reduction in *dDA1* transcripts following sleep deprivation is also observed when mRNA is extracted from neurons (Figure 6A, green). Together these data

indicate that evaluating mRNA extracted from whole heads, brains, and neurons may be effective in revealing transcripts which are differentially expressed in different cellular compartment following sleep disruption.

We then focused our analysis on AMPs genes. As seen in Figure 6B, blue, *Mtk*, *dro*, *AttB* and *drs* levels are dramatically increased in whole-heads following sleep deprivation consistent with previous reports [34]. However, when we examined mRNAs extracted from brains, we observed that while *Mtk* and *dro* levels are strongly increased following sleep deprivation, the magnitude of the increase is smaller than that observed in whole-heads. These data reveal, perhaps predictably, the contribution of other head tissues, especially the fat body to increased AMP transcripts following sleep loss [18]. Interestingly, *AttB* is only modestly increased in brains following sleep deprivation while *drs* remains unchanged. Surprisingly, when we examined mRNAs extracted from neurons using the TRAP method, we found that *Mtk* transcripts are not increased by sleep deprivation (Figure 6B, green). These data suggest that the increase in *Mtk* transcripts found in mRNA extracted from brains may be due to an upregulation of *Mtk* in glia. In contrast to *Mtk*, *dro* levels are increased in neurons following sleep deprivation in a manner very similar to what we observed in brains (Figure 6B, red). This latter result suggests the possibility that sleep deprivation increases *dro* expression in neurons and may have less of an impact on glia. Finally, neither *AttB* nor *drs* levels are dramatically altered in either brains or neurons following sleep deprivation suggesting that the elevated levels of *AttB* and *drs* may be due to the effects of sleep loss on the fat body.

It is important to emphasize that while the expression profiles presented in Figure 6 are intriguing, the data are correlative. Nonetheless, the results lead to two hypotheses: The first hypothesis is that increasing *Mtk* in glia, but not neurons, will disrupt STM. The second hypothesis is that increasing *dro* in neurons, but not glia will result in cognitive impairments. To test these hypotheses, we expressed *Mtk* and *dro* in neurons using the *elav-GAL4* driver and in glia using the *repo-GAL4* driver. As seen in Figure 7A–B, daytime and nighttime sleep are not changed in *elav-GAL4/+>UAS-Mtk/+* flies (green) compared with both parental controls (blue). Interestingly, the intensity of waking locomotor activity is increased in *elav-GAL4/+>UAS-Mtk/+* flies (Figure 7C). When *Mtk* is specifically expressed in glia using the *repo-GAL4* driver, daytime sleep is significantly increased but nighttime sleep is unaffected (Figure 7D, E). Importantly, the intensity of waking locomotor activity is increased in *repo-GAL4/+>UAS-Mtk/+* flies indicating that the increased sleep is not due to a sick or lethargic fly (Figure 7F). Consistent with the effects on sleep, we found that *Mtk* expression in neurons did not alter STM (Figure 7G, left), while expression of *Mtk* in glia using *repo-GAL4* substantially disrupted performance (Figure 7G, right). Neither photosensitivity nor quinine sensitivity are altered in *elav-GAL4/+>UAS-Mtk/+ or repo-GAL4>UAS-Mtk* flies compared with parental controls indicating that the impaired performance in the APS is not due to changes in sensory thresholds (Table S1). Thus, the expression of *Mtk* in neurons and glia differentially modulates both sleep and STM.

We next tested the hypothesis that increasing *dro* in neurons will disrupt STM. As seen in Figure 7H, daytime sleep in *elav-GAL4/+>UAS-dro/+* flies does not differ from both parental controls. However, nighttime sleep is significantly reduced in *elav-GAL4/+>UAS-*

dro/+ flies compared with both *elav-GAL4*/+ and *UAS-dro*/+ flies (Figure 7I); the intensity of waking locomotor activity is not different in *elav-GAL4*/+>*UAS-dro*/+ flies (Figure 7J). In contrast to the reduction in nighttime sleep seen when *dro* is expressed in neurons, no changes in either daytime or nighttime sleep were observed when *dro* was expressed in glia using the *repo-GAL4* driver (Figure 7K–L). However, the intensity of waking locomotor activity is increased in *repo-GAL4>UAS-dro* flies compared to both parental controls (Figure 7M). Finally, we examined STM in flies expressing *dro* in neurons using *elav-GAL4*. As seen in Figure 7N, neuronal expression of *dro* significantly disrupts memory (left panel) while the expression of *dro* in glia does not affect STM formation (right panel). As above, neither photosensitivity nor quinine sensitivity are altered in *elav-GAL4*/+>*UAS-dro*/+ or *repo-GAL4*/+>*UAS-dro*/+ flies compared with parental controls indicating that the impaired performance in the APS is not due to changes in sensory thresholds (Table S1).

4. Discussion

Resilience/vulnerability is largely defined as a *Gene X Environment* outcome. Unfortunately, research has tended to focus on the “environment” variable in the equation. Given the importance in understanding how humans succeed when adversity strikes, a broader approach, including genetic analyses, needs to be developed. Although sleep is rarely considered as a potential factor in studies examining resilience/vulnerability, sleep disruptions are known to exacerbate a number of neurological and psychiatric illnesses [41]. Interestingly, while individual differences are widely observed in humans studies of resilience/vulnerability, individual differences are rarely studied in the genetic model organism *Drosophila melanogaster* [42]. With this in mind, we used the natural variability in the response to sleep loss that is found in wild-type populations to identify candidate genes mediating resilience/vulnerability. Follow up genetic studies suggest the possibility that the differential activation of immune factors in neurons and glia may contribute to individual differences in resilience/vulnerability to sleep disruption in flies.

It is surprising that very few studies have exploited individual differences in flies given the long standing assertion that behavior is extremely variable. This observation is even more stunning given that so many scientists attribute genetic background as a cause for the observed variability. Indeed, studies investigating an assortment of behaviors, including sleep, long term memory, circadian rhythms, etc., present mean data collected from tens, if not hundreds of individuals per condition. Outliers are routinely ignored as they have little impact on the population mean. Interestingly, when individuals have been studied, the goal of the analysis is frequently to rule out the possibility that individuals in a group ignore salient stimuli and simply follow the aggregate behavioral choices of the group (i.e. group behavior may be confounded) [43–45]. Thus, while individuals are known to vary greatly in an assortment of behavioral tasks little has been done to identify the underlying genetic causes.

