

Time of Day Effects on Neurobehavioral Performance During Chronic Sleep Restriction

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Introduction: Chronic nocturnal sleep restriction results in accumulation of neurobehavioral impairment across days. The purpose of this study was to determine whether time of day modulates the effects of sleep restriction on objective daytime performance deficits and subjective sleepiness across days of chronic sleep restriction. **Methods:** There were $N = 90$ healthy adults (21–49 yr; 38 women) who participated in a 14-d laboratory protocol involving randomization to 1 of 18 schedules of restricted nocturnal sleep with and without a diurnal nap for 10 consecutive days. The total time available for daily sleep ranged from 4.2 h to 8.2 h across conditions. Performance lapses on the psychomotor vigilance test (PVT) and subjective sleepiness were measured each day every 2 h during scheduled wakefulness. Nonlinear mixed-effects regression was used to test the hypothesis that there would be an interaction between time of day and the accumulation (slope across days) of neurobehavioral sleepiness. **Results:** In agreement with earlier studies, less sleep time resulted in faster accumulation of deficits across days. Time of day significantly affected this relationship for both PVT lapses and subjective sleepiness. The build-up rate of cumulative neurobehavioral deficits across days was largest at 0800 and became progressively smaller across the hours of the day, especially between 1600 and 2000. Following 8 d of sleep restricted to 4 h/d, subjects averaged 8.3 more PVT performance lapses at 0800 than at 1800. **Discussion:** This study provides evidence that the circadian system has a substantial modulatory effect on cumulative impairment from chronic sleep restriction and that it facilitates a period of relatively protected alertness in the late afternoon/early evening hours when nocturnal sleep is chronically restricted.

Keywords: psychomotor vigilance, neurobehavioral impairment, sleepiness, circadian rhythm, wake maintenance zone.

AT LEAST TWO DISTINCT sleep/wake-related physiological processes can be distinguished as regulating alertness and neurobehavioral performance. In the two-process model, these factors are conceptualized as a homeostatic sleep-dependent process and an endogenous circadian process (4,7). While the two-process model provides a theoretical framework within which the effects of acute total sleep deprivation on sleep and daytime alertness can be predicted (5), the model fails to accurately predict the cumulative neurobehavioral deficits that result from consecutive days of sleep restriction (27,29). This build-up of cumulative deficits occurs in a sleep dose-dependent manner—the more sleep is restricted the faster deficits accumulate over days of sleep restriction (2,22,29). It has been suggested that another, longer homeostatic process is needed to accurately model the effects of chronic sleep restriction on neurobehavioral function (16,20).

Support for a longer homeostatic process derives from sleep dose response and response surface mapping experiments involving many different sleep restriction conditions. These studies suggest that a basic model founded on total daily amount of sleep adequately describes the progression of cognitive deficits across days of sleep restriction (29), regardless of whether daily sleep is consolidated or split into a nocturnal anchor sleep period and a daytime nap (22). However, these experiments have all focused on day-average neurobehavioral outcomes, averaging out the circadian contribution to cumulative deficits.

There is reason to hypothesize that the circadian system modulates the slope of cumulative neurobehavioral deficits across sleep-restriction days, even during wakefulness that occurs in the diurnal portion of the circadian cycle. Experiments using ultra-short (ultradian) sleep-wake schedules (17), spontaneous internal desynchrony data (25), and forced desynchrony (8,32) have demonstrated that the wake-promoting effects of the circadian system are most evident in the late afternoon and evening, prior to habitual sleep onset, and up to the initial endogenous secretion of melatonin. In the present study, we sought to investigate the effect of time of day on the build-up of neurobehavioral deficits across days of chronic sleep restriction and to determine whether the expression of cumulative neurobehavioral deficits during chronic sleep restriction are attenuated during the afternoon in the period leading up to a zone of minimal sleep tendency previously described as the forbidden zone for sleep (17), or wake maintenance zone (25).

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METHODS

Subjects

A total of 90 healthy adults (52 men, 38 women; mean age 29.5 yr, age range 21–49 yr) participated in a 14-d laboratory study involving strict schedules for time in bed (TIB). Subjects were screened to ensure they had no medical, psychiatric, or sleep-related disorders and were drug-free. This was determined by history, physical examination, and questionnaires, and by blood and urine laboratory tests and toxicological screening. Subjects could not have worked regular or rotating shift work within the past 2 yr and they could not have traveled across time zones in the 3 mo prior to the experiments. They were required to be in bed for approximately 8 h daily during the week preceding the study, as verified by wrist actigraphy combined with daily diary reports and time-stamped phone records for time to bed and time awake. The Institutional Review Board of the University of Pennsylvania reviewed and approved the study and each subject gave written informed consent.

Procedure and Study Design

Following two baseline nights of 8.2 h TIB (2154 to 0606), subjects were randomly assigned to 1 of 18 sleep-restriction conditions, each involving a specific sleep regimen maintained for 10 consecutive days. The final day of the study included a recovery sleep period of 14 h TIB (2154 to 1154). The 10-d sleep restriction assignments involved randomization to one of four nocturnal sleep conditions (4.2, 5.2, 6.2, or 8.2 h TIB) centered around 0200, and one of seven diurnal nap sleep conditions (0.0, 0.4, 0.8, 1.2, 1.6, 2.0, or 2.4 h TIB) centered around 1400. These different anchor and nap sleep durations were crossed to yield a total of 4 anchor-sleep-only conditions and 14 anchor-plus-nap-sleep conditions (Table I). Five subjects were assigned to each of the experimental conditions so that the total sample size was $N = 90$.

Throughout all scheduled waking periods during the 14-d experiment, subjects underwent neurobehavioral assessments of cognitive performance, sleepiness, and mood every 2 h. Between test bouts they were allowed to read, watch movies, and interact with laboratory staff

TABLE I. CONDITIONS STUDIED IN THE SPLIT-SLEEP, DOSE-RESPONSE EXPERIMENT OF CHRONIC SLEEP RESTRICTION.

