Industrial Health 2019, **57**, 118–132

Original Article

Cardiac autonomic activity during simulated shift work

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Received February 22, 2018 and accepted July 3, 2018
Published online in J-STAGE August 8, 2018

Abstract: Shift work leads to adverse health outcomes including increased risk of cardiovascular disease. Heart rate (HR) and heart rate variability (HRV) are measures of cardiac autonomic activity and markers of cardiovascular disease and mortality. To investigate the effects of shift work on cardiac autonomic activity, we assessed the influence of simulated night work on HR and HRV, and dissociated the direct effects of circadian misalignment from those of sleep displacement and altered physical activity patterns. A total of 29 subjects each participated in one of two in-laboratory, simulated shift work studies. In both studies, EKG was continuously monitored via Holter monitors to measure HR and the high frequency (HF) component of HRV (HF-HRV). We found endogenous circadian rhythmicity in HR and HF-HRV. Sleep and waking physical activity, both displaced during simulated night work, had more substantial, and opposite, effects on HR and HF-HRV. Our findings show systematic but complex, interacting effects of time of day, sleep/wake state, and physical activity on cardiac autonomic activity. These effects need to be taken into account when evaluating HR and HRV in shift work settings and when interpreting these measures of cardiac autonomic activity as markers of cardiovascular disease.

Key words: Night shift, Human sleep, Circadian misalignment, Parasympathetic nervous system, Sympathetic nervous system, Vagal tone, Heart rate variability

Introduction

Shift work has substantial negative effects on long-term health[1)]. Shift work is associated with hypertension, myocardial infarction, diabetes, and obesity[2)]. Furthermore,

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shift work increases the risk for cardiovascular disease[3, 4] —a leading cause of mortality in the United States[5].

The health consequences of shift work are partly due to the circadian misalignment associated with these work schedules[6, 7]. The circadian pacemaker, located in the suprachiasmatic nuclei (SCN) of the hypothalamus, functions as an internal biological clock, maintaining a wide range of biological processes on a (near-) 24-h rhythm[8]. The SCN sends projections to other areas of the hypothalamus, including the subparaventricular zone and the dorsomedial nucleus of the hypothalamus. During the daytime hours, projections from these areas cause activation of the nuclei of the ascending arousal system, which promote wakefulness; and inhibition of the ventrolateral preoptic (VLPO) nucleus of the hypothalamus, which promotes sleep[8]. As such, the circadian pacemaker exerts a drive for wakefulness during the daytime hours, which it withdraws during the nighttime hours, thereby promoting sleep[9]. In night shift workers, who must be awake at night and sleep during the day, the behaviorally driven timing of wakefulness and sleep thus conflicts with the biologically driven timing of pressure for wakefulness and sleep. This conflict is central to understanding the health consequences of shift work[10].

Heart rate (HR) and heart rate variability (HRV) are measures of cardiac autonomic nervous system activity and markers of cardiovascular health and mortality[11]. Reduced parasympathetic activity, as indicated by higher HR and lower HRV, is associated with increased risk of cardiovascular disease, increased risk of all-cause mortality, and overall degraded health[11, 12]. Given the relationship between cardiac autonomic activity and cardiovascular disease on the one hand, and the association between shift work and cardiovascular disease on the other, studies in shift work settings have used HR and HRV to investigate the risk of cardiovascular disease associated with shift work[13–17]. However, HR and HRV are dynamically influenced by endogenous circadian rhythmicity[18–23] and by the timing of sleep[19, 22, 23], as well as by waking physical activity, exercise, and posture[24–26]. Since shift work schedules produce misalignment between endogenous circadian rhythmicity and the timing of sleep and wakefulness, it is important to understand the impact of this misalignment on HR and HRV.

We set out to study these aspects of circadian misalignment on cardiac autonomic activity in two laboratory-based, simulated shift work studies. Study 1 aimed to dissociate the effects of endogenous circadian rhythmicity, sleep, waking physical activity, and exercise on HR and HRV. Study 2 aimed to provide an integrated view of the factors dissociated in study 1.

Methods

Subjects

A total of 29 healthy young adults each participated in one of two laboratory-based, simulated shift work studies. In study 1, N=14 healthy young adults (ages 22–34 yr, 4 female) completed a seven-day, six-night laboratory study. Subjects were assigned to either a day shift condition or a night shift condition. In study 2, N=15 healthy young adult males (ages 18–29 yr) completed a six-day, five-night laboratory study. Subjects were assigned to one of four Naval shift schedules.

Subjects were physically and psychologically healthy as assessed by history, questionnaires, and physical examination. They had no sleep or circadian disorders as verified by history, questionnaires, and wrist actigraphy (Actiwatch-2; Respironics, Bend, OR); and, in study 1 only, by baseline polysomnography. Subjects were free of traces of drugs and alcohol as assessed by blood and urine chemistry, and were non-smokers. They did not travel across time zones within one month of entering the study, and were not exposed to shift work within three months of entering the study.

