Sex differences in the circadian regulation of sleep and waking cognition in humans

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Edited by Joseph S. Takahashi, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, and approved March 7, 2016 (received for review November 3, 2015)

The sleep–wake cycle and circadian rhythmicity both contribute to brain function, but whether this contribution differs between men and women and how it varies across cognitive domains and subjective dimensions has not been established. We examined the circadian and sleep–wake-dependent regulation of cognition in 16 men and 18 women in a forced desynchrony protocol and quantified the separate contributions of circadian phase, prior sleep, and elapsed time awake on cognition and sleep. The largest circadian effects were observed for reported sleepiness, mood, and reported effort; the effects on working memory and temporal processing were smaller. Although these effects were seen in both men and women, there were quantitative differences. The amplitude of the circadian modulation was larger in women in 11 of 39 performance measures so that their performance was more impaired in the early morning hours. Principal components analysis of the performance measures yielded three factors, accuracy, effort, and speed, which reflect core performance characteristics in a range of cognitive tasks and therefore are likely to be important for everyday performance. The largest circadian modulation was observed for effort, whereas accuracy exhibited the largest sex difference in circadian modulation. The sex differences in the circadian modulation of cognition could not be explained by sex differences in the circadian amplitude of plasma melatonin and electroencephalographic slow-wave activity. These data establish the impact of circadian rhythmicity and sex on waking cognition and have implications for understanding the regulation of brain function, cognition, and affect in shift-work, jetlag, and aging.

cognition | biological rhythms | gender | slow wave sleep | melatonin

Circadian rhythms are generated by a set of core "clock" genes and are present in nearly every cell of the body and brain (1). These local clocks and rhythms are synchronized by neural and endocrine pathways originating from the master circadian pacemaker located in the suprachiasmatic nucleus (SCN) (2, 3). In addition, the timing of behaviors such as food intake and sleep and associated changes in local and systemic cues contribute to the temporal organization in peripheral tissues outside the SCN (4, 5). In view of the pervasiveness of circadian rhythms, it is not surprising that they affect many aspects of physiology, behavior, and cognition in health and in disease. Indeed, circadian abnormalities have been implicated in disorders of sleep, mood, and cognition (6), in deleterious responses to shift work (7), depression (8), and Alzheimer's disease (9). The prevalence of most of these disorders is higher in women than in men (10), and their impact on psychological functions and quality of life of patients differs between the sexes (11).

Women are underrepresented in both circadian and sleep research (12), although sex differences in human circadian and sleep characteristics are emerging from the few studies that contrast men and women. (Note: Throughout this paper we refer to contrasts between biologically male and female research participants by the term sex difference, rather than the term gender difference, which connotes the assignment of, or preference for, different social roles.) Reported sex differences are earlier timing of clock gene rhythms in the brain (13), a shorter intrinsic circadian period of body temperature and melatonin rhythms (14, 15), earlier timing and larger amplitude of the melatonin rhythm (16), earlier timing and longer duration of sleep, and more slow-wave sleep (SWS) in women (15, 17–19). Whether these sex differences extend to the contribution of sleep and circadian rhythmicity in subjective and objective measures of waking function has not been documented.

Sleep contributes to subjective and objective measures of waking function as evidenced by the deleterious effects of insufficient sleep on alertness, mood, sustained attention, working memory, and other behavioral markers of brain function (20–22). The separate and combined contribution of circadian and sleep–wake cycles remains undocumented for most commonly used laboratory tasks. It is generally accepted that the sleep–wake cycle constitutes a rhythm of recovery and deterioration of brain function, although whether all cognitive functions are affected similarly continues to be debated. The molecular and cellular mechanism underlying this deterioration and recovery and in particular the role of SWS in synaptic homeostasis and cognition are areas of intense investigation (23, 24).

The contribution of the circadian timing system to performance while awake is twofold. First, it regulates the structure and timing of sleep so that the recovery and deterioration of functional capacity normally occur during the night and day, respectively (25). Second, circadian rhythmicity contributes directly to brain function independent of the timing of sleeping and waking. This independence has been shown by uncoupling

Significance

Circadian rhythms affect our physiology and psychology, in health and disease. Most of our knowledge about the human circadian timing system is based on research in men. Some circadian characteristics, such as the intrinsic frequency of the circadian clock and the amplitude of the melatonin rhythm, have been shown to differ between men and women. Whether the circadian regulation of mental functions differs between men and women is unknown. Here we show that circadian rhythmicity in mental functions exhibits sex differences so that the night-time impairment in cognitive performance is greater in women than in men. These findings are significant in view of shift-work–related cognitive deficits and disturbances of mood, which are more prevalent in women.

Author contributions: J.A.G. and D.-J.D. designed research; D.-J.D. directed the research; N.S., A.S.L., and J.C.L. performed research; J.A.G. contributed new reagents/analytic tools; N.S., A.S.L., P.J.M., and J.A.G. analyzed data; and N.S., A.S.L., P.J.M., J.A.G., and D.-J.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1521637113/-/DCSupplemental.

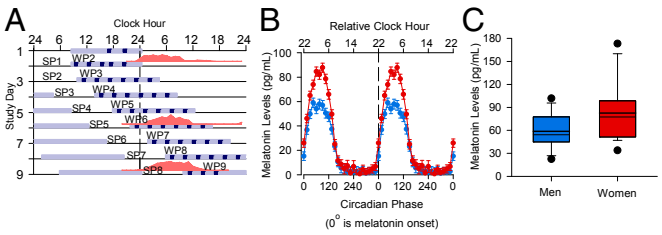

Fig. 1. (*A*) Schematic representation of the forced desynchrony protocol. SP, sleep period; WP, wake period. The single-plotted cognitive testing sessions are shown as blue boxes, and the red area plots are melatonin profiles from the three sampling cycles. (*B*) Average melatonin profiles (least-square means and SEMs) for men (blue) and women (red). All data were aligned to melatonin onset before averaging. (*C*) Box-and-whisker plot of melatonin amplitude (mean ± SEM) in men (59.49 ± 5.95 pg/mL) and women (82.62 ± 8.09 pg/mL). The whiskers show the 10th and 90th percentiles, and the circles represent outliers.

the sleep–wake cycle from SCN-driven rhythms, such as the melatonin rhythm. Under these conditions of forced desynchrony, various aspects of waking performance continue to display nearly a 24-h rhythmicity in synchrony with the melatonin rhythm in addition to the rhythmicity associated with the sleep–wake cycle (26–29). Some of the putative mechanisms by which circadian rhythmicity may contribute to brain function, e.g., the circadian modulation of synaptic plasticity independent of vigilance, have been identified (30).

How the sleep–wake-dependent and circadian regulation of waking performance vary among individuals has been documented to some extent within the context of age-related changes in cognition (31), but, to our knowledge, sex differences in the circadian regulation of waking performance have not been investigated. However, these differences are of considerable interest, given sex differences in cognition, circadian rhythmicity, and sleep, as well as the widespread presence of sex hormone receptors within the neural structures of the circadian timing system (32) and sex differences in cognition (33).

Here we used a forced desynchrony protocol to establish and quantify sex differences in circadian and sleep–wake-dependent regulation in subjective sleepiness, mood, task demand, attention, working memory, motor control, and temporal processing in healthy men and women. To measure these functions, we chose tasks with established validity, retest reliability, and, crucially, sensitivity to circadian and sleep–wake-dependent effects (22, 34, 35). The sensitivity to circadian and sleep–wake-dependent effects was key, because the aim was to determine whether there are sex differences in the circadian and sleep–wake-dependent modulation of cognition. Given the sex differences in SWS/slow-wave activity (SWA) and that these variables are primarily a marker of sleep homeostasis and are implicated in the regulation of cognition (36), we may expect sex differences in the homeostatic (wake-dependent) component of cognition. On the other hand, given the sex differences in melatonin amplitude and that melatonin is a marker of the circadian process, we may predict a greater circadian modulation of cognition in women.

