Modeling Daily and Subdaily Cycles in Rat Sleep Data

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SUMMARY

We developed a statistical model for rat sleep-wake behavior over the 24-hour day which could be used for animals exposed to shorter cycles of light and dark. Rat behavior was classified either as wake or sleep as determined by analysis of electrophysiological data. The proposed model consists of three parts: the first two explain cyclic effects relating to the lighting conditions, whereas the last part reflects any acyclic effect. Hypothesis tests were conducted on the magnitude of the parts. The model also accounts for correlated errors.

1 Introduction

Sleep-wake behavior in mammals is determined by a combination of extrinsic and intrinsic factors. "Uncomfortable" environmental conditions can interfere with sleep, as can loud noises and other abrupt changes. Somewhat apart from these extrinsic stimuli, endogenous daily or circadian rhythms are generated in the brain by the suprachiasmatic nucleus of the hypothalamus. These rhythms are not entirely independent of external stimuli; the purely intrinsic rhythms are only approximately 24 hours long, and are adjusted to match the length of the day and the phase of external stimuli. This process, called entrainment, can be driven by a number of factors including exercise and social cues. The most potent of these is light,

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by far the most important regulator of circadian rhythm entrainment in both natural and experimental situations.

In addition, light has important effects on sleep-wake states which are not the result of the changes it induces in endogenous rhythms. For example, in diurnal mammals such as humans, bright light exposure during the normal sleep period (i.e., the dark) tends to induce wakefulness, whereas dark exposure during the daytime tends to be soporific. The opposite is seen in nocturnal mammals such as rats, who tend to go to sleep when exposed to light during their normal active (i.e., dark) period. Light can thus affect sleep-wake state through regulation of circadian rhythms as well as through non-rhythmic shifts in behavioral state following changes between light and dark conditions. See Benca *et al* (1993) for more background on the relation of sleep stages to lighting conditions.

This article describes the analysis of an experiment which was designed to measure the effects of light-dark cycles of varying lengths on sleep patterns in rats. Two inbred strains of rats, albino Lewis and pigmented Brown Norway, were exposed to one of two types of experimental lighting conditions. The first condition, "Baseline," was a standard 24-hour schedule of 12 hours lights on followed by 12 hours lights off; the second condition, "Test," exposed animals to a continuous 3 hour lights on/3 hour lights off schedule. Illuminance level when lights were on varied among three conditions: high (1,000 lux), normal (150 lux) and low (50 lux). Continuous electrophysiological recordings to determine sleep and wake states were performed using standard techniques. Sleep or wake state was determined for each 30 second epoch throughout the experiment. Every 10 consecutive readings were averaged, yielding percentages of time asleep for each day's 288 5-minute intervals. These percentages were in turn averaged over three days of experimentation for each group of rats of the same strain subjected to the same conditions. In this paper, we will concentrate on modeling the percentage of time asleep, which corresponds to total sleep time.

The purpose of this analysis is to develop a statistical model for sleep-wake patterns in response to light-dark cycles of varying lengths, which would take into account both the rhythmic circadian component and stimulus-induced shifts in behavior. Such a model could also be used to describe other physiological parameters which may include both rhythmic and acute components, such as temperature and hormone secretion. Studies assessing the effects of acute changes in light conditions often report average data over the period of time in question. Although this approach may be satisfactory to demonstrate large and/or persistent shifts in behavior, it cannot fully describe time-dependent patterns which may be short in duration or quickly followed by rebound effects. Sine/cosine wave functions are traditionally used (e.g., Strogatz 1986) for modeling circadian systems, but do not necessarily fit. Trigonometric wave functions are particularly inadequate for analyzing data obtained in light-dark cycles because of the inability to incorporate abrupt shifts which may occur at transitions between light and dark. Wang and Brown (1996) studied human circadian rhythms by suggesting a periodic spline model. That model cannot be used in our case because it does not separately fit the three components which are of interest here and are described below.