In this study, we assessed the ability of individual flies to form STM following 3 different types of sleep disruption: 12 h of sleep deprivation, spontaneous sleep fragmentation, and flies that were deprived of sleep on their first day of adult life and allowed to rest unperturbed for 5 days (developmental sleep deprivation). Our analysis showed that in each

category of flies examined, there are individuals that form STM in the face of sleep loss (resilient) while other individuals show dramatic declines in cognitive behavior (vulnerable). Importantly, we ruled out the possibility that differences in cognitive performances were due to pre-existing differences in sleep. Furthermore, we began by demonstrating that the ability to form STM is a stable trait for an individual but can vary between individual siblings taken from the same population. Although we did not evaluate the stability of STM following multiple exposures to sleep deprivation, it should be noted that such protocols are likely to introduce additional confounds and are thus beyond the scope of the current investigation. In any event, we have developed a novel protocol in which the natural variation observed for individual flies can be exploited to identify genes that may confer resilience/vulnerability to sleep disruption. These studies revealed a potential role for genes coding for Antimicrobial Peptides, which are involved in innate immunity. Specifically, our data suggest that when sleep is disrupted, cognitive resilience or vulnerability is associated with different levels of AMP transcript expression.

Since gene profiling is inherently correlative, we used genetics to determine whether any of the candidate genes could indeed alter cognitive behavior. We were intrigued by a number of immune related transcripts due to previous studies linking inflammation with vulnerability to sleep loss, and the well-established link between immunity and sleep [17, 18, 20, 22, 34]. Thus, we expressed each AMP in adult flies using an inducible GAL4-driver that expresses in all tissues. Surprisingly, while increasing *Mtk* increased sleep and disrupted STM, STM was also impaired following the expression of AMPs that did not alter baseline sleep.

Since the cellular identity underlying these cognitive impairments could not be determined using a ubiquitous driver, we evaluated transcripts from heads (which include eyes, cuticle, fat body, neurons and glia), brains (glia and neurons) and neurons (using the TRAP method [40]). Interestingly, we discovered that *Mtk*, *dro* and *Att* transcript levels seemed to be differentially increased by sleep deprivation in glia (*Mtk*), neurons (*dro*) or primarily in the head fat body (*Att*). Follow-up genetic studies confirmed that expressing *Mtk* in glia but not neurons, and expressing *dro* in neurons but not glia, disrupted memory while modulating sleep in opposite directions. It is worth noting that the AMPs investigated are believed to signal through the fat body or hemocytes. Thus, the observation that tissue-specific expression of AMPs in neurons or glia can impact sleep and STM does not rule out the possibility that the AMPs can influence sleep and STM by signaling through the fat body and/or other tissues. The results with *Mtk* are reminiscent of previous studies emphasizing the role of glia in sleep regulation [29, 46]. However, while the previous studies have identified genetic manipulations in glia that protect flies from cognitive deficits following sleep deprivation, our data suggest that activating an immune factor in glia can disrupt STM. The observation that expressing *Mtk* increases sleep and disrupts STM suggests that *Mtk* expression in glia reduces sleep efficiency thereby necessitating that animals compensate by sleeping more. Indeed, administering lipopolysaccharide in mice with deficient glia signaling reduces delta power and increases sleep time [47]. Thus, it seems that disrupting glia signaling protects animals from sleep deprivation while also increasing their vulnerability to immune challenge. These data indicate that various factors within glia can

contribute to individual differences in resilience/vulnerability to qualitatively different challenges.

It is interesting to note that a fly model of the neurodegenerative disease ataxia-telangiectasia is associated with an increase expression of AMPs in glia [48]. A major difference between our sleep deprivation results, and the results reported by Petersen et al., 2012, is that *Mtk*, *dro*, *drs*, and *Att* are each elevated in glia in ataxia-telangiectasia flies while sleep deprivation seems to preferentially increase glial expression of *Mtk*. These data emphasize that while a particular tissue has the potential to increase AMP production, it will only do so when activated by the appropriate stimulus. Thus, while glia may be able to increase AMP expression in response to a variety of challenges, sleep deprivation does not uniformly activate all AMPs. This observation allows for an extra layer of complexity in elucidating the genetic mechanisms underlying individual differences in resilience/vulnerability to different kinds of challenges. Indeed, similar conclusions have been noted previously. For example, polymorphisms that provide resilience in the response to sleep deprivation may result in vulnerability to other challenges (e.g. Starvation) [14]. Thus one must be cautious in generalizing the role of a 'resilience/vulnerability' factors to different challenges as they may play different roles in alternate circumstances.

The increase in glial AMP expression in ataxia-telangiectasia suggests the possibility that the STM impairments observed in this study may be due to neurodegeneration. While we did not evaluate neurodegeneration directly, this possibility is not likely for several reasons. First, as mentioned above, all AMPs are expressed in glia during ataxia-telangiectasia, which is not the case following sleep deprivation. Secondly, flies expressing AMPs constitutively throughout development and into early adulthood do not show degeneration; degeneration is only observed in 25-day old flies [38]. We activated AMPs for only 2-days using the GeneSwitch system, 10 days less than in the Cao et al. 2013, study. Thus, our data suggests that STM impairments can exist prior to the time when neurodegeneration might occur.

Given the well-documented observation that individuals vary greatly in their response to sleep loss [11, 49], it seems likely that the individual's resilience/vulnerability to sleep disruption could serve to modulate his or her ability to negotiate the environment when exposed to a variety of common challenges. In other words, an ability to tolerate sleep deprivation might represent a protective factor, and in this manner allow an individual to cope with potentially difficult or traumatic events. By exploiting individual differences in the ability of flies to maintain cognitive behavior during sleep deprivation we have developed a protocol that may allow us to reveal molecular mechanisms relevant for human health and disease. In conclusion, our data suggest that future studies may benefit from investigating the microbiome as a possible source for individual variation in levels of immune factors which, could in turn, affect sleep and/or resiliency/vulnerability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Rolf Bodmer and Marc Tatar for comments and advice and Yasuko Suzuki for helpful contributions. This work was funded by grants from the USA National Institutes of Health (R01 NS051305 to P.J.S., 1 P01 AG033561-01 to N.S. & P.J.S).