Results

Melatonin. The imposed 28-h day (Fig. 1*A*) successfully desynchronized the sleep–wake cycle from the melatonin rhythm in 16 men and 18 women (see Table 1 for demographics). The period of the melatonin rhythm ($P = 0.67$) and timing of melatonin onset ($P = 0.33$), which was used to determine circadian phase (37), were not significantly different between women and men in this sample (38). However, the amplitude of the melatonin rhythm (*SI Appendix, SI Methods*) was significantly larger in women ($P < 0.035$) (Fig. 1 *B* and *C*).

Sleep Parameters in Men and Women. Habitual bed and waking times (with the exception of waking time on workdays), assessed during screening using the Munich Chronotype Questionnaire (39, 40), were significantly earlier in women than in men (Table 1). The habitual time spent in bed, sleep duration, and sleep quality score [Pittsburgh Sleep Quality Index (41)] were not significantly different between the sexes (Table 1). Because the duration and quality of sleep preceding a wake period may impact the circadian variation of performance, we first consider how sleep is influenced by circadian phase and time asleep separately and then consider their interaction.

Circadian phase. The circadian modulation of sleep efficiency was evident from the variation in total sleep time (TST) per sleep episode, in that the maximum TST averaged across participants was observed for the seventh sleep period (SP) (mean ± SEM: 525.67 ± 5.60 min), and the minimum TST was observed for the fifth SP (mean ± SEM: 424.89 ± 12.9 min). In SP 7, the average bedtime (~20:10) considerably preceded the average dim-light melatonin onset (DLMO) time (~23:30, the third blood-sampling cycle). These results are in accordance with earlier studies (25) showing that the sleep episodes initiated before DLMO are the longest. Analyzed across all circadian phases, the circadian rhythm of sleep efficiency, i.e., TST/time available for sleep, reached its minimum near the rise of melatonin (at ~22:00) and its maximum on the falling limb of the melatonin rhythm (at ~06:00) (Fig. 2). The circadian rhythm in rapid-eye-movement (REM) sleep, expressed as a percentage of TST (REM%), oscillated in phase with the sleep-efficiency rhythm.

SWA, i.e., EEG power density in the 0.5–4 Hz range, displayed a statistically significant, albeit low-amplitude, circadian rhythm with higher values during the biological day and lower values during the biological night and early day (Fig. 3, *Left*). In view of the hypothesized role of slow waves in recovery of brain function, we analyzed the topographical aspects of its circadian regulation and sex differences therein. Unlike other sleep parameters, the circadian modulation of SWA differed between men and women (*SI Appendix*, Table S1). Women exhibited more SWA than men at the DLMO (i.e., close to habitual bedtime), and this difference was significant over the posterior ($P < 0.01$) and central ($P < 0.03$) brain regions.

Topographical analyses showed that the interaction between sex and circadian phase was most pronounced over the central and posterior brain regions (Fig. 3, *Left* and *SI Appendix*, Table S1). SWA was higher in these regions in women during the biological night, suggesting an overall lower circadian SWA amplitude than in men. To investigate this difference further, we performed two analyses. In the first, a circadian waveform

Table 1. Participant demographics

Variable	Women	Men	*P*
Age, y	26.67 ± 0.83	24.54 ± 0.72	0.06
Bedtime on workdays	23:07 ± 00:12	23:56 ± 00:20	0.05
Wake time on workdays	07:20 ± 00:16	08:09 ± 00:19	0.06
Bedtime on free days	23:51 ± 00:16	01:02 ± 00:18	0.01
Wake time on free days	08:35 ± 00:14	09:56 ± 00:21	0.003
Habitual sleep duration	07:55 ± 00:13	07:44 ± 00:12	0.54
Sleep quality	3 ± 0.40	4 ± 0.41	0.88
Diurnal preference	52.88 ± 1.48	46 ± 2.27	0.02
Melatonin onset	22:24 ± 00:20	22:12 ± 00:19	0.33
Phase angle	−1:53 ± 00:22	−2:10 ± 00:20	0.58
N	18	16	

The data are mean ± SEM. The *P* value is based on an independent sample two-tailed Student's *t* test assuming unequal variance. Bedtimes and wake times on workdays and free days are from the Munich Chronotype Questionnaire (39), habitual sleep duration and sleep quality are from the Pittsburgh Sleep Quality Index (41), and diurnal preference is from the Horne–Ostberg Questionnaire (40). Melatonin onset was estimated from the melatonin rhythm as described in *Methods*. Phase angle was computed as melatonin onset − habitual bedtime; a negative phase angle denotes that melatonin onset occurred before bedtime. Note: All times are in hh:mm.

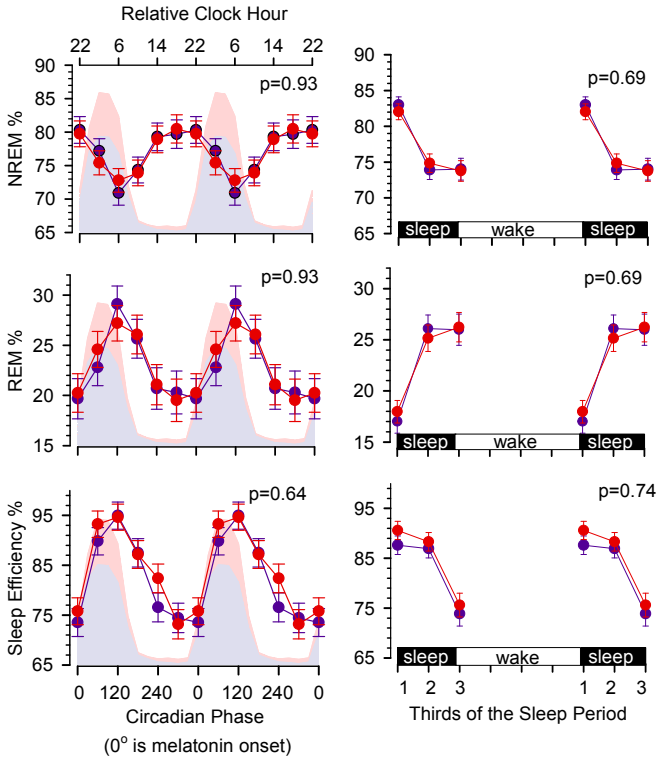

Fig. 2. Circadian (*Left*) and sleep-dependent (*Right*) modulation of sleep parameters in men (blue) and women (red). The double-plotted data represent least-square means and SEMs from the linear mixed-model analyses. The clock hour above the *x* axis is shown relative to melatonin onset. Purple and red backgrounds are the average melatonin profiles of men and women, respectively. NREM%: non-REM sleep as a percentage of TST; REM%: rapid eye movement sleep as a percentage of total sleep time (% of TST); sleep efficiency%: 100 × TST/available time for sleep. *P* values are for the sex*circadian and sex*time asleep interactions.

educed by a nonlinear fit showed a statistically nonsignificant higher amplitude in men than in women in the frontal (mean ± 1 SD; men: 47.54 ± 28.87; women: 27.95 ± 33.51; $\chi^2 = 3.1$; $P = 0.08$), central (men: 26.73 ± 19.14; women: 16.15 ± 16.63; $\chi^2 = 2$; $P = 0.16$), and posterior regions (men: 22.84 ± 18.61; women: 15.15 ± 14.47; $\chi^2 = 1.72$; $P = 0.18$). In the second, the average circadian SWA waveform, educed without assuming a sinusoid, exhibited a trough at 120° (morning) and a maximum at 300° (early evening) (Fig. 3, *Left*). A comparison of the distance (amplitude) between these two circadian bins (computed separately for all participants across all three brain regions) indicated a significantly higher amplitude in men over the frontal (men: 42.03 ± 21.61; women: 25.89 ± 20.26; $\chi^2 = 7.1$, $P = 0.008$) and posterior (men: 17.05 ± 11.9; women: 10.2 ± 7.41; $\chi^2 = 4$, $P = 0.045$) brain regions and a trend toward a higher amplitude in men over the central brain region (men: 22.14 ± 13.65; women: 13.76 ± 8.10; $\chi^2 = 3.1$, $P = 0.079$). This sex difference in the circadian amplitude of SWA is the opposite of the sex difference in the circadian amplitude of cognition (detailed below).

