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FKBP14 regulates sleep in response to starvation and oxidative stress

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ABSTRACT

Animals modulate sleep in response to stresses that include hypoxia, aging, diet and starvation; however, the molecular basis for integrating stress and sleep remain unclear. As animals age, they experience an increase in oxidative damage and loss of sleep quality. Moreover, both oxidative stress and poor sleep quality contribute to shortened lifespans in Drosophila and other animals. FKBP14 is a member of a highly conserved family of FK506-binding proteins (FKBPs) that bind to the immunosuppressant drug FK506. FKBP14 has recently been identified as a Presenilin (Psn)-dependent mediator of Notch signaling. Psn plays a pivotal role in the formation of Amyloid β (Aβ) plaques that contribute to Alzheimer's disease, and recently sleep loss has been associated with these plaques in the brain. We have identified *Fkbp14* as a potent inhibitor of sleep during starvation and found that it is necessary in the peptidergic neurons of the fly brain for this response, suggesting that FKBP14 regulates sleep in the brain during starvation.

Oxidative stress also contributes to Aβ plaques and we find that *Fkbp14* mutants are resistant to changes in sleep when fed the free-radical inducing drug, Paraquat, which is known to impair sleep quality and shorten lifespan. The possible role of FKBP14 in mediating Notch signaling to regulate sleep in response to oxidative stress exemplifies the importance of sleep quality and may provide insight into age and stress-related perturbations in sleep that contribute to Alzheimer's disease.

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INTRODUCTION

Animals modulate sleep in response to stresses that include hypoxia, aging, diet and starvation; however, the molecular basis for integrating stress and sleep remain unclear. We have identified *Fkbp14*, a member of the conserved family of FK506 binding proteins (FKBPs) that have prolyl-isomerase activity, as a potent inhibitor of sleep during starvation. We found that FKBP14 is necessary in the peptidergic neurons of the fly brain for this response, suggesting that FKBP14 regulates sleep in these neurons during starvation. FKBP14 mediates Presenilin (Psn)-dependent Notch signaling, and that regulation of Notch mediates sleep homeostasis following deprivation. Psn, oxidative stress, and sleep loss play pivotal roles in the formation of Amyloid β (Aβ) plaques that contribute to Alzheimer's disease [1-7]. Interestingly, *Fkbp14* males are resistant to changes in sleep during oxidative stress but remain highly sensitive to dietary protein, both of which shorten lifespan and induce sleep fragmentation [8, 9]. This suggests that FKBP14 regulates sleep quality specifically in response to oxidative stress. The possible role of FKBP14 in mediating Notch signaling to regulate sleep exemplifies the importance of sleep quality and may provide insight into age and stress-related perturbations in sleep that contribute to Alzheimer's disease.

RESULTS AND DISCUSSION

Fruit flies display similar hallmarks of sleep as mammals that include relaxed posture, extended periods of behavioral quiescence with increased arousal threshold, and homeostatic rebound following sleep deprivation [10, 11]. As in mammals, sleep in fruit flies is affected by hypnotics or stimulants such as caffeine, as well as by age, diet, and starvation [9, 12, 13].

During starvation, animals will suppress sleep amount although sleep quality remains unaffected. This response is evolutionarily conserved in *Drosophila* and reflects a balance between sleep and caloric need presumably to promote foraging, and for a fruit fly that can only survive 1-2 days without food, balancing energy with sleep requirements is imperative [9, 14, 15]. Unlike sleep deprivation or insomnia, which shortens lifespan and impairs learning [16, 17], periodic starvation is known to increase lifespan and suppress sleep without cognitive effects [18-20]. Moreover, during starvation sleep quality is maintained as determined by sleep duration, which when fragmented, is the parameter in sleep disorders that shorten lifespan— and not total sleep per se [21, 22].

FKBP14 regulates starvation-induced sleep suppression

Fkbp14 belongs to a highly conserved family of proteins that bind to the immunosuppressant drugs FK506. FKBPs comprise a large family of conserved immunophilins that have peptidyl-prolyl isomerase activity, which convert cis-trans of peptide bonds— a rate limiting step in protein folding [23]. The mammalian brain is enriched in FKBPs, and FKBP14 interacts antagonistically with AP-1 to regulate synaptic size at the neuromuscular junction in *Drosophila* larvae [24, 25]. *Fkbp14* is also expressed in the adult brain, fat body, and other tissues [24, 26].

FKBP14 was identified in an independent sleep-suppression screen that included over 1,000 RNAi lines (data not shown). To confirm that FKBP14 regulates sleep during starvation, we starved a hypomorph mutant, $Fkbp14^{EP}$, which carries a P-element insertion in the 3' untranslated region, and found that these mutants fail to suppress sleep during starvation (Figure 1A). Similar to wild-type, *Fkbp14* did not change its sleep duration in response to starvation although their sleep bouts were longer in general (Fig. 1B). Two other *Fkbp14*

hypomorphic alleles were also screened, and when starved either failed to suppress sleep or suppressed sleep significantly less than wild type (Figure S1A through C).

We then asked whether FKBP14 regulates the response to hunger, which would explain the lack of sleep suppression during 24 h starvation. To determine if these flies could sense hunger and respond accordingly, we performed a blue dye assay as previously described, which measures the rate of relative food intake [27]. Starved and fed flies were allowed to feed on standard food containing blue dye for 30 minutes and were then flash-frozen and analyzed by spectrophotometry. We found that *Fkbp14* mutants, like wild-type, significantly increase food intake following starvation, suggesting that loss of FKBP14 does not affect feeding or hunger and thus was not the reason for the lack of sleep suppression in *Fkbp14* mutants (Figure 1C).

We then asked whether FKBP14 regulates starvation-specific pathways to affect sleep or if loss of FKBP14 affects general sleep pathways and therefore fails to suppress sleep in *Fkbp14* mutants. Caffeine induces wakefulness via the *Drosophila* D1 dopamine receptor (D1DA) in the mushroom bodies (MBs), although MBs were found to be dispensable for starvation-induced sleep suppression [14, 28]. To determine if *Fkbp14* mutants were able to suppress sleep pharmacologically, we fed flies caffeine as previously described and measured sleep for 48 h [13]. We found that *Fkbp14* mutants were able to suppress sleep in response to caffeine similar to wild-type (Figure 1C). This suggests that Fkbp14 regulates sleep amount specifically through metabolic or starvation stress.

FKBP14 is required in peptidergic neurons to regulate sleep

Since sleep is regulated in the fly brain and FKBP14 regulates synaptic size at the neuromuscular junction in fruit fly larvae, we asked whether FKBP14 functions in the neurons and adult brain

to regulate sleep. To determine this, we used a pan-neuronal GAL4 driver to induce RNAi of *Fkbp14*. We found that flies failed to suppress sleep when *Fkbp14* was knocked down in these cells (Figure 2A). To determine if FKBP14 functions in the peptidergic neurons, which comprise about 70% of the fly brain, we knocked down *Fkbp14* in these cells. We found that FKBP14 was necessary in these cells to regulate sleep in response to starvation (Figure 2A). However, overexpressing *Fkbp14* in wild-type flies using the same neuronal drivers did not appear to induce a hypersensitive sleep response to starvation (Figure S4), suggesting that increasing FKBP14 in the brain does not cause an exaggerated phenotype. Knockdown of *Fkbp14* will need to be confirmed via quantitative PCR. We previously found that expressing a constitutively-active insulin (InR) in the insulin-producing cells (IPCs) fails to suppress sleep in flies when starved, similar to the *Fkbp14* mutants (results not shown). However, knocking down *Fkbp14* in the IPCs or in the insulin-producing median Neurosecretory cells (mNSCs), which overlap with the IPCs, does not affect sleep (Figures 2C-D and S2).