In this paper, we suggest modeling the sleep data using a three-component model: 1. A piecewisely continuous regression model, which may have a discontinuity at the time the lights change, is used to fit the baseline data. The fitted regression function is then used as a covariate in modeling the test data, explaining the 24-hour cycle effect in the 6-hour lighting condition. 2. The 6-hour cycle effect is explained using a periodic piecewise polynomial function with period equal to 6 hours. In each 6-hour period, the function may have a discontinuity at the time the lights change. 3. The third part of the model, which we call an "acyclic" term is used to explain any effect other than the 24-hour or 6-hour cyclic effects and is fitted by using a nonparametric smoothing operator on the residuals of the first two parts. Thus our model has three parts; the first two are parametric and the last one is

nonparametric; the whole model can then be regarded a semiparametric. This model allows for more flexibility in describing sleep patterns than the traditional sine/cosine model. The model is introduced in Section 2, and in Section 3 hypothesis tests are conducted to test the significance of each part in the model. For semiparametric models, an iterative algorithm is used in Section 4 to refine both the parametric and the nonparametric components. In Section 5, we discuss a possible correlation in the error terms and suggest an adjustment. The model is summarized and discussed in Section 6.

2 Our Model

To take into account the 24-hour cycle effects, which should be comprised of endogenous circadian influences, entrainment, homeostatic influences and the effects of light and dark, we model the baseline data first with a piecewisely continuous regression function $f_{24}(x)$, where x represents time in hours. According to experience and the experimental results, the percentage of time in sleep (SLEEP hereafter) increases sharply at x=12 when the light is switched from on to off. For that reason, $f_{24}(x)$ is allowed to have a discontinuity at x=12. There are many nonparametric smoothing methods in the literature (e.g., kernel method, Härdle 1990; local polynomial kernel method, Wand and Jones 1995; smoothing spline method, Wahba 1991). Recently the local polynomial kernel method has been demonstrated to have some preferable properties (see e.g., Fan and Gijbels 1996). Therefore we use this method to fit $f_{24}(x)$ by applying the S-plus function loess.smooth() with span=.3 which is in the recommended range (see Section 7.11 of the Splus Manual (1995)). Four combinations of strain and light intensity, namely, (Brown Norway, High), (Brown Norway, Normal), (Lewis, Normal) and (Lewis, Low), are considered in this paper. For (Brown Norway, High), the baseline data of SLEEP and the fitted $f_{24}(x)$ are plotted in Figure 1(a) with dot points and solid curves.

We then use the fitted function from the baseline data as a covariate in modeling the test data. That is, the functional form from the baseline data is used to model the 24-hour cycle effect in the test data, with the magnitude of the effect to be estimated. To explain the 6-hour cycle effect, a periodic piecewise polynomial function, $f_6(x)$, is used with a period of 6 hours. In each 6-hour interval, $f_6(x)$ is defined to be a piecewise polynomial with a possible discontinuity in the middle of the interval. The order of the polynomial is determined by the backward model selection procedure with significance level .05 and with the hierarchy principle (namely, if x^k is in the model, then all lowers of x should be in the model whether they are significant or not). Combining these two parts, we are actually fitting the following model

$$SLEEP(x) = c\widehat{f}_{24}(x) + f_6(x) + e^*(x), \tag{2.1}$$

where $\widehat{f}_{24}(x)$ is the fitted regression function from the baseline data and $e^*(x)$ denotes the noise. The value of the coefficient c reflects the effect of the 24-hour cycle – the bigger, the more important. Its estimated values are .162, .374, .775 and .540, respectively, in the four cases considered. The fitted $f_6(x)$ for (Brown Norway, High) is plotted in Figure 1(c).

Model (2.1) is based on the assumption that SLEEP could be completely explained but for random noise by the 24-hour cycle effect and the 6-hour cycle effect. The validity of this assumption is unknown; therefore, we fit the residuals of (2.1) by a nonparametric smoother. This induces the following modification of (2.1):

$$SLEEP(x) = c\widehat{f}_{24}(x) + f_6(x) + f_a(x) + e(x), \tag{2.2}$$

where $f_a(x)$ is a nonparametric function and $e(x)$ is the noise.

