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A mathematical model describes Drosophila sleep behavior in w1118 controls and in a hyposomnolent \textit{insomniac} line

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I propose a mathematical model, produced by least-squares nonlinear regression to fit the form \( Y = aX^b \), which can explain sleep behavior in the healthy animal as well as previously-reported changes in total sleep and sleep architecture in hyposomnolent mutants.

This model, fit to sleep data, yields coefficient of determination R squared, which describes goodness of fit. R squared is lower in hyposomnolent mutant \textit{insomniac} as compared to control, indicating a poorer fit of the model to the data in \textit{insomniac}. R squared also tends to be lower in daytime sleep as compared to nighttime sleep.

My findings raise the possibility that low R squared is a feature of all hyposomnolent mutants, not just \textit{insomniac}. If this were the case, R squared could emerge as a novel means by which sleep researchers might assess sleep dysfunction.
A mathematical model describes Drosophila sleep behavior in w1118 controls and in a hyposomnolent insomniac line

Running title: A Model for Drosophila Sleep

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Conflict of interest statement

My research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Summary

The conserved nature of sleep in Drosophila has allowed the fruit fly to emerge in the last decade as a powerful model organism in which to study sleep.

Recent sleep studies in Drosophila have focused on the discovery and characterization of hyposomnolent mutants. One common feature of these animals is a change in sleep architecture: sleep bout count tends to be greater, and sleep bout length lower, in hyposomnolent mutants.

I propose a mathematical model, produced by least-squares nonlinear regression to fit the form $Y = aX^b$, which can explain sleep behavior in the healthy animal as well as previously-reported changes in total sleep and sleep architecture in hyposomnolent mutants.

This model, fit to sleep data, yields coefficient of determination $R$ squared, which describes goodness of fit. $R$ squared is lower in hyposomnolent mutant *insomniac* as compared to control, indicating a poorer fit of the model to the data in *insomniac*. $R$ squared also tends to be lower in daytime sleep as compared to nighttime sleep.

My findings raise the possibility that low $R$ squared is a feature of all hyposomnolent mutants, not just *insomniac*. If this were the case, $R$ squared could emerge as a novel means by which sleep researchers might assess sleep dysfunction.

Keywords: Drosophila, sleep, activity, waking, *insomniac*, homeostasis, architecture, consolidation, least-squares, nonlinear regression
1. **Introduction**

Sleep in Drosophila exhibits many characteristics that are seen also in mammalian sleep, including extended periods of quiescence, reduced arousal threshold, and hyper-consolidation of sleep after sleep deprivation [1]. Further, drugs that alter mammalian sleep have analogous effects in Drosophila, suggesting conserved neural and biochemical mechanisms [2]. The conserved nature of sleep in Drosophila has allowed the animal to emerge in the last decade as a powerful model for the study of sleep.

Much recent work in Drosophila has been focused on the discovery and study of certain hyposomnolent mutants [3] [4] [5] [6] [7].

In addition to reduced total sleep, hyposomnolent mutants also demonstrate altered sleep architecture. Sleep is poorly consolidated: bout length is reduced as compared to control [3] [5] [7] [8]. In some of these cases bout count is also reduced [5], but more frequently it is elevated [3] [7] [8]. One such example is *Insomniac* [3] [8], which is the basis of much of the modeling work in this study.

The goal of this study is to produce a mathematical model that describes sleep behavior in control animals. Further, I will examine the extent to which this model also holds true in the hyposomnolent mutant *insomniac*.

My results may establish a new paradigm for analysis of sleep dysfunction in hyposomnolent mutants. These techniques could also be used on higher animals, including humans.

2. **Methods**

All animals came from the Bloomington Stock Center at Indiana University. *Insomniac* corresponds to stock number 18307. w1118 was used as control.

*Insomniac* was outcrossed for 8 generations to an isogenic w1118 line to control for genetic background. Only males were used in this experiment, for mutants and controls. Animals were 1-5 days old.