Fkbp14 mediates Psn-dependent Notch signaling by interacting with Psn in the ER presumably to mediate protein folding [1], and Notch signaling in the Mushroom Bodies (MBs) regulates sleep homeostasis of the fruit fly. A mutation in *bunched*, a negative regulator of Notch signaling, as well as a gain-of-function Notch allele, both confer resistance to sleep deprivation as indicated by a lack of sleep rebound but with no impairment in learning or markers of sleepiness after deprivation [20, 29]. The MBs are pivotal cells necessary for learning and memory as well as sleep, although the MBs appear to be dispensable for starvation-induced sleep suppression [14, 30, 31]. An *Fkbp14* reporter gene was found to localize in glomeruli of the antennal lobe that innervates the MB calyx [32], but whether it functions in the

MBs is not known. To determine if FKBP14 functions to regulate sleep in the MBs, we knocked down *Fkbp14* using the c739-GAL4 driver, which is expressed in the α/β lobes of the MBs [33]. However, knocking down *Fkbp14* in these cells did not appear to affect sleep under starvation, although the results were not conclusive (Figure S2A and B). Notwithstanding, it is clear that FKBP14 is required in the brain to regulate sleep but further studies will be needed to determine distinct neuronal subpopulations that require FKBP14 to mediate this response.

FKBP14 regulates sleep in response to oxidative stress

Another potential role for FKBP14-mediated Notch signaling is during environmental stress. A negative regulator of Notch, *bunched*, is upregulated in flies during oxidative and mechanical stress [2]. Feeding flies Paraquat, a free-radical generator that induces oxidative stress, causes sleep fragmentation and shortens lifespan [2, 8]. *Fkbp14* regulates lifespan, because a mutation in *Fkbp14* increases lifespan of male flies [3]. Since lifespan genes confer resistance to oxidative stress [3, 34], it is possible that FKBP14 mediates stress-specific pathways given our findings that FKBP14 regulates sleep in response to starvation stress.

To determine if *Fkbp14* mutants are resistant to oxidative stress, we fed the drug Paraquat to WT and *Fkbp14* mutant males as previously described [35] until most flies were dead, which was 7 days. As predicted, *Fkbp14* mutant males are more resistant to oxidative stress than wild type (Figure 3A and B). It is possible that *Fkbp14* mutants have reduced activity levels, which may explain their increased lifespan due to a lower rate-of-living. We measured their activity levels by number of beam crossings per waking minute using the DAMS, and we found that their activity level, although higher than wild-type, are similarly affected by Paraquat (Figure 3C and D).

Oxidative stress also decreases sleep quality as is characteristic of aging animals across phyla. Paraquat-fed flies display increased sleep fragmentation as well as sleep loss [8, 22]. Interestingly, starvation or calorie restriction increases sub-lethal levels of reactive oxygen species (ROS) but without inducing oxidative damage [36, 37]. Induction of sub-lethal amounts of ROS are necessary for cell signaling in response to starvation or calorie restriction, but sub-lethal amounts of Paraquat do not affect sleep in WT until the last week of life [8]. To determine if *Fkbp14* mutants are resistant to changes in sleep quality during oxidative stress, we fed flies a higher dose of [20 mM] Paraquat as previously described [35], and measured total sleep, sleep duration, and number of sleep bouts using the DAMS. We found that *Fkbp14* flies are resistant to changes in total sleep and do not experience fragmented sleep as measured by sleep duration and number of sleep bouts (Figure 4A-C). The *Fkbp14* mutants do not sleep more due to lethargy, since we show that their activity levels are higher than wild-type, although similarly affected by Paraquat (Figure 4D). Taken together, it appears that FKBP14 modulates sleep in response to starvation and oxidative stress pathways.

Dietary protein causes sleep fragmentation but not by inducing oxidative stress

Nutritional parameters that regulate lifespan also regulate sleep. In flies and mammals, prior studies show that protein restriction increases lifespan and this effect is independent of caloric content [38, 39]. In Drosophila, dietary yeast is a source of amino acids, and when combined with sucrose induces sleep fragmentation compared to flies fed sucrose alone. Increasing concentrations of sucrose up to 35% did not increase sleep fragmentation, but the effect was specifically due to dietary yeast extract. Even though total sleep in yeast-fed flies is higher compared to flies fed sucrose alone, the yeast-fed flies experience shorter sleep bout durations

as well as increased sleep bouts, suggesting an overall reduction in sleep quality [12]. Moreover, as in yeast-fed flies, total sleep is increased while sleep duration is fragmented in aged flies, suggesting an increased sleep need presumably in response to lower sleep quality [8].

As animals age, they experience an increase in oxidative damage and fragmented sleep. However, it is unclear whether age or dietary yeast reduce lifespan because of increased sleep fragmentation, or if these factors, including sleep fragmentation, independently contribute to shorter lifespans. Dietary methionine alone is responsible for increasing lifespan in flies and mammals, and methionine restriction lowers mitochondrial reactive oxygen species [40-46]. Since it is possible that dietary protein affects sleep by increasing oxidative damage, we asked whether *Fkbp14* mutants are resistant to yeast-induced changes in sleep quality. We fed flies 5% sucrose in agar (AS), or the same diet but with 5% yeast extract (ASY) instead of 2% yeast as previously described [12]. Interestingly, both male and female *Fkbp14* mutants were highly sensitive to dietary yeast, and sleep in *Fkbp14* males was significantly more fragmented than wild-type (Figure 5). This suggests that dietary protein, although a possible cause of oxidative damage and therefore aging, does not affect sleep by inducing oxidative stress.

FKBP14 may mediate Notch signaling pathways in stress and sleep

FKBP14 acts downstream of Notch signaling by mediating presenilin (Psn)-dependent cleavage of the intracellular domain of Notch by γ-secretase [1]. Loss of FKBP14 results in reduced levels of Amyloid β (Aβ) fragments in the brain of flies that express a human C99 fragment of APP, and FKBP14 is also maintains protein levels of Psn [1]. Psn is a transmembrane protein necessary for the cleavage of the amyloid β-protein, a primary component of amyloid plaques associated

with Alzheimer's disease [47-49]. Sleep deprivation causes accumulation of Aβ in the interstitial fluid in the brain of mice, and recent studies in mice and human showed that Aβ plaques are associated with poor sleep quality [4-6]. Also, sleep onset in mice rapidly increases interstitial space and drive the clearance of metabolites, including Aβ, from the brain [7]. Thus, the role of sleep in Alzheimer's disease is becoming increasingly clear, as is a role for FKBP14.