References

1. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology. 2013; 38:1858–1873. [PubMed: 23838101]
2. Gillespie CF, Phifer J, Bradley B, Ressler KJ. Risk and resilience: genetic and environmental influences on development of the stress response. Depression and anxiety. 2009; 26:984–992. [PubMed: 19750552]
3. Stevens HE, Leckman JF, Coplan JD, Suomi SJ. Risk and resilience: early manipulation of macaque social experience and persistent behavioral and neurophysiological outcomes. Journal of the American Academy of Child and Adolescent Psychiatry. 2009; 48:114–127. [PubMed: 19127170]
4. Cicchetti D, Blender JA. A multiple-levels-of-analysis perspective on resilience: implications for the developing brain, neural plasticity, and preventive interventions. Annals of the New York Academy of Sciences. 2006; 1094:248–258. [PubMed: 17347356]
5. Chuah YM, Venkatraman V, Dinges DF, Chee MW. The neural basis of interindividual variability in inhibitory efficiency after sleep deprivation. J Neurosci. 2006; 26:7156–7162. [PubMed: 16822972]
6. Van Dongen HP, Vitellaro KM, Dinges DF. Individual differences in adult human sleep and wakefulness: Leitmotif for a research agenda. Sleep. 2005; 28:479–496. [PubMed: 16171293]
7. Rogers NL, Dorrian J, Dinges DF. Sleep, waking and neurobehavioural performance. Front Biosci. 2003; 8:s1056–1067. [PubMed: 12957855]
8. Goldstein AN, Greer SM, Saletin JM, Harvey AG, Nitschke JB, Walker MP. Tired and apprehensive: anxiety amplifies the impact of sleep loss on aversive brain anticipation. J Neurosci. 2013; 33:10607–10615. [PubMed: 23804084]
9. Killgore WD, Balkin TJ, Wesensten NJ. Impaired decision making following 49 h of sleep deprivation. Journal of sleep research. 2006; 15:7–13. [PubMed: 16489997]
10. Tesler N, Gerstenberg M, Huber R. Developmental changes in sleep and their relationships to psychiatric illnesses. Current opinion in psychiatry. 2013; 26:572–579. [PubMed: 24060918]
11. Van Dongen HP, Baynard MD, Maislin G, Dinges DF. Systematic interindividual differences in neurobehavioral impairment from sleep loss: evidence of trait-like differential vulnerability. Sleep. 2004; 27:423–433. [PubMed: 15164894]
12. Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ. PER3 polymorphism predicts sleep structure and waking performance. Curr Biol. 2007; 17:613–618. [PubMed: 17346965]
13. Bachmann V, Klaus F, Bodenmann S, Schafer N, Brugger P, Huber S, Berger W, Landolt HP. Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. Cerebral cortex. 2012; 22:962–970. [PubMed: 21734253]
14. Donlea J, Leahy A, Thimgan MS, Suzuki Y, Hughson BN, Sokolowski MB, Shaw PJ. Foraging alters resilience/vulnerability to sleep disruption and starvation in Drosophila. Proc Natl Acad Sci U S A. 2012; 109:2613–2618. [PubMed: 22308351]
15. Almli LM, Fani N, Smith AK, Ressler KJ. Genetic approaches to understanding post-traumatic stress disorder. The international journal of neuropsychopharmacology/official scientific journal of the Collegium Internationale Neuropsychopharmacologicum. 2014; 17:355–370.
16. McGrath LM, Cornelis MC, Lee PH, Robinson EB, Duncan LE, Barnett JH, Huang J, Gerber G, Sklar P, Sullivan P, et al. Genetic predictors of risk and resilience in psychiatric disorders: a cross-disorder genome-wide association study of functional impairment in major depressive disorder, bipolar disorder, and schizophrenia. American journal of medical genetics Part B,

Neuropsychiatric genetics: the official publication of the International Society of Psychiatric Genetics. 2013; 162B:779–788.

17. Gozal D, Crabtree VM, Sans Capdevila O, Witcher LA, Kheirandish-Gozal L. C-reactive protein, obstructive sleep apnea, and cognitive dysfunction in school-aged children. American journal of respiratory and critical care medicine. 2007; 176:188–193. [PubMed: 17400731]
18. Williams JA, Sathyanarayanan S, Hendricks JC, Sehgal A. Interaction between sleep and the immune response in Drosophila: a role for the NFkappaB relish. Sleep. 2007; 30:389–400. [PubMed: 17520783]
19. Opp MR. Sleep and psychoneuroimmunology. Immunology and allergy clinics of North America. 2009; 29:295–307. [PubMed: 19389583]
20. Toth LA, Tolley EA, Krueger JM. Sleep as a prognostic indicator during infectious disease in rabbits. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1993; 203:179–192.
21. Krueger JM, Clinton JM, Winters BD, Zielinski MR, Taishi P, Jewett KA, Davis CJ. Involvement of cytokines in slow wave sleep. Progress in brain research. 2011; 193:39–47. [PubMed: 21854954]
22. Imeri L, Opp MR. How (and why) the immune system makes us sleep. Nat Rev Neurosci. 2009; 10:199–210. [PubMed: 19209176]
23. Zielinski MR, Krueger JM. Sleep and innate immunity. Front Biosci (Schol Ed). 2011; 3:632–642. [PubMed: 21196401]
24. Marin I, Kipnis J. Learning and memory … and the immune system. Learn Mem. 2013; 20:601–606. [PubMed: 24051097]
25. Donlea JM, Thimgan MS, Suzuki Y, Gottschalk L, Shaw PJ. Inducing sleep by remote control facilitates memory consolidation in Drosophila. Science. 2011; 332:1571–1576. [PubMed: 21700877]
26. Seugnet L, Suzuki Y, Vine L, Gottschalk L, Shaw PJ. D1 receptor activation in the mushroom bodies rescues sleep-loss-induced learning impairments in Drosophila. Curr Biol. 2008; 18:1110–1117. [PubMed: 18674913]
27. Seugnet L, Suzuki Y, Stidd R, Shaw PJ. Aversive phototaxic suppression: evaluation of a short-term memory assay in Drosophila melanogaster. Genes Brain Behav. 2009; 8:377–389. [PubMed: 19220479]
28. Le Bourg E, Buecher C. Learned suppression of photopositive tendencies in Drosophila melanogaster. Anim Learn Behav. 2002; 30:330–341. [PubMed: 12593325]
29. Seugnet L, Suzuki Y, Merlin G, Gottschalk L, Duntley SP, Shaw PJ. Notch signaling modulates sleep homeostasis and learning after sleep deprivation in Drosophila. Curr Biol. 2011; 21:835–840. [PubMed: 21549599]
30. Seugnet L, Suzuki Y, Donlea JM, Gottschalk L, Shaw PJ. Sleep deprivation during early-adult development results in long-lasting learning deficits in adult Drosophila. Sleep. 2011; 34:137–146. [PubMed: 21286249]
31. Thimgan MS, Suzuki Y, Seugnet L, Gottschalk L, Shaw PJ. The perilipin homologue, lipid storage droplet 2, regulates sleep homeostasis and prevents learning impairments following sleep loss. PLoS Biol. 2010; 8
32. Seugnet L, Galvin JE, Suzuki Y, Gottschalk L, Shaw PJ. Persistent short-term memory defects following sleep deprivation in a drosophila model of Parkinson disease. Sleep. 2009; 32:984–992. [PubMed: 19725249]
33. Seugnet L, Suzuki Y, Thimgan M, Donlea J, Gimbel SI, Gottschalk L, Duntley SP, Shaw PJ. Identifying sleep regulatory genes using a Drosophila model of insomnia. J Neurosci. 2009; 29:7148–7157. [PubMed: 19494137]
34. Thimgan MS, Gottschalk L, Toedebusch C, McLeland J, Rechtschaffen A, Gilliland-Roberts M, Duntley SP, Shaw PJ. Cross-translational studies in human and Drosophila identify markers of sleep loss. PLoS One. 2013; 8:e61016. [PubMed: 23637783]
35. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature. 2002; 417:287–291. [PubMed: 12015603]

