A probabilistic model of sleep structure suggests a primary role for wake probability in overall sleep in *Drosophila melanogaster*

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ABSTRACT

In humans and flies, sleep is a highly organized behavior, occurring in discrete bouts that are consolidated at night but have a more fragmented structure during the day. Longer episodes correlate with transitions to deeper sleep states for both species, and sleep fragmentation has been shown to have deleterious outcomes for a large number of physiological processes. In mammals and birds, EEG provides an excellent measure of sleep structure and state. In invertebrates, assessment of sleep structure has relied on measures of episode duration, which can fail to capture important aspects of sleep architecture. Here we model *Drosophila* sleep as a probabilistic behavior and develop analytical tools to measure transition probabilities and the organization of sleep episodes within a defined analysis window. We show that these tools provide insight into the underlying mechanisms of normal sleep and into how they are altered in pathological conditions. The probability of transition to the wake state, P(Wake) is a major driver of normal sleep and responsive to dopaminergic tone, suggesting it is a measure of arousal. Our method for assessing sleep fragmentation adds important information about the organization of sleep bouts that is missing in measures of episode duration alone. Importantly, the ability to obtain a measure of the probability structure underlying sleep in a particular condition or genotype provides a way to compare the state of brain sleep circuits across conditions that utilizes information from all of the many processes that influence sleep.

INTRODUCTION

Sleep is a broadly conserved, restorative, and essential behavior. In many animals, sleep progresses through a sequence of stages with successively higher arousal thresholds (for reveiw see Eban-Rothschild and Bloch, 2012). The length of time spent continuously asleep determines the likelihood of reaching deeper sleep states, which are associated with restorative function (Scammell et al., 2017). Conditions which disrupt long episodes of sleep in mammals are associated with cognitive disruption and poor health outcomes (Baud et al., 2013; Lim et al., 2012; Qian et al., 2016; Saint Martin et al., 2016). In the fruit fly *Drosophila melanogaster* there is also evidence for deeper sleep stages that are only reached during long sleep bouts (Nitz et al., 2002; van Alphen et al., 2013; Yap et al., 2017), and sleep has been shown to be important for memory formation (Berry et al., 2015; Donlea et al., 2011). The neural mechanisms that regulate sleep episode duration and the transitions between sleep and wake are therefore of significant clinical and basic science interest.

Transitions between sleep and wake can appear sharp and switch-like. In the early 20th century, von Economo (von Economo, 1930) argued that the transition between sleep and wake was localized to a single “nervous center.” In contemporary literature, localization of function is still expressed using the idea of "sleep-promoting" or “wake-promoting” cells or neurons. However, far from arising from a single center, there are many interconnected cell groups that participate in sleep transitions in the mammalian brain (Scammell et al., 2017). Even in *Drosophila*, which have much smaller brains, the localization of sleep initiation is complicated, with dozens of cellular loci that can, in specific contexts, drive sleep/wake transitions (Tomita et al., 2017). While the

idea of a single, localized sleep switch is not tenable, understanding the relationship between the many neurons that have been shown to be able to affect sleep has been challenging.

One obstacle to obtaining a more holistic view of the distributed sleep circuit is that there is not a metric for comparison of the function of different brain areas or genotypes which captures the structure of sleep or the probabilistic nature of sleep transitions. In this paper we develop new analysis tools that will be useful for assessing both sleep structure and the probabilistic nature of behavioral transitions. We provide a new way to measure sleep fragmentation (the Hamming Index) that captures information about the organization of episodes as well as their duration. We also develop a method for obtaining a global assessment of the state of the sleep system expressed as probabilities of behavioral transitions. This is useful due to the complex nature of sleep circuits/genes and provides a way to compare the “state” of the system between genotypes or treatments. Lastly, we provide evidence that state transition probabilities can be tied to biological processes. We find that P(Wake) is strongly influenced by dopamine, the major neurochemical involved in arousal in *Drosophila* (Andretic et al., 2005; Kume et al., 2005).

EXPERIMENTAL PROCEDURES

Animals

Flies were raised on cornmeal-dextrose-yeast food in a 25°C incubator. The incubator maintained a 12 hour:12 hour light:dark cycle. Flies were collected 0-2 days post-eclosion and housed in mixed-sex, low-density vials until the beginning of the

experiment (typically 12 male and 12 female flies). All flies used in behavioral experiments were females that were co-housed with males, and were therefore presumably mated. For experiments in which the age of the flies is not specified, flies were 6 – 10 days old at the beginning of data collection. Transgenic flies were as follows: *per*^{01}(CS) – gift of Michael Rosbash, *w*^{1118} and *w*^{1118}; *dat*^{fmn} – gift of Amita Sehgal, PBac{WH}*inc*^{f00285}, *w*^{1118} (*inc*^{2}; Bloomington Stock Center # 18307).

Sleep Measurements

Flies were briefly anesthetized with CO_2, loaded into glass tubes containing 5% agarose/2% sucrose food, and tubes were mounted in *Drosophila* Activity Monitor (DAM) boards (Trikinetics, Waltham, MA). Experiments were carried out at 25°C with a 12 hour:12 hour light:dark cycle, unless otherwise specified. Flies were given a day to acclimate to the DAM system before data collection began. Sleep deprivation was performed by securing the DAM systems to a motorized vortexer and shaking the flies with an inter-pulse period of 10 seconds and a pulse duration of 2 seconds. Unshaken control flies were housed in the same incubator, but not attached to the vortexer.

Generation of In Silico data

Simulation data was generated by custom programs written in Matlab (MathWorks, Natick, MA). The Markov chain simulation accepted as input the number of simulations, the duration (in time steps) of each simulation, and the values of P(Wake) and P(Doze) for the *in silico* flies. The simulation was initialized in a random state with equal probability of wake and sleep at *time* = 1. At each subsequent time step *i*, the

simulator: 1) Generated a random number between 0 – 1, 2) Checked activity at *time*(*i* – 1), 3) Compared the random number with the behavioral transition probability. If the fly was active at *time*(*i* – 1), it was compared with P(Doze). If the fly was inactive, it is compared with P(Wake). 4) If the random number was less than or equal to the appropriate transition probability, the fly switches states. 5) The simulation proceeded to the next time step. The first 100 time steps were discarded to minimize the contribution of the random initialization state to the behavioral output. The Power-Law simulation proceeded along similar lines, however rather than a single P(Wake) and P(Doze), the power-law function was solved for the behavioral state duration at time = *i*, and the result was compared with the random number 0 – 1. Activity was coded as 1, inactivity was coded as 0.