All subjects reported to be good sleepers, habitually sleeping between 6 and 10 h daily with regular bedtimes and typical wake times between 06:00 and 09:00. Subjects were asked to avoid napping and to maintain their habitual sleep schedules during the seven days before the laboratory experiment. Compliance with this part of the study was verified by means of wrist actigraphy and sleep/wake diary, and subjects reported their bedtimes and rising times on a time-stamped voice recorder. Subjects were also instructed to refrain from caffeine or alcohol consumption, and to avoid drugs (including tobacco products) during the seven days before the laboratory experiment. Compliance was verified with urine and breathalyzer tests immediately prior to entering the laboratory.

Experimental design

Study 1

Figure 1 shows a schematic of the experimental design for study 1. Subjects were in the laboratory continuously for seven days (six nights) with up to two other subjects in the laboratory at the same time. For each study run, subjects were assigned to either a day shift (DS) condition (n=7; ages 24.0 ± 2.2 yr) or to a night shift (NS) condition (n=7; ages 27.6 ± 3.2 yr). The total amount of scheduled sleep and wakefulness was the same for these two conditions.

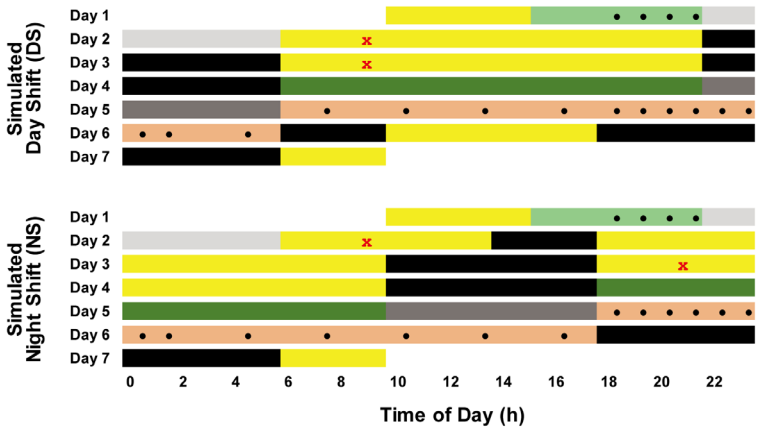

Fig. 1. Schematic of the design of study 1, showing the day shift (top) and night shift (bottom) conditions simulated in the laboratory. In each panel, days progress from top to bottom, and time of day progresses from left to right. Orange, 24-h constant routine period; dark green, wakefulness during third shift day used for analyses; dark gray, sleep opportunity during third shift day used for analyses; light green, baseline wakefulness period used for analyses; light gray, baseline sleep opportunity used for analyses; yellow, other scheduled wakefulness; black, other scheduled sleep opportunities. Red markings, stepping exercise; filled black circles, blood sampling times.

On the first day, subjects entered the laboratory at 10:00. Meals were provided at 13:00 and 19:00. This day included an 8-h nighttime sleep opportunity (22:00–06:00).

At 8:45 on the second day, shortly after awakening from baseline sleep, subjects completed an exercise protocol. This protocol encompassed a 15-min stepping exercise, which involved stepping on and off an 8-inch step bench to the sound of a beat (50 bpm). For 30 min before and after the stepping exercise, subjects were seated in a controlled posture (upright with feet flat on the floor, hands in their lap, and back flat against the back rest of the chair) while avoiding physical movements as much as possible.

Condition assignment (DS or NS) was announced at 11:50 on the second day. Time in the study up until condition announcement served as the baseline period.

Subjects assigned to the DS condition followed a simulated day shift schedule for three days, with daytime wakefulness (06:00–22:00) and nighttime sleep opportunities (22:00–06:00). Meals were provided at 07:00, 13:00 and 19:00 each simulated DS day. At 08:45 on the second simulated DS day, subjects again completed the exercise protocol described above.

Subjects assigned to the NS condition first received a 4-h prophylactic nap opportunity (14:00–18:00) on the second day in the laboratory, in order to transition to a simulated night shift schedule. They then followed the night shift schedule for three days, with nighttime wakefulness (18:00–10:00) and daytime sleep opportunities (10:00–18:00). Meals were provided at 19:00, 01:00 and 07:00 each simulated NS day. At 20:45 on the second simulated NS day, subjects again completed the exercise protocol described above.