36. Imler JL, Bulet P. Antimicrobial peptides in Drosophila: structures, activities and gene regulation. Chemical immunology and allergy. 2005; 86:1–21. [PubMed: 15976485]

37. Silverman N, Paquette N, Aggarwal K. Specificity and signaling in the Drosophila immune response. Invertebrate survival journal: ISJ. 2009; 6:163–174. [PubMed: 21625362]

38. Cao Y, Chtarbanova S, Petersen AJ, Ganetzky B. Dnr1 mutations cause neurodegeneration in Drosophila by activating the innate immune response in the brain. Proc Natl Acad Sci U S A. 2013; 110:E1752–1760. [PubMed: 23613578]

39. Andretic R, Shaw PJ. Essentials of sleep recordings in Drosophila: moving beyond sleep time. Methods Enzymol. 2005; 393:759–772. [PubMed: 15817323]

40. Thomas A, Lee PJ, Dalton JE, Nomie KJ, Stoica L, Costa-Mattioli M, Chang P, Nuzhdin S, Arbeitman MN, Dierick HA. A versatile method for cell-specific profiling of translated mRNAs in Drosophila. PLoS One. 2012; 7:e40276. [PubMed: 22792260]

41. Harvey AG. A transdiagnostic approach to treating sleep disturbance in psychiatric disorders. Cognitive behaviour therapy. 2009; 38(Suppl 1):35–42. [PubMed: 19697179]

42. Kain JS, Stokes C, de Bivort BL. Phototactic personality in fruit flies and its suppression by serotonin and white. Proc Natl Acad Sci U S A. 2012; 109:19834–19839. [PubMed: 23150588]

43. Ofstad TA, Zuker CS, Reiser MB. Visual place learning in Drosophila melanogaster. Nature. 2011; 474:204–207. [PubMed: 21654803]

44. Chabaud MA, Preat T, Kaiser L. Behavioral characterization of individual olfactory memory retrieval in Drosophila melanogaster. Frontiers in behavioral neuroscience. 2010; 4:192. [PubMed: 21258642]

45. Chabaud MA, Isabel G, Kaiser L, Preat T. Social facilitation of long-lasting memory retrieval in Drosophila. Curr Biol. 2009; 19:1654–1659. [PubMed: 19781943]

46. Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron. 2009; 61:213–219. [PubMed: 19186164]

47. Nadjar A, Blutstein T, Aubert A, Laye S, Haydon PG. Astrocyte-derived adenosine modulates increased sleep pressure during inflammatory response. Glia. 2013; 61:724–731. [PubMed: 23378051]

48. Petersen AJ, Rimkus SA, Wassarman DA. ATM kinase inhibition in glial cells activates the innate immune response and causes neurodegeneration in Drosophila. Proc Natl Acad Sci U S A. 2012; 109:E656–664. [PubMed: 22355133]

49. Rupp TL, Wesensten NJ, Balkin TJ. Trait-like vulnerability to total and partial sleep loss. Sleep. 2012; 35:1163–1172. [PubMed: 22851812]

Highlights

- Individual differences in vulnerability to stress pose significant societal costs.
- The ability to form STM during sleep disruption is a stable individual trait.
- Immune factors contribute to individual differences in vulnerability to sleep loss.
- Distinct immune factors in glia and neurons impact the vulnerability to sleep loss.
- The microbiome may be a source for individual variation in cognitive vulnerability.

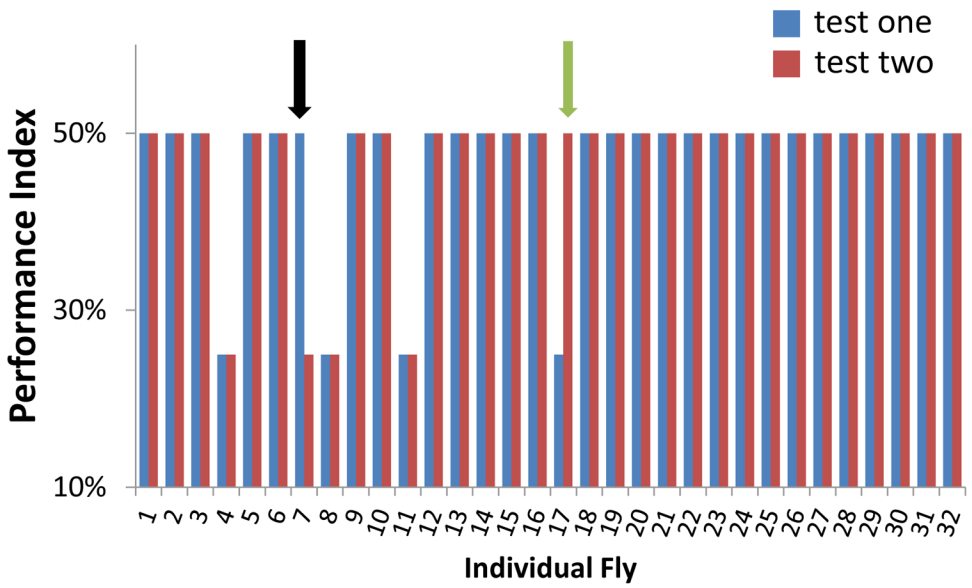

Figure 1. Individual flies show stable short-term memory over repeated trials

Individual *Cs* flies were tested in the APS (Test-1, blue) and then re-tested 2-days later (Test-2; red). Flies displayed stable performance over the two trials. Arrows indicate flies whose score changed between Test-1 and Test-2.