Diurnal Nap Sleep TIB (h)	Nocturnal Anchor Sleep TIB (h)			
	4.2	5.2	6.2	8.2
0.0	4.2	5.2	6.2	8.2
0.4	4.6	5.6	6.6	
0.8	5.0	6.0	7.0	
1.2	5.4	6.4	7.4	
1.6	5.8	6.8		
2.0	6.2	7.2		
2.4	6.6			

The table shows the total time in bed (TIB) per 24 h across the 10 sleep restriction days as a function of nocturnal anchor sleep TIB and diurnal nap sleep TIB for each of the 18 conditions.

to help them stay awake, but no vigorous activities were permitted. Ambient light levels were less than 50 lux during scheduled wake times; during scheduled sleep times, all lights were off (≤ 0.1 lux).

Neurobehavioral Measurements

Neurobehavioral functions were assayed using a number of cognitive performance and subjective sleepiness measures. The primary outcomes for evaluating cumulative deficits in alertness were the 10-min Psychomotor Vigilance Test (PVT) of sustained attention performance (10–12) and the Stanford Sleepiness Scale (SSS) (13). These measures were selected for analysis because they are free of learning effects and thus a priori most suitable for characterization of physiologically driven temporal changes in performance and sleepiness. PVT lapses of attention (reaction times greater than 500 ms) were assessed for each test bout to measure impairments in behavioral alertness. SSS scores (on a 7-point scale) were recorded for each test bout to measure subjective sleepiness. The data from the days of sleep restriction were expressed relative to the subjects' baseline (day 2) scores.

Neurobehavioral assessments were made every day at 0400, 0600, 0800, 1000, 1200, 1400, 1600, 1800, 2000, and 2200. Subjects in the 8.2-h control condition did not perform the 0400 and 2200 test bouts; in the other conditions these test bouts were, therefore, excluded from the present analyses. Further, test bouts occurring in some conditions immediately upon awakening from a nocturnal sleep period or a diurnal nap (i.e., 0600 and 1400) were excluded to avoid confounds from sleep inertia effects (9,30). Thus, our analyses focused on the six test bouts occurring at 0800, 1000, 1200, 1600, 1800, and 2000.

Other Measurements

Polysomnographic measurements were taken for the nocturnal sleep periods and the diurnal naps during the baseline days and the 10 sleep restriction days, except for the diurnal naps on days 2 and 7 and the subsequent nocturnal sleep periods on days 3 and 8. During these two 24-h periods subjects were without electrodes to reduce any skin irritation and to permit subjects to shower. The polysomnographic data of this study have been reported elsewhere (21).

In a subset of 60 subjects, blood serum melatonin was collected hourly over a 24-h interval via an indwelling venous catheter on two occasions during the laboratory experiment. The first 24-h interval commenced on the day following the first baseline night (day 1) at 1630 and the second 24-h interval commenced on the day preceding the final sleep restriction night (day 12) at 1630. These data were used to determine circadian phase before and after the sleep restriction days, as estimated by dim light melatonin onset (DLMO). DLMO was operationally defined as the instant that the linear interpolation between data points on the melatonin concentration curve crossed the $10 \text{ pg} \cdot \text{ml}^{-1}$ threshold (18).

Of the 60 subjects that had blood drawn, data meeting quality control standards (i.e., no missing data points in the vicinity of DLMO) were available for 36 subjects. Phase shifts resulting from the experimental protocol were assessed by comparing the timing of the DLMO on the final sleep restriction day versus baseline as a function of experimental condition. For the purpose of this particular analysis, subjects were grouped by nocturnal sleep restriction assignment: 8.2 h ($N = 3$), 6.2 h ($N = 4$), 5.2 h ($N = 13$), or 4.2 h ($N = 16$).

Data Analyses

Analyses of neurobehavioral function were accomplished by fitting nonlinear mixed-effects response surface maps (RSM) to examine the time-of-day-specific progression of neurobehavioral impairment across sleep restriction days for each of the 18 conditions. RSM allows the investigation of the relationship of a dependent measure to more than one experimentally varied independent measure (23), taking advantage of the full sample size ($N = 90$). Thus, the technique is suitable for investigating the build-up rate of neurobehavioral impairment across days of sleep restriction as a function of time of day across the 18 experimentally varied combinations of anchor and nap sleep in the present experiment.

In a subset of 60 subjects, blood samples were acquired every 15 min using an indwelling catheter and blood pump for analysis of melatonin and other humoral factors (e.g., cortisol, growth hormone—not analyzed here). The blood draw equipment prevented any neurobehavioral performance data acquisition during the 24-h period before Baseline day 2 and the 24-h period beginning at 1630 on sleep restriction day 9. Therefore, all analyses focused on sleep-restriction days 1 through 8, which involved a total of 4320 test bouts (90 subjects by 8 d of sleep restriction by 6 times of day). Missing neurobehavioral data in days 1 through 8 (due to miscellaneous technical failures in testing equipment or treatment of minor symptoms such as backaches) constituted less than 3% of the data. Our data analysis techniques were suitable for a data set with a small amount of randomly occurring missing data points (15,30).

A series of models were developed that describe the experimental data in mathematical terms, where each subsequent model introduced progressively simplifying assumptions. The models were tested statistically by evaluating how adequately the simpler model matched the experimental data relative to the more complex model, accounting for the difference in complexity. Specifically, for both PVT lapses and SSS sleepiness scores, RSM models of progressively decreasing complexity (degrees of freedom) were developed to test hypotheses about the relationship between the build-up of neurobehavioral impairment and the durations of nocturnal anchor and diurnal nap sleep as a function of time of day. Hypotheses were tested with the likelihood ratio test for nested models (χ^2 statistic).