Sleep Data Analysis

Both DAM system and *in silico* data were analyzed with a common set of modular programs written in Matlab. Raw beam crossing data from the DAM was trimmed to the appropriate experimental time period and binned to 1 minute intervals using DAMFileScan (TriKinetics). The experimental design and fly genotype metadata were transcribed into a text file. The Matlab program imported the DAM data and metadata. Flies with 13 or more hours of continuous inactivity at the end of an experiment were excluded as potentially dead. Continuous periods of inactivity lasting 5 minutes or longer were classified as sleep. Standard sleep measures were calculated as follows: Activity while awake was the total beam breaks divided by the number

minutes with activity. Total Sleep was the count of bins classified as sleep. Mean episode duration was the total sleep divided by the number of episodes.

The Hamming index was calculated as follows: the Hamming distance was calculated between the experimental data and every possible fully consolidated equivalent. A fully consolidated equivalent is a sleep record with the same total sleep as the experimental data, but only one sleep episode. The fully consolidated templates were generated iteratively by creating an empty variable of equal length to the experimental window, placing an episode with duration equal to total sleep at the first bin of that variable, and moving the episode through the duration of the variable one bin at a time. The minimum value of Hamming distance between the experimental data and a fully consolidated equivalent is the Hamming Index for that fly. If there is no sleep, or if the fly exclusively sleeps during the analysis window, the Hamming Index is undefined.

Probability of wake, or P(Wake), was calculated as follows: 1) Count every bin of inactivity except the last one. This is the denominator, the total number state transitions from inactivity. 2) For every bin of inactivity, count the number of times the fly is active in the subsequent bin. This is the numerator, the total number of state transitions from inactive to active. 3) Divide the total state transitions from inactive to active by the total state transitions from inactive. If there were no bins where the fly does not move (*i.e.*, denominator is zero), the transition probability is undefined. Probability of doze, or P(Doze), was calculated identically, but with activity and inactivity reversed.

Curve fitting was performed using the Matlab curve fitting toolbox using the least absolute residual method. Fit parameters are reported ± the 95% confidence interval. Statistical analysis was performed using the Matlab statistics toolbox, data was tested

for main effects using ANOVA, and post-hoc t-tests were corrected using Tukey's Honestly Significant Difference Procedure (hsd).

RESULTS

Conditional Probability and Information Theory Measures of Sleep

One approach to quantifying the structure of fly sleep is to consider an animal's behavior as switching between two states: active and inactive. Between each observation, the fly may choose to either remain in its current state or switch. The activity pattern of the fly reveals the overall probability of choosing each state, and the conditional probability of switching e.g. from active to inactive. We propose to call these conditional probabilities P(Wake), the probability that the fly will switch from the inactive state to the active state, and P(Doze), the probability that the fly will switch from the active state to the inactive state (see *Experimental Procedures* and Fig. 1 for detailed explanation).

We also propose a new method for measuring sleep fragmentation that we call the Hamming distance sleep fragmentation index, or Hamming Index. The most commonly used method for analyzing sleep fragmentation is based on sleep episode duration or episode count. Episode duration and number of episodes are important features of sleep structure, but information about when the episodes occur is also important. The Hamming Index is the minimum Hamming distance (number of mismatches divided by sequence length, Hamming, 1950) between the behavioral output of the fly and any fully consolidated equivalent – a sleep pattern with the same total duration of sleep that is organized into a single episode. The Hamming index

therefore captures data about both the degree to which sleep occurs in long episodes and the clumping of those episodes within an analysis window. Three example activity patterns with the same total sleep duration but different sleep structures demonstrate some properties of these measures (Fig. 1).

Circadian and Environmental Regulation of Sleep Structure

In order to assess the usefulness of our approach to quantifying fly sleep structure, we analyzed sleep data acquired with the well-characterized *Drosophila* Activity Monitor (DAM) system (Trikinetics, Waltham, MA). Sleep in female wildtype (WT, *Canton Special*, n = 60) and circadian defective (per^{01} mutant, n = 57) flies was measured for 3 days in a 12:12 light:dark cycle (LD), after which the lights were set to off, and sleep was measured for 6 days in constant darkness (DD) (Fig. 2A-B). There is a significant main effect of day (ZT0-12) v. night (ZT12-24) and a significant interaction between time of day and genotype for all measures of sleep quantified (Repeated measures ANOVA; all $p < 0.0001$). Flies in DD without a functional circadian clock produce identical sleep during the subjective day and night: the total duration, mean episode duration, level of fragmentation, and probability of behavioral state transition do not differ significantly (all $p > 0.3$; Fig. 2C-G). Light alone is a potent cue for differentiating daytime and nighttime sleep. In per^{01} flies lacking an internal circadian clock, light causes significant decreases in total sleep and mean episode duration, and significant increases in sleep fragmentation, P(Wake), and P(Doze) (all $p < 0.0001$; Fig. 2C-G). The circadian clock also drives significant differences between daytime and nighttime sleep in the absence of light. WT flies in DD conditions have less total sleep,

higher sleep fragmentation, and a higher P(Wake) during subjective day than night (all $p < 0.0001$; Fig. 2C-G). Mean sleep episode duration and P(Doze) are regulated by environmental light alone (all $p < 0.0001$), but not by the circadian clock in DD (all $p > 0.06$; Fig. 2D,G). Finally, when flies have two sources of information about time of day, both an LD cycle and circadian clock, flies sleep more with longer mean episode duration at night (all $p < 0.0001$). However, sleep fragmentation as measured by Hamming Index, is significantly higher at night than during the day ($p < 0.0001$). P(Wake) is significantly lower at night in WT flies in LD ($p < 0.0001$), while P(Doze) is not significantly regulated by time of day ($p = 0.067$).

These group comparisons lead to three overall conclusions. First, P(Wake) is regulated more strongly by environmental light than by the circadian clock. This is consistent with studies in which LD cycles outside the ability of the clock to entrain are presented (e.g. Chiesa et al., 2007; Madrid et al., 1998). Second, P(Doze) is not strongly regulated by light or by the circadian clock. This suggests that P(Wake) may be the major regulator of total sleep and strongly influence mean episode duration. Lastly, the regulation of sleep fragmentation appears to be more complex than the regulation of total sleep.

In order test these group-level associations, we measured both linear and non-linear relationships between the behavioral state transition probabilities P(Wake) and P(Doze), and measures of sleep structure (Table 1). Across conditions and genotypes, P(Wake) is strongly correlated with both total sleep and mean episode duration, but is not significantly correlated with sleep fragmentation. P(Doze) is moderately, but significantly, correlated with total sleep, mean episode duration, and sleep

fragmentation. P(Wake) and P(Doze) are not correlated with one another. While these correlations are suggestive, they cannot unambiguously demonstrate causation, especially due to the complexity of sleep regulation in flies (Tomita et al., 2017). An *in silico* approach to sleep offers a more controlled arena in which to investigate the potential causal relationships among these behavioral parameters.