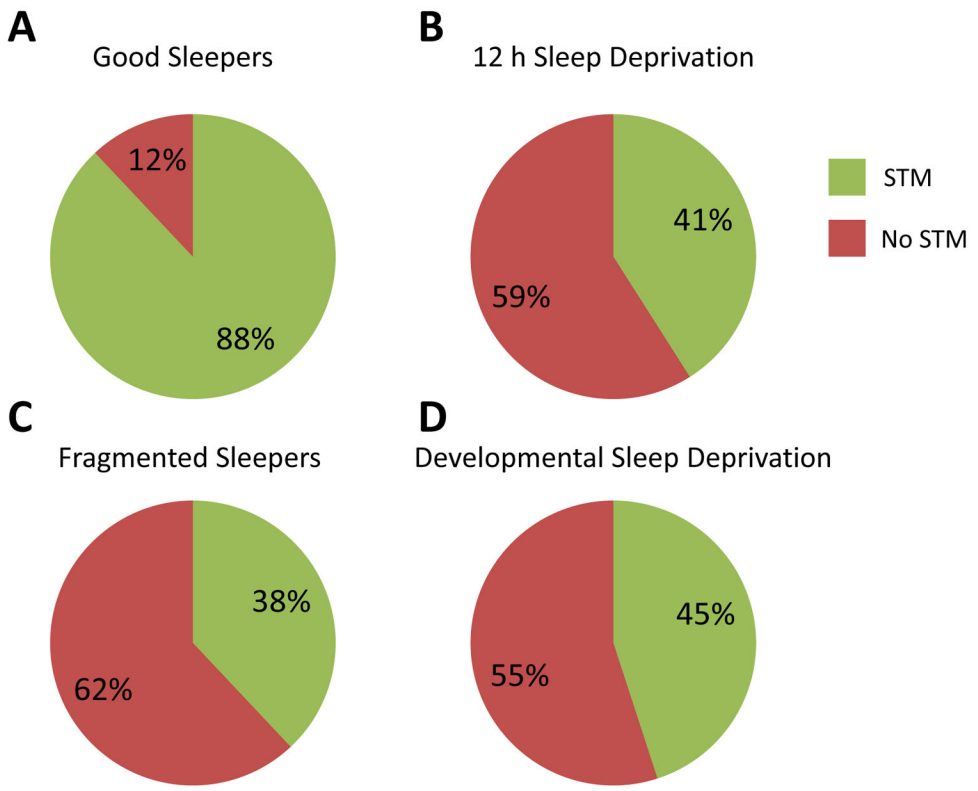

Figure 2. Individual flies show resilience or vulnerability to sleep loss

Short-term memory (STM) was assessed using APS in 4 different groups of *Cs* female flies. **(A)** In flies that are good sleepers (i.e. normal sleeping time that is consolidated). **(B)** Following 12 h of sleep-deprivation. **(C)** In flies that have normal sleeping time but sleep is fragmented. **(D)** In flies that have been developmentally sleep-deprived for 12 h during the first night of their adult life and allowed to rest unperturbed for 5 days before being evaluated for STM.

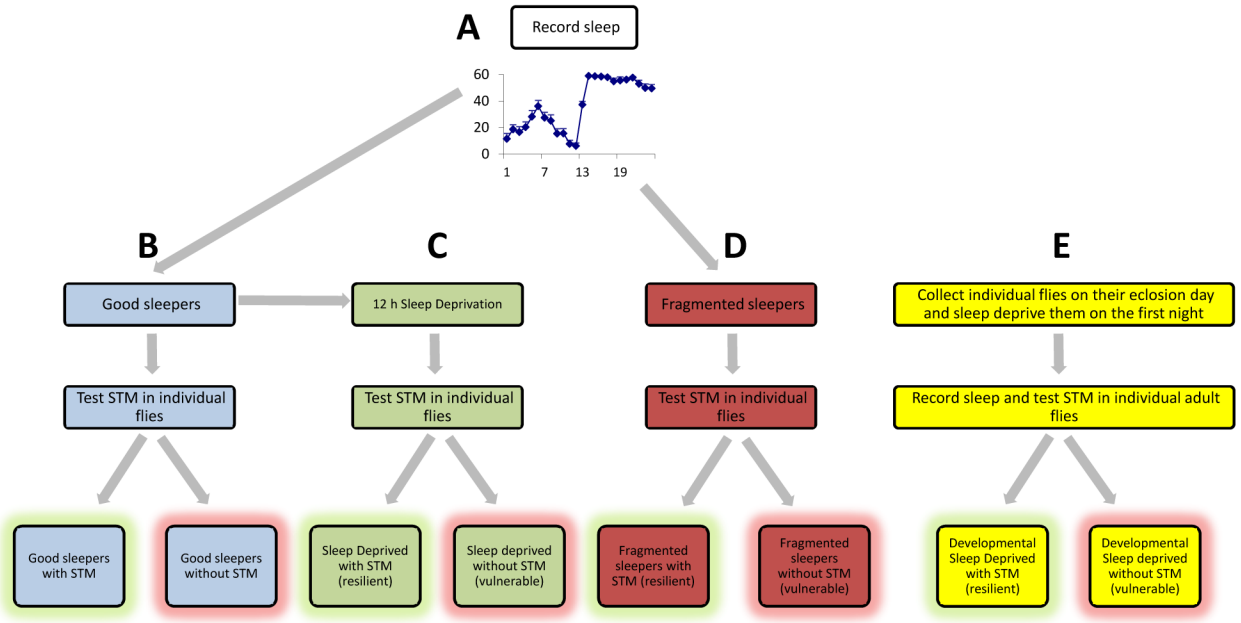

Figure 3. Protocol used for RNA extraction

(A) Sleep was recorded in individual female *Cs* flies. Short-term memory (STM) was evaluated using the APS in four different groups of flies: **B)** good sleepers **(C)**, good sleepers that were subjected to 12 h of sleep deprivation the night preceding the STM assay **(D)**, Flies that spontaneously exhibit fragmented sleepers and **(E)** flies that were sleep deprived on the first night of their adult life and allowed to rest un-perturbed for 5 days before being evaluated for STM. Within each of the four groups, two subgroups of flies were identified, flies that have STM (resilient) and those that don't (vulnerable). Resilient and vulnerable individual flies within each of the four groups were pooled together in groups of 20 and RNA was extracted from heads.