On the fifth day in the laboratory, all subjects were exposed to a 24-h constant routine protocol. This protocol allowed for measuring the endogenous circadian rhythm in HR and HRV[18–20]. In the DS condition, the 24-h constant routine started at 6:00; in the NS condition, it started at 18:00. During the constant routine, subjects were kept awake at all times. They remained seated in a semi-reclined position, with the exception of brief bathroom breaks. They received hourly equicaloric snacks (80 calories per snack; 40% carbohydrate, 30% protein, 30% dietary fat). One subject in the DS condition did not consume the hourly snacks during the last 6 h of the constant routine; this subject's HR and HRV data recorded during this period were not used for analyses.

After the constant routine, subjects in the DS condition received a 4-h nap opportunity (06:00–10:00), followed by a 12-h nighttime recovery sleep period (18:00–06:00). Subjects in the NS condition had a 12-h nighttime recovery sleep period right after the constant routine. All subjects were discharged from the laboratory on the seventh day at 10:00.

Blood samples were collected at 1-h intervals across a 3-h period right before bedtime (18:30–21:30) on the baseline day. Blood samples were also collected at 3-h intervals throughout the 24-h constant routine, and at 1 h intervals during a 7-h portion of the constant routine (18:30–01:30; Fig. 1). Blood samples were analyzed for markers of endogenous circadian timing; melatonin levels

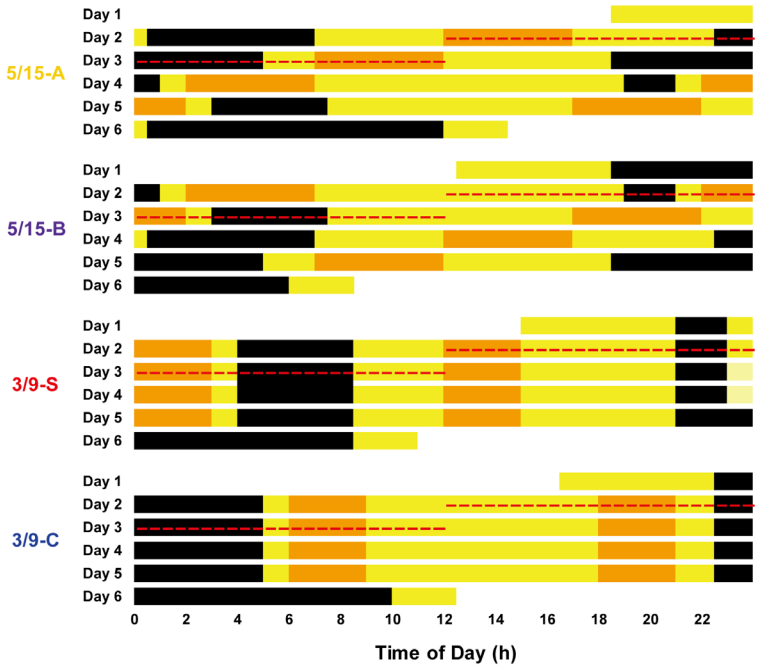

Fig. 2. Schematic of the design of study 2, showing each of the four watch sections simulated in the laboratory. In each panel, days progress from top to bottom and time of day progresses from left to right. Black, scheduled sleep opportunities; orange, watchstanding periods; yellow, other scheduled wakefulness periods. Red dotted lines, 24-h period used for analyses.

were quantified using RIA (IB88111, KMI Diagnostics Inc., Minneapolis, MN, USA), and cortisol levels were quantified using ELISA (ADI-900-097, Enzo Life Sciences, Farmingdale, NY, USA). During the baseline blood sampling period and throughout the 24-h constant routine, subjects were seated in a semi-reclined position.

In both conditions, sleep and nap opportunities were recorded with digital polysomnography (Nihon Kohden, Foothill Ranch, CA, USA). Activity levels were recorded continuously using wrist actigraphy (Actiwatch-2; Respironics, Bend, OR, USA).

Study 2

Figure 2 shows a schematic of the experimental design for study 2. Subjects were in the laboratory continuously for six days (five nights) with up to seven other subjects in the laboratory at the same time. Simulating around-the-clock Naval shift schedules (watch sections), subjects were assigned to one of four conditions as previously described[27]:

5/15-A: a "5/15" backward rotating watch section, with 6.5-h sleep opportunities beginning at 00:30 on Day 2, 22:30 on Day 2, 18:30 on Day 3, and split sleep at 19:00 (2 h) on Day 4 and 03:00 (4.5 h) on Day 5 (n=4; ages 25.0 ± 2.2 yr);

5/15-B: a "5/15" backward rotating watch section equivalent to the 5/15-A watch section, but shifted by two days in the four-day rotation cycle (n=4; ages 22.3 ± 3.8 yr);

3/9-S: a "3/9" non-rotating watch section, with 6.5-h split sleep opportunities beginning at 21:00 (2 h) and 4:00 (4.5 h) each day (n=3; ages 23.7 ± 1.5 yr);

3/9-C: a "3/9" non-rotating watch section, with 6.5-h consolidated sleep opportunities beginning at 22:30 each day (n=4; 24.0 ± 3.4 yr).