In Silico Sleep Recapitulates Features of In Vivo Fly Sleep

We used an agent-based simulation paradigm to generate *in silico* sleep data. Each *in silico* fly was initialized in a random state (either active or inactive) and with a P(Wake) and P(Doze). At each subsequent time step, the simulation used the current state of the fly and the behavioral transition probability to determine the new state of the fly. WT and per^{01} fly sleep data (from Fig. 2) were used to generate empirical heat maps of the relationship between P(Doze), P(Wake), and sleep parameters (Fig. 3A). Corresponding *in silico* sleep data was generated for each combination of P(Doze) and P(Wake) (range: 0-1 binned to 25 steps, n = 100 simulations per bin; Fig. 3B). Comparison of the *in vivo* and *in silico* datasets shows qualitative similarity with some notable quantitative differences, especially in mean sleep episode duration (Fig. 3A,B, *middle column*). One possible explanation for this quantitative discrepancy is that our model of state transition probability is too simple. For example, previous reports have shown that sensory thresholds and neural activity change as a function of the duration that the fly has been asleep (Nitz et al., 2002; van Alphen et al., 2013; Yap et al., 2017). This is similar to the situation in humans, where transition models have to consider sleep sub-states (Perez-Atencio et al., 2018).

In order to integrate this information into our models, we calculated P(Wake) and P(Doze) as a function of behavioral state duration (Fig. 3C,D). We found both P(Wake) and P(Doze) have a power-law relationship with the duration of activity state. We then used the values for P(Wake) and P(Doze), and the best-fit power-law functions, to generate new *in silico* data matched to LD day, LD night, DD day, and DD night in WT flies. We then compared the deviation of our simulations from the *in vivo* WT fly data (n = 100 *in silico* flies per condition; Fig. 3E). We refer to the simulations with a fixed P(Wake) and P(Doze) as "Markov Chain simulations," because they are a special case of a Markov chain. Both the Markov chain and power law *in silico* flies accurately reproduce total sleep of their matched *in vivo* group (Fig. 3E, *left column*). The Markov chain simulations systematically underestimate sleep episode duration, while the power law simulations match the *in vivo* data (Fig. 3E, *middle column*). Both the Markov and power law simulations produce more fragmented *in silico* sleep than shown in the *in vivo* data (Fig. 3E, *right column*). Across groups, the majority of both the Markov chain and power law simulations show small standardized differences from *in vivo* data ($|Z\text{-score}| < 2$), indicating that the relatively low complexity simulations are capable of capturing a meaningful fraction of the *in vivo* behavior.

Our simulation approach assumes that population-wide fit of behavioral state transition probability can be used to simulate *in silico* individuals. We tested the assumption that individual fly behavior is faithfully represented by population means using two approaches (Fig. 4). First, we tested the hypothesis that variation of a single fly's behavior over time is comparable to the variation in behavior between flies (Fig. 4A,B). We found instead that for both P(Wake) and P(Doze), there is significantly

greater variability between flies than within a single fly across time (two tailed *T*-test; $p < 0.0001$). Second, we tested the hypothesis that estimates of P(Wake) and P(Doze) derived from each individual fly would converge toward the population estimate of these measures. Population estimates of P(Wake) and P(Doze) are centrally located within the distribution of individual fly derived estimates (Fig. 4C,D). Population estimates of the power law distribution parameters of P(Wake) and P(Doze) are also relatively faithfully reproduced by the distribution of individual fly estimates, with the notable exception of the lower-bound parameter of P(Wake) (Fig. 4E-J). In all individual flies, the lower bound of P(Wake) was estimated to be zero, while as a population the lower bound was 0.5% (Fig. 4I). This discrepancy is likely due to the difficulty of getting a good estimate of low frequency events from limited data, and would be most apparent when estimating the frequency of long duration sleep episodes. While it is in principle possible to estimate the single fly P(Wake) and P(Doze), fitting a power law function to individual fly data is limited by the ability to accurately assess long-duration episode frequency. The distribution of P(Wake) and P(Doze) and their power law fit parameters are unimodal, allowing population-based averages to approximate a “typical” fly.

Effect of Increased Homeostatic Sleep Pressure on Behavioral State Transitions

Sleep deprivation in flies, like in other animals, leads to increased subsequent sleep (Shaw et al., 2000). A common model for the generation of increased sleep after release from sleep deprivation is that there is an increase in sleep “pressure” which alters sleep drive (Fuller et al., 2006). In order to quantify how sleep pressure affects behavioral state transitions, we performed a sleep deprivation experiment during which

WT flies were shaken periodically for 12 hours overnight (*n* = 120 shaken, 117 control; Fig. 5A). Compared with a control group of flies, flies that were shaken slept less while the shaker was on, and more the following day (Fig. 5A). We defined two time windows: PRE, ZT0-6 the morning prior to sleep deprivation, and POST, ZT0-6 the morning following sleep deprivation. Sleep deprived flies, but not control flies, slept significantly more POST than PRE and had significantly less sleep fragmentation as assessed both by Hamming Index (all $p < 0.0001$; Fig. 5B,C) and mean episode duration (in min, for shaken group, PRE = 40.9 (SD 44.4), POST = 206.8 (SD 137.9), $p < 0.0001$, for control group, PRE = 40.2 (SD 49.1), POST = 52.2 (SD 61.7), $p = 0.2$). P(Wake) was significantly decreased and P(Doze) was significantly increased in sleep deprived flies (all $p < 0.0001$), but not control flies, during the POST interval (Fig. 5D,E).

While both P(Wake) and P(Doze) are significantly different following sleep deprivation, three lines of evidence suggest that P(Wake) is principally responsible for the change in behavior: 1) change in total sleep during POST v. PRE is more strongly correlated with change in P(Wake) ($R = -0.97$, $p < 0.0001$) than change in P(Doze) ($R = 0.19$, $p = 0.06$), 2) The effect size of the change in P(Wake) is larger than the effect size of P(Doze) (Cohen's d = -1.46 and 0.81, respectively, (Cohen, 1992)) and 3) Superimposing the observed change in P(Wake) and P(Doze) on simulated data, the change along the P(Wake) axis is more consequential than the change along the P(Doze) axis (Fig. 5F,G).