Time asleep. Time asleep (sleep-dependent modulation) had a significant effect on sleep parameters, as expected, with a reduction in sleep efficiency and increase in REM% and a reduction in both non-REM% (NREM%) and SWA as sleep progressed. No significant sex*time asleep interactions were observed for any of these sleep parameters (Figs. 2 and 3, *Right* and *SI Appendix*, Table S1).

Interaction between circadian phase and time asleep. The interaction between circadian phase and time asleep was significant for sleep efficiency, in that the circadian disruption of sleep became stronger as sleep progressed, particularly in the evening hours. No significant interactions between circadian phase and time asleep or between sex, circadian phase, and time asleep were observed for NREM%, REM%, or sleep efficiency (*SI Appendix*, Fig. S9*A* and Table S1).

Waking Performance. During the scheduled awake episode, subjects completed a 40-min performance test battery consisting of 16 subjective rating scales and objective performance tests (see ref. 22 and *SI Appendix, SI Methods* for a detailed description) at ~3-h intervals. These assessments included measuring subjective sleepiness, mood, task demand, attention, working memory, motor control, and temporal processing.

Linear mixed-model analyses with circadian phase, time awake, and sex represented as categorical variables and TST in the preceding sleep period as a covariate revealed a significant effect [false-discovery rate (FDR)-adjusted *P* value] of circadian phase for 32 measures, of time awake for 24 measures, and of time awake*circadian phase interaction for one measure (*SI Appendix*, Tables S2 and S3). There was no significant main effect of sex (FDR-adjusted *P* value) (*SI Appendix*, Table S4) for any measure, although, the unadjusted *P* value suggested a nominal effect of sex for six measures from the pursuit tracking, temporal processing, and spatial and integrated one-back tasks (*SI Appendix*, Table S4). Four measures showed a significant sex*circadian phase interaction, but none showed a significant sex*time awake interaction (*SI Appendix*, Table S5). A nominal effect (unadjusted *P* value; see *SI Appendix*, Tables S2, S3, and S5) of circadian phase was seen in four more measures, of time awake in seven more measures, of a circadian*time awake interaction in seven more measures, of sex*circadian phase interaction in 10 more measures, and of sex*time awake interaction in four

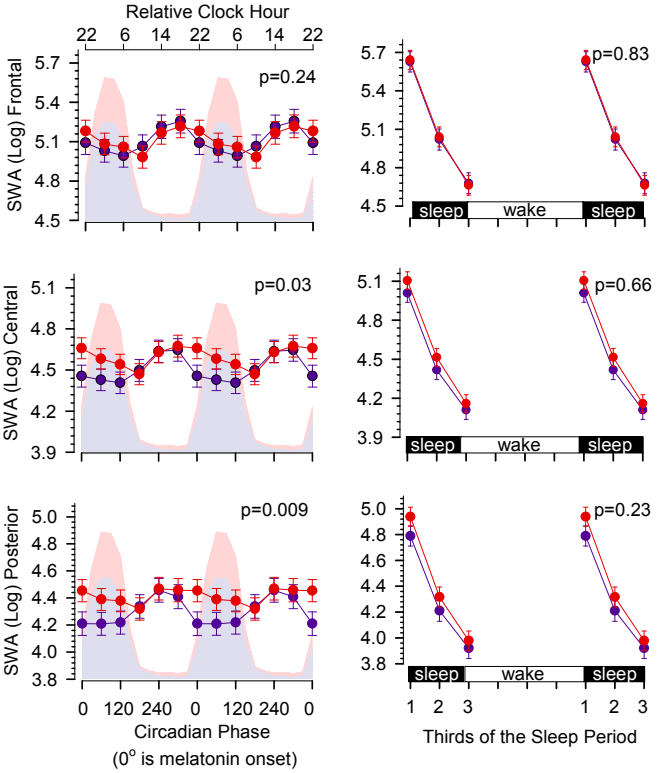

Fig. 3. Topographical analysis of sex difference in circadian variation of SWA in men (blue) and women (red). Circadian (*Left*) and sleep-dependent (*Right*) modulation of SWA (log values) in NREM sleep in men and women in the frontal, central, and posterior areas. The double-plotted data are the least-square means and SEMs from the linear mixed-model analyses. The clock hour above the *x* axis is shown relative to melatonin onset. Purple and red backgrounds are the average melatonin profiles of men and women, respectively. *P* values are for the sex*circadian and sex*time asleep interactions.

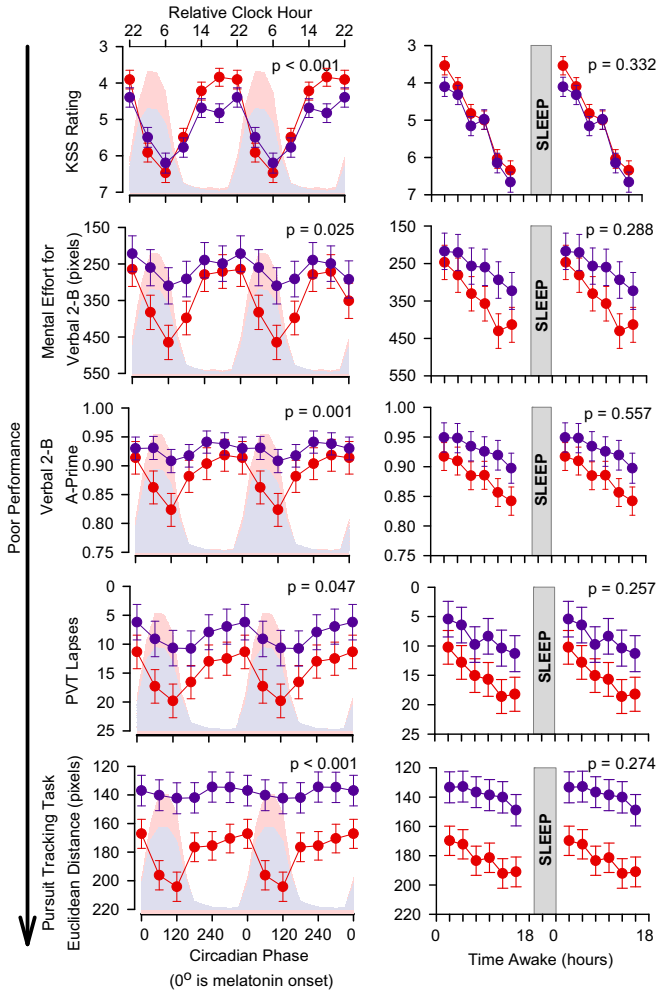

Fig. 4. Circadian (*Left*) and time awake-dependent (*Right*) modulation of two subjective and three objective measures in men (blue) and women (red). The clock hour above the *x* axis is shown relative to melatonin onset. Purple and red backgrounds are the average melatonin profiles of men and women, respectively. The double-plotted data are the least-square means and SEM from the linear mixed model, in which the TST in the preceding sleep episode was a covariate. KSS: Karolinska sleepiness scale; PVT: psychomotor vigilance tasks; verbal-2B: verbal two-back working memory task. *P* values are for the sex*circadian and sex*time awake interactions. Note: For all measures the direction of poor performance is down.

measures. Because TST in the preceding sleep period was a covariate in the model and was significant in 21 of 39 tests (*SI Appendix*, Table S6), the observed circadian variation and the interaction with sex cannot be explained by a differential sleep debt alone.