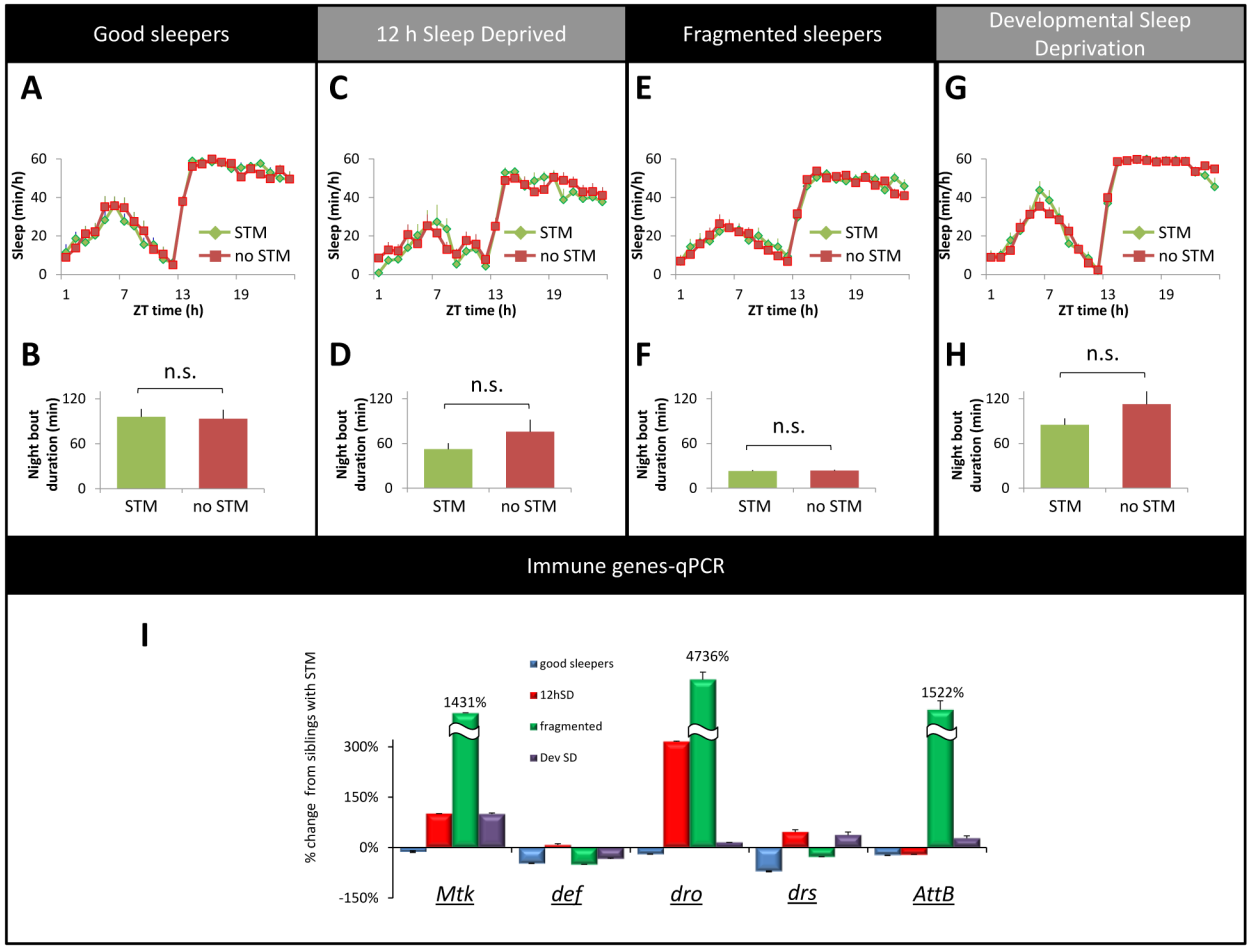

Figure 4.

(A) Good sleepers *Cs* flies that exhibit STM sleep the same as their memory impaired siblings. A 2 × 24 h repeated measures ANOVAs did not yield a significant condition (STM, No STM) x Time (24 h) interaction $F_{[23,736]}$=0.993, p= 0.455; n=17 and 23/group). **(B)** Nighttime sleep consolidation does not differ between good sleeping flies that form STM and their cognitively impaired siblings (t-test, p=0.88). **(C)** Baseline sleep does not differ between sleep deprived flies that exhibit STM deficits and their resilient siblings that maintain STM following sleep loss. A repeated measures ANOVAs did not yield a significant condition (STM, No STM) x Time (24 h) interaction $F_{[23,437]}$=1.138, p= 0.334; n=15 and 22/group). **(D)** Night bout duration on the day before sleep deprivation is not different between resilient (STM) and their impaired siblings (t-test, p=0.27). **(E)** Memory impaired *Cs* flies that have spontaneously fragmented sleep display the same sleep pattern as their resilient siblings: A repeated measures ANOVAs did not yield a significant condition (STM, No STM) x Time (24 h) interaction $F_{[23,805]}$=0.606, p= 0.829; n=17 and 26/group). **(F)** Night bout duration is not different between fragmented sleepers that have STM and their resilient siblings (t-test, p=0.76). **(G)** *Cs* flies that were sleep deprived on their first day of adult life, and allowed to rest unperturbed for 5 days yet continue to display wild-type STM do not sleep different than their impaired siblings. A repeated measures ANOVAs did

not yield a significant condition (STM, No STM) x Time (24 h) interaction $F_{[23,805]}$=0.998, p= 0.442; n=17 and 21/group). **(H)** Night bout duration is not different between developmentally sleep deprived flies that have STM and those that don't (t-test, p=0.19). **(I)** qPCR for *Metchnikowin* (*Mtk*), *defensin* (*def*), *drosocin* (*dro*), *drosomycin* (*drs*) and *AttacinB* (*AttB*) were performed on heads from flies that have STM and those that don't within each sleeping category (i.e. good sleepers,12 h of Sleep Deprivation (12 h SD), fragmented sleepers and Developmental Sleep Deprivation (Dev SD)). The data are presented as the % change from siblings that have STM.