Examining the history-dependence of P(Wake) and P(Doze) provides additional insight into how sleep pressure modifies behavioral transitions (Fig. 5H,I). Neither the initial value (PRE: 1.24 ± 0.08, POST:1.30 ± 0.06) nor the long-term lower bound of

P(Wake) (PRE: 0, POST: 0) differs between PRE and POST, but the power law exponent of decay is substantially faster in POST (PRE: 1.03 ± 0.04, POST: 1.20 ± 0.04; Fig. 5H). The shape of P(Doze) diverges between PRE and POST during movement episodes longer than 6 minutes (Fig. 5I) but, because fewer than 20% of wake episodes last longer than 6 minutes, this divergence does not result in decreased overall P(Doze) (Fig. 5E). In WT flies, it appears that the primary mechanism generating homeostatic rebound sleep is a decreased probability of waking up during a sleep episode rather than the initiation of additional episodes. The change in P(Wake) does not result from a generalized decrease, but from the flies more rapidly entering deep sleep after initiating a sleep episode. It is clear from the heat maps (Fig. 5F,G), however, that if a fly's initial position (i.e. their respective P(Wake) and P(Doze)) was in the lowest left part of the map, the effect of changes in P(Doze) might be behaviorally relevant. The fact that both mechanisms are engaged could be important in animals, e.g. mutants, whose initial sleep parameters are abnormal.

Mutations Affecting Sleep Act Through Both Wake and Doze Probability

Sleep is regulated by a multitude of molecular processes, and many null and hypomorphic mutations disrupt sleep in flies (Helfrich-Forster, 2018). We examined how two known sleep mutants, *fumin* (dat^{fmn}; n = 39 mated female flies; Kume et al., 2005) and *insomniac* (inc^{2}; n = 33; Stavropoulos and Young, 2011), affect behavioral state transition probabilities (Fig. 6). We found that *fumin* flies show the previously demonstrated decreased total sleep relative to control flies with the same genetic background (w^{1118}; n = 40; $p < 0.0001$; Fig.6A,B). Female *insomniac* flies, in our

experimental conditions, unexpectedly have increased daytime total sleep and unchanged nighttime sleep ($p < 0.0001$ and $p = 0.16$, respectively; Fig. 6A,B). Interestingly, we find that *insomniac* flies are significantly hyperactive when awake ($p < 0.0001$; Fig. 6C) in addition to being hyper-somnolent. Both *fumin* and *insomniac* flies have significantly shorter mean sleep episode duration during the day ($p < 0.012$), but only *fumin* flies have shorter sleep episodes at night ($p < 0.0001$; Fig. 6D). Finally, during the day *fumin* flies have significantly less fragmented sleep while *insomniac* flies have significantly more fragmented sleep ($p < 0.0001$). There are no significant differences in sleep fragmentation at night as assessed by Hamming Index ($p > 0.7$). Visual examination of individual sleep rasters confirms that despite shorter mean sleep episodes, sleep and activity in *fumin* flies is not substantially more disorganized than in wild type (Fig. S1). In summary, *fumin* flies sleep less than WT and in shorter episodes both during the day and night. *Insomniac* sleep only differs from WT sleep during the day, during which they sleep more, in shorter episodes, with greater sleep fragmentation.

Both the *fumin* and *insomniac* phenotypes can be explained by changes in P(Wake) and P(Doze) (Fig. 6F-G). *Fumin* flies have increased P(Wake) and decreased P(Doze) both in the day and night ($p < 0.002$), which our simulations predict would cause the observed phenotypes (Fig. 6H,G). The *insomniac* mutation does not significantly alter P(Wake) ($p > 0.25$), but significantly increases P(Doze) both during the day and night ($p < 0.0001$), and shows behavior that consistent with our *in silico* predictions (Fig. 6H,I). The power law functions of P(Wake) and P(Doze) provide additional insight into this process (Fig. 6J,K). The increase in P(Wake) between WT

and *fumin* flies is driven by a difference between the respective power law exponents of decay (WT: 1.16 ± 0.03, *fumin*: 0.83 ± 0.07; Fig. 6H). The increase in P(Doze) between WT and *insomniac* flies is driven by differences between both the initial value of P(Doze) (WT: 0.62 ± 0.03, *insomniac*: 1.06 ± 0.31) and the respective power law exponents of decay (WT: 0.66 ± 0.02, *insomniac*: 0.47 ± 0.16). The divergent effects of these two mutations, while complex, are well explained by a relatively simple model of behavioral state transition probability.

DISCUSSSION

Sleep serves many physiological purposes, so it is unsurprising that it should be responsive to many different variables. Competing or interacting switches which are organized hierarchically can allow many factors to be integrated, but how to express the global state of such a complex system is not clear. In this paper we model sleep in terms of the probabilities of state transitions. We also introduce a new measure of sleep fragmentation, the Hamming Index, that gives a quantitative assessment of the organization of the sleep episodes in the epoch being studied.

Sleep as a probabilistic behavior has been extensively modelled in both rodents and humans (Bianchi et al., 2012; Perez-Atencio et al., 2018; Stephenson et al., 2016; Yang and Hursch, 1973). Previous descriptions of *Drosophila* sleep structure have focused on describing the relative frequency of sleep episodes of different durations (Ueno et al., 2012; Diamond, 2016). These reports each found that fly sleep follows a power-law distribution of sleep episode duration, and that the distribution is affected by a variety of factors, including environment and dopamine levels. Each report also

suggested a metric for quantifying sleep structure: coefficient of determination (Diamond, 2016), and power-law fit coefficients (Ueno et al., 2012). This report both agrees with and builds upon this previous work. We find that P(Wake) is strongly correlated with mean episode duration (R = -0.89) and that both P(Wake) and P(Doze) follow a power-law dependence upon duration of sleep and wake, respectively (Fig. 3). We also find that dopamine profoundly affects P(Wake), consistent with a shift in the distribution of episode duration (Ueno et al., 2012). By focusing not on sleep episodes themselves, but on the state transitions that define sleep episodes, we also gain insight into the mechanisms that generate the observed distribution of sleep episode duration. This mechanistic advantage is most apparent in the generation of *in silico* data for a wide range of conditional probabilities, and allows us to plot the relative positions of flies in a conditional probability space.

Fragmentation of sleep is a critical human health issue, and the major way that this is assessed in patient populations is by use of measures analogous to sleep bout duration (sleep stage percentages and sleep efficacy) from polysomnography records. These methods, however, can fail to capture fragmentation even in patients with obstructive sleep apnea (Swihart et al., 2008). Models that extract information about transitions and the underlying organization of episodes have been shown to be better predictors of fragmentation and morbidity (Bianchi et al., 2010; Bianchi et al., 2012; Swihart et al., 2008). For *Drosophila* sleep the bout length serves as the major metric for fragmentation. Episode duration, however, can fail to identify changes in consolidation. For example in wildtype animals in DD there is no difference between

episode duration in subjective day and night, but there are clock-dependent processes consolidating sleep that can be detected in the Hamming Index (Fig. 2).