Notch regulates sleep homeostasis in flies and plays a role in lethargy, a sleep-like state, in the nematode *Caenorhabditis elegans.* Moreover, environmental stressors reduce Notch signaling in both flies and worms. Oxidative stress inhibits Notch signaling in flies by inducing the Notch repressor, *bunched*, a gene that is also upregulated in response to sleep deprivation and promotes sleep rebound [29, 50, 51]. Interestingly, both FKBP14 and Notch signaling negatively regulate c-Jun N-terminal Kinase (JNK) pathways. The Notch intracellular domain inhibits scaffold activity of JNK signaling, and Psn-1 negatively regulate SAPK/JNK pathways. Moreover, FKBP14 interacts with the GSK-3β homologue in Drosophila to antagonistically impinge on AP-1-dependent transcription by modulating JNK signaling pathways [24, 52, 53]. Further supporting the role of FKBP14 in regulating sleep downstream of Notch signaling is that knockdown of JNK in the brain of flies reduces both sleep and longevity [54]. Given these findings, it is likely that FKBP14 mediates Notch signaling to suppress sleep, but this interaction has not been elucidated.

FUTURE DIRECTIONS

Further investigation will be needed to localize neuronal subpopulations where FKBP14 functions to regulate sleep. Once these neurons are identified, we will attempt to rescue the mutant phenotype be expressing FKBP14 in the *Fkbp14* mutant background. This will determine

if expressing FKBP14 in these cells is sufficient to regulate sleep. We also will confirm the validity of RNAi by measuring transcripts of other FKBP family members to determine if there are off-target effects, as well as confirming RNAi of *Fkbp14* in dissected brains using quantitative PCR. To determine relative protection against oxidative stress, we will assay the activity of Superoxide Dismutase in dissected brain tissue against different concentrations of Paraquat in wild-type and in *Fkbp14* mutants. Further studies will also be needed to investigate molecular mechanisms between FKBP14 and Psn-mediated Notch signaling that impair sleep in response to oxidative or starvation stress. To determine this, we will attempt to rescue to mutant phenotype by expression Notch target genes that are downregulated in the *Fkbp14* mutant. Since flies with overactive Notch signaling fail to rebound following sleep deprivation [29], we can knock down *Fkbp14* in these flies to see if FKBP14 is necessary for Notch-mediated regulation of sleep following deprivation.

METHODS

Genetic Screen

Over 1,000 RNAi lines from Vienna *Drosophila* Resource Depertment (VDRC) were independently screened for starvation-induced sleep suppression using a ubiquitous expression driver, *da-GAL4* (Bloomington Stock No. 8641). The results of this screen are not included.

Fly Stocks

Fly stocks were maintained in a 12 h:12 h light:dark cycle at 25°C in conical vials containing standard Jazz-Mix, prepared according to manufacturer's instructions (Applied Scientific, cat # AS153). The following stocks were used in this study: W^{1118} wild type flies supplied by J. DiAngelo. Canton-S wild-type flies supplied by P. Masek, and White Canton-S (WCS)

(Bloomington).

Collection name	*Chromosome*	*Stock #*	*Insertion*
Fkbp14^{EP}	2R	20145	P{EPgy2}
Fkbp14^{PBac}	2R	18553	PBac{WH}
Fkbp14^{Mi}	2R	34181	Mi{MIC}
UAS-Fkbp14 RNAi	3	V12864	P{GD4819}
UAS-Fkbp14 RNAi	3	V39071	P{GD4819}
Collection name	***Chromosome***	***Stock #***	***Expression pattern***
Da-GAL4	3	8641	*Daughterless* (ubiquitous)
Nsyb-GAL4	3	51635	All neurons
C929-GAL4	2	25373	Peptidergic cells
C739-GAL4	2	7362	α/β lobes of MBs
Dilp2-GAL4	2	37516	Adult IPCs
Dilp3-GAL4	Obtained from M. Tatar of Brown Univ.		Larvae and Adult IPCs
Dsk-GAL4	3	51981	MNSCs (overlap w/ IPCs)

Sleep and Activity Recording

In all behavioral experiments, activity and sleep were recorded using the *Drosophila* Activity Monitoring System (Trikinetics, Waltham, MA) in a 12 h: 12 h light:dark cycle at 25°C. Recordings were analyzed with the Excel-based 'Sleep Counting Macro' as previously described [Pittman] [31]. All experiments were maintained in a 25°C incubator with 12:12 LD cycles.

Starvation assay

To measure starvation-induced sleep suppression, 5-7 day old mated females were anesthetized on CO2 and loaded into DAMS tubes containing standard food. After one day of acclimation, baseline sleep was recorded for 24 hours starting at ZT0. At ZT0 of day 2, all flies were transferred to tubes containing 1% agar (Fisher cat # BP1423), and activity/sleep was recorded for another 24 h. Data represents 16 flies per group from at least two or independent

experiments.

Caffeine-feeding

To induce sleep loss, caffeine anhydrous (Sigma) was dissolved in dH20 to [20g/mL] and then applied to 100 mL of standard food for a final concentration of 1 mg/mL. Control food was prepared similarly without caffeine. Flies were housed on standard food and allowed to hatch for 1 day on standard food and adults were transferred to fresh food until aged 5-6 days. Male W^{1118} and *Fkbp14*^{EP} flies were immobilized on a chilled platform over ice and then loaded into DAMS tubes containing either [1 mg/mL] caffeine or food alone. Previous findings from two different wild type lines (WCS and W^{1118}) showed that females fail to suppress sleep on 1, 2, and 4 [mg/mL] caffeine (not shown). Thus, only males were used in this experiment.

Survival and sleep under oxidative stress

For oxidative-stress, Paraquat Dichloride (Sigma, cat # 36541) was dissolved in water to a concentration of [200 mM] and then dissolved in standard food that was previously heated and cooled to 55-60°C, to a final concentration of [20 mM]. Control food was prepared similarly without Paraquat. A small amount of standard food dye (red and green, respectively) was applied to each by dipping the corner of a spatula in the dye and then stirring into 20 mL of Paraquat or control food. 4-day old wild-type male Canton-S and male *Fkbp14*^{EP} flies were loaded onto a chilled platform over ice and then loaded into DAMS tubes containing either [20 mM] Paraquat-food or food alone. W^{1118} and females of each group were loaded at the same time but the majority of each group failed to survive 24 hours and so were not included in the analysis. Survival was measured concurrently using the same flies wherein sleep was analyzed (Figures 3 and 4). Flies that were alive were transferred to fresh food or Paraquat food every 3

days until dead.

Dietary yeast-induced sleep fragmentation

For the AS diet, 5% $^{w/v}$ sucrose (Fisher cat # S0389) was applied to heated 1% agar and stirred until dissolved. For the ASY diet, 5% $^{w/v}$ Yeast extract (Fisher cat # BP1422) was added to half of the prepared AS food and stirred until dissolved. Male and female wild type W^{1118} and *Fkbp14*^{EP} flies were housed on standard food and allowed to hatch for 24 hours before transferring to four separate tubes per group, until aged for 2 more days on standard food. At 3-4 days old, flies were immobilized on a chilled platform over ice and then loaded into DAMS tubes containing either AS or ASY food. Flies were flipped to fresh AS or ASY food every 3-4 days for the duration of the experiment. Data represents 32 flies for each group per each of 2 experiments that ran concurrently. A previous study that demonstrated yeast-induced sleep fragmentation used a concentration of 2% instead of 5%, yet our method repeated their results in W^{1118} flies [12].