Fig. 4 profiles these sex differences with plots of least-square means of the sex*circadian phase (left panels) and sex*time awake (right panels) interactions for sleepiness, subjective mental effort, verbal two-back accuracy (A-prime), lapses in attention measured in the psychomotor vigilance task (PVT), and motor-tracking accuracy. These plots cover the spectrum of subjective dimensions and cognitive domains examined and include tasks [the Karolinska sleepiness scale (KSS) and the PVT] commonly used in sleep and circadian research. Performance deteriorated with time awake for all measures, with no evidence of major differences between the sexes. From a circadian perspective, performance was worst in the early morning hours, on the falling limb of the melatonin rhythm, and was best in the evening hours just before the onset of melatonin secretion, for both sexes. The circadian nadir in performance was more pronounced for women in all displayed measures except subjective sleepiness. For this measure women were less sleepy than men in the biological afternoon and evening.

Comparison of the Circadian and Time Awake Effects on Waking Performance. To quantify further the effects of circadian phase, time awake, and interactions with sex, we computed effect sizes for every single measure (Fig. 5 and *SI Appendix*, Tables S2, S3, and S5). The largest effect sizes for circadian phase (Fig. 5*A* and *SI Appendix*, Table S2) were observed for subjective measures such as sleepiness, mood, and effort as well as the slowest reaction times and lapses on the PVT (reaction time >500 ms). However, effect sizes for measures of working memory were smaller; furthermore, they did not increase monotonically from one-back to two-back to three-back tasks, even though the last has a higher executive load and is considered more challenging. For time awake, also, subjective measures displayed larger effect sizes than objective measures, and within the objective measures the largest effects were observed for slow-response times and lapses on the PVT (Fig. 5*A* and *SI Appendix*, Table S2). These time awake effect sizes were numerically larger than the circadian effect sizes in 29 of 39 measures, whereas circadian effect sizes were numerically larger for 10 of 39 measures (signed rank test for this difference: $P = 0.0012$). The supremacy for time awake effects was seen in subjective measures such as sleepiness, task demand, and effort as well as in slow-response times on the PVT and false alarms on the sustained attention response task (SART). The supremacy for circadian effects was most pronounced for SART (misses), response times in the random interval repetition (RIR), and some working memory tasks (Fig. 5*A* and *SI Appendix*, Table S2).

Effect of Sex on the Circadian and Time Awake-Dependent Regulation of Waking Performance. Overall the sex, sex*circadian phase, and sex*time awake effects were smaller than the time awake and

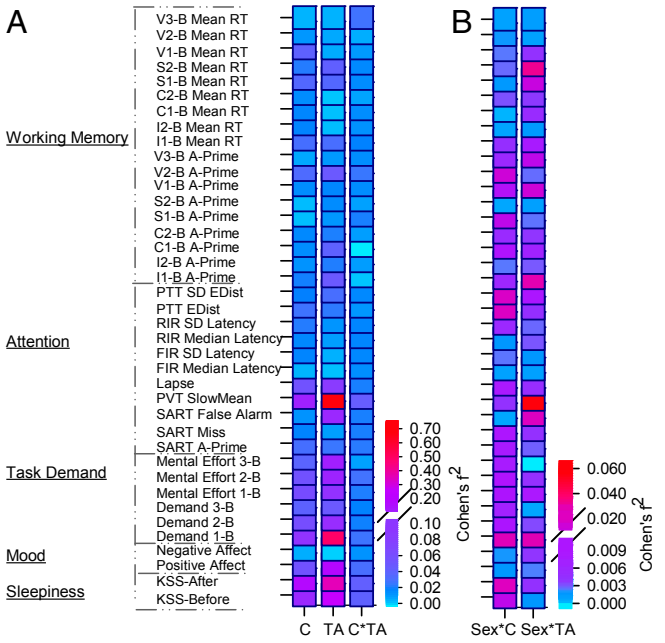

Fig. 5. (*A*) Effect sizes (Cohen's f^2) for circadian (C), time awake (TA), and circadian*time awake (C*TA) effects as derived from the linear mixed-model analyses. (*B*) Effect sizes (Cohen's f^2) for sex*circadian (Sex*C) and sex*time awake (Sex*TA) interactions as derived from the linear mixed-model analyses. C1, C2: color one-back and two-back; EDist: Euclidean distance; FIR: fixed interval repetition; I1, I2: integrated one-back and two-back; KSS: Karolinska sleepiness scale; PTT: pursuit tracking task; PVT: psychomotor vigilance task; RIR: random interval repetition; SART: sustained attention response task; S1, S2: spatial one-back and two-back; V1, V2, V3: verbal one-back, two-back, and three-back. Note: The scales in *A* and *B* are intentionally different to reflect differences in the effects of circadian phase, time awake, and their interaction with sex.

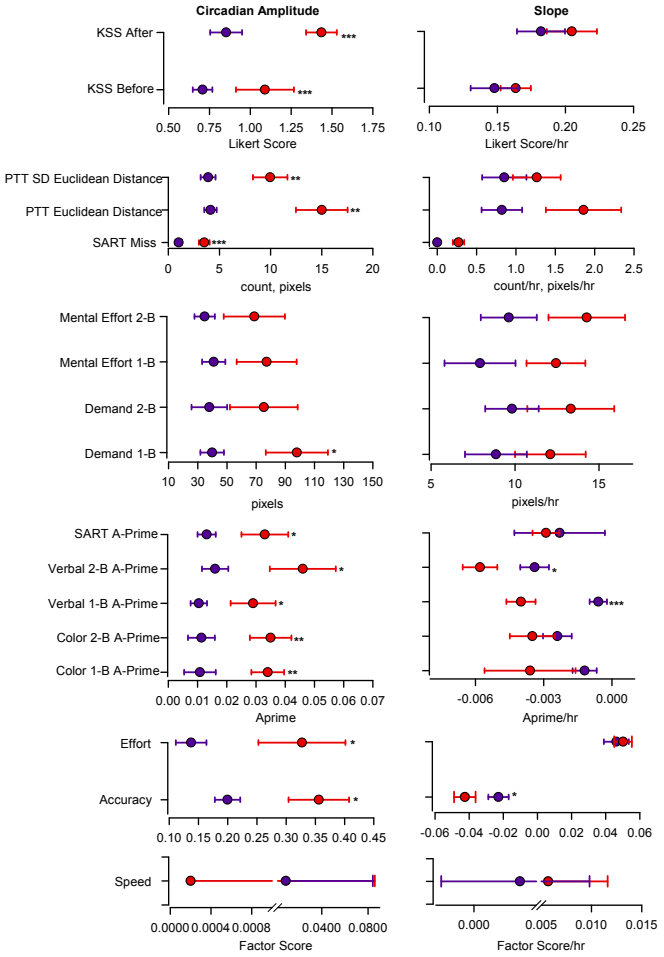

Fig. 6. (*Left*) The average ($\beta_2 \pm$ SEM) circadian amplitude at wake time as estimated by the nonlinear mixed model for men (blue) and women (red). (*Right*) The average ($\beta_1 \pm$ SEM) slope per hour as estimated by the nonlinear mixed model for men (blue) and women (red). Sex differences in these circadian amplitudes and slopes were tested with an independent sample *t* test (assuming unequal variance). Significant differences (Satterthwaite approximation) are shown as $*P < 0.05$, $**P < 0.01$, and $***P < 0.0001$.

circadian effects on cognition (*SI Appendix*, Tables S3, S4, and S5). Of the tasks (pursuit tracking, temporal processing, and spatial and integrated one-back tasks) for which the main effect of sex was nominally significant (unadjusted *P* value) (*SI Appendix*, Table S4), performance on the pursuit tracking task (PTT) showed a significant sex*circadian effect so that performance in the early hours of the morning was more impaired in women than in men (Fig. 4 and *SI Appendix*, Table S5). The effect sizes for the sex*circadian phase interaction were largest for sleepiness, subjective mental effort, motor tracking performance, and A-prime on the verbal two-back task (Fig. 5*B* and *SI Appendix*, Table S5). The effect size for the significant time awake*sex interaction was largest for the PVT slow mean and verbal one-back and three-back A-prime (*SI Appendix*, Table S5), with performance deteriorating more rapidly in women (see *SI Appendix*, Table S8 for slope). This effect was minimal for the performance measures on all other tasks. The effect sizes of the sex*time awake and sex*circadian interactions showed a pattern that was somewhat different from the time awake and circadian main effects. For example, the effect sizes for time awake were largest for subjective measures, in particular the KSS, whereas the sex*time awake effect size was much smaller for subjective measures than for some objective measures. Likewise, the circadian effect size for the PVT slow mean was relatively large, but the sex*circadian effect size for this measure was relatively small.