After four simulated watch section days, all subjects received an 11.5-h recovery sleep opportunity. Subjects were discharged from the laboratory on Day 6 (Fig. 2).

Subjects assigned to the 5/15-A and 5/15-B watch sections were in the laboratory at the same time. Likewise, subjects assigned to the 3/9-S and 3/9-C watch sections were in the laboratory at the same time. Condition assignment was announced at the beginning of the study. The total amount of scheduled sleep and wakefulness was the same for these four conditions.

Subjects slept in bunk beds in a shared sleeping area; they were instructed to try to sleep during scheduled sleep opportunities and to minimize the sleep disturbance of others. They received three meals and a snack each watch section day. Subjects were scheduled to stand simulated watch duties (i.e., simulated watchstanding) for an average of 6 h per watch section day (Fig. 2). During simulated

watchstanding, subjects performed continuous, cognitively demanding computer tasks while seated at a desk, including tests on psychomotor vigilance, reaction time, perseveration, memory, response inhibition, and decision-making processes. In all four conditions, activity levels were recorded continuously using wrist actigraphy (Actiwatch-2; Respironics, Bend, OR, USA) to assess sleep/wake patterns (reported elsewhere)[27].

Both studies

In both studies, to enable experimental control over the simulated shift work conditions, subjects remained isolated from the outside world. They had no exposure to natural daylight and no visitors, phone calls, e-mail, internet access, live television, radio, or other contact with the external environment. Caffeine, alcohol, and tobacco were not allowed. During scheduled wakefulness, light exposure was fixed, with illuminance set below 50 lux in study 1 and below 100 lux in study 2. Ambient temperature was kept at 22 ± 1 °C (mean ± SD) during both studies, except during the constant routine in study 1 when it was 24 ± 1 °C.

Cognitive performance tests were administered at least every 2 h during scheduled wakefulness. Subjects performed the same types and the same number of performance tests regardless of condition assignment in study 1, and likewise in study 2. Between performance tests, meals, and sleep opportunities, subjects were allowed to read, watch movies, play games, and talk with other study participants and research staff. Beside the stepping exercise in study 1, subjects were not allowed to engage in vigorous physical activity. Trained research assistants carefully monitored the subjects 24 h per day and kept them awake during scheduled waking periods.

Both studies conformed with the Recommendations from the Declaration of Helsinki of 1983. The studies were approved by the Institutional Review Board of Washington State University. All subjects gave written, informed consent, and were financially compensated for their time.

Measurements

HR and HRV

In both studies, electrocardiography (EKG) was recorded at 4,096 Hz with a Holter monitor (DMS 300-3A; Bravo, Huntington Beach, CA, USA) using standard 5-lead electrode placement. The EKG was recorded continuously, with the exception of a portion of the second simulated shift day in study 1 (not relevant for data analyses) and brief periods around scheduled shower opportunities. The EKG was manually reviewed and ectopic beats were removed. The EKG records were then analyzed using CardioScan software (version 11.4; Stateline, NV, USA). EKG data were binned into 5-min epochs. Epochs with movement artifact were removed.

For each epoch, the average HR and the high frequency (HF) component of the HRV power spectrum were calculated. The HF component of HRV (HF-HRV) included frequencies ranging from 0.15 to 0.40 Hz, as proposed by the American Heart Association[28]. There is broad concensus that HF-HRV represents parasympathetic activity[29]. We did not consider a low frequency (LF) component of the HRV power spectrum, previously believed to reflect mostly sympathetic activity[30], as there is mounting uncertainty regarding the reliability of that interpretation[29, 31].

In study 1, the EKG data from 15:00 to 22:00 on the first day in the laboratory were used to determine baseline HR and HF-HRV during wakefulness (Fig. 1, light green bars). The EKG data from 22:00 on the first day to 06:00 on the second day were used to determine baseline HR and HF-HRV during sleep (Fig. 1, light gray bars). The EKG data from the third simulated shift day were used to compare HR and HF-HRV between the simulated DS or NS schedules. Data from 06:00 to 22:00 in the DS condition and from 18:00 to 10:00 in the NS condition were used to determine HR and HF-HRV during simulated shift work when subjects were awake (Fig. 1, dark green bars). Data from 22:00 to 6:00 in the DS condition and from 10:00 to 18:00 in the NS condition were used to determine HR and HF-HRV during simulated shift work when subjects were scheduled to sleep (Fig. 1, dark gray bars). The EKG recordings obtained during the 24-h constant routine were used to assess the endogenous circadian rhythm in HR and HF-HRV. Epochs recorded during the constant routine that overlapped with neurobehavioral testing (15 min every 2 h, beginning 2 h after waking), intravenous (iv) catheter insertion or removal for blood sampling, or bathroom breaks (and 5 min intervals after bathroom breaks) were discarded.