The function $f_a(x)$ in (2.2) represents the remaining systematic effect after the 24-hour cycle and the 6-hour cycle effects have been explained. We call this term the *acyclic* effect. Again, the Splus function loess.smooth() is used to fit $f_a(x)$. The fitted acyclic part of (2.2) for (Brown Norway, High) is presented in Figure 1(d). We then combine three estimated

parts of (2.2) as the fitted model of SLEEP, shown in Figure 1(b) with the test data for the case of (Brown Norway, High). We can see that it fits the data well.

Next we make several remarks on the above model building procedure. First, the reason why we use a periodic piecewise polynomial (instead of a nonparametric function) to fit $f_6(x)$ is to formulate the problem in a semiparametric framework (c.f. model (2.2)). If both $f_6(x)$ and $f_a(x)$ are nonparametric, then they might not be identifiable. For example, $\{f_{24}(x), f_6(x), f_a(x)\}$ and $\{f_{24}(x), f_6(x) + g(x), f_a(x) - g(x)\}$ determine the same mean function for the response variable, where $g(x)$ is any periodic but continuous function with period equal to 6 hours. Second, the test data may include some information about $f_{24}(x)$. It is still unknown to us how to make use of this information in efficiently estimating $f_{24}(x)$. Fortunately, the sample size of the baseline data is moderately large ($n = 288$), and the estimator of $f_{24}(x)$ from the baseline data alone should be reasonably good. Third, the data used in this paper are averages over: (1) 10 consecutive readings in each 5-minute interval; (2) three days of experimentation; and (3) a group of rats. By performing the first averaging, we actually assumed that the probability for rats in sleep state was a constant in each 5-minute interval. Therefore our model is designed for describing large and/or persistent shifts in behavior such as the 24-hour cycle and 6-hour cycle effects, but not for describing transient pattens of brief duration. The second and third averagings simplify the error structure of the data.

If we prefer not to perform the first averaging in order to check some possible short-in-duration patterns, then the observations are binary (denoting "sleep" or "not sleep" in each 30-second epoch) and model (2.2) could be replaced by the following semiparametric logistic regression model (see Cox and O'Sullivan (1990) for related discussion):

$$\log\{\frac{p(x)}{1-p(x)}\} = c^* \widehat{f}_{24}(x) + f_6^*(x) + f_a^*(x), \tag{2.3}$$

where $p(x)$ denotes the probability of a rat in sleep at time x, c^*, $f_6^*(x)$ and $f_a^*(x)$ are defined

similarly to c, $f_6(x)$ and $f_a(x)$ in (2.2). If the third averaging is not performed, then (2.3) needs to be further generalized by including a random effect term to explain the variability among different rats. How to fit the resulting model is an open problem at this time.

3 Hypothesis Tests

3.1 Testing the Acyclic Term

Using the model introduced in Section 2, we can attempt to answer our major questions; namely, how much variation in the test data is due to the 24-hour cycle effect? How much can be explained by the 6-hour cycle effect? Can the data be explained completely but for random noise by the 24-hour and 6-hour cycle effects? First, we consider hypotheses $H_0 : f_a(x) \equiv 0 \ \ vs \ \ H_a : f_a(x) \neq 0$, for some x, where $f_a(x)$ is the acyclic term of (2.2). H_0 is saying that SLEEP can be completely explained by the 24-hour cycle and the 6-hour cycle effects.

Using the "extra sum of squares" principle, a natural testing statistic of H_0 is the F statistic which is defined as follows:

$$F = \frac{(SSE_{H_0} - SSE_{H_a})/(df(Error)_{H_0} - df(Error)_{H_a})}{SSE_{H_a}/df(Error)_{H_a}} \tag{3.1}$$

In (3.1), SSE is the sum of squares of error and df(Error) is the degree of freedom of error. The quantity $df(Error)_{H_0} - df(Error)_{H_a}$ is actually the degree of freedom of the acyclic term (which is denoted as df_a hereafter) and $df(Error)_{H_a}$ can be calculated by the formula: $df(Error)_{H_a} = \#$ observations $- \#$ parameters in the first two parts of $(2.2) - df_a$. Hence the essential part of calculating F is to calculate df_a.