Linear modeling. To investigate whether the sex*circadian interactions in performance could be explained by sex differences in melatonin amplitude, SWA, or age, we entered each of these as a covariate in separate analyses. With the exception of response times in the verbal one-back, spatial one- and two-back, color, and integrated two-back tasks (for melatonin aptitude), and A-prime for the integrated two-back task (for age), these covariates had no significant effect on any of the measures (*SI Appendix*, Table S6), suggesting that sex differences in melatonin amplitude, SWA, or age do not account for the sex differences observed in performance.

Nonlinear modeling of the effects of circadian phase and time awake on cognition in men and women. Although the linear mixed model and derived effect sizes provide some insight into the nature of the circadian and time awake effects and their interaction with sex on performance, they are categorical variables in the model. To quantify the circadian amplitude and slope of the rate of deterioration with time awake in cognition, we fitted a nonlinear mixed model to the data. In contrast to the linear model in which time awake and circadian phase are discrete factors, this approach fits them as continuous factors. Circadian modulation was represented as a sine function with a period derived from the participants' melatonin rhythm, and circadian phase and amplitude were free parameters. The effects of time awake were represented as a linear function. Amplitude and slope were random factors (see *Methods* for details). Estimates of circadian amplitude were indeed significantly larger in women for sleepiness, effort (demand one-back), A-prime and misses on the SART, Euclidean distance measures on the PTT, and A-prime on the color and verbal one-back and two-back tasks (see Fig. 6 and *SI Appendix*, Table S7). Estimates of the slope of the deterioration with time awake were significantly different between the sexes for A-prime in the integrated one-back and verbal one-back, two-back, and three-back

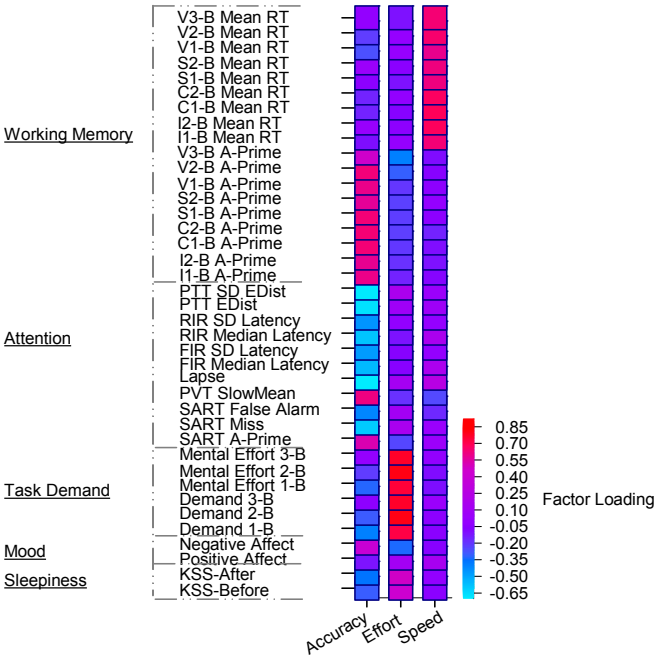

Fig. 7. The contribution of the subjective and objective cognitive measures toward three factors (accuracy, effort, and speed) derived from a PCA, using the varimax rotation method with Kaiser normalization. C1, C2: color one-back and two-back; EDist: Euclidean distance; FIR: fixed interval repetition; I1, I2: integrated one-back and two-back; KSS: Karolinska sleepiness scale; PTT: pursuit tracking task; PVT: psychomotor vigilance task; RIR: random interval repetition; SART: sustained attention response task; S1, S2: spatial one-back and two-back; V1, V2, V3: verbal one-back, two-back, and three-back.

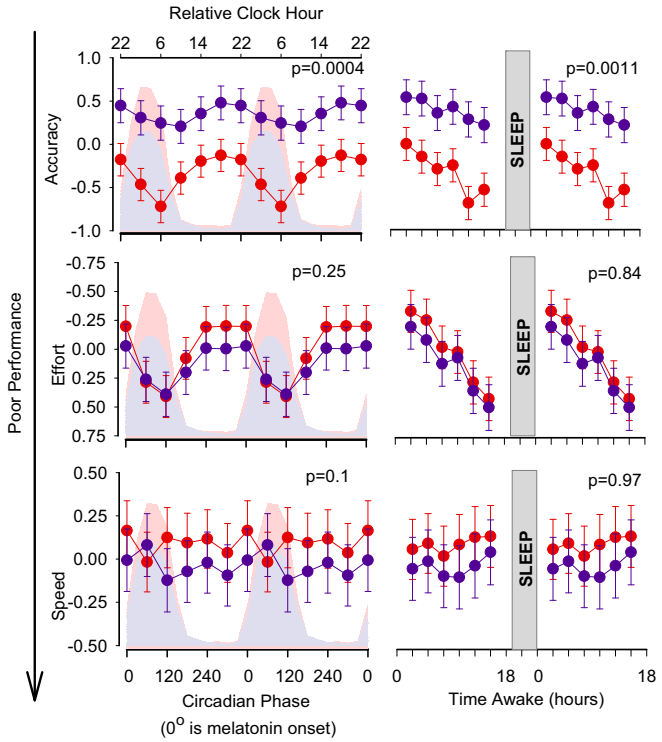

Fig. 8. Circadian (*Left*) and time awake-dependent (*Right*) modulation in factor scores (accuracy, effort, and speed) in men (blue) and women (red). The double-plotted data are least square means and SEMs from the linear mixed-model analyses. The clock hour above the *x* axis is shown relative to melatonin onset. Purple and red backgrounds are the average melatonin profiles of men and women, respectively. *P* values are for sex*circadian and sex*time awake interactions.

tasks such that women showed a greater decline in A-prime with time awake (Fig. 6 and *SI Appendix*, Table S8; two of these slopes, those corresponding to the circadian amplitudes in the left panel, are shown in the figure).

Factor Analyses of Waking Performance. These analyses based on a large number of single performance variables suggest that subjective measures and some attention and accuracy measures are substantially affected by circadian phase and time awake and that these effects are modulated by sex. To explore the nature of these effects further and to reduce the multiplicity problem, we applied principal components analysis (PCA) using a varimax rotation with Kaiser normalization to the data (details are given in *SI Appendix*, *SI Methods*). Three main factors accounting for 46% of the common variance emerged (factor 1: 19.748%; factor 2: 13.719%; factor 3: 12.784%) and were labeled "accuracy," "effort," and "speed," based on the PCA loadings of the 39 contributing measures (Fig. 7). These factors reflect the underlying basis of performance in all subjective and objective tasks, regardless of the specific cognitive functions they probe. Linear mixed-model analysis indicated a significant effect of circadian phase and time awake and a significant interaction between these factors for accuracy and effort (Table 2). The main effect of sex was significant for accuracy, which was lower in women. The sex*circadian phase and sex*time awake interactions for this factor were significant (Fig. 8 and Table 3), and the three-way interaction was marginally significant ($P = 0.051$). These interactions appear to reflect a larger circadian amplitude and more rapid deterioration of accuracy with time awake in women. We confirmed these results with nonlinear modeling of the factor scores, which yielded a larger circadian amplitude and a steeper time awake-dependent slope, especially for accuracy (Fig. 6 and Table 4).