In study 2, only the 24-h period from 12:00 on the second day until 12:00 on the third day was used for analysis of HR and HF-HRV in each of the four watch sections (Fig. 2, red dotted lines). This 24-h period is representative of the differences between the watch sections, and serves to illustrate the complex interactions of multiple factors influencing cardiac autonomic activity. Due to technical failure, the data from one subject in the 3/9-S watch section was lost. With regard to the EKG recordings, therefore, the sample size for study 2 was N=14.

Activity levels and sleep

Actigraphic data were analyzed using Actiware software (version 6.0; Respironics, Bend, OR, USA). The number of activity counts, recorded in 1-min epochs, was averaged into 5-min bins. For study 1, the 5-min bins from 15:00 until 22:00 on the first day in the laboratory were used as estimates of waking physical activity at baseline (Fig. 1, light green bars). The 5-min bins from the third simulated shift day were used as estimates of waking physical activity during shift work: from 06:00 to 22:00 in the DS condition, and from 18:00 to 10:00 in the NS condition (Fig. 1, dark green bars).

Polysomnographic recordings of sleep periods in study 1 were scored according to the criteria of the American Academy of Sleep Medicine[32]. Total sleep time (TST) was assessed for the baseline night (Fig. 1, light gray bars) and for the sleep period of the third simulated shift day (Fig. 1, dark gray bars).

Statistical analysis

Statistical analyses were performed using SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA). Unless noted otherwise, data were analyzed with mixed-effects analysis of variance[33] (ANOVA). For study 1, subject-specific baseline values for wake (average over 15:00 to 22:00 on day 1; Fig. 1, light green bars) and sleep (average over 22:00 day 1 to 06:00 day 2; Fig. 1, light gray bars) were included as a covariate in analyses of HR and HF-HRV for wakefulness and sleep, respectively, during simulated shift work. Analyses focused on physical activity included the 5-min averages of activity counts from actigraphy as covariate. For study 2 analyses, watch section (5/15-A, 5/15-B, 3/9-S, 3/9-C) was included as a covariate. All mixed-effects analyses included a random effect on the intercept over subjects to account for idiosyncratic inter-individual differences in the magnitude of the signals analyzed. Estimates are provided as marginal mean ± SE (unless otherwise noted). Figures show raw hourly means and SEs by condition.

Circadian rhythmicity was analyzed with a non-linear, mixed-effects regression implementation of cosinor analysis[34]; the 5% data with the most extreme residuals were excluded. Rhythm parameters, which included amplitude, acrophase (timing of the peak), and mesor (center value), were tested and compared between conditions with t tests embedded in the cosinor analysis. Statistical testing for an effect of time awake was done using linear mixed-effects regression; this analysis was applied to the residuals of the cosinor analysis across the two conditions in order to avoid colinearity in parameter estimation.

HR and HF-HRV data were analyzed based on 5-min epochs. However, for data from the stepping exercise in study 1, the three 5-min epochs covering each exercise session were averaged into a single 15-min bin, and the data collected prior to each exercise session were also averaged into 15-min bins, beginning 15 min after scheduled awakening (in order to exclude potential confounds due to sleep inertia[35]). Reactivity to the stepping exercise was quantified as the difference between the 15-min bin containing the exercise session and the 15-min bin immediately pre-exercise.

Results

Effects of simulated shift work on HR and HF-HRV during constant routine

The constant routine protocol in study 1 allowed for measuring the endogenous circadian rhythms of HR and HRV. Before focusing on the HR and HF-HRV data, we considered the effect of the simulated shift work that preceded the constant routine protocol (Fig. 1) on the timing of the circadian pacemaker. We found that markers of the circadian pacemaker, melatonin and cortisol, did not show a substantial shift in timing comparing DS to NS[36].

Despite the constant conditions under which the HR and HF-HRV data were measured, these data showed pronounced rhythms in both conditions (Fig. 3). Regardless of condition, HR was high during the afternoon hours and low during the nighttime hours. HF-HRV peaked earlier in the day than HR—especially in the NS condition, which showed an HF-HRV rhythm that was approximately inverse to the HR rhythm. Cosinor analysis confirmed significant 24-h rhythmicity in HR in the DS condition (t_{13}=6.67, p<0.001) and the NS condition (t_{13}=19.77, p<0.001). The amplitude of the 24-h rhythm was 1.0 bpm (± 0.2 bpm) for the DS condition and 3.0 bpm (± 0.2 bpm) for the NS condition. The difference between conditions of 2.0 bpm was statistically significant (t_{12}=9.27, p<0.001). The mesor (center value) was 64.6 bpm (± 3.4 bpm) in the DS condition and 64.4 bpm (± 3.4 bpm) in the NS condition, which was not significantly different (t_{12}=0.03, p=0.97).The acrophase (peak) of the 24-h rhythm in HR occurred at 14:34 (± 34 min) in the DS condition and at 16:43 (± 10 min) in the NS condition. The 129 min delay in the NS condition relative to the DS condition was statistically significant (t_{12}=3.61, p=0.004). After accounting for 24-h rhythmicity, there was no evidence of an effect of time awake in the HR data of the two conditions (t_{13}=0.37, p=0.72).