Sleep was monitored using TriKinetics’ DAM2 Drosophila Activity Monitors, as previously described [9]. Briefly, animals were placed inside activity tubes containing food made of 5% sucrose and 2% agarose and then housed in an incubator with 12-hour:12-hour day:night cycles at 25 degrees C and 85% humidity. Animals were given three days to acclimate to the day/night cycle before data collection began. After the acclimation period, data collection lasted 4 full 24-hour periods. Sleep is defined as 5 minutes of inactivity [10]. Animals that died or showed significant loss of health during the course of the experiment were automatically excluded from the results. Data was processed using SleepLab, custom Matlab-based software provided by Dr. William Joiner (UCSD).

All daytime data have been separated from nighttime data, but otherwise all data have been combined together over four days for each genotype. Statistical analysis was handled with GraphPad PRISM 6.
In analysis of sleep behavior, ordinary (unweighted) least-squares nonlinear regression is used to produce lines of fit, constrained to the equation $Y = aX^b$. In each line of fit, the independent variable $X$ represents the sleep bout count of the animal-time period pair, while the observed response variable $Y$ represents the mean sleep bout length in that same animal-time period pair.

Similar lines of fit are produced using activity bout data. In activity bout data analysis, independent variable $X$ represents activity bout count, and observed response variable $Y$ represents mean activity bout length.

Nonlinear regression assumes that the pool of residuals is drawn from a Gaussian distribution. The D’Agostino & Pearson omnibus K2 test is used to test for attainment of this requirement.

$R^2$ is computed based on the data’s adherence to the same ordinary least-squares nonlinear regression line discussed previously. Specifically, $R^2 = 1 – (SSres/SStot)$, where $SSreg$ is the sum of the squares of all distances along the y-axis between data points and the best-fit curve, and $SStot$ equals the sum of square of all distances along the y-axis between data points and the horizontal line that runs through the mean of all y-values.

### 3. Results

$n = 31$ per genotype and 64 total. Since each animal slept for four 24-hour periods, including four days and four nights, we consider sets of 124 observations.

#### 3.1 Characterization of wild type sleep

Sleep in wild type animals is consistent with that seen in the literature, in terms of both total time slept and sleep architecture [3] [8].

#### 3.2 Characterization of insomniac sleep

*Insomniac* demonstrates a robust phenotype in terms of total time slept. *Insomniac* animals tested in this experiment sleep significantly less than controls in the 24-hour period. According to a two-tailed, two-sample heteroscedastic (allowing for unequal variance) Student’s T-test, probability that measures of total sleep per 24 hours in *insomniac* and controls came from the same distribution is given by $p < 0.0001$.

*Insomniac* also demonstrates a strong phenotype in sleep architecture. Bout length is shorter and bout count greater in *insomniac* as compared to its control. According to the same Student’s t-test performed above, $p < 0.0001$ for both mean sleep bout length per 24 hours and bout count per 24 hours.

Sleep in *insomniac* is compared to control in Fig. 1. Decreased total sleep is seen in the nighttime only in *insomniac* as compared to control. Decreased mean sleep bout length and increased mean sleep bout count is seen in both daytime and nighttime in *insomniac* as compared to control.

**Figure 1 here**
That sleep in **insomniac** is poorly consolidated can be observed qualitatively. **Fig. 2** represents activity in **insomniac** and control. We see that, in the case of **insomniac**, activity is distributed throughout periods in which control flies normally sleep.

**Figure 2 here**

### 3.3 Production of a mathematical model

Past work has generally studied average total sleep per 24 hours, taken over all animals in a given experimental condition and over the entire duration of the experiment. Instead, I consider daytime and nighttime sleep separately, and I consider the sleep behavior of individual animals during single days or nights. By considering the sum of individual animal-day and animal-night pairs, I can produce from these data a mathematical model that describes sleep behavior.