TIB was used in the analysis as a proxy for total sleep time (TST). There were 368 polysomnographic records

available to confirm TST across sleep restriction days 1 through 8 except for days 3 and 8 (these two 24-h periods were without electrodes to permit subjects to shower). The overall correlation between TIB and TST was 0.85. Across the 18 different sleep restriction conditions, the sleep efficiency (i.e., TST/TIB) ranged from 0.91 to 0.80 in a dose-dependent linear relationship. For every hour of additional TIB per 24 h, sleep efficiency dropped by $1.6\% \pm 0.4\%$ (mean \pm SEM) (see 21 for a more detailed analysis of sleep variables from this study). The suitability of TIB as a proxy for TST was tested by repeating the analysis using TST to confirm that the results did not substantively change.

Our RSM models were based on earlier work (22,29) in which we showed that day-average cumulative impairment across days of sleep restriction could be adequately described by a model of the general form:

$$y_{\text{avg}} \sim \beta t^{\theta},$$

where y_{avg} is the day-average level of impairment, and t stands for time (i.e., days of sleep restriction). Here θ describes the curvature of the build-up over days (3,29), which is typically in the sublinear range (from 0 for flat up to 1 for linear). The parameter of greatest interest in this general model is β (sometimes called a nonlinear slope), which represents the rate of the build-up of impairment over days. A normally distributed random effect was placed on β to account for systematic individual differences in vulnerability to sleep loss (28,29).

This nonlinear mixed-effects model was adapted for the present paper, focusing now on time-of-day specific outcomes (y_{tod}) instead of day-average values (y_{avg}). Of the models tested here, the model with the highest number of degrees of freedom assumed that the build-up rate β of neurobehavioral impairment across days was condition-specific and idiosyncratic, without systematic relationship to the anchor and nap sleep durations (similar to a two-way analysis of variance over the different anchor sleep and nap sleep conditions with interaction terms). In this model, the parameter β_{cond} is thus a condition-specific parameter (i.e., 18 degrees of freedom):

$$\text{Model 1: } y_{\text{tod}} \sim \beta_{\text{cond}} t^{\theta}.$$

Model 1 is useful for drawing three-dimensional RSM plots with the condition-specific nonlinear slope (i.e., build-up rate of impairment across days of sleep restriction) on the z-axis plotted against anchor sleep TIB and nap sleep TIB on the x-axis and y-axis, respectively.

Following estimation of the full Model 1 with condition-specific nonlinear slope parameters, various reduced versions of the model were fitted. The first reduction involved removing any interaction between anchor sleep duration and nap sleep duration. That is, the nonlinear slope is no longer idiosyncratic for each condition, but depends on anchor sleep condition independent of nap sleep duration (i.e., β_{anchor}) and on nap sleep condition independent of anchor sleep duration (i.e., γ_{nap}):

$$\text{Model 2: } y_{\text{tod}} \sim (\beta_{\text{anchor}} + \gamma_{\text{nap}}) t^{\theta}.$$

Note that in Model 2, β_{cond} (18 degrees of freedom) was replaced by β_{anchor} (4 degrees of freedom) and γ_{nap} (7 degrees of freedom), reducing the complexity of the model by 7 degrees of freedom.

A further reduced model assumed that the build-up rate of performance impairment across days of sleep restriction is linearly related to anchor sleep duration and also linearly related to nap sleep duration:

$$\text{Model 3: } y_{\text{tod}} \sim (\alpha + \beta \text{TIB}_{\text{anchor}} + \gamma \text{TIB}_{\text{nap}}) t^{\theta},$$

where α is a basal slope level (offset) common to all conditions. Note that β and γ are now rate constants expressing the build-up rate per hour of TIB. Thus, for every hour of additional anchor sleep, the rate of change of neurobehavioral function across sleep restriction days is altered by β , and for every hour of additional nap sleep, the rate of change of neurobehavioral function across sleep restriction days is altered by γ . Compared to Model 2, Model 3 reduced the complexity of the RSM by another 8 degrees of freedom.

A subsequent reduction of the model considered only the combined anchor and nap TIB during the 24 h of the day:

$$\text{Model 4: } y_{\text{tod}} \sim (\alpha + \delta \text{TIB}_{\text{total}}) t^{\theta},$$

where $\text{TIB}_{\text{total}} = \text{TIB}_{\text{anchor}} + \text{TIB}_{\text{nap}}$ (see Table I). Here the parameter δ represents the rate of change of neurobehavioral function across days per hour of total daily TIB. This model includes only one independent variable ($\text{TIB}_{\text{total}}$) and thus the RSM approach is now reduced to a univariate regression that can be plotted in conventional two-dimensional graphs. Model 4 had one degree of freedom less than Model 3.

The most reduced model we used, which served as a null model for hypothesis testing, assumes that the build-up of neurobehavioral impairment across days is constant (i.e., independent of daily TIB):

$$\text{Model 5: } y_{\text{tod}} \sim \alpha t^{\theta},$$

where the parameter α is the overall nonlinear slope. Model 5 had again one degree of freedom less than the previous Model 4.

These models were fitted to the neurobehavioral performance data using nonlinear mixed effects regression in order to examine the effect of time of day (30). Analyses were performed first for each individual test time (i.e., unique slope and intercept estimates at each testing time), and then in a pooled analysis that included all six test times (i.e., unique slopes and intercepts) for direct comparisons between times of day. The pooled analysis used a single parameter for the curvature θ and for the variance of the random effect on the nonlinear slope.