At any given point in time, performance is determined by elapsed time awake, circadian phase, and their interaction. A display of this interaction for accuracy demonstrates how in both men and women performance is determined by time awake and circadian phase, with the magnitude of circadian variation increasing with time awake (Fig. 9; see *SI Appendix*, Fig. S9*B* for effort and speed). However, this increase is much more pronounced in women than in men, especially for accuracy. In other words, women are much more affected by the combination of long times awake and an adverse circadian phase, i.e., the early morning hours. In real life this detriment would occur during 12-h shifts, which are common in many occupational settings such as medical professions/services, even when working-time directives are complied with.

Discussion

The data confirm that sleep and almost all aspects of waking function are modulated by the sleep–wake cycle and circadian phase and are qualitatively similar in men and women. With the exception of a general additional slowing of impaired vigilance (PVT slow responses), in both men and women the effects of circadian phase and time awake on waking function are more pronounced for subjective measures of sleepiness and effort/task demand than for objective measures of attention and working memory. Quantitative differences between the sexes indicate that the circadian amplitudes in effort and accuracy (Table 4) are larger in women and that accuracy deteriorates more in women than in men when a long time awake is combined with an adverse circadian phase.

Sleep. The later sleep time of men during free days and the larger melatonin amplitude in women confirm previous reports on sex differences related to sleep timing and circadian rhythmicity in young adults (16, 42). The circadian melatonin period was similar to the reported values in a larger forced desynchrony study (14), but in this sample the sex difference was not statistically different. The modulation of sleep efficiency, REM sleep, and NREM sleep by circadian phase (as indexed by the melatonin rhythm) and by homeostatic sleep pressure (as indexed by time asleep) was considerable, in accordance with previous reports (25, 34, 43), but did not differ between the sexes. Only SWA, which has been reported to differ between men and women (17, 44) and is regulated primarily by time asleep but also to some extent by circadian phase (25), displayed a sex difference which was limited to the circadian modulation of SWA over the posterior region. This intriguing

Table 2. Effects of circadian phase, time awake, and their interaction for factor scores (accuracy, effort, and speed)

	Circadian					Time awake				Circadian*time awake				
Measures	Num df	den df	F	f^2	*P*	Den df	F	f^2	*P*	Num df	den df	F	f^2	*P*
Accuracy	5	1,067	13.17	0.062	<0.0001	924	22.03	0.119	<0.0001	25	1,023	2.56	0.063	<0.0001
Effort	5	768	20.46	0.133	<0.0001	203	31.26	0.77	<0.0001	25	627	2.06	0.082	0.002
Speed	5	1,037	0.83	0.004	0.531	922	0.96	0.005	0.444	25	949	0.75	0.02	0.811

Results from the general linear mixed-model ANOVA on the factor scores derived from PCA. An autoregressive covariance matrix was used in all of the analyses, except for effect, in which an unstructured component was used instead. Den df: denominator degrees of freedom. F: F-statistic from the ANOVA; f^2: effect size (Cohen's f^2); Num df: numerator degrees of freedom; *P*: *P* value from the ANOVA.

Table 3. Effects of sex and the sex × circadian phase, sex × time awake, and the sex*circadian*time awake interactions on factor scores (accuracy, effort, and speed)

	Sex					Sex*circadian					Sex*time awake				Sex*circadian*time awake				
Measures	Num df	Den df	F	f^2	*P*	Num df	Den df	F	f^2	*P*	Den df	F	f^2	*P*	Num df	Den df	F	f^2	*P*
Accuracy	1	32.1	6.8	0.212	0.014	5	1,058	4.57	0.022	0.0004	926	4.11	0.022	0.001	25	1,053	1.51	0.036	0.051
Effort	1	32.1	0.17	0.005	0.679	5	727	1.33	0.009	0.251	205	0.41	0.01	0.839	25	588	0.8	0.034	0.743
Speed	1	32.4	0.3	0.009	0.590	5	1,063	1.88	0.009	0.095	925	0.18	0.001	0.970	25	1,001	0.73	0.018	0.831

Results from the general linear mixed-model ANOVA on the factor scores derived from PCA. An autoregressive covariance matrix was used in all of the analyses, except for effect, in which an unstructured component was used instead. Only accuracy had significant interactions. Den df: denominator degrees of freedom; F: F-statistic from the ANOVA; f^2: effect size (Cohen's f^2); Num df: numerator degrees of freedom; *P*: *P* value from the ANOVA.

finding indicates a sex-specific difference in the physiological regulatory mechanism of SWA with a local aspect. The circadian modulation of the wake EEG (45) and of slow waves during sleep (46) has been shown to be more dominant in posterior than in frontal regions, which appear to be regulated primarily by sleep–wake pressure (homeostatic). Importantly, the overall circadian modulation of SWA was lower in women than in men, and this effect was not limited to the posterior brain region but was present over the frontal region as well. Thus, our finding may suggest a sex difference in the local regulation of sleep (47, 48).

SWA decreases with age, and the women in our study were 2 y older on average than the men. An analysis for SWA controlled for age showed that the reported effects persisted. Thus, the basic aspects of the circadian and homeostatic regulation of sleep are qualitatively similar in men and women. It is interesting that the sex difference in the circadian modulation of SWA, with men displaying a larger amplitude, is the opposite of the sex differences in the circadian amplitude of cognition.

Circadian and Time Awake-Dependent Regulation of Waking Function. Although we assessed subjective and objective characteristics across tasks that reflect different modalities of working memory, attention, motor control, and temporal and spatial processing, we show that three dimensions of performance (accuracy, speed, and effort) underlie this wide range of functioning. These dimensions of performance are probably most relevant in the real world. Critically, in our analyses of the direct circadian modulation of waking cognition, we controlled for the confounding effects of sleep duration (49) as well as SWA in the preceding sleep period. Although nearly all aspects were affected by circadian phase and time awake, quantitative differences emerged. Subjective judgments/evaluations and attention measures were more affected than working memory or aspects of executive function by both time awake and circadian phase. For example, the largest effect sizes were observed for subjective sleepiness, task demand, and accuracy of verbal working memory. Previously, subjective measures of effort, mood, and sleepiness and measures of sustained attention were shown to be more affected by total sleep deprivation and repeated partial sleep deprivation than were measures of working memory and executive function (20–22). In the present study, the deterioration of brain function was observed even though time awake varied only between 0 and ∼19 h. This deterioration was modulated by circadian phase so that it was more pronounced when wakefulness coincided with the phase of melatonin secretion, i.e., the biological night. In one previous study with a number of tasks similar to that in our study, circadian effect sizes for sleepiness and attention were relatively pronounced, in accordance with the current study (26). However, inhibition was more affected by circadian phase in that study than in our study, where aspects of inhibition assessed in SART did not show comparably large circadian effects. Our data indicate that both time awake and circadian phase primarily affect measures related to self-reported effort for completing a working memory task and the effort required to stay awake.

In this study we document the effect of sex, time awake, sleep, and circadian phase on three underlying characteristics of human performance: accuracy, speed, and effort. This approach emphasizes what the tasks have in common rather than the particular cognitive requirements of specific tasks. It largely confirms what is observed when specific tasks are considered: The largest circadian and time awake effect sizes were found for effort and accuracy, whereas speed was not much affected. This latter observation may be related to our tasks being timed rather than untimed. Research suggests that timed tasks favor men and are a disadvantage for women (50). This consideration of what tasks have in common, rather their particular nuances, may make these results more relevant to real-world tasks than to single laboratory tasks.