There has been considerable discussion about computing the degree of freedom of a nonparametric term like $f_a(x)$. Suppose that the predicted values of this function at the design points can be expressed as $\underline{\hat{\mathbf{f}}}_a = \mathbf{S}\underline{\mathbf{y}}$ where $\underline{\hat{\mathbf{f}}}_a = (\hat{f}_a(x_1), \hat{f}_a(x_2), \cdots, \hat{f}_a(x_n))'$, $\underline{\mathbf{y}} = (y_1, y_2, \cdots . y_n)'$, $\{x_i\}$ are the design points, $\{y_i\}$ are the observations (residuals of (2.1) in our case) and $n = 288$ is the sample size. Most nonparametric smoothers, including the one we used, have this expression since they all provide linear estimates.

In this paper, we use the formula $df_a = tr(2\mathbf{S} - \mathbf{S}'\mathbf{S})$ which was suggested by several authors including Hastie and Tibshirani (1987). To obtain $\mathbf{S}$, we apply the nonparametric smoother to a sequence of response vectors $\underline{\mathbf{e}}_1 = (1, 0, 0, \cdots, 0)'$, $\underline{\mathbf{e}}_2 = (0, 1, 0, \cdots, 0)'$, $\cdots$, $\underline{\mathbf{e}}_n = (0, 0, 0, \cdots, 1)'$. The corresponding predicted vectors are the columns of $\mathbf{S}$. The value of df_a is then calculated to be 12.185.

Wang and Brown (1996) used the bootstrap method to get the empirical distribution of F and then to perform the F-test, since F is not exactly F-distributed. But Cleveland and Devlin (1988) showed that the distribution of F is fairly close to a F-distribution. Based on that approximation, our test results show that the acyclic term is statistically significant for all four combinations of rat strain and light intensity.

3.2 Testing the 24-hour Cycle and the 6-hour Cycle Effects

We now test the significance of the 24-hour and the 6-hour cycle effects. Since the acyclic term is significant, model (2.2) will be used. We first test hypotheses $H_0 : c = 0 \ \ vs \ \ H_a : c \neq 0$. H_0 means that there is no 24-hour cycle effect under the 6-hour cycle lighting condition. From the model building procedure, $\hat{c}$ is a linear combination of the test data. Hence it is appropriate to use a t-test here. The value of $\hat{c}$ is obtained by fitting (2.1). Its standard error is computed by formula $s.e.(\hat{c}) = s.e.^*(\hat{c})\sqrt{MSE_{(2.2)}/MSE_{(2.1)}}$, where $s.e.^*(\hat{c})$ is the standard error of $\hat{c}$ from (2.1), $MSE_{(2.1)}$ and $MSE_{(2.2)}$ are the mean squared errors of (2.1) and (2.2),

respectively. The adjustment factor $\sqrt{MSE_{(2.2)}/MSE_{(2.1)}}$ used in the above formula accounts for the variability explained by the acyclic term in model (2.2). The test results are presented in Table 1. These results demonstrate that the 24-hour cycle effect is significant under the 6-hour cycle lighting condition for both rat strains and all light intensities.

The value of c can be interpreted as the percentage of the baseline pattern remaining in the data under the 6-hour cycle lighting condition. In addition to the hypothesis tests performed above, it is also interesting to compare c values of different rat strains and lighting conditions. To accomplish this, we consider contrasts $\sum_{i=1}^{4} \lambda_i c_i$ where λ_i's are coefficients satisfying $\sum_{i=1}^{4} \lambda_i = 0$ and the subscript i denotes different groups in the order of (Brown Norway, High), (Brown Norway, Normal), (Lewis, Normal) and (Lewis, Low). A natural test statistic for $H_0 : \sum_{i=1}^{4} \lambda_i c_i = 0 \ \ vs \ \ H_a : \sum_{i=1}^{4} \lambda_i c_i \neq 0$ is $Z = (\sum_{i=1}^{4} \lambda_i \widehat{c}_i)/\sqrt{\sum_{i=1}^{4} \lambda_i^2 Var(\widehat{c}_i)}$.