Finally, in order to compare impairment build-up rates between the six different times, a common intercept (α) for all six testing times was needed. To accomplish this, a previously published, nonlinear mixed-effects regression model was considered that uses cumulative wake extension as a continuous independent variable (29). Cumulative wake extension was calculated by summing the difference between scheduled TIB and the estimated sleep need (parameter λ) over the sleep restriction days. In this model formulation, sleep need was estimated as the daily TIB that would be needed to maintain optimal performance across the experimental days. This model takes the following form:

$$\text{Model 6: } y_{\text{tod}} \sim \gamma (\lambda t - \sum_t \text{TIB})^{\theta}.$$

Here γ represents the rate of change of neurobehavioral performance across cumulative hours of wake extension (rather than across days of sleep restriction), while t enumerates the number of successive days of sleep restriction. As with the nonlinear slopes (β) in the previous models, estimation of γ included a normally distributed random effect to account for individual differences. Model parameters were simultaneously estimated for a global λ parameter and test-time specific γ parameters using all six diurnal testing times.

RESULTS

There were no significant baseline (day 2) differences among the 18 experimental conditions for PVT performance (one-way ANOVA, $F_{17,72} = 0.85$, n.s.) or SSS scores ($F_{17,72} = 0.82$, n.s.). Furthermore, there were no significant differences at baseline between the six different testing times for PVT performance ($F_{5,85} = 1.05$, n.s.) or SSS scores ($F_{5,85} = 0.31$, n.s.).

Fig. 1 displays time-of-day specific RSM of PVT lapses across sleep restriction days, with the build-up rates estimated for each condition specifically. In these RSM, the rate of change across days is shown as a function of anchor sleep duration and nap sleep duration (Model 1). In the RSM for the morning tests (0800, 1000, and 1200; left panels), the impact of restricting anchor and/or nap sleep duration is readily noticeable. For the afternoon tests (1600, 1800, and 2000; right panels), however, the impact of restricting anchor and/or nap sleep duration appears to be dampened, particularly for the tests taken at 1800.

To investigate these effects in statistical terms we fitted Models 1 through 6 described in the Methods section to the PVT performance data. The value of the curvature parameter θ in Model 1 was 0.92 ± 0.06 (estimate \pm SE), indicating a near-linear build-up of performance impairment across days of sleep restriction, which was consistent with previous findings (29). The build-up of PVT lapses across days was not significant in the control condition (8.2 h nocturnal sleep) for psychomotor vigilance tests taken at any of the six times of day considered (all $t \leq 0.88$, n.s.).

For every test time except 2000, it was found that without significant loss of information, the build-up of