There are, however, cases where Hamming Index can indicate no fragmentation even when there is a decrease mean episode duration. Total sleep in *fumin* animals, which have high dopaminergic tone (Kume et al., 2005), is severely decreased, as is mean episode duration. Hamming Index for this genotype is actually decreased during the day and unchanged at night. This is likely due to the fact that there are very few sleep episodes, which decreases the degrees of freedom for the organization of sleep in that window. Examination of the sleep rasters of these genotypes (Fig. S1), however, suggests that there are differences in organization that are not picked up when considering the entire night as one analysis block. Sleep during the night is likely not regulated by a single process- early night sleep and late night sleep show differences in bout length and structure in both in humans (Norman et al., 2006; Yang and Hursch, 1973) and flies (Kunst et al., 2014). A sliding window analysis of Hamming Index (or of bout length) would likely better capture nighttime sleep structure and provide a better platform for identifying fragmentation. In most cases, however, even when considering the night as a single analysis window, mean episode duration and Hamming Index are in agreement and since they provide complimentary information, the use of Hamming Index will increase confidence in the assessment of relative consolidation.

The utility of these new analysis tools is demonstrated by using them on sleep data from wild type and clockless (per^{01}) mutants. We find that both light and the circadian clock can alter the structure of sleep. For flies without a functioning clock, the structure of sleep is completely dependent on daily exposure to light. Light drives the

majority of daily sleep into the night and also reduces its fragmentation as measured by both mean episode duration and Hamming Index. For wildtype flies, light during the day also appears to promote nighttime sleep. Most of the difference between day and night sleep can be attributed to changes in P(Wake). This could be explained by actions of dopamine, which have been shown to influence the sleep function of the clock in LD (Shang et al., 2011).

Pathological changes in sleep secondary to acute deprivation or mutations can also be described using our metrics. Homeostatic rebound sleep in wild type animals, like normal sleep, appears to be controlled primarily by P(Wake) and results in a significant decrease in fragmentation as measured by the Hamming Index and mean episode duration.

The two sleep mutants we examined appear to affect sleep via changes in different underlying parameters. *Fumin*, which is a mutation in the dopamine reuptake transporter, drastically increases P(Wake), in line with the idea that dopamine is a major determinant of arousal. *Insomniac* flies on the other hand increase P(Doze) during both the day and night. The analysis of transition probabilities suggests that while there are similarities in these two genotypes at a macroscopic scale, the underlying molecular and circuit processes they affect must be different.

Arousal state has a major influence on sleep amount and architecture (Ouyang et al., 2004; Phillips et al., 2013; Scammell et al., 2017; Tagliazucchi and van Someren, 2017) and may be one of the key underlying processes that coordinate the actions of the disparate sleep circuits. Using our new analysis tools, we show for wild type flies, the major determinant of sleep structure is P(Wake), and we posit this is a direct

measure of arousal. P(Wake) is tied to the activity of dopaminergic neurons since *fumin* mutants, which have an increase in dopaminergic tone (Kume et al., 2005) have a greatly increased P(Wake). This suggests that P(Wake) may be an important parameter to measure when assessing the global state of the distributed sleep circuit, arousal and the involvement of dopaminergic systems in a phenotype.

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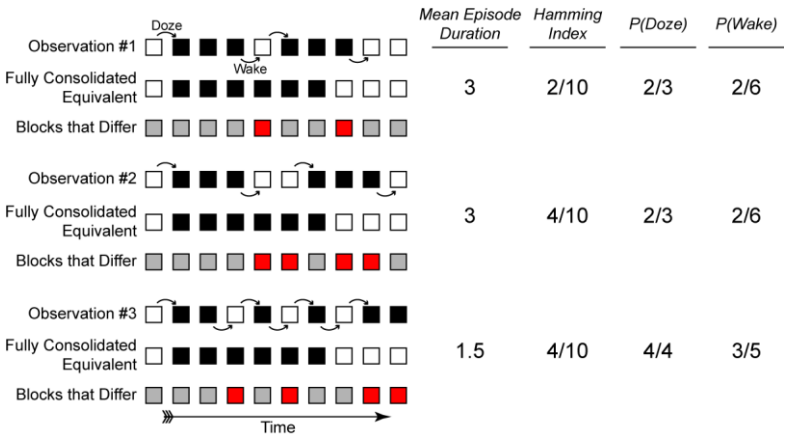

Figure 1 – Conditional Probability and Information Theory Measures of Sleep. Three exemplar sleep patterns (Observation #1-3) are shown that have the same total sleep and median time of sleep, but different structure. White squares: wake, Black squares: sleep. The fully consolidated equivalent of each of these observations is the same (middle rows). During the calculation of Hamming Index, the Hamming distance to all possible fully consolidated equivalents is calculated to find the minimum value. Blocks that differ between each observation and the fully consolidated equivalent are red, blocks that are the same are gray. Transitions between behavioral states are indicated by curved arrows. Transitions from active to inactive (Doze) are above the blocks, transitions from inactive to active (Wake) are below. The calculation for P(Wake) is as follows: Count every measurement bin where the fly does not move except the last one. This the total number of transitions *from* inactive. For every measurement bin where the fly does not move, count the number where the fly moves at the next bin. This is the number of transitions *from* inactive *to* active. Divide the transitions *from* inactive *to* active by the total number of transitions to calculate the transition probability of inactive to active, or P(Wake). The calculation for P(Doze) proceeds in the same way,

but with “active” and “inactive” switched. See *Experimental Procedures* for additional details.