Blue Dye feeding assay

Flies of each group were either fed or starved for 24 h on standard food, after which they were allowed to feed for 30 min. on standard food mixed with equal concentrations of FD&C Blue #1 (Spectrum Chemical cat # 3844-45-9) and flash-frozen. Each of 4 flies per group were individually homogenized in 200 µL of PBS buffer and 3 serial dilutions of each were made. Blue-dye standards were also prepared. The absorbance of each sample was measured by spectrophotometry at 655 nm λ using the iMark microplate reader (BIO-RAD, Philadelphia). Data represents one experiment, n=4 per group.

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FIGURES

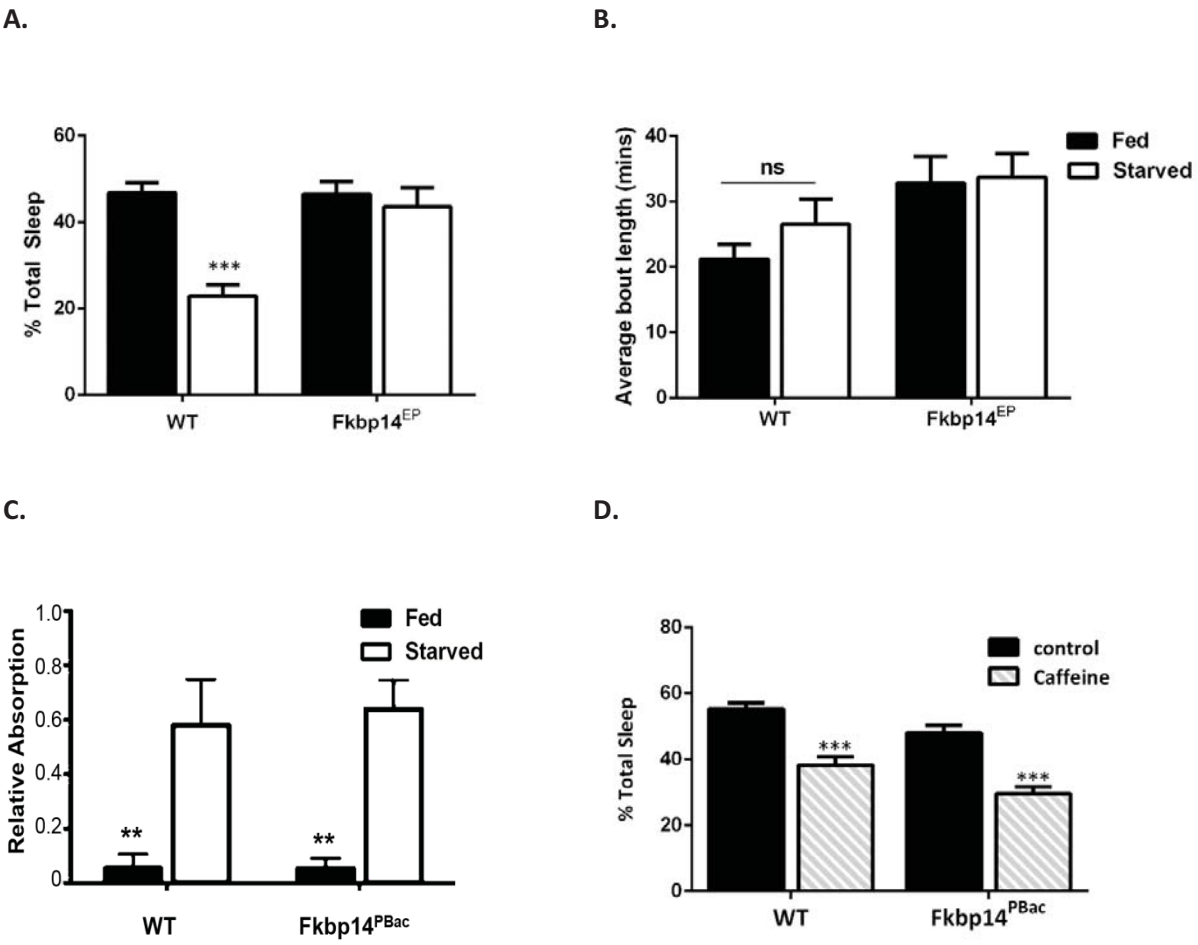


Figure 1. FKBP14 regulates sleep in response to starvation. *Fkbp14* mutants do not suppress sleep (A, B) but increase feeding (C) in response to 24 hr starvation. (D) *Fkbp14* mutants are able to suppress sleep over 48 hours on [1 mg/mL] caffeine. (C) White Canton-S wild-type (WT), n=32, one experiment. *** denotes P-value <0.0001; ** denotes P-value <0.001; one-tailed, paired (A and B) and unpaired (C and D) student *t*-test (Fed/control vs. treatment).