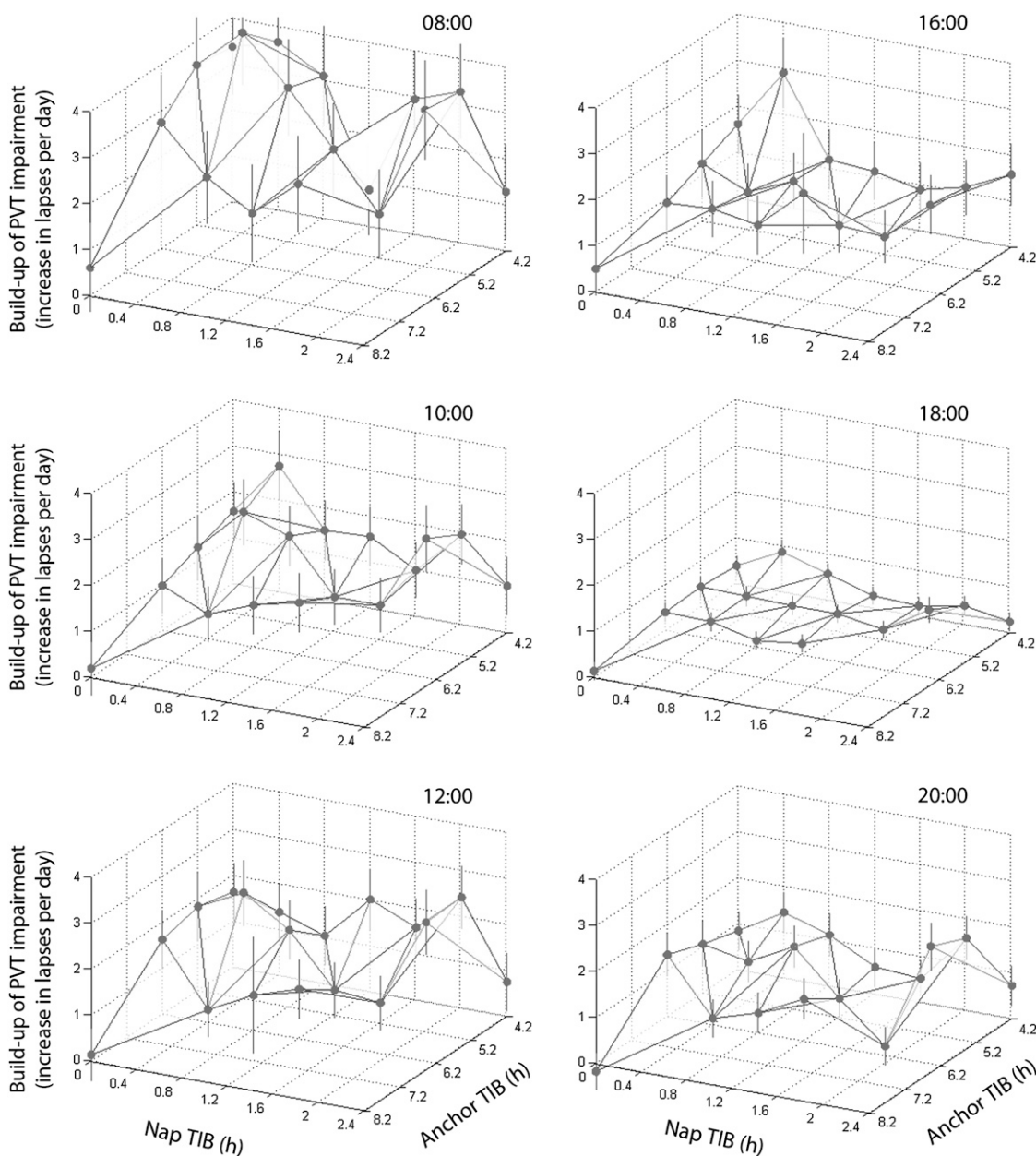


Fig. 1. Response surface maps of the time-of-day specific daily rate of change in psychomotor vigilance performance lapses (reaction times greater than 500 ms) across days of sleep restriction (indicated on the z-axis), plotted against nocturnal anchor sleep TIB (x-axis) and diurnal nap sleep TIB (y-axis). Dots indicate rate of change (nonlinear slope β) estimated for each of the 18 different conditions (Model 1). Upwards on the z-axis indicates greater performance impairment build-up (\pm SEM).

PVT lapses across sleep restriction days could be expressed as a linear function of daily total TIB (Model 4). This model captured at least 40.7% of the variance in the data for all test times [likelihood ratio test between Model 1 and Model 4: all $\chi^2(16) \leq 20.5$, n.s.]. In each case, less total TIB per 24 h resulted in a faster build-up of PVT lapses. However, the build-up rate was dependent on the time of day, as determined using Model 4, is illustrated for conditions involving sleep restriction to a total of 4.2 h, 5.2 h, 6.2 h, or 8.2 h TIB per day in Fig. 2 (top panel).

At 1800 the effect of sleep restriction was so small and consistent across sleep conditions that the RSM could be

reduced further to a single constant, independent of daily total TIB [likelihood ratio test between Model 4 and Model 5: $\chi^2(1) = 2.8$, n.s.]. This TIB-independent model captured 54.2% of the variance for the 1800 test bout (same amount of variance explained as Model 4).

The RSM for the 2000 test time had more complexity than could be adequately captured by Model 4. The model that most adequately described the build-up of PVT lapses across sleep restriction days for the 2000 test time was Model 2, which specifically accounted for nocturnal sleep condition and daytime nap sleep condition [likelihood ratio test between Model 2 and Model 4: $\chi^2(9) = 21.8$, $P < 0.05$]. This model had eight more parameters than Model 4, but only explained 0.3% more

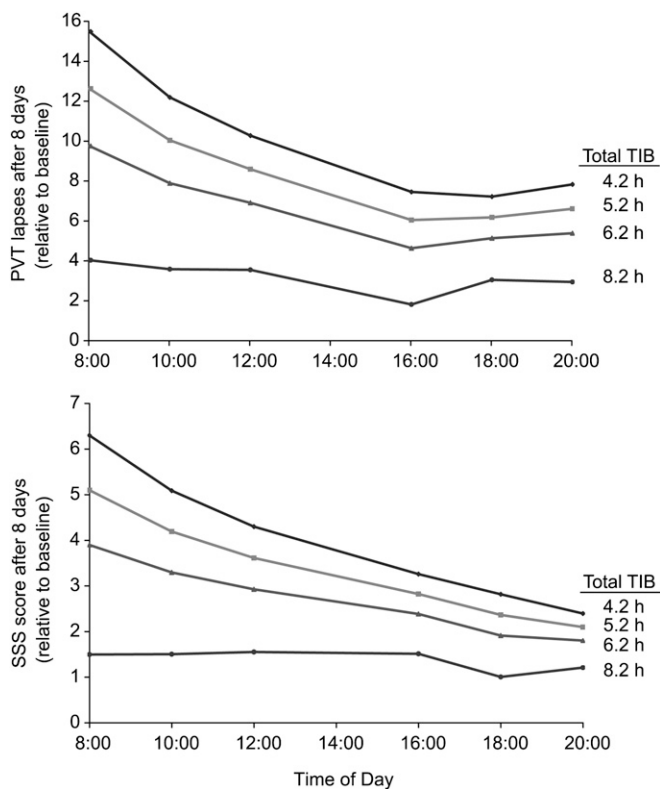


Fig. 2. Model-based projections of neurobehavioral impairment level as a function of testing time of day after 8 d of sleep restriction to either 4.2 h, 5.2 h, 6.2 h, or 8.2 h total daily TIB (Model 4 estimates). Time of day (hh:mm) is indicated on the abscissas, PVT lapses (change from baseline) are indicated on the upper panel, and Stanford Sleepiness Scale (SSS) subjective sleepiness ratings (relative to baseline) are indicated on the lower panel. Previously reported data from $N = 13$ subjects during total sleep deprivation indicated comparable increases in impairment levels (changes relative to baseline averaged across daytime testing times, i.e., 0800, 1000, 1200, 1600, 1800, 2000) of 10.2 ± 1.3 lapses and 1.1 ± 0.1 sleepiness units after 1 d of total sleep deprivation (29).

variance. Thus, for the current analysis regarding the effect of time of day, the additional complexity does not appear to be needed and Model 4 suffices.

To shed further light on the nature of the build-up rate of PVT performance impairment across days of sleep restriction as a function of time of day, a nonlinear regression of performance changes across days of sleep restriction was conducted that involved simultaneously estimating time-of-day specific parameters of Model 4 for all six testing times examined (pooled analysis). The effect of testing time was found to be a significant factor in determining the relationship between TIB and the build-up rate of performance impairment across days of sleep restriction ($F_{5,89} = 10.21, P < 0.001$). The build-up rate was systematically largest during the morning test bouts, while it was significantly diminished in the afternoon. When the analysis was repeated using total sleep time as the independent variable, the results did not substantively change ($F_{5,89} = 5.14, P < 0.001$).