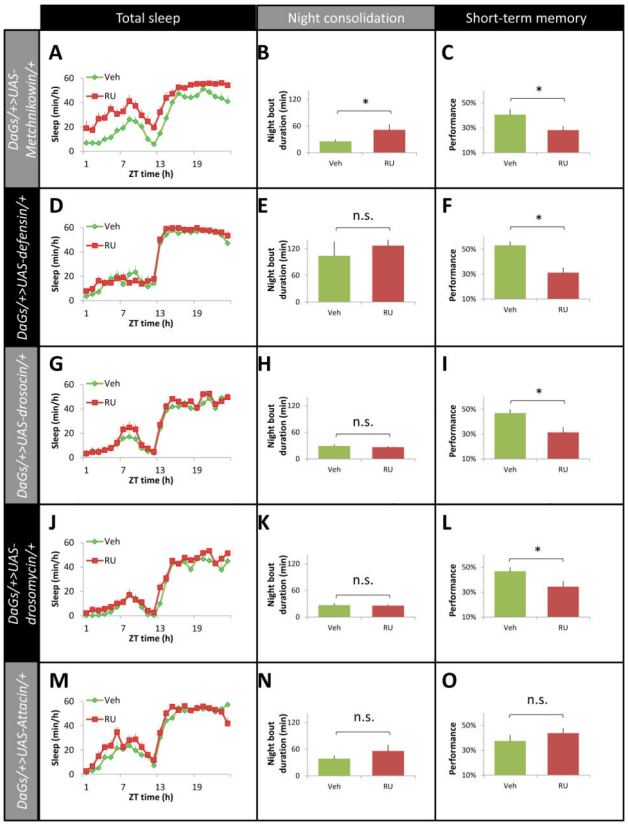

Figure 5. Overexpression of AMPs genes in adult flies impairs short-term memory

(A) *DaGs/+>UAS-Mtk/+* flies fed RU486 (RU) sleep more than their vehicle (Veh) fed siblings; A 2 (Drug: RU, VEH) X Time (24 h) repeated measures ANOVAs yielded a significant main effect for Drug (RU, Veh) ANOVA $F_{[1,30]} = 14.48$; p=0.001) and since RU-fed *DaGs/+>UAS-Mtk/+* sleep more than controls during both the day and night no Drug X Time interaction ANOVA $F_{[23,690]} = 1.17$; p=0.26, n=16 flies/group). **(B)** The increase in sleep in RU-fed *DaGs/+>UAS-Mtk/+* is accompanied by an increase in nighttime sleep bout duration (t-test, p=0.04). **(C)** STM is impaired in RU-fed *DaGs/+>UAS-Mtk/+* flies compared with their vehicle-fed siblings (t-test, p=0.02, n=8–9 flies/group). **(D)** RU-fed *DaGs/+>UAS-def/+* flies sleep similarly to their vehicle-fed siblings. A 2 × 24 h repeated measures ANOVAs did not yield a main effect for Drug (ANOVA $F_{[1,26]} = 0.84$; p=0.36) or a significant Drug x Time interaction (ANOVA $F_{[23,598]} = 0.85$; p=0.65, n=14–15 flies/group). **(E)** Night bout duration is not different in RU-fed *DaGs/+>UAS-def/+* flies compared with their vehicle-fed siblings (t-test, p=0.51). **(F)** STM is impaired in RU-fed *DaGs/+>UAS-def/+* flies compared with their vehicle-fed siblings (t-test, p=0.0004, n=8 flies/group). **(G)** RU and Veh-fed *DaGs/+>UAS-dro/+* flies sleep similarly. A 2 × 24 repeated measures ANOVA did not yield a Main effect for Drug (ANOVA $F_{[1,30]} = 0.82$; p=0.37) or a Drug X Time interaction (ANOVA $F_{[23,690]} = 0.63$; p=0.90, n=16 flies/group). **(H)** Night bout duration does not differ between RU and Veh fed *DaGs/+>UAS-dro/+* flies (t-test, p=0.56). **(I)** STM is impaired in RU-fed *DaGs/+>UAS-dro/+* flies compared with their Veh-fed siblings (t-test, p=0.004, n=8 flies/group). **(J)** RU and Veh fed *DaGs/+>UAS-drs/+* siblings sleep similarly. A 2 × 24 repeated measures ANOVA did not yield a Main

effect for Drug (ANOVA $F_{[1,28]} = 3.46$; p=0.07) or a Drug X Time interaction (ANOVA $F_{[23,644]} = 0.89$; p=0.60, n= 15 flies/group). **(K)** Night bout duration does not differ in RU and Veh-fed *DaGs/+>UAS-drs/+* siblings (t-test, p=0.80). **(L)** STM is impaired in RU-fed *DaGs/+>UAS-drs/+* flies compared with their Veh-fed siblings (t-test, p=0.02, n= 8 flies/group). **(M)** RU and Veh fed *DaGs/+>UAS-Att/+* siblings sleep similarly. A 2 × 24 repeated measures ANOVA did not yield a Main effect for Drug (ANOVA $F_{[1,30]} = 2.49$; p=0.13) but a significant Drug X Time interaction (ANOVA $F_{[23,690]} = 1.86$; p=0.009, n= 16 flies/group). **(N)** Night bout duration did not differ in *DaGs/+>UAS-Att/+* flies fed RU486 compared with their vehicle-fed siblings (t-test, p=0.27). **(O)** STM is not impaired in RU-fed *DaGs/+>UAS-Att/+* flies compared with their vehicle-fed siblings (t-test, p=0.17, n=8 flies/group).

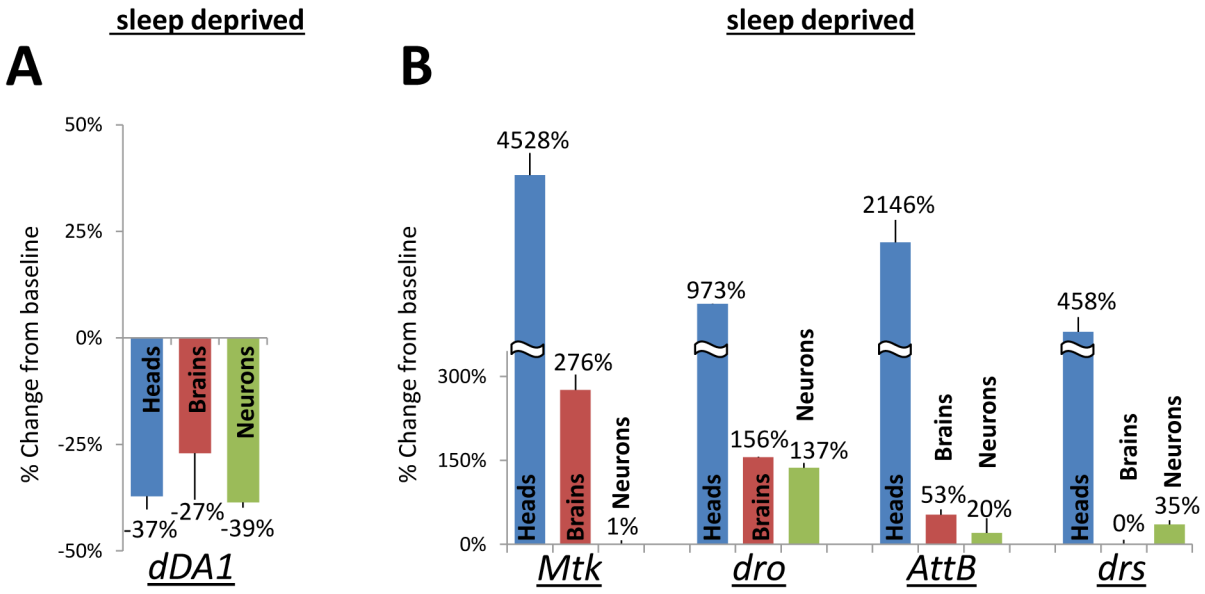

Figure 6. Sleep deprivation increases level of *Metchnikowin* and *drosocin* in different cellular compartments