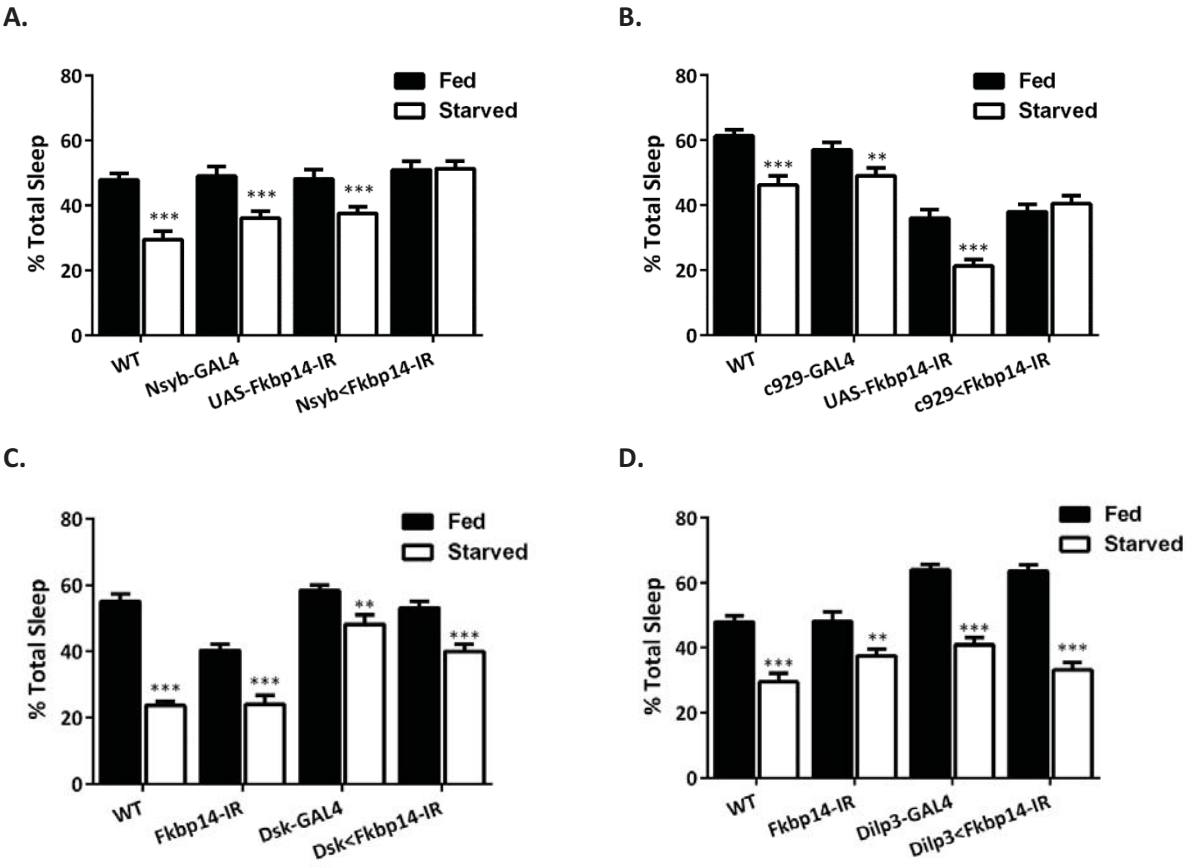


Figure 2. Fkbp14 regulates starvation-induced sleep suppression in the brain. Tissue-specific RNAi of Fkbp14 using GAL4/UAS system. (A) Fkbp14 is required in neuronal (Nsyb-GAL4) and (B) in peptidergic neurons (c929-GAL4) of the brain. (C) Fkbp14 is not required in the insulin-producing median neurosecretory cells (mNSCs) (Dsk-GAL4) or in the Insulin Producing cells (IPCs) (Dilp3-GAL4) of the brain (D). (C) WCS wild-type and (D) Canton-S wild type. One-tailed, paired student *t*-test (Fed/control vs. treatment). *** denotes P-value <0.0001; ** denotes P-value <0.001.

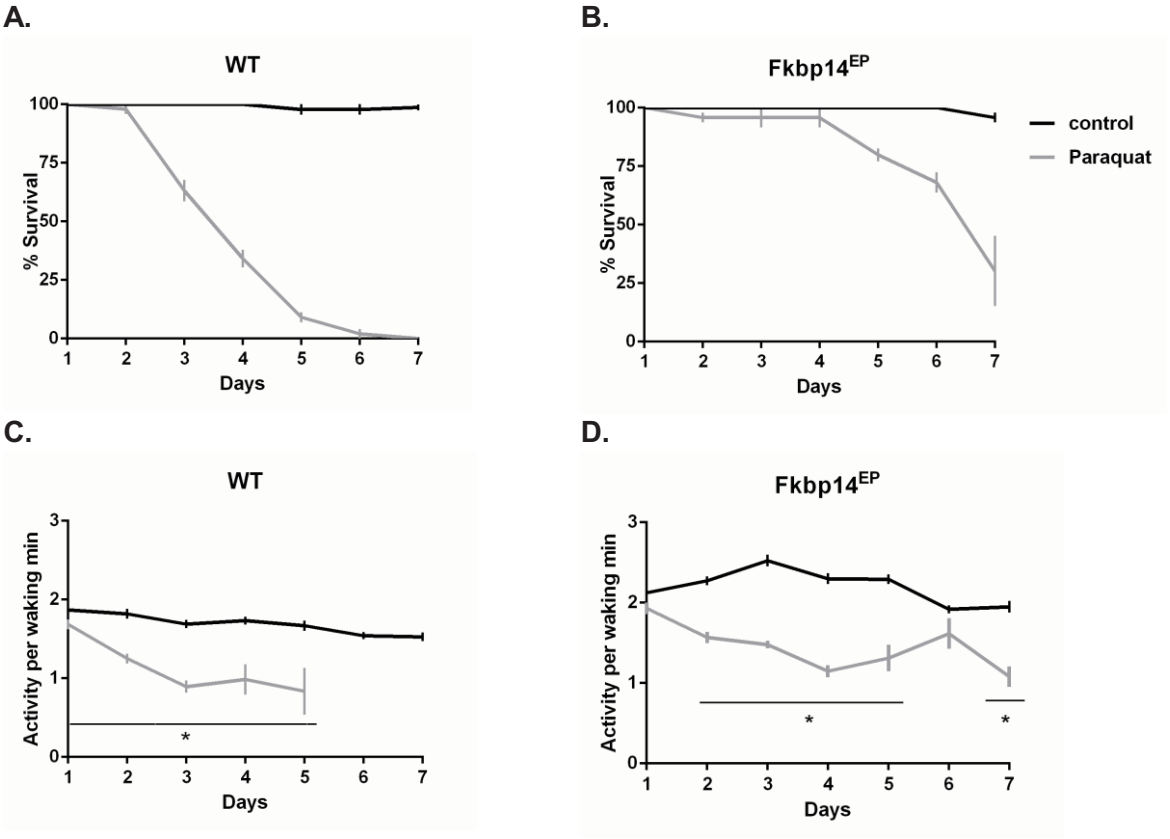


Figure 3. *Fkbp14^{EP}* survive longer under oxidative stress than wild type. Male flies were fed [20 mM] Paraquat on standard food for 7 days. (A) *Fkbp14^{EP}* survive longer than wild type. (B) *Fkbp14^{EP}* reduce activity similar to wild type. * denotes P-value <0.05; one-tailed, unpaired student T-test; treatment vs. control (for each day).