To rule out the possibility that the effect of testing time in the above analysis was due to differences between test-time specific intercept values rather than slopes across days, a further nonlinear mixed-effects regression

of performance changes across days of sleep restriction was conducted that involved estimating a common intercept for all six testing times using cumulative wake extension as the independent variable (Model 6). The estimated daily sleep need in this analysis was $\lambda = 9.5 \pm 0.7$ h of TIB. The effect of testing time was again found to be a significant factor in determining the relationship between TIB and the build-up rate of performance impairment across days of sleep restriction ($F_{5,89} = 2.72, P < 0.05$). **Fig. 3** (top panel) shows the build-up rate of PVT impairment across days of sleep restriction as a function of cumulative wake extension for each different time of day.

Fig. 4 displays time-of-day specific RSM of the build-up of SSS subjective sleepiness across sleep restriction days estimated for each condition specifically. In each RSM, the rate of change across days is shown as a function of anchor sleep duration and nap sleep duration (Model 1). The same statistical analysis techniques used to examine these effects for PVT lapses were applied to subjective sleepiness. The value of the curvature parameter θ in Model 1 was 0.48 ± 0.05 (estimate \pm SE), indicating a moderately nonlinear build-up of sleepiness across days of sleep restriction. The build-up of subjective sleepiness across days was not significant in the control condition (8.2 h nocturnal sleep) for any of the six times of day considered (all $t \leq 1.83, n.s.$).

For every test time except 1200, it was found that without significant loss of information, the build-up of

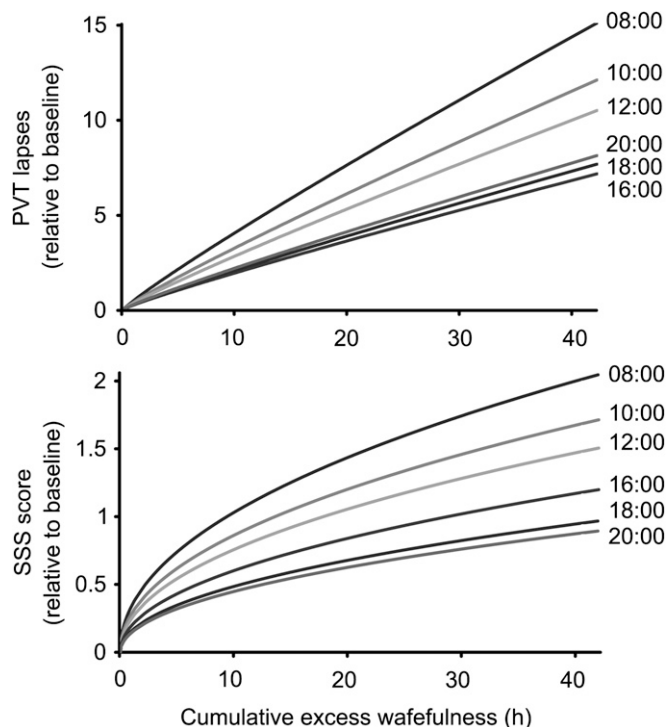


Fig. 3. Model-based projections of time-of-day specific performance impairment plotted against cumulative excess wakefulness. The top panel displays cumulative hours of excess wakefulness on the abscissa and PVT lapses (relative to baseline) on the ordinate. The bottom panel displays Stanford Sleepiness Scale (SSS) subjective sleepiness (relative to baseline) on the ordinate.