mRNA was extracted from whole heads (containing eyes, cuticle, fat body, glia and neurons) and brains (glia and neurons) of *Cs* flies under baseline and after 12 h of sleep deprivation (SD). In addition, we used the TRAP technique [40] and extracted mRNA from neurons only in *MJ85b-GAL4>UAS-GFP::RpL10A* flies, during baseline and after SD. The data are expressed as a percentage change in mRNA during SD relative to baseline. **(A)** Transcript levels of the *Drosophila D1 dopamine receptor* (*dDA1*) are down-regulated in heads, brains and in neurons following SD. **(B)** Transcript levels for *Mtk*, *dro*, *drs*, and *AttB* from heads (blue), brains (red) and neurons (green) are differentially regulated by sleep deprivation following 12 h of SD.

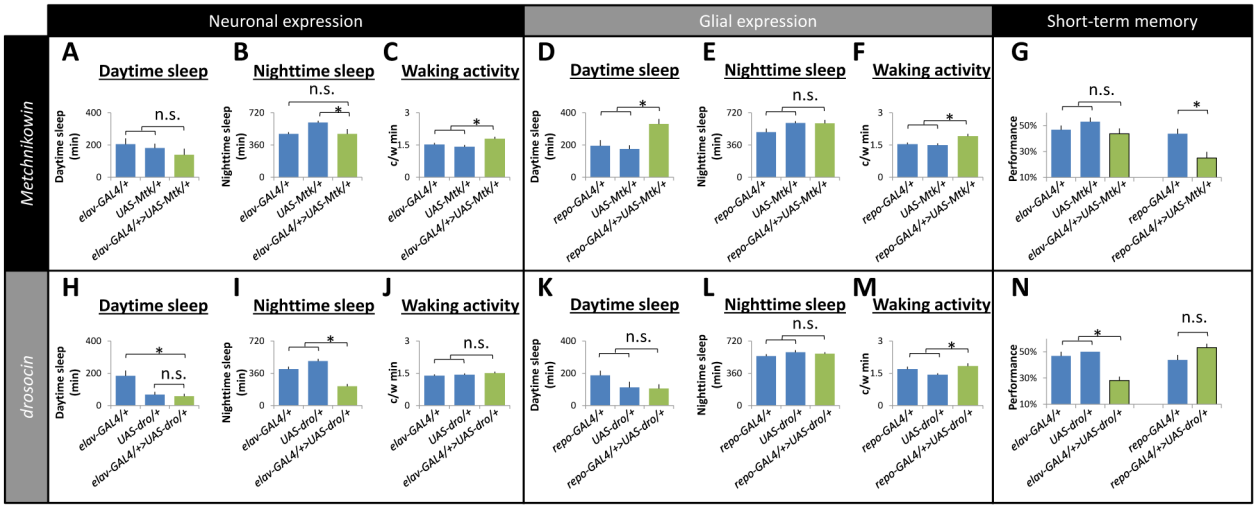

Figure 7. Expression of *Mtk* and *dro* in neurons and glia differentially impact STM

(A, B) Neuronal expression of *Mtk* does not alter daytime sleep or nighttime sleep compared to both *elav-GAL4/+* and *UAS-Mtk/+* parental controls (blue) (One way ANOVA $F_{[2,40]}$ = 0.88; p=0.42 and One way ANOVA $F_{[2,40]}$ = 6.70; p=0.003, respectively; *p<0.05 modified Bonferroni test). **(C)** Neuronal expression of *Mtk* increases the intensity of waking locomotor activity compared to parental controls (One way ANOVA $F_{[2,40]}$ = 4.72; p=0.01). **(D, E)** Glial expression of *Mtk* increases daytime sleep but does not alter nighttime sleep compared to controls (One way ANOVA $F_{[2,42]}$ = 7.41; p=0.001 and ANOVA $F_{[2,42]}$ = 3.08; p=0.06, respectively; *p<0.05 modified Bonferroni test). **(F)** Glial expression of *Mtk* increases the intensity of waking locomotor activity compared to genetic controls (One way ANOVA $F_{[2,42]}$ = 7.04; p=0.002). **(G)** Neuronal expression of *Mtk* does not alter STM compared to parental controls (left panel, blue) but glial expression of *Mtk* disrupts STM (One way ANOVA $F_{[4,36]}$ = 7.51; p=0.0001, *p<0.05 modified Bonferroni test). **(H)** Neuronal expression of *dro* does not alter daytime sleep when compared to both *elav-GAL4/+* and *UAS-dro/+* parental controls (One way ANOVA $F_{[2,45]}$ = 9.23; p=0.0004), *p<0.05 modified Bonferroni test). **(I)** Neuronal expression of *dro* reduces nighttime sleep compared with parental controls (One way ANOVA $F_{[2,45]}$ = 27.99; p= 1.26^{E-08}). **(J)** The intensity of waking locomotor activity is not changed in *elav GAL4>UAS-dro* flies (One way ANOVA $F_{[2,45]}$ = 1.00; p=0.38). **(K, L)** Glial expression of *dro* does not change daytime or nighttime sleep compared to parental controls (One way ANOVA $F_{[2,45]}$ = 2.26; p=0.11 and One way ANOVA $F_{[2,45]}$ = 1.11; p=0.34, respectively). **(M)** Glial expression of *dro* increases waking activity (One way ANOVA $F_{[2,45]}$ = 3.88; p=0.03). **(N)** Neuronal expression of *dro*, but not glial expression disrupts STM compared to parental controls (One way ANOVA $F_{[4,36]}$ = 10.42; p= 1.03^{E-05}, *p<0.05 modified Bonferroni test).