**Fig. 3** shows the individual animal data.

Sleep behavior is most regular in the case of control night (**Fig. 3c**). To this set of data, I fit the model

\[ y = a \cdot x^b \]  

**Equation 1**

where \( y \) corresponds to mean sleep bout length, for an individual animal, over the course of a single night; and \( x \) corresponds to sleep bout count for that same individual animal over the course of a single night. Coefficient of determination \( R^2 \) is 0.993 in the case of control night. In **Fig. 3**, **Eq. 1** is fit to all experimental conditions. \( R^2 \) is not as high in other experimental conditions as it is in control night, indicating a worse fit to the model in these other experimental conditions.

**Figure 3 here**

**Eq. 1**, the parameters that comprise it, and the \( R^2 \) coefficient might provide valuable insight towards the analysis of sleep behavior in Drosophila, even in experimental conditions where \( R^2 \) is relatively low.

The parameter \( b \) is negative in experimental conditions. This indicates that, as bout count rises, mean sleep bout length falls. Further, \( b \) tends to reside near \(-1\).

In **Eq. 1**, \( a \) tends to estimate total sleep. For example, in **Fig. 3c**, \( a = 682.9 \). Consistent with this prediction, measured sleep for this genotype and timeframe is 673 minutes. Given the form of **Eq. 1**, that \( a \) estimates total sleep should make sense. Suppose, in the regression **Eq. 1**, it so happens that \( b = -1 \) exactly. Then we can re-express the equation as

\[ y \cdot x = a \]  

**Equation 2**

**Eq. 2** shows that (in the case \( b = -1 \)) the best regression generates a fixed constant \( a \) with the special property that the product of any pair of values attained by the variables \( x \) and \( y \) tends to fall close to \( a \). These values in turn correspond to the bout count and mean bout lengths, respectively, of the animals. And, we know that, in an individual animal-time period pair, mean bout length times bout count equals total sleep for that time period. Thus we see why, when \( b \) falls close to \(-1\), \( a \) estimates total sleep.
As $b$ deviates from $-1$, $a$ becomes a worse estimate of mean total sleep. For example, in Fig. 3b, $a = 68$. This drastically underestimates total sleep for this genotype and timeframe. For $b < -1$, $a$ is an underestimate of mean total sleep. For $b > -1$, $a$ is an overestimate. The tendency of $a$ to estimate total sleep, as well as the relationship between $b$ and $a$ I have just described, holds in both control animals and in insomniac. In insomniac, $a$ may not be as good an estimate of total sleep, in part because $b$ may stray further from $-1$.

The coefficient of determination $R^2$ may be of use. As described earlier, $R^2$ is greatest in the setting of control sleep behavior at night. $R^2$ close to 1 indicates that the mathematical model closely fits the data.

$R^2$ is closer to 1 in the nighttime, as compared to the daytime, with genotype controlled for. In other words, control night has greater $R^2$ than control day; meanwhile, insomniac night has greater $R^2$ than insomniac day. Additionally, $R^2$ is farther from 1 in insomniac, as compared to control, with time of day controlled for. Insomniac night has lower $R^2$ than control night; insomniac day has lower $R^2$ than control day.

So, in the daytime, and in insomniac, the model tends to fit the data less well.

Under conditions where $R^2$ is relatively low, such as insomniac day, 95% confidence intervals for parameters $a$ and $b$ tend to be wider relative to the absolute value of these parameters. Also, 95% confidence bands tend to be wider as well in conditions with low $R^2$.

3.4 Application of the model to activity data

I conducted a similar statistical analysis on the behavior of the animals used in this experiment, except considering activity bouts as opposed to sleep bouts.

Eq. 1 does not fit the activity bout data as well as it fits the sleep bout data. $R^2$ is 0.608 at maximum.

Like in the case of the sleep bout data, $R^2$ is higher in control than it is in insomniac. $R^2$ values are 0.608 and 0.582 in control, nighttime and daytime, respectively, compared to 0.270 and 0.299 in insomniac.

Note that, in contrast with the sleep bout data, it is not the case in the activity bout data that $R^2$ changes in daytime as compared to nighttime. Within a given genotype, daytime and nighttime $R^2$ values are nearly identical.