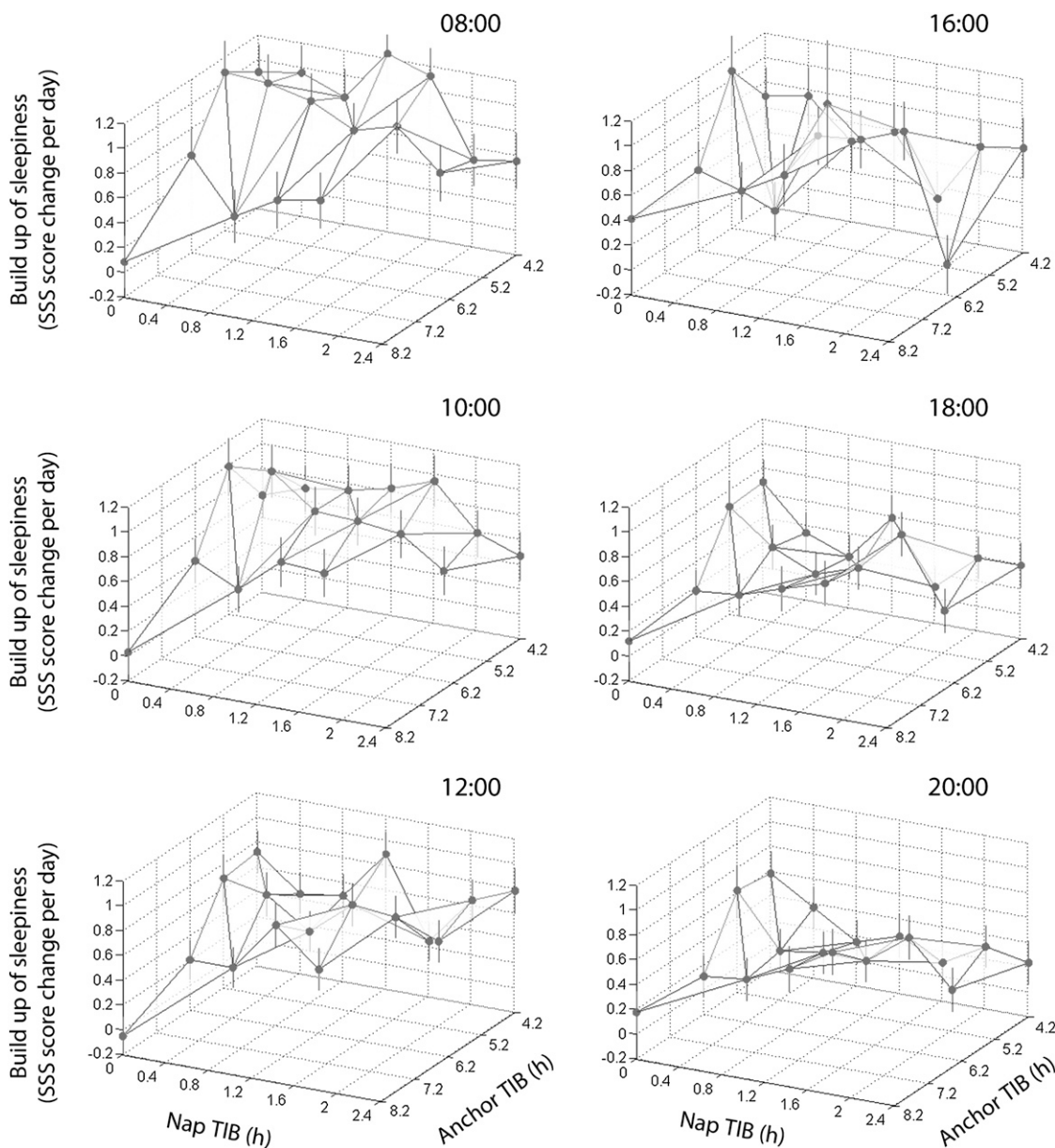


Fig. 4. Response surface maps of the daily rate of change in subjective sleepiness (on a scale from 1 to 7) across days of sleep restriction (indicated on the z-axis) plotted against nocturnal anchor sleep TIB (x-axis) and diurnal nap sleep TIB (y-axis). Dots indicate rate of change (nonlinear slope β) estimated for each of the 18 different conditions (Model 1). Upwards on the z-axis indicates greater sleepiness build-up (\pm SEM).

subjective sleepiness across sleep restriction days could be expressed as a linear function of daily total TIB (Model 4). This model captured at least 34.9% of the variance in the data for all test times [likelihood ratio test between Model 1 and Model 4: all $\chi^2(16) \leq 25.1$, n.s.]. In each case, less total TIB per 24 h resulted in a faster build-up of subjective sleepiness. However, this build-up was dependent on the time of day at which testing occurred. The effect of time of day on the build-up rate of subjective sleepiness, as determined using Model 4, is illustrated for conditions involving sleep restriction to a total of 4.2 h, 5.2 h, 6.2 h, or 8.2 h TIB per day in Fig. 2 (bottom panel).

For the afternoon test times (i.e., 1600, 1800, 2000), the RSM could be reduced further to a single constant, independent of daily total TIB [likelihood ratio tests between

Model 4 and Model 5: $\chi^2(1) = 1.6$, n.s. for 1600; $\chi^2(1) = 3.0$, n.s. for 1800; and $\chi^2(1) = 1.2$, n.s. for 2000]. The reduction in the amount of variance explained by the simpler TIB-independent model was less than 0.6% for all three afternoon test times. The RSM for the 1200 test time had more complexity than could be adequately captured by Model 4. The model that most adequately described the build-up of subjective sleepiness across sleep restriction days for the 1200 test time was Model 1, which accounted for each experimental condition separately [likelihood ratio tests between Model 1 and Model 4: $\chi^2(16) = 26.6$, $P < 0.05$]. This model had 16 more parameters than Model 4, but explained only 2.9% more variance. Thus the more complex model does not appear to be needed here and Model 4 suffices for the examination of time-of-day effects.

A pooled nonlinear regression of sleepiness changes across days of sleep restriction, simultaneously estimating time-of-day specific parameters of Model 4 for all six testing times, showed that testing time was a significant factor in determining the relationship between TIB and the build-up rate of sleepiness across days of sleep restriction ($F_{5,89} = 13.66, P < 0.001$). Similar to the results found for PVT lapses, the rate of subjective sleepiness build-up was largest during the morning test bouts. When the analysis was repeated using total sleep time as the independent variable the results did not substantively change ($F_{5,89} = 8.40, P < 0.001$).

To rule out the possibility that the effect of testing time in the above analysis was due to differences between test-time specific intercept values rather than slopes across days, Model 6 was applied using cumulative wake extension as the independent variable and containing a common intercept. The estimated daily sleep need in this analysis was $\lambda = 8.5 \text{ h} \pm 0.2 \text{ h}$ of TIB. The analysis confirmed that testing time was a significant factor in determining the relationship between TIB and the build-up rate of sleepiness across days of sleep restriction ($F_{5,89} = 16.25, P < 0.001$). Fig. 3 (bottom panel) shows the build-up rate of subjective sleepiness across days of sleep restriction as a function of cumulative wake extension for each different time of day. In order to display subjective sleepiness on the same time scale as PVT performance the sleep need parameter (λ) was held constant ($\lambda = 9.5 \text{ h}$) for both measures.

The timing of the DLMO at baseline and the circadian phase shifts detected following 10 d of sleep restriction are described in **Table II** as a function of nocturnal anchor sleep condition (8.2 h, 6.2 h, 5.2 h, or 4.2 h) (see 24 for a detailed description of the melatonin data analysis). At baseline, there were significant differences in the timing of the DLMO between the four nocturnal sleep conditions as indicated by one-way ANOVA ($F_{3,32} = 3.20, P < 0.05$). Following 10 d of sleep restriction, significant phase delays were observed for subjects scheduled to 4.2 h nocturnal TIB (average phase delay of 86.6 min) or 5.2 h nocturnal TIB (average phase delay of 83.2 min). Smaller, non-significant phase delays were observed in subjects scheduled to 6.2 h or 8.2 h nocturnal TIB. These data indicate that during the sleep restriction days, performance tests at 2000 occurred on average within 4 h of DLMO.