Since the sample size (288 in our case) of each group is moderately large, we could reasonably assume that Z has an approximate $N(0,1)$ distribution. The results of testing several contrasts are presented in Table 2. We can see that all pairwise differences among 4 groups are significant. Lewis rats are affected by the 24-hour cycle effect more significantly than the Brown Norway rats. The interaction between rat strain and light intensity is significant.

By using the same F statistic (3.1), we can test the significance of the 6-hour cycle effect. Our test results show that it is significant for all combinations of rat strain and light intensity, which is clearly shown in Figure 1(c) for the case of (Brown Norway, High).

4 An Iterative Algorithm

As mentioned before, (2.2) is a semiparametric model. It is natural to use an iterative algorithm to refine both the parametric and nonparametric parts. The algorithm can be

described as follows:

An Iterative Algorithm of Model Building

1. *Initial model fitting as described in Section 2.*
2. *At the ith iteration, $i \geq 1$, fit model (2.1) on the residuals of the previous fit.*
3. *Apply the kernel smoothing operator to the residuals of the fitted model in step 2 and calculate the residual sum of squares $SSE^{(i)}$.*
4. *If $|SSE^{(i)} - SSE^{(i-1)}|/SSE^{(i-1)} < \varepsilon$ where ε is a prespecified constant, then stop. Otherwise, let $i = i + 1$ and return to step 2.*

Our results indicate that this algorithm does not improve the fit much. The improvement (which is measured by $|SSE^{(i)} - SSE^{(i-1)}|/SSE^{(i-1)}$) of the first iteration is less than 3% for the Brown Norway rats and less than 8% for the Lewis rats. The improvement of the second iteration is less than 0.9% and 1.5%, respectively, for the two rat strains.

5 Consideration of Possible Correlation in Error Terms

The analysis presented above is based on the assumption that the observations are independent of each other. This assumption may be suspect for sleep data. Suppose that the noise follows the following k-th order autoregression model

$$e(i) = \rho_1 e(i-1) + \rho_2 e(i-2) + \cdots + \rho_k e(i-k) + \epsilon(i), \tag{5.1}$$

where $\epsilon(i)$ is a white noise process with zero mean and variance σ_ϵ^2. If $k = 0$, then no correlation exists in $e(i)$.

Akaike's Information Criterion (AIC) is a very popular criterion to determine the value of k in (5.1). The best value of k, according to that criterion, is the one that induces the minimum of the AIC function $AIC(k) = n\log(\widehat{\sigma}^2_{\epsilon,k}) + 2k$, where $\widehat{\sigma}^2_{\epsilon,k}$ is some estimator of σ^2_ϵ. We find that autocorrelation does exist in the residual values of model (2.2), and that model (5.1) with $k = 1$ or $k = 2$ explains the majority of such autocorrelation.

When the error term follows the autoregression model (5.1), generalized least squares (GLS) estimation will be an appropriate method to estimate the parameters in (2.2) (see e.g., Section 5.2, Judge *et al* 1980). The GLS estimation, however, can be accomplished by using the least squares (LS) estimation on the transformed data introduced below. For cases of $k = 1$ and $k = 2$, let

$$\mathbf{P}_1 = \begin{bmatrix} \sqrt{1-\rho_1^2} & 0 & 0 & \cdots & 0 & 0 \\ -\rho_1 & 1 & 0 & \cdots & 0 & 0 \\ 0 & -\rho_1 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 1 & 0 \\ 0 & 0 & 0 & \cdots & -\rho_1 & 1 \end{bmatrix}; \quad \mathbf{P}_2 = \begin{bmatrix} \alpha_1 & 0 & 0 & 0 & \cdots & 0 & 0 \\ \alpha_2 & \sqrt{1-\rho_2^2} & 0 & 0 & \cdots & 0 & 0 \\ -\rho_2 & -\rho_1 & 1 & 0 & \cdots & 0 & 0 \\ 0 & -\rho_2 & -\rho_1 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ 0 & 0 & 0 & 0 & \cdots & 1 & 0 \\ 0 & 0 & 0 & 0 & \cdots & -\rho_1 & 1 \end{bmatrix}$$