In general, time awake effects on performance were larger than the circadian effects, especially for subjective measures such as sleepiness and task demand. This finding is in accordance with the results obtained in a similar forced desynchrony protocol (26) and in a quantitative analysis of the effects of 36 h of wakefulness across nearly 1.5 circadian cycles (51). In accordance with previous observations (27–29), both the linear and nonlinear analyses demonstrated that the effects of time awake and circadian phase interacted: The deterioration with time awake is most pronounced in the early morning; in other words, the effect of circadian phase on performance very much depends on time awake. This result has important implications for understanding performance in shift work: Circadian phase cannot be manipulated easily, whereas time awake can.

Sex Differences in the Circadian and Time Awake-Dependent Regulation of Waking Function. A main effect of sex was observed only for the factor accuracy, which was lower in women, particularly for Euclidean distance measures in PTT and A-prime in the spatial one-back and integrated one-back tasks, which require spatial and color memory. Similar sex differences in accuracy on spatiotemporal tasks have been reported, although it has been pointed out that these differences, especially with respect to spatial skills, are lower when tests are untimed rather than timed (33). All our tasks in which these differences were observed were time constrained,

Table 4. Circadian amplitude (at wake time) and slope of the change with time awake in factor scores

Factor scores	Women	Men	t-Statistic (*P* value)
Amplitude			
Accuracy	0.356 ± 0.052	0.200 ± 0.022	2.78 (0.011)
Effort	0.327 ± 0.075	0.137 ± 0.026	2.4 (0.036)
Speed	0.0002 ± 0.086	−0.009 ± 0.075	0.08 (0.94)
Slope			
Accuracy	−0.043 ± 0.006	−0.023 ± 0.006	2.27 (0.029)
Effort	0.050 ± 0.005	0.046 ± 0.007	0.44 (0.665)
Speed	0.006 ± 0.006	0.004 ± 0.006	0.24 (0.81)

The amplitudes (mean ± SEM) and time awake slopes (mean ± SEM) are from the nonlinear mixed-model analyses for the three factor scores in women and men. Sex differences were tested using an independent sample *t* test assuming unequal variance (Welch's *t*-statistic and Welch–Satterthwaite *P* value).

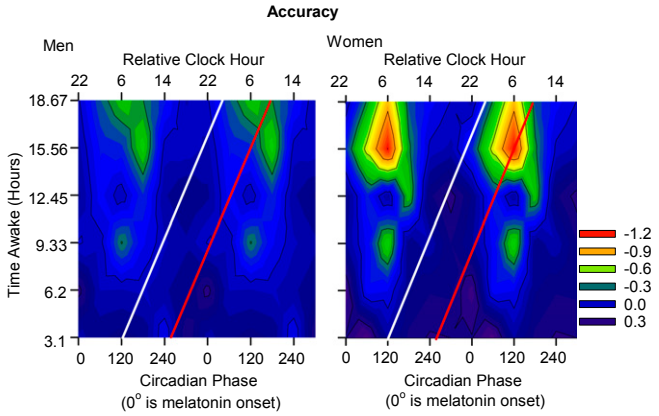

Fig. 9. The impact of circadian phase–time awake interaction on deterioration of accuracy ($P = 0.05$) in men (*Left*) and women (*Right*). Accuracy is shown as a deviation from the average accuracy factor score computed separately for men and women. Warmer colors indicate worse performance. The clock hour above the *x* axis is shown relative to melatonin onset. The *y* axis indicates the upper limit of wake-episode intervals. The two trajectories represent a waking day that includes a day shift (white) and a waking day that includes a late/night shift (red). Note that the starting point for the two trajectories is at the lower limit of the first interval of the *y* axis.

as are many real-world tasks in which coordinating the components of performance require that precise temporal constraints are met [e.g., driving, during which gear-shifting, braking, acceleration, and steering must be coordinated to maintain control of the vehicle or to deal with other road users or obstacles (52)].

The effects of circadian phase interacted with sex so that the circadian amplitude in general was larger in women and in particular for accuracy and effort. Although the sex differences in the circadian regulation in waking performance were seen primarily in the impairment during the biological night, subjective sleepiness was an exception. Interestingly, men and women did not differ in their subjective report of sleepiness during the night, but women rated themselves as significantly less sleepy than men during the daytime (Fig. 4). The large circadian amplitude in women was expressed as reduced effort in the wake maintenance zone, i.e., just before the onset of melatonin, and as reduced accuracy in the morning hours. Although time awake effects were not as greatly affected by sex, accuracy, with its steeper slope in women, was an exception: It deteriorated more in women than in men with time awake. Given that time awake effects and circadian phase effects interact, it is not surprising that waking performance, especially accuracy, deteriorates more in women than in men when a long time awake is combined with an adverse circadian phase.

Interpretation of Sex Differences in the Circadian and Time Awake-Dependent Regulation of Waking Function. The data imply that sex differences in the circadian and time awake-dependent regulation of waking function are quantitative rather than qualitative in nature (50, 53). The basic aspects of this regulation, i.e., time awake and circadian phase effects and their interaction, are present in both men and women. The larger circadian amplitude in cognition in women could not be explained by the sex differences in SWA or melatonin amplitude, the classical markers of the homeostatic and circadian process, respectively. In general, sex differences in the circadian timing system are interpreted within a neuroendocrine context, in which sex hormones have both long-lasting and acute effects (54). Of course, potential mediators of the observed sex differences can be very diverse, ranging from structural and connectome differences (53) to differences in neuromodulators and local brain clocks (55). Sex differences in circadian amplitude have been reported for rhythmicity in gene expression in several brain areas in rodents, with the direction of the difference depending on brain region (56).

Limitations of the Present Study. Although our battery of cognitive tasks was extensive and allowed a comprehensive assessment of several aspects of waking performance, it was designed primarily for sleep and circadian studies and not for detecting sex differences per se. Many of our tasks had time limits, a condition that may be advantageous for men and disadvantageous for women (50), although it should be noted that the effort–judgment tasks, which were self-paced, still reflected sex differences. Future studies investigating circadian and homeostatic effects on cognition should take into account task-dependent advantages/disadvantages for men and women.

The length of our protocol made it impractical to control for phase of the menstrual cycle, which modulates cognitive functioning at both a neural and behavioral level, and our approved protocol did not allow for collection of data on the menstrual phase of the women during the study. Attentional processing, integration of emotion, response to reward, and performance in spatial tasks are all affected by the menstrual phase (57). Hormonal changes linked to the menstrual cycle also interact with the circadian effect on cognition (58). Some aspects of sleep, such as spindles, are affected by the menstrual phase, whereas others, such as SWS, are not (59). Because this 2-y study included a random sample of women it is likely that all phases of the menstrual cycle were covered. Thus our sample is representative of women; i.e., it is not based on only the luteal or follicular phases. Hormonal contraceptives also influence cognitive functions such as verbal fluency, spatial processing, and emotional processing (60). They were not exclusionary in our study, and nine women used this form of contraception. This small number made it impractical for us to explore the influence of these contraceptives in any meaningful way. Even though the modest sample size in our study made it difficult to assess the interactive effects of the above-mentioned factors, to our knowledge ours is the largest study to date investigating sex differences in the sensitivity to adverse circadian phase and time awake.

Implications. Extrapolation of these laboratory findings to the real world would suggest that women are more affected by night-shift work than men. Indeed, reports that have looked at sex differences in working hours, work shifts, and occupational injuries show that women seem to be at increased risk for occupational injuries during extended work shifts, nonstandard shifts, and changing shifts (61). This difference may in part reflect social factors such as family and childcare responsibilities that lead women to work longer hours and to sleep less on days off than men (62). It is clear that physiological, cognitive, and social factors interact to create a landscape of cognitive vulnerability in men and women. Our findings provide insight into the factors contributing to sex differences in sensitivity to the acute effects of adverse circadian phase and extended wakefulness on cognition. These findings also may be relevant in view of the cognitive deficits observed in chronic shiftwork (7) and jetlag (63).