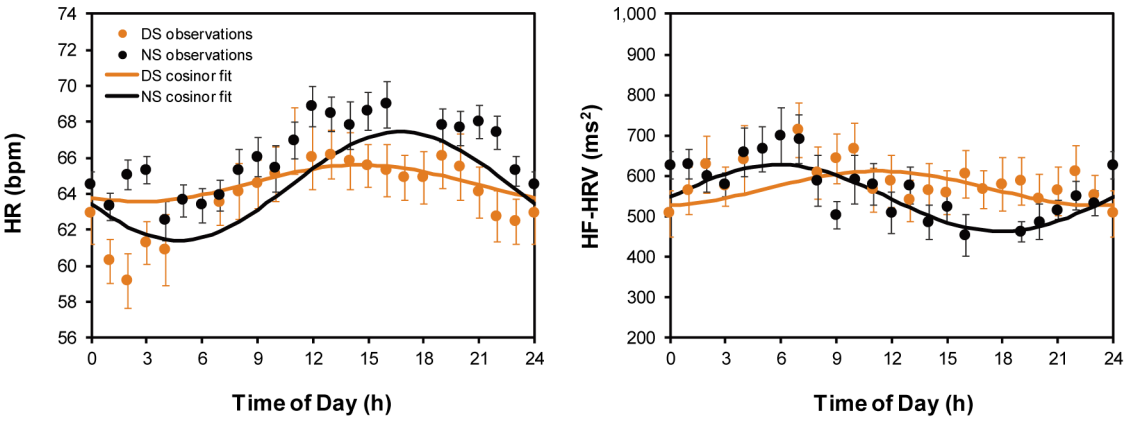

Fig. 3. HR (left) and HF-HRV (right) during the 24-h constant routine for the day shift (DS) and night shift (NS) conditions. Dots represent observed means; error bars indicate ± 1 standard error. Curves represent fitted 24-h rhythms. Data and curves are projected onto a 24-h axis from midnight to midnight.

Cosinor analysis also confirmed significant 24-h circadian rhythmicity in HF-HRV in the DS condition (t_{13}=4.81, p=0.001) and the NS condition (t_{13}=8.32, p<0.001). The amplitude of the 24-h rhythm was 42.4 ms^2 (± 8.8 ms^2) for the DS condition and 82.2 ms^2 (± 9.9 ms^2) for the NS condition. The difference between conditions of 39.8 ms^2 was statistically significant (t_{12}=3.01, p=0.011). The mesor (center value) was 569.5 ms^2 (± 117.9 ms^2) in the DS condition and 545.3 ms^2 (± 117.9 ms^2) in the NS condition, which was not significantly different (t_{12}=0.15, p=0.89). The acrophase (peak) of the 24-h rhythm of HF-HRV occurred at 11:16 (± 53 min) in the DS condition and at 05:53 (± 24 min) in the NS condition. The 323 min advance in the NS condition relative to the DS condition was statistically significant (t_{12}=5.50, p<0.001). The difference in the acrophase is substantial, but visual inspection of the data (Fig. 3) suggests that some outliers that remained in the data may have skewed the cosinor fit in the DS condition, resulting in an apparent delay of the estimate of the acrophase of HF-HRV in that condition. Potentially connected, after accounting for 24-h rhythmicity, there was a small but statistically significant effect of time awake in the HF-HRV data (t_{13}=2.21, p=0.046), with HF-HRV decreasing by 1.6 ms^2 (± 0.7 ms^2) for every hour awake.

Effects of simulated shift work on HR and HF-HRV during wakefulness and sleep

The simulated shift work period preceding the constant routine protocol in study 1 allowed for measuring the effects of shifted wakefulness and sleep periods on HR and HRV. Before investigating the effects of wakefulness and sleep, we examined the polysomnographic records of nighttime baseline sleep (Fig. 1, light gray bars) and nighttime or daytime sleep at the end of the three-day simulated shift work period (Fig. 1, dark gray bars). Baseline TST was 416.1 min (± 15.5 min) for the DS condition and 431.4 min (± 15.5 min) for the NS condition, which was not significantly different ($F_{1,12}$=0.49, p=0.50). TST for the third simulated shift day was 441.1 min (± 16.6 min) in the DS condition (i.e., nighttime sleep) and 392.4 min (± 16.6 min) in the NS condition (i.e., daytime sleep). Thus, daytime sleep in the NS condition was 48.7 min (± 23.5) min shorter than nighttime sleep in the DS condition; the difference approached statistical significance ($F_{1,12}$=4.32, p=0.060).