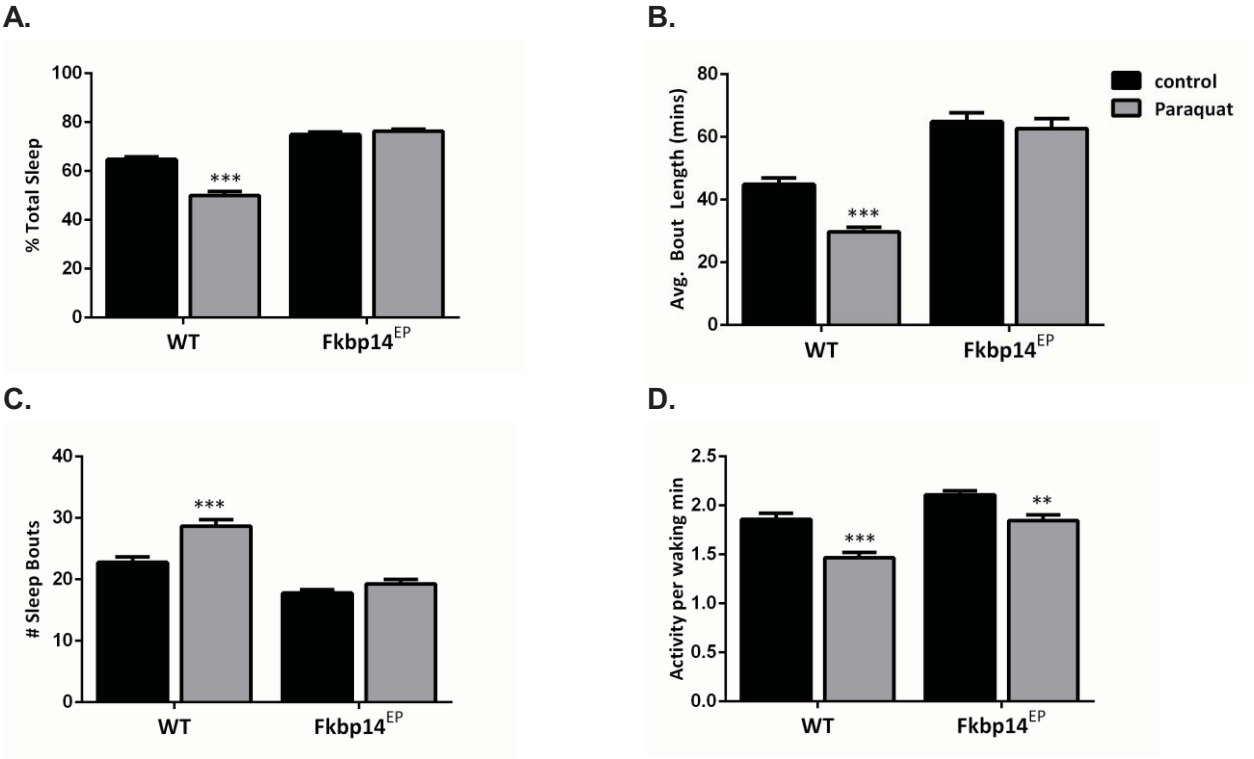


Figure 4. Fkbp14^{EP} maintains sleep quality under oxidative stress. Male flies were fed [20 mM] Paraquat on standard food for 48 hours and their activity recorded using DAMS. (A) Fkbp p14^{EP} mutants do not suppress total sleep (A) and sleep quality is unaffected in Fkbp14^{EP} mutants as measured by (B) average sleep bout length and (C) number of sleep bouts. (D) Fkbp14^{EP} remain sensitive to Paraquat as determined by awake activity. Data represented in Fig. 3. One-tailed, unpaired student *t*-tests (control vs. treatment) *** denotes P-value <0.0001, ** denotes P-value <0.001. Each bar represents group averages across 2 days.

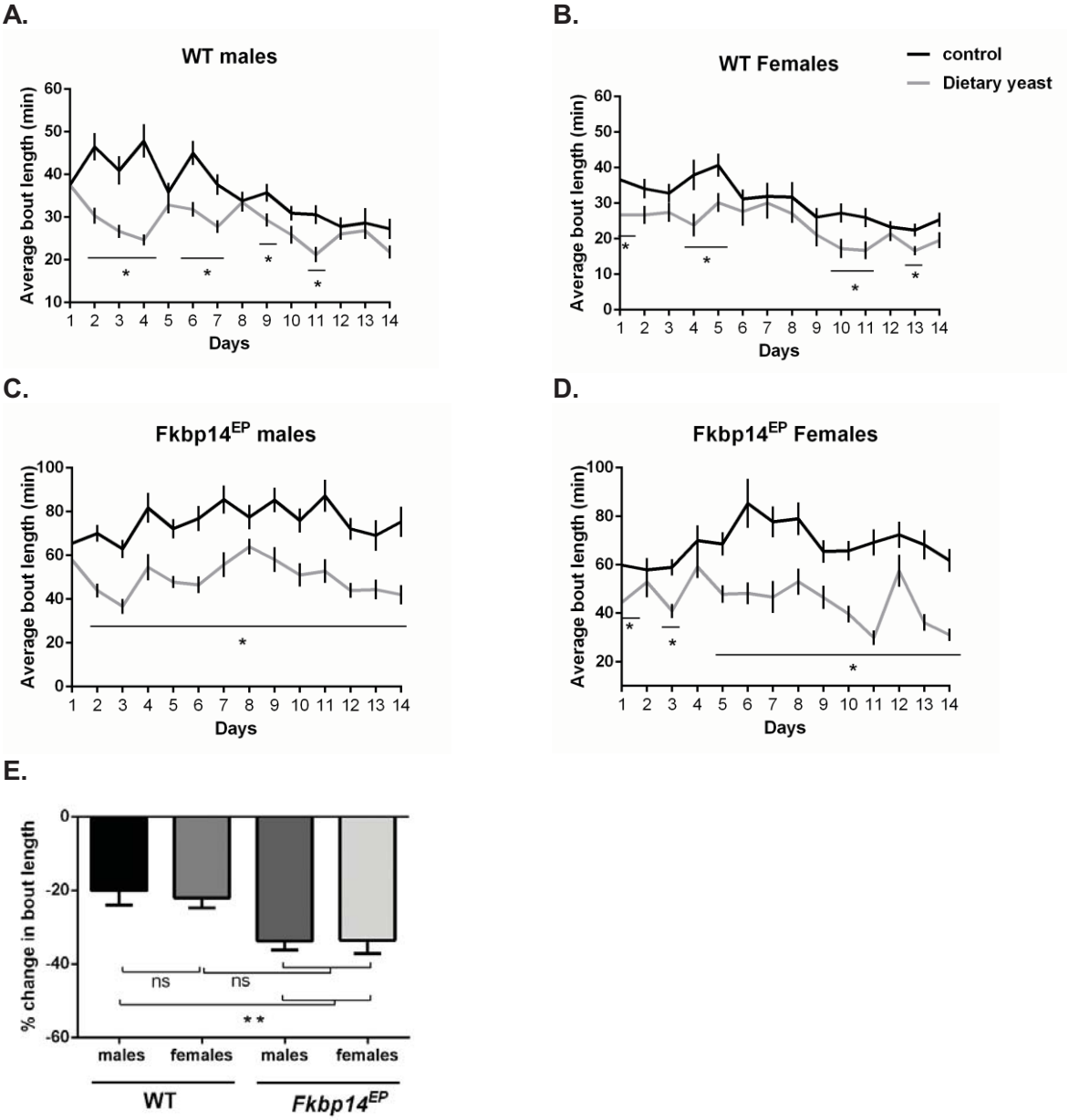


Figure 5. Fkbp14^{EP} remain sensitive to yeast-induced changes in sleep duration. Flies were fed a diet of 5% sucrose and 5% yeast extract in 1% agar (ASY) or the same diet without yeast (AS). Sleep quality as determined by average sleep-bout length, was recorded over 2-weeks. Two-tailed unpaired *t* test. (E) *Fkbp14^{EP}* flies were significantly different from WT males but not significantly different from WT females. One-way ANOVA between daily group averages of AS vs. ASY per day over two weeks. Percent change for each day was calculated as follows: ((group average on ASY)-(group average on AS))/(group average on AS))*100. Tukey's multiple comparison tests. ** denotes P-value <0.001, * denotes P-value <0.05.