Methods

Ethics and Participants. The research protocol received a favorable opinion from the University of Surrey Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before any study-related procedure. Data from 34 participants (18 women) are presented here. Participants were in good health as assessed by medical history, physical examination, and standard biochemistry/hematology and had no sleep complaints as indicated by questionnaires and clinical polysomnography (PSG) on the baseline night. A history of shift-work in the preceding year, travel across more than one time zone in the preceding 2 mo, blood donation in the preceding 6 mo, consumption of ≥300 mg of caffeine/d and/or ≥14 units of alcohol/wk, smoking, and pregnancy in women were all exclusionary. Nine women were taking contraceptive medication, which was not exclusionary. The men and women in our study were well matched in age, bedtime and wake times on workdays and free days (Munich

Chronotype Questionnaire), melatonin onset, and intrinsic circadian period. These data come from the same participants as in Lazar et al. (38); our data come from 16 men, whereas those in Lazar et al. (38) are from 17 men (one male participant was excluded from all analyses because of significant loss of PSG data). Genomic DNA was extracted from buccal swab samples to analyze the variable number tandem-repeat polymorphisms in *PERIOD3* (men: $PERIOD3^{4/4} = 7$; $PERIOD3^{4/5} = 3$; $PERIOD3^{5/5} = 6$; women: $PERIOD3^{4/4} = 6$; $PERIOD3^{4/5} = 5$; $PERIOD3^{5/5} = 7$). The distribution of these genotypes across the sexes did not differ significantly from uniformity ($P < 0.76$), and the effect of this polymorphism is not discussed here. Its effect on the melatonin rhythm and sleep are presented elsewhere (38, 64, 65).

The laboratory protocol was a slightly different implementation of the 28-h forced desynchrony protocol (25) described previously (38, 64). It was conducted at the Surrey Clinical Research Centre and lasted 10 calendar days (i.e., one beat cycle). Following a baseline 8-h/16-h sleep/wake episode at habitual bedtime [from sleep diary and wrist actigraphy data obtained during the 2 wk preceding the laboratory session (64)], the sleep-wake cycle and meals were rescheduled to a 28-h period (sleep: 9 h:20 min; and wake: 18 h:40 min) starting with sleep at habitual bedtime on SP 2 (Fig. 1*A*). To minimize relative coordination between the 28-h day and endogenous circadian rhythm, the laboratory environment was kept in low light (<5 lux) during all wake episodes (including baseline) and in darkness during sleep episodes and was free of time cues. Melatonin levels were measured at 1- to 2-h intervals during three 28-h periods at the beginning, middle, and end of the protocol, without disrupting sleep (Fig. 1*A*). Cognitive performance and polysomnography were measured throughout the forced desynchrony.

Plasma Melatonin Sampling. Melatonin levels were determined from blood samples collected at 1- to 2-h intervals via an in-dwelling i.v. cannula during three 28-h periods at the beginning, in the middle, and at the end of the protocol (Fig. 1*A*). During sleep periods, collection was done remotely without entering the bedrooms to avoid disrupting sleep. On the first and third occasions, sleep started at the habitual bedtime; on the second it started 12 h out of phase with habitual bedtime. The samples were frozen at −20 °C until melatonin quantification, which was done with an RIA (Stockgrand Ltd.). The limit of detection for the assay was 3.4 pg/mL, with interassay coefficients of variation (mean ± SD) of 21.9% at 8.5 ± 1.9 pg/mL, 13.4% at 36.6 ± 4.9 pg/mL, 13.5% at 81.06 ± 10.9 pg/mL, and 11.7% at 123.5 ± 14.0 pg/m. We derived the melatonin amplitude, circadian phase, and circadian period from these plasma samples. See *SI Appendix, SI Methods* for details of how melatonin parameters were computed.

Sleep parameters were derived from PSG as described by Lazar et al. (46). Briefly, EEG signals were derived from a 12-channel EEG montage (Fp1-A2, Fp2-A1, F3-A2, F4-A1, T3-A2, T4-A1, C3-A2, C4-A1, P3-A2, P4-A1, O1-A2, and O2-A1) according to the 10–20 system. Eye movement, muscle tone, and heart rate were recorded through left and right EOG, submental EMG, and ECG electrodes which were referenced to A2 and A1, respectively. The ground and reference electrodes were placed at FPz and Pz, respectively. Sleep staging was performed in 30-s epochs according to the Rechtschaffen and Kales criteria (66).

Spectral Analyses of Sleep. Spectral analyses were performed as described by Lazar et al. (46). All artifact-free EEG segments of the sleep stages of interest (NREM and stages 2, 3, and 4) were concatenated within consecutive 20-min intervals between lights out and lights on. Spectral power values were calculated as averages over detrended, Hanning windowed 4-s epochs with 50% overlap using Welch's periodogram method as implemented in the NumPy [version (ver.) 1.10.0], SciPy (ver. 0.13.3), and Matplotlib (ver. 1.3.1) numeric libraries for Python (ver. 2.7.6), all available at www.scipy.org and https://www.python.org. Power in the SWA band 0.5–4 Hz (sometimes referred to as "delta activity") was calculated for NREM sleep. This range is in accordance with other studies (46, 67), and applying slightly different definitions, such as 0.5–4.5 Hz, did not affect our results substantially.

Cognitive performance was assessed at ∼3-hourly intervals during each wake episode (Fig. 1*A*) with a 40-min battery of 16 tasks spanning subjective sleepiness (KSS), mood (positive and negative affect scale), effort/task demand (visual analog scale), attention (PVT; SART), motor control (PTT), temporal processing (fixed/random interval tasks; FIR-RIR), and nonverbal and verbal working memory (spatial, color, integrated spatio-color, and verbal n-back tasks). The tasks were chosen for cognitive validity, reliability, and, importantly, sensitivity to circadian and sleep–wake-dependent effects (22, 34, 35). The test battery was computerized using Active X, C#, and Exactics code and was presented on monitors with screen refresh rates of 60 Hz (22, 68). The tasks were administered in one of three orders, as described by Lo et al. (22), and were fixed for each participant for the duration of the forced desynchrony. Where speed and accuracy were measured, participants were told that both were equally important. Across subjects the data loss was 4.3 + 12.4% (mean + 1 SD), and across tasks the range was 3.74–7.62%.

Cognitive Testing Battery. See *SI Appendix, SI Methods* for details.

Statistical Analyses. We assessed the sex difference in our demographic variables with a Student's *t* test using PROC TTEST in SAS 9.2 (SAS Institute Inc., Cary, NC).

Linear approach. We used Procedure MIXED (SAS 9.2) to implement the general linear mixed-model ANOVA and corrected for multiplicity using the Benjamini–Yekutieli procedure (69). Cohen's f^2 was the effect size index (70). See *SI Appendix, SI Methods* for details.

Nonlinear approach. Cognitive performance was modeled as a linear function of time awake, with TST added to it (49), and a cyclical (sine) function of circadian phase, with time awake and circadian phase being continuous factors, using Procedure NLMixed (SAS 9.2). The circadian amplitude of SWA was estimated with a sine wave fit using the scipy.optimize.curve_fit Python function. See *SI Appendix, SI Methods* for details.

PCA. See *SI Appendix, SI Methods* for a detailed description of PCA.

ACKNOWLEDGMENTS. We thank Sigurd Johnsen for statistical advice; Zsolt I. Lazar for programming assistance; Sibah Hasan and Ana Slak for help with data acquisition; the research nurses, research officers, program managers, and study physicians of the Surrey Clinical Research Centre for the clinical conduct of the study; Simon Archer, Malcolm von Schantz, Jonathan Johnston, Colin Smith, Emma Laing, and Giselda Bucca for helpful discussions; and Benita Middleton for melatonin assays. The primary support for this research was provided by Biotechnology and Biological Sciences Research Council Grant BB/F022883. A.S.L. is supported by Grant RG77329 from the Jacques and Gloria Gossweiler Foundation. D.-J.D. is supported by a Royal Society Wolfson Research Merit Award.

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