where $\alpha_1 = \sqrt{[(1+\rho_2)\{(1-\rho_2)^2 - \rho_1^2\}]/(1-\rho_2)}$ and $\alpha_2 = -\rho_1\sqrt{1-\rho_2^2}/(1-\rho_2)$. Then GLS estimates of the parameters in (2.2) are the LS estimates after we left-multiply both the observation vector and the design matrix corresponding to the parametric part of (2.2) by $\mathbf{P}_k$. To estimate the nonparametric part of (2.2), we first need to make an inverse transformation of $\mathbf{P}_k$ on the residuals of the LS estimation and then apply the nonparametric smoothing operator.

We applied this procedure with $k = 1$ and 2 to the sleep habit data. SSE values with and without the autocorrelation transformation are all calculated. In Table 3, the

values $(SSE - SSE^*)/SSE$ are presented where SSE^* and SSE represent the SSE values with and without the autocorrelation consideration, respectively. These results show that autocorrelation consideration with $k = 1$ slightly improves the model fitting.

6 Concluding Remarks

We have presented a method for modeling sleep patterns under different light-dark cycles. Besides the issues raised at the end of Section 2, we are currently concerned about several points. First, we assume in our model that discontinuities may exist at the time points when we change lighting conditions. But it is not obvious whether there is a shift in the positions of the discontinuities. The apparent magnitude of the 24-hour component may be attenuated by changes in the timing of the discontinuities which are similar to phase shifts described under trigonometric wave models. Thus models which fail to account for shifts in discontinuities may underestimate c. Second, it is not clear how to compare the second and the third parts of the model between different rat strains and light intensities. Nevertheless, the proposed analytical methods may be useful for looking at the effects of stimuli on data which may include rhythmic components.

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Table 1: *Hypothesis testing results of* $H_0 : c = 0$ *vs* $H_a : c \neq 0$.

	Brown Norway, High	Brown Norway, Normal	Lewis,Normal	Lewis,Low
$\widehat{c}$	0.162	0.374	0.775	0.540
$s.e.(\widehat{c})$	0.024	0.023	0.056	0.036
t-value	6.750	16.261	13.839	15.000
p-value	<.0001	<.0001	<.0001	<.0001

Table 2: *Hypothesis testing results of* $H_0 : \sum_{i=1}^{4} \lambda_i c_i = 0$ *vs* $H_a : \sum_{i=1}^{4} \lambda_i c_i \neq 0$. $Contrast_{1-2}$ *denotes contrast for comparing group 1 with group 2, etc.*

Contrast	λ_1	λ_2	λ_3	λ_4	Z	p-value
$Contrast_{1-2}$	1	-1	0	0	-6.378	<.0001
$Contrast_{1-3}$	1	0	-1	0	-10.061	<.0001
$Contrast_{1-4}$	1	0	0	-1	-8.737	<.0001
$Contrast_{2-3}$	0	1	-1	0	-6.624	<.0001
$Contrast_{2-4}$	0	1	0	-1	-3.886	.0001
$Contrast_{3-4}$	0	0	1	-1	3.530	.0004
$Contrast_{12-34}$	1	1	-1	-1	-10.469	<.0001
$Contrast_{13-24}$	1	-1	1	-1	.309	.7573
$Contrast_{14-23}$	1	-1	-1	1	-6.007	<.0001

Table 3: *Results from an algorithm with autocorrelation modeled. The values are* $(SSE - SSE^*)/SSE$ *where* SSE^* *and* SSE *represent the SSE values with and without autocorrelation respectively.*

k	Brown Norway, High	Brown Norway, Normal	Lewis,Normal	Lewis,Low
1	0.0271	-0.0285	0.0172	0.0372
2	-0.0732	-0.1222	-0.0913	-0.1533