SUPPLEMENTARY FIGURES

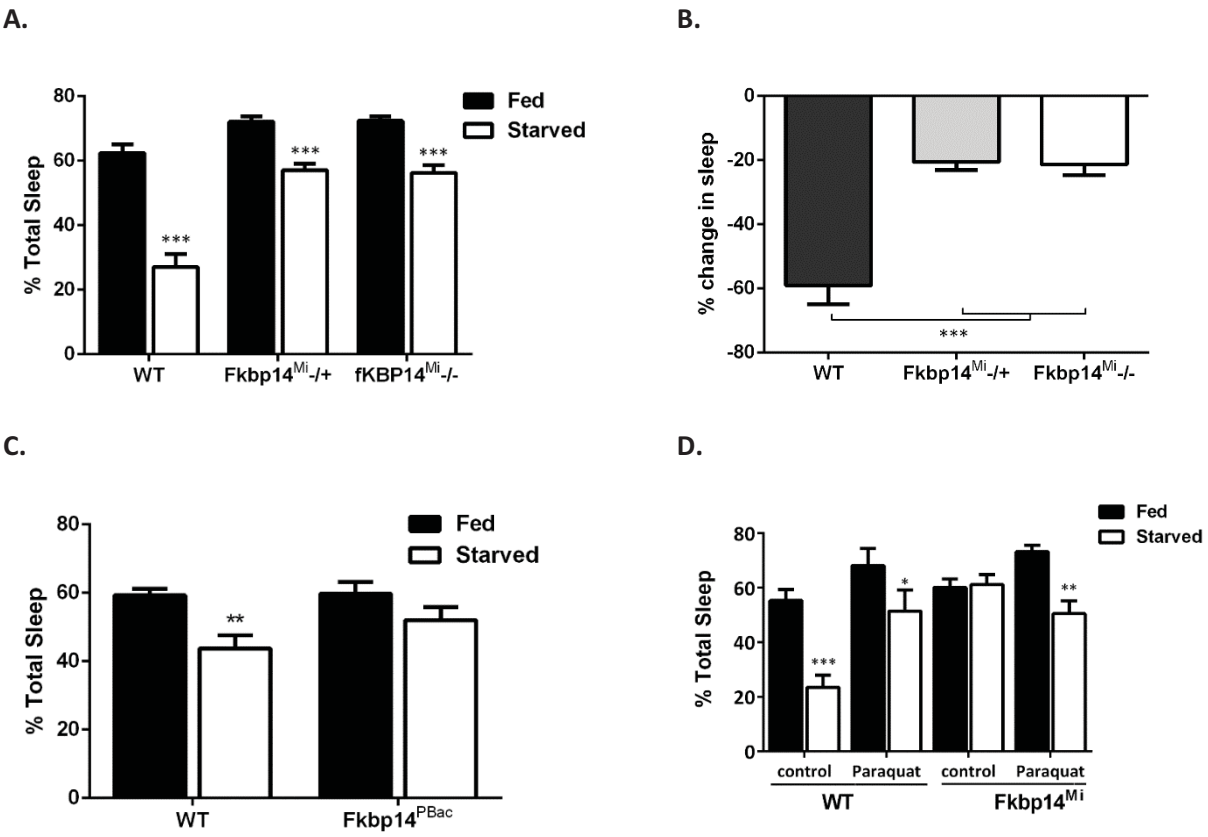


Figure S1. Fkbp14 regulates starvation-induced sleep suppression. (A) Fkbp14^{Mi} or Fkbp14^{Mi} heterozygotes suppressed sleep when starved but (B) significantly less than WCS wild type flies. 1 experiment, n=16 per group. (C) Fkbp14^{PBac} did not suppress sleep when starved. (D) WT and Fkbp14^{Mi} flies were fed [5 mM] Paraquat for 24 hours and then starved for 24 hours. Fkbp14^{Mi} failed to suppress sleep when starved but significantly reduced sleep after combining oxidative and starvation stress. 1 experiment, n=16, 5, 13 and 12, respectively. Note only 5 WCS survived treatment. Paired student *t*-test (A, C and D); One-way ANOVA with Tukey's multiple comparison tests (B). *** denotes P-value <0.0001, ** denotes P-value <0.001; * denotes P-value <0.05.

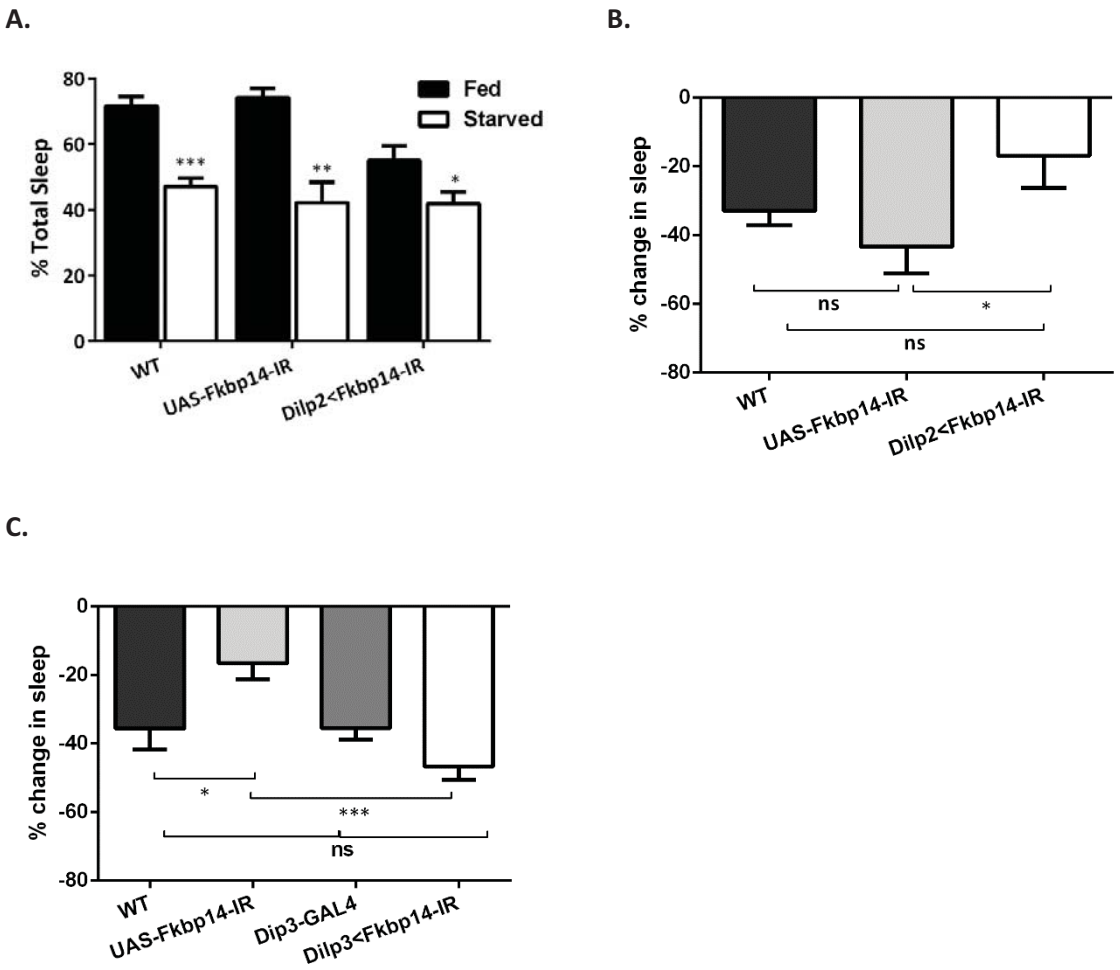


Figure S2. Fkbp14 does not function in IPCs to regulate sleep during starvation. (A and B) RNAi of Fkbp14 in the Insulin Producing Cells (IPCs) during adulthood using the Dilp2-GAL4 driver, which is expressed in adults but not during development, did not affect sleep during starvation. Canton-S flies, 1 experiment n=16. (C) Percent change in sleep in Fkbp14 RNAi in IPCs throughout development is not greater in experiment compared to W^{1118} controls (data from Fig. 2D). (A) Paired student *t*-test (Fed vs starved) and (B and C) One-way ANOVA with Tukey's multiple comparison tests. *** denotes P-value <0.0001; ** denotes P-value <0.001.