3.5 Statistical tests for the appropriateness of the mathematical model

Dependency between parameters $a$ and $b$ as they fit to the sleep data ranges from 0.822 to 0.984.

The sleep data do not pass the D’Agostino & Pearson omnibus K2 test of normalcy test in any genotype or timeframe, including control day, control night, insomniac day, and insomniac night.

Dependency and normalcy in the activity data is not reported, because I do not make the claim that Eq. 1 is an appropriate model for the activity data.

4. Discussion
4.1 Evaluation of sleep behavior

Sleep behavior in the control is similar to that seen in past work. Sleep behavior in control is normal quantitatively (Fig. 1) and qualitatively (Fig. 2). This indicates that my sleep system is in good working order. Further, the sleep phenotype I have demonstrated in insomniac mutants, which is characterized by reduced total sleep and poor consolidation, is consistent with past reports [3] [8].

4.2 The value of consideration of daytime and nighttime sleep

Past work has usually reported only on total sleep and sleep architecture during the 24-hour period. Splitting sleep behavior into daytime and nighttime, as I have, is useful. Consideration of daytime and nighttime sleep provides detail which may be missed if only 24-hour sleep is considered. For example, as seen in Fig. 1a, only total nighttime sleep is significantly different in insomniac as compared to control; total daytime sleep is not. This is missed when, as has been done in previous work, only 24-hour sleep values are compared.

Perhaps more importantly, splitting daytime and nighttime sleep allows the mathematical model to be fit to each group separately. Sleep behavior is different in daytime versus nighttime, and so combining these two pools of data would reduce signal. Separate consideration of daytime and nighttime allows more information, and more precise information, to be drawn from these data.

4.3 Merits of the mathematical model

Coefficient of determination R^2, which measures goodness of fit to the mathematical model described in Eq. 1, is as high as 0.993. This serves to validate the mathematical model: at least in some circumstances, the model describes behavior very well. Even in conditions where R^2 is not as high, the model appears to describe the behavior reasonably well considering the higher degree of variability within those data. Future study should examine whether Eq. 1 describes sleep behavior in other genotypes besides w1118 and insomniac.

As mentioned in the results, R^2 is lower in insomniac. Future research might investigate whether or not R^2 is also diminished in other hyposomnolent mutants. If this were the case, R^2 could emerge as a novel means by which sleep dysregulation might be measured. A lower R^2 could indicate a greater degree of dysregulation. R^2 could then be used to assess sleep dysfunction in other Drosophila lines, and in other animals, including humans.

Note that R^2 constitutes a measure of sleep behavior independent of those measures usually studied in Drosophila sleep research, namely, total sleep, mean bout length, and mean bout count. Any of these measures could be changed in a Drosophila line, without change in R^2. Likewise, R^2 could theoretically change without corresponding change in total sleep, mean bout length, or mean bout count. Thus the R^2 measure offers novelty.

4.4 Other measures provided by the mathematical model

As mentioned in the results, where R^2 is relatively low, the 95% confidence intervals for parameters a and b tends to be wide relative to the absolute value of these parameters. Further research should investigate whether or not the 95% confidence intervals for parameters a and b...
are also wider than control in other hyposomnolent mutants besides *insomniac*. Eq. 1 parameter confidence interval width, like $R^2$, could serve as a novel measure of sleep dysregulation.

In situations with low $R^2$, confidence bands also tend to be wider. Width of the confidence band could also be evaluated as a potential measure of sleep dysregulation.

### 4.5 Application of the model to the activity data

Fit of Eq. 1 to the activity data tends to be poorer than fit of Eq. 1 to the sleep data. This might suggest that total sleep is more tightly regulated during sleep than total activity is during waking.

Whatever the reason, it seems that Eq. 1 may not be as appropriate a model for waking behavior as it is for sleep behavior.

### 4.6 Limitations of the model as applied to the sleep data

Dependency between parameters can be as high as 0.984, which indicates that $a$ and $b$ may be redundant. If a simpler model is desired, Eq. 2 would suffice. However, the inclusion of $b$ seems to be merited, because production of a model conforming to Eq. 1 is not difficult, and $b$ still improves goodness of fit.