DISCUSSION

Our findings suggest that PVT performance deficits and subjective sleepiness progressively worsen in a sleep-

dose dependent and time-of-day dependent manner across days of sleep restriction. While the dose-dependent effect has been reported previously (2,22,29), the time-of-day dependence on the build-up rate of neurobehavioral impairment with increasing cumulative sleep loss across days is documented here for the first time. The results indicate significant sleep-dose-dependent performance deficits in the morning hours (see Fig. 1, 0800 RSM), followed by a period during the late afternoon-early evening when sleepiness and performance capability remain relatively stable even in the face of chronic sleep restriction in the range studied (see Fig. 1, 1800 RSM). This observation is congruent with the existence of a “wake maintenance zone” (25) or “forbidden zone” (17), a zone of minimal sleep tendency (6,8) in the late afternoon to early evening hours. Subtle performance deficits appear to be noticeable again at the 2000 test time, although there were no statistically significant differences between the 1800 and 2000 time points.

For PVT performance, the curvature parameter θ in Model 1 was in the linear range (i.e., a value close to 1), indicating a near-linear build-up of PVT performance impairment across days of sleep restriction. This is consistent with previous reports from two chronic partial sleep restriction studies that reported near-linear curvatures (22,29). The curvature parameter for sleepiness ratings was in the sublinear range (values less than 1), indicating a gradual dampening of subjective responsiveness to sleep loss with increasing days of sustained sleep restriction (see Fig. 3). This result is also consistent with the earlier reports, confirming that subjective measures do not accurately track objective performance impairment across successive days of sleep restriction.

The current analysis was focused on the interaction between cumulative sleep loss (i.e., across days) and time of day (i.e., within days) during a split-sleep, dose-response sleep restriction experiment. Nocturnal sleep bouts were centered around 0200 and afternoon naps were centered around 1400. Thus, subjects receiving less nocturnal sleep woke up earlier and were consequently awake longer at given test times in the morning and, depending on nap duration, also in the afternoon. The RSM analyses indicated that it was not justified statistically to distinguish between different sleep restriction conditions with equivalent amounts of total TIB for all but the PVT scores measured at 2000 and subjective sleepiness measured at 1200. PVT scores measured at 2000 and the sleepiness measured at 1200 were better described by more complex models that specifically accounted for nocturnal sleep condition and daytime nap

TABLE II. TIMING OF DLMO AT BASELINE AND AFTER 10 d OF SLEEP RESTRICTION (SHOWN AS hh:mm) AND THE NET PHASE DELAY FROM BASELINE TO AFTER SLEEP RESTRICTION AS A FUNCTION OF DAILY NOCTURNAL ANCHOR TIME IN BED (TIB).

Anchor TIB (h)	Number of Subjects with DLMO Data	DLMO at Baseline	DLMO After Restriction	Phase Delay (min)	t Statistic for Phase Delay	P-Value
4.2	16	22:28	23:54	86.6	4.75	< 0.001
5.2	13	21:53	23:16	83.2	3.70	< 0.01
6.2	4	21:05	21:36	31.0	0.56	n.s.
8.2	3	22:10	22:49	39.7	0.82	n.s.

sleep condition. However, the additional variance explained by the more complex models was small (0.3% for PVT at 2000 and 2.7% for sleepiness at 1200). As such, the observed interaction between cumulative sleep loss and time of day cannot likely be explained solely by differences in the amount of time since awakening or how sleep was divided between nocturnal sleep and nap sleep.

Differences were observed in the timing of DLMO among the distinct anchor sleep conditions, likely due to differences in the timing of light and dark exposure resulting from different nocturnal sleep conditions (24). These differences may have contributed to the finding that time of day impacts the expression of cumulative performance impairment across days of chronic sleep restriction. However, the neurobehavioral performance effects of time of day had a dose-response relationship with anchor sleep TIB, whereas timing of DLMO did not, suggesting that difference in DLMO alone cannot explain the neurobehavioral results.

In previous reports, performance impairment in the morning hours has often been attributed to sleep inertia (26). As test bouts occurring immediately upon awakening were excluded from the present analyses, sleep inertia was not likely to be involved substantially in the observed morning impairments. In this context, the suggestion from an earlier study that caffeine intake in the morning, as many people habitually do, serves to counteract sleep inertia (31) may need to be reconsidered. The present findings suggest that caffeine use may be common in the morning because it could serve to counter the detrimental effects of chronic sleep loss experienced most strongly during those hours of the day.

The present results have implications for biomathematical models of fatigue and performance (19). Such models attribute the time of day fluctuation in neurobehavioral function to a circadian process typically modeled as a near-sinusoidal function or Van der Pol oscillator (e.g., 1). Our laboratory experiments have suggested there may be a distinct, novel, regulatory process for waking neurobehavioral function that involves the accumulation of impairment across days of sleep restriction (23,29). The present findings suggest that, under conditions of chronic sleep restriction, there are sleep-dose dependent interactions between the circadian process and this long-term chronic sleep restriction homeostatic process. Thus, biomathematical models developed to characterize and predict performance during chronic sleep restriction need to either be augmented or reformulated to capture this interaction (20).

For operational settings, the present results suggest that when sleep is chronically restricted, morning operations are at higher risk of incidents due to fatigue-related human error than late afternoon or early evening operations. Consistent with this suggestion, a recent field study of train engineers reported that the morning shift was associated with increased sleep restriction, higher levels of sleepiness, and more fatigue-related performance errors than afternoon and evening shifts (14). We expect that nocturnal times (e.g., 0000 to 0700) would

have an even greater modulatory effect on the accumulation of neurobehavioral deficits from chronic sleep restriction.

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