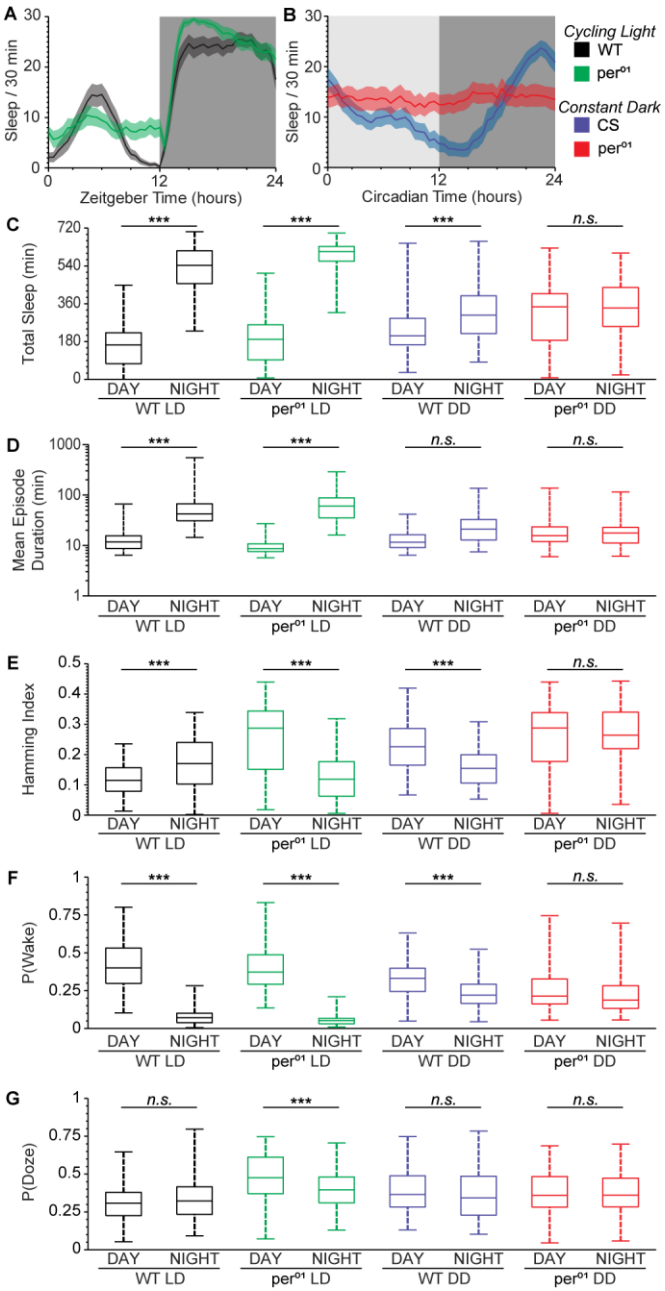

Figure 2 – Circadian and Environmental Regulation of Sleep Structure. (A-B) Circadian profile of sleep in a 12:12 Light:Dark cycle (LD; A) and in constant darkness (DD; B). Shaded regions indicate lights on and lights off times (A) or subjective day and night (B). Lines are the population average, shaded area is the 95% confidence interval of the mean. Individual profiles are the average of 3 days of behavior. DD sleep

measured on days 4-6 of constant darkness. (C-G) Box and whisker plots of total sleep (C), mean sleep episode duration (D), Hamming index (E), P(Wake) (F), and P(Doze) (G). Boxes show the 25/50/75th percentiles for each group, whiskers indicate the minimum and maximum. *n.s.* indicates no significant difference, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

TABLE 1 – Spearman Correlations Among Sleep Measures

	P(Wake) Rho	P(Wake) p-value	P(Doze) Rho	P(Doze) p-value
Total Sleep	-0.9804	$< 10^{-10}$	0.2221	1.2129e-06
Mean Episode Duration	-0.8869	$< 10^{-10}$	-0.1588	5.7062e-04
Hamming Index	-0.0249	0.5918	0.4665	$< 10^{-10}$
P(Doze)	-0.0769	0.0966		

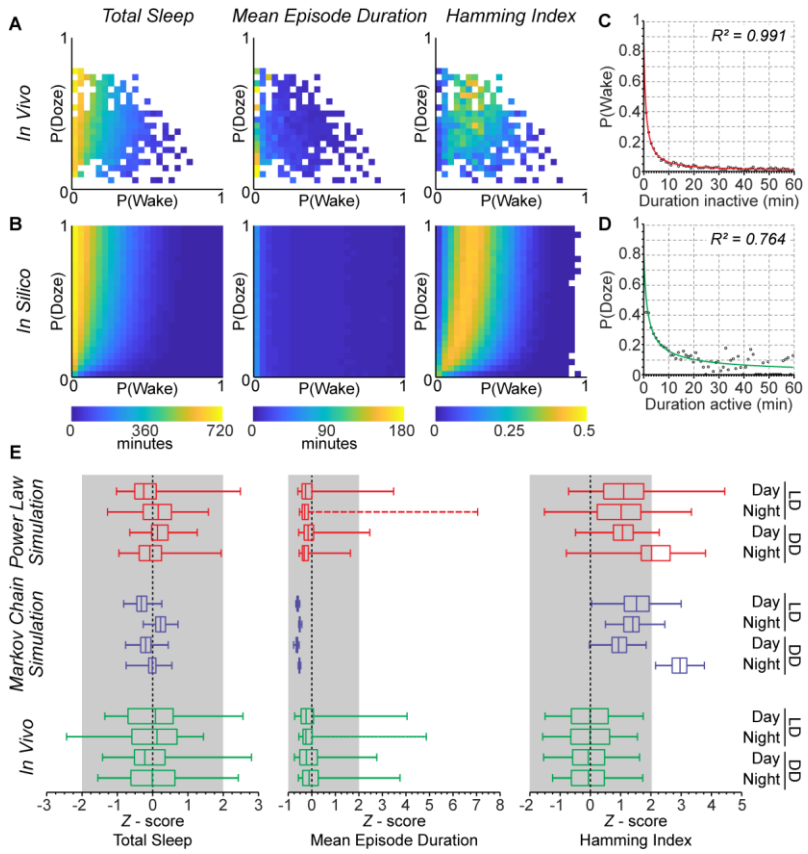

Figure 3 – *In Silico* Sleep Recapitulates Features of *In Vivo* Fly Sleep. (A, B) Heatmaps of *in vivo* (A) and *in silico* (B) sleep properties. (A) Each *In vivo* fly was assigned to one of 25 bins from 0 to 1 based upon it's respective P(Doze) and P(Wake). The mean value of total sleep (*left*), mean sleep episode duration (*center*), and Hamming index (*right*) was calculated for all samples in the bin, and the bin was assigned a color according to the legend (*bottom*). Coordinates without any samples are displayed as white. (B) For the central value of each P(Doze) and P(Wake) bin, 100 *in silico* flies were simulated. The mean value of each parameter of these simulations is displayed using the same color map as the *in vivo* data. Coordinates coded white for *in silico* Hamming index have an undefined value because no behavioral transitions were generated for the duration of the epoch. (C, D) P(Wake) and P(Doze) plotted as a function of the duration of inactivity (C) and activity (D), respectively. These data are

derived from the night epoch of CS flies in LD conditions. Data was fit using the equation $a(x + 1)^{-b} + c$, where *a*, *b*, and *c* are free parameters. Black points are experimental data, red and green lines are the power law functions that best fit the data. (E) *Z*-scores of *in vivo* and *in silico* sleep. The population-derived values of P(Doze) and P(Wake), and their power-law equivalents, were derived for each listed *in vivo* experimental condition and used to simulate sleep behavior. *Z*-scores were calculated using the sample mean and standard deviation of the corresponding *in vivo* experimental group.