That the sleep data universally fail the D'Agostino & Pearson omnibus K2 test under all circumstances might be cause for concern. Regardless of this finding, though, my model still appears to have merit, discussed in 4.3. Further, failure of this test need not indicate that nonlinear regression is an inappropriate strategy. Especially in large data sets, deviations from normalcy may reach statistical significance without corresponding to real practical meaning [11]. So, it appears that my least-squares nonlinear regression procedure may be resistant to violations of the standard that underlying distributions be Gaussian [11]. Nevertheless, future work could look at the use of robust nonlinear regression models, as opposed to the least-squares nonlinear model used here. These are less distorted by data sets whose residuals come from non-Gaussian distributions [11].

Note also that, if mean sleep bout length values are weighted by $1/y^2$, performance on the D’Agostino & Pearson omnibus K2 normalcy test is improved but still poor.

### 5. Acknowledgments

Dion Dickman provided lab space and resources. Hui Yang conducted the genetics for this experiment, including outcrossing. William Joiner provided advice for this project and supplied the computer program I used for analysis of sleep behavior. Shi Xing, Benjamin Diamond, John A. Jane Sr. and Bobby Starke provided further feedback.

### 6. References


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Characterization of sleep in *insomniac* versus control

Error bars represent the standard error measurement. Each experiment consists of 31 animals, all of which survived for four days. So, each genotype-time period pair represents an average across $n = 124$ measurements. (A) Total sleep in insomniac versus control. Values shown represent mean total sleep, across the four days of the experiment. I have distinguished between insomniac and control, and within these distinctions, I have distinguished again between daytime and nighttime. (B) Sleep bout length in insomniac versus control. Values represent averages across the length of the experiment. (C) Mean number of sleep bouts across the length of the experiment. *$p < 0.0001$* according to two-tailed, two-sample heteroscedastic Student’s T-test.
Representative actograms for control and *insomniac*

(A) control. (B) insomniac. Each panel represents the sleep/wake activity of a single animal. So, three animals are shown for each genotype, and six are represented in total. Note disorganized sleep/wake behavior in *insomniac*, including extensive activity during lights-off 12-hour periods. Day one (not shown) as well as days two and three (shown in **Fig. 2**) were not considered in data analysis.
3

Relationship between bout count mean bout length in individual animals

Each dot represents a single animal-day pair (panels A and B) or animal-night pair (panels C and D). The y axis represents the mean number of bouts slept during each animal-time period pair, and the x axis represents the amount of bouts slept in that same animal-time period pair. Thus n for each figure is equal to 31 * 4 = 124 animal-time period pairs. Each panel contains an inset, which lists, from to bottom: the equation of the line of fit, in the format $Y = aX^b$; the coefficient of determination $R^2$; the 95% confidence interval for the $a$ parameter; and the 95% confidence interval for the $b$ parameter. Dotted lines represent the upper and lower margins of the 95% confidence band. The chances are 95% that the true line of fit lies between these upper and lower margins.
[A] Control day

\[ y = 304.2 \times x^{-0.899} \]
\[ R^2 = 0.741 \]
\[ 241.4 < a < 366.9 \]
\[ -0.991 < b < -0.807 \]

[B] Insomniac day

\[ y = 67.58 \times x^{-0.446} \]
\[ R^2 = 0.142 \]
\[ 25.80 < a < 109.4 \]
\[ -0.654 < b < -0.238 \]

[C] Control night

\[ y = 682.9 \times x^{-1.094} \]
\[ R^2 = 0.993 \]
\[ 671.4 < a < 694.5 \]
\[ -1.023 < b < -0.985 \]

[D] Insomniac night

\[ y = 415.9 \times x^{-1.011} \]
\[ R^2 = 0.604 \]
\[ 279.9 < a < 551.9 \]
\[ -1.115 < b < -0.867 \]