The temporal profiles of HR and HF-HRV during the baseline day and night (Fig. 1, light green and light gray bars, respectively) and during the wakefulness and sleep periods of the third simulated shift day (Fig. 1, dark green and dark gray bars, respectively) are shown in Fig. 4. Mixed-effects ANOVA of the HR data during baseline wakefulness, as a function of condition and time of day, showed no significant main effect of condition ($F_{1,986}$=0.01, p=0.92). There was, however, a significant main effect of time of day ($F_{83,986}$=13.64, p<0.001), with substantial changes in HR over time in both conditions. HR was lowest around hours 17:00 and 18:00, when subjects were mostly seated for baseline blood sampling procedures. The interaction of condition by time of day was also significant ($F_{83,986}$=1.45, p=0.007). HR was slightly greater in the NS condition around hour 15:00. Overall, HR during baseline wakefulness was comparable between the two conditions.

For scheduled sleep at baseline, there was no significant main effect of condition on HR ($F_{1,1067}$=1.85, p=0.17),

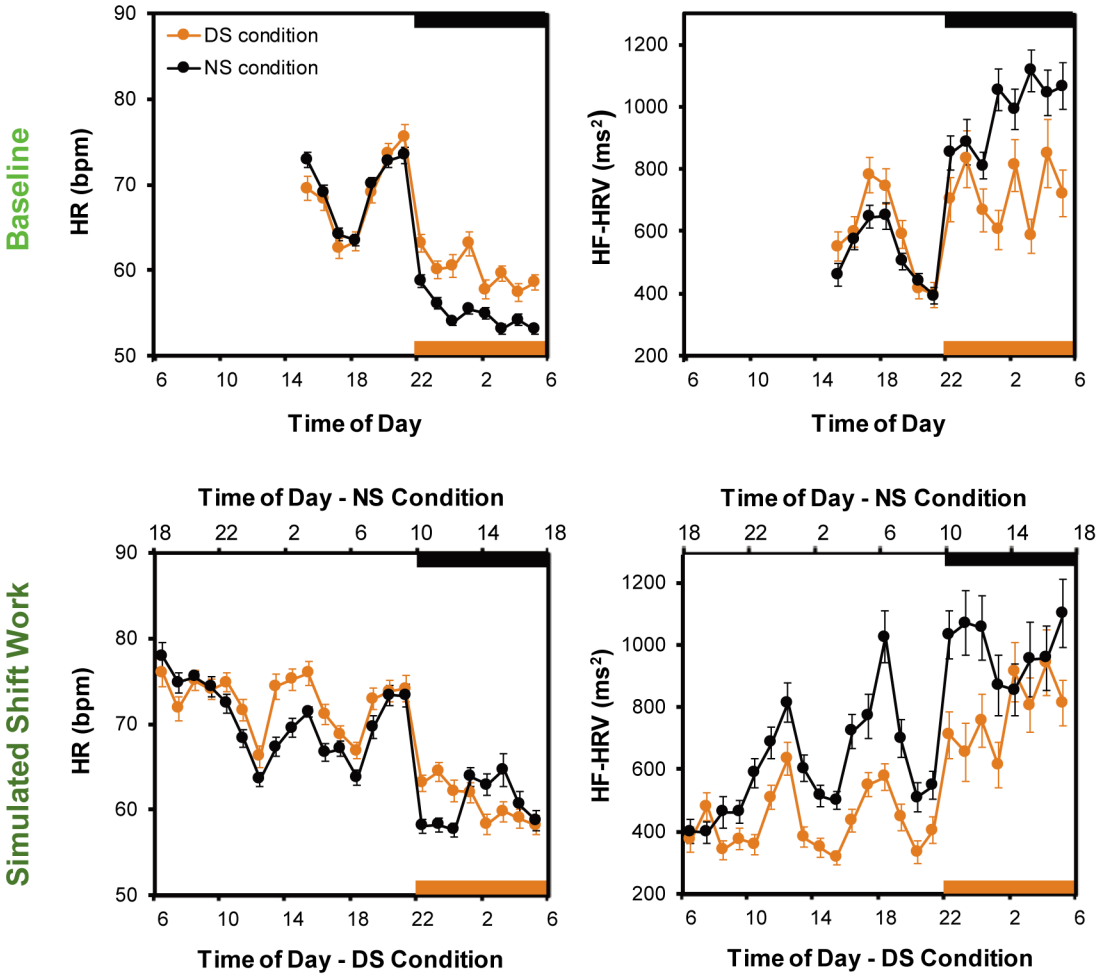

Fig. 4. HR (left panels) and HF-HRV (right panels) for the day shift (DS; orange) and night shift (NS; black) conditions at baseline (top panels) and during simulated shift work (bottom panels). For the bottom panels, the top axis indicates time of day for the night shift condition, and the bottom axis indicates time of day for the day shift condition. Dots represent observed means; error bars indicate ± 1 standard error. Orange horizontal bar, scheduled sleep opportunity for the DS condition; black horizontal bar, scheduled sleep opportunity for the NS condition.