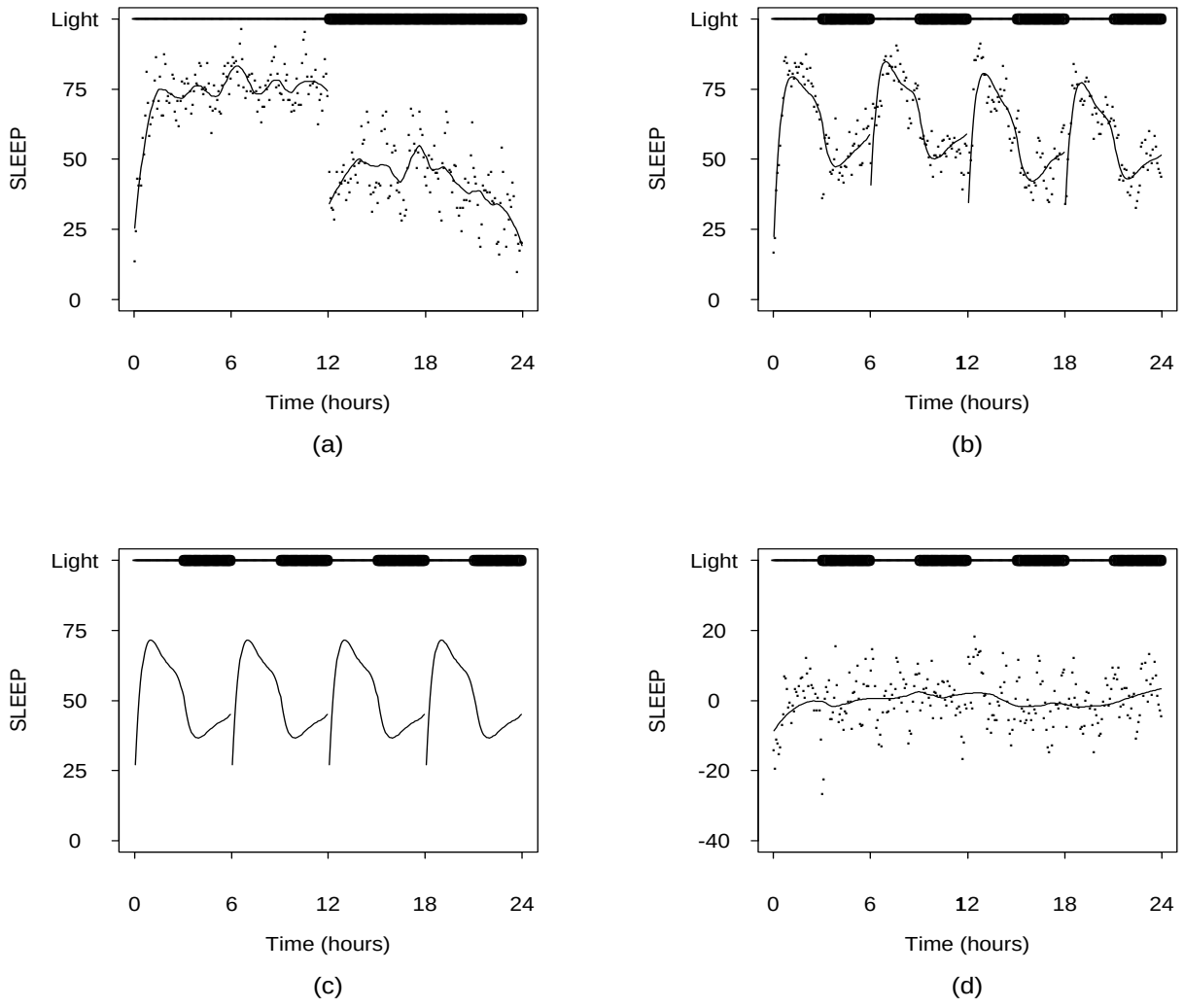

Figure 1: Data and fitted models for Brown Norway rats under high level lighting condition. (a) Fitted model of the baseline data; (b) fitted model of the test data; (c) estimated function of $f_6(x)$; (d) estimated function of $f_a(x)$ and residuals of model (2.1). In each plot, thin lines at top indicate periods of light. Thick lines represent darkness.