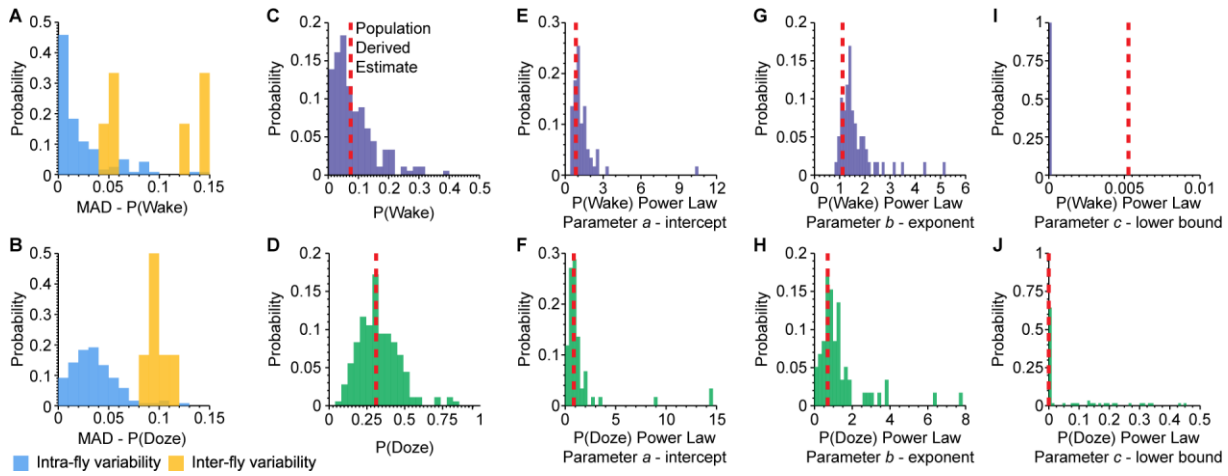

Figure 4 – Population and Individually Derived Estimates of Behavioral State Transition Probability. (A, B) Histograms of median absolute deviation (MAD) of P(Wake) (A) and P(Doze) (B). Intra-fly variability is calculated based on 3 day epochs and 3 night epochs of behavior from each fly. Inter-fly variability is based on the MAD of the population during each epoch (*n* = 60 WT flies). Low sample size of inter-fly variability in (A) precludes concluding if the data are bimodally distributed. (C, D) Histograms of P(Wake) and P(Doze) calculated for each fly. The population derived estimate is shown as a dashed red line. (E-J) Histograms of the estimates of each parameter, *a, b,* and *c*, of a power law fit using the equation $a(x + 1)^{-b} + c$ of individual fly behavior. Data in (E-J) are from the nighttime behavior of CS flies in LD conditions.

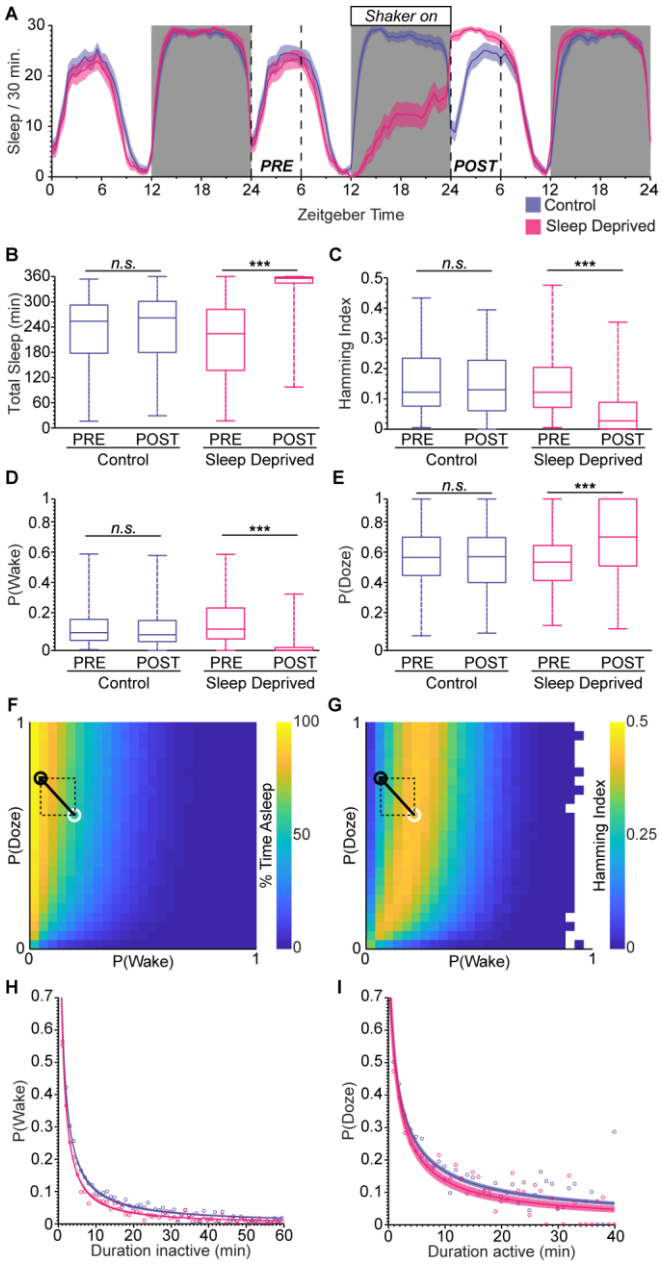

Figure 5 - Effect of Increased Homeostatic Sleep Pressure on Behavioral State Transitions. (A) Circadian profile of sleep over 3 days, including one night of sleep deprivation (labeled “Shaker on”). Shaded regions indicate lights on and lights off times. Analysis windows for PRE and POST are indicated. Mean sleep is indicated by lines, shaded region is the 95% confidence interval. (B-E) Box and whisker plots of total sleep (B), Hamming index (C), P(Wake) (D), and P(Doze) (E). Boxes indicate the 25/50/75th

percentiles for each group, whiskers indicate the minimum and maximum. (F,G) Heatmaps of simulated sleep at each level of P(Wake) and P(Doze), reproduced from Fig. 3. Circles indicate the coordinates of the mean P(Wake) and P(Doze) of sleep deprivation group before sleep deprivation (white circles) and following sleep deprivation (black circles). Dashed lines show the change along each dimension. (H,I) Power law function fits of P(Wake) and P(Doze) during PRE and POST. PRE: blue, POST: magenta. Open circles are the empirical data, lines are the best fit, and shaded regions are the 95% prediction bounds for the fit. *n.s.* indicates no significant difference, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