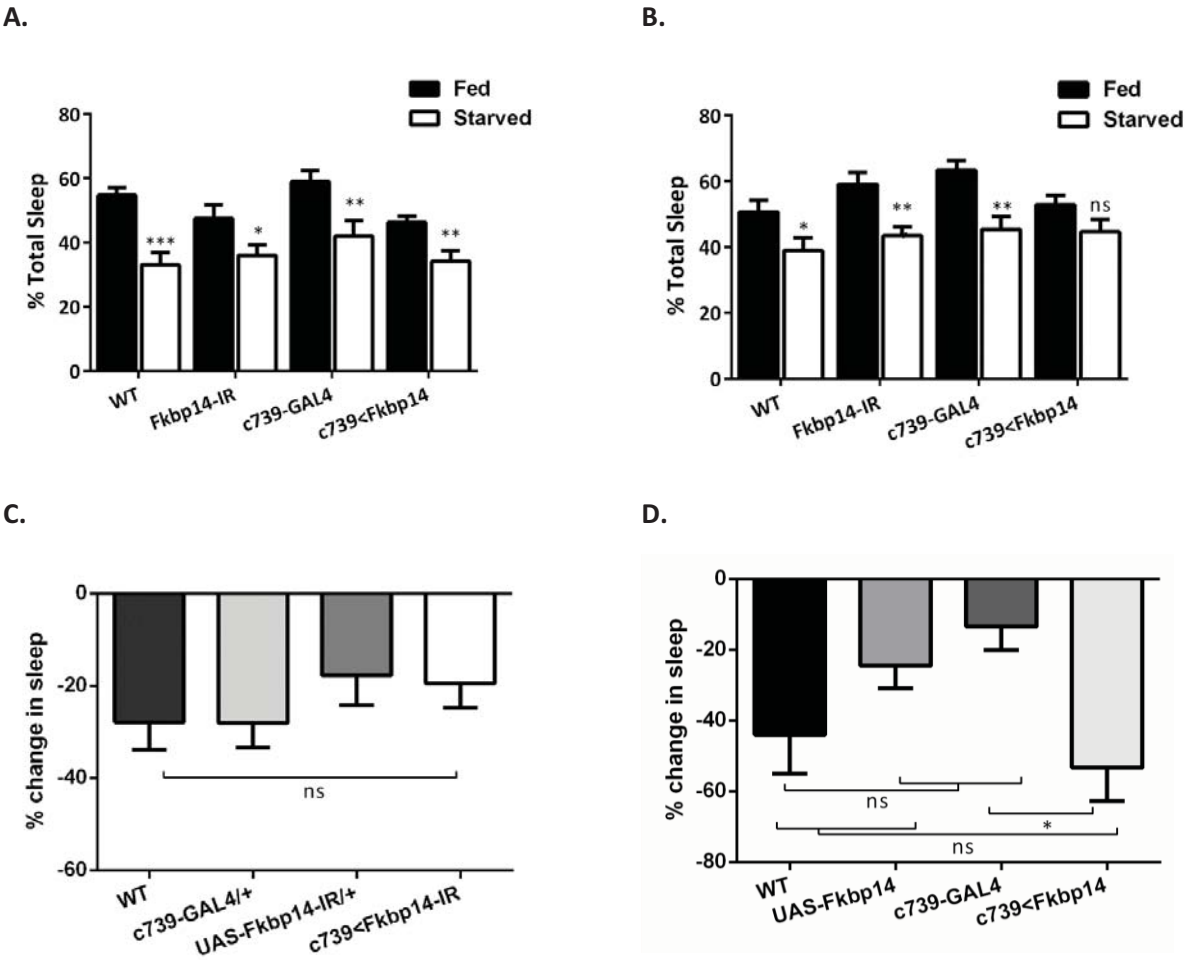
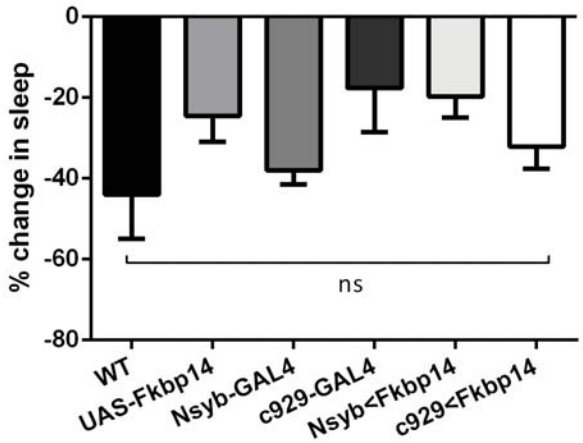


Figure S3. Fkbp14 does not appear to regulate sleep in the α/β lobes of the Mushroom Bodies (MBs). Tissue-specific RNAi of Fkbp14 using GAL4/UAS system. (A and B) RNAi of Fkbp14 using c739-GAL4, which mimics expression of *dimm* in the MB, does not yield consistent results between 2 different experiments of n=16. (C) One-way ANOVA of combined experiments (from A and B) did not show differences in percent change in sleep during starvation. Tukey's multiple comparison tests. (D) Overexpression of Fkbp14 in the MBs does not appear to induce a significant response, n=16 per group. *** denotes P-value <0.0001; ** denotes P-value <0.001; ; * denotes P-value <0.05.

A.



B.

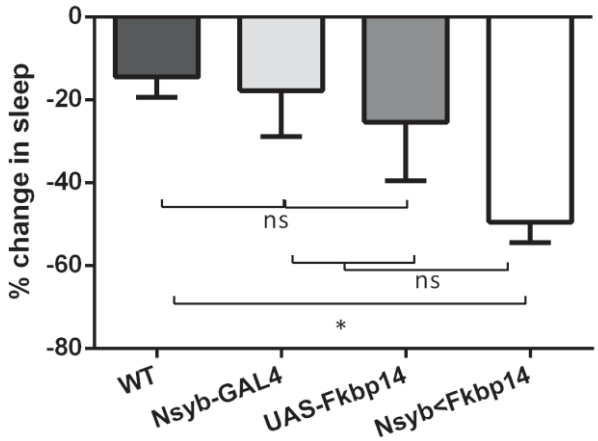


Figure S4. Overexpression of Fkbp14 in the neurons or peptidergic neurons of the brain does not appear to induce hypersensitivity to starvation. Tissue-specific overexpression of Fkbp14 using Nsyb-GAL4 (neuronal) and c929-GAL4 (peptidergic cells). n=16 for each group (A and B). (B) Canton-S wild type, One-way ANOVA did not show differences in percent change in sleep during starvation. Tukey's multiple comparison tests. (D) Overexpression of Fkbp14 in the MBs does not appear to induce a significant response, n=16 per group. *** denotes P-value <0.0001; ** denotes P-value <0.001; ; * denotes P-value <0.05.