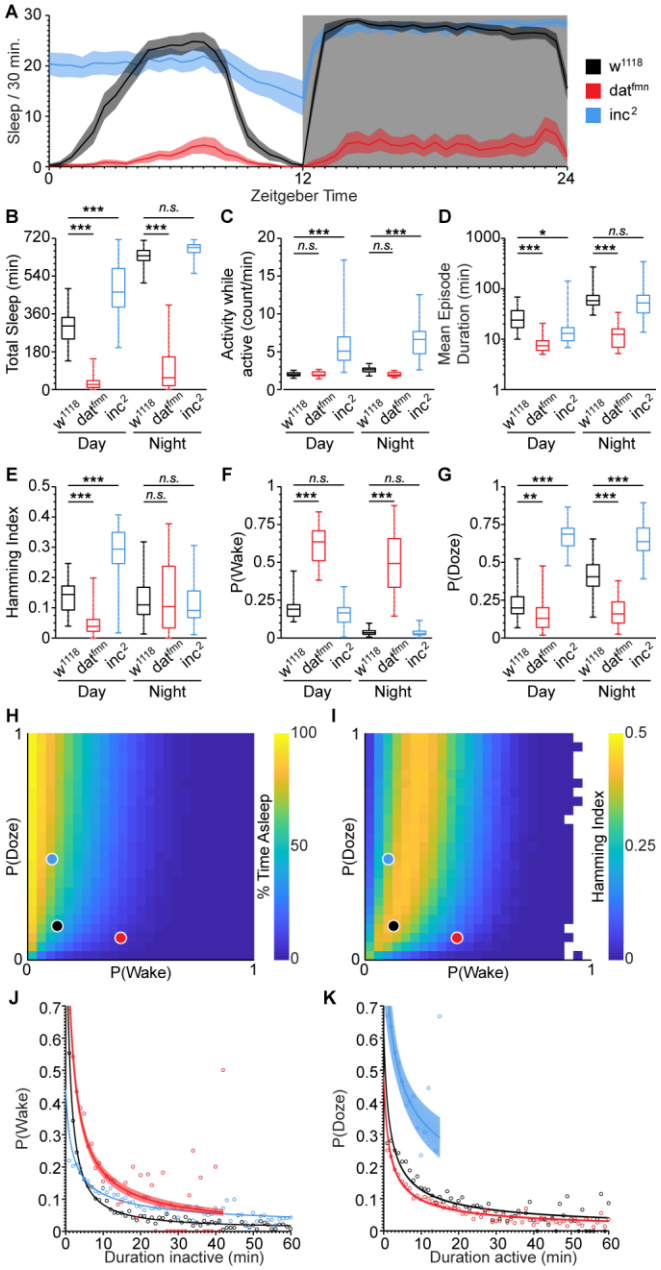

Figure 6 - Mutations Affecting Sleep Act Through Both Wake and Doze Probability. (A) Circadian profile of sleep in a 12:12 Light:Dark cycle. Shaded regions indicate lights on and lights off times. Lines are the population average, shaded area is the 95% confidence interval of the mean. Individual profiles are the average of 3 days of behavior. (B-G) Box and whisker plots of total sleep (B), activity while awake (C), mean sleep episode duration (D), Hamming index (E), P(Wake) (F), and P(Doze) (G). Boxes

indicate the 25/50/75th percentiles for each group, whiskers indicate the minimum and maximum. (H,I) Heatmaps of simulated sleep at each level of P(Wake) and P(Doze), reproduced from Fig. 2. Circles indicate the coordinates of the mean P(Wake) and P(Doze) of the isotype control (w^{1118}; black), *fumin* (red), and *insomniac* (blue) flies during daytime. (J,K) Power law function fits of P(Wake) and P(Doze) of each genotype during daytime. w^{1118}: black, *fumin*: red, *insomniac*: blue. Open circles are the empirical data, lines are the best fit, and shaded regions are the 95% prediction bounds for the fit. The P(Wake) *fumin* fit and P(Doze) *insomniac* fit do not extend across the full range of x-axis because fits were only evaluated in the range for which 2 empirical data points were collected, and no flies of those genotypes produced sufficiently long episodes of inactivity or activity, respectively. *n.s.* indicates no significant difference, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

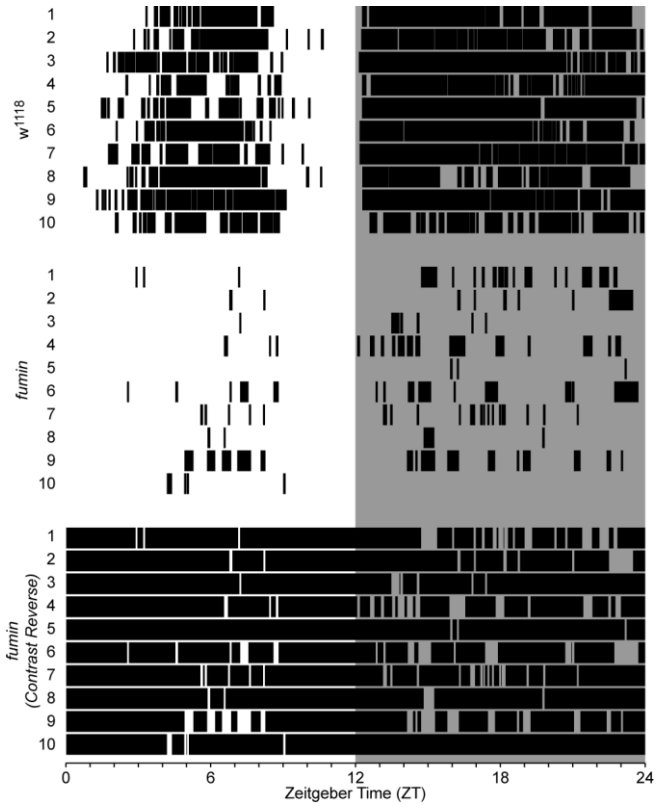

Figure S1 – Sleep Rasters of Control and *fumin* flies. 24 hours sleep rasters of ten w^{1118} flies (Top) and ten *fumin* flies (Middle and Bottom). In the top two sets of rasters, white is wake, black is sleep. In the bottom set of rasters, white is sleep and black is wake. The gray filled area is night.