but there was a significant main effect of time in bed ($F_{95,1067}$=3.82, p<0.001), with HR gradually decreasing over time in bed. The interaction of condition by time in bed was also significant ($F_{95,1067}$=1.30, p=0.032). The NS condition displayed a greater decrease in HR than the DS condition as time in bed progressed (Fig. 4, top left).

During wakefulness on the third day of simulated shift work, there was a trend for a main effect of condition on HR after controlling for baseline ($F_{1,2173}$=3.15, p=0.076). HR was greater in the DS condition (73.2 ± 1.5 bpm) than in the NS condition (69.5 ± 1.5 bpm). There was also a significant main effect of time awake ($F_{191,2173}$=10.10, p<0.001). As during baseline, there were substantial changes in HR over time that were common to both conditions. The interaction of condition by time awake was also significant ($F_{190,2173}$=1.59, p<0.001). The DS condition had greater HR than the NS condition about halfway through the simulated work day.

For scheduled sleep during the third day of simulated shift work, there was a trend for a main effect of condition on HR after controlling for baseline ($F_{1,1055}$=3.01, p=0.083). There was also a significant main effect of time in bed ($F_{95,1055}$=1.28, p=0.043) and a significant interaction of condition by time in bed ($F_{95,1055}$=2.15, p<0.001). HR decreased across the nighttime sleep period in the DS condition, whereas it initially increased and then decreased during the daytime sleep period in the NS condition (Fig. 4, bottom left).

The HF-HRV data were essentially a mirror image of the HR data. Mixed-effects ANOVA of the HF-HRV data during baseline wakefulness, as a function of condition and time of day, showed no significant main effect of con-

dition on HF-HRV ($F_{1,986}$=0.15, p=0.70). There was, however, a significant main effect of time of day ($F_{83,986}$=4.24, p<0.001), with substantial changes in HF-HRV over time in both conditions. HF-HRV was highest around hours 17:00 and 18:00, when subjects were mostly seated for baseline blood sampling procedures. The interaction of condition by time of day was not significant ($F_{83,986}$=0.81, p=0.89). HF-HRV during baseline wakefulness was comparable between conditions.

For HF-HRV during scheduled sleep at baseline, there was no significant main effect of condition ($F_{1,1067}$=0.54, p=0.46) and no significant main effect of time in bed ($F_{95,1067}$=0.86, p=0.82). There was, however, a significant interaction of condition by time in bed ($F_{95,1067}$=1.41, p=0.008). The NS condition displayed a greater increase in HF-HRV than the DS condition as time in bed progressed (Fig. 4, top right).

During wakefulness on the third day of simulated shift work, there was a significant main effect of condition on HF-HRV after controlling for baseline ($F_{1,2174}$=5.32, p=0.021) and a significant main effect of time awake ($F_{191,2174}$=4.83, p<0.001). As during baseline, there were substantial changes in HF-HRV over time that were common to both conditions. The interaction of condition by time awake was also significant ($F_{190,2174}$=1.62, p<0.001). The DS condition had lower HF-HRV than the NS condition for the larger part of the simulated shift day, beginning a few hours after awakening.

For scheduled sleep during the third day of simulated shift work, there was no significant main effect of condition on HF-HRV after controlling for baseline ($F_{1,1055}$=0.18, p=0.67). There was also no significant main effect of time in bed ($F_{95,1055}$=0.82, p=0.89) and no significant interaction of condition by time in bed ($F_{95,1055}$=0.87, p=0.80). Despite the lack of statistical significance when accounting for baseline differences, the pattern of HF-HRV during sleep on the third shift day was similar (but mirrored) to that of HR for both conditions (Fig. 4, bottom right).

Effects of physical activity on HR and HF-HRV

Figure 5 shows the average number of activity counts per minute during wakefulness for the DS and NS conditions, at baseline (Fig. 1, light green bars) and during simulated shift work (Fig. 1, dark green bars). Mixed-effects ANOVA of the activity data during baseline wakefulness, as a function of condition and time of day, showed no significant main effect of condition ($F_{1,996}$=2.42, p=0.12). There was, however, a significant main effect of time of day ($F_{83,996}$=15.11, p<0.001) and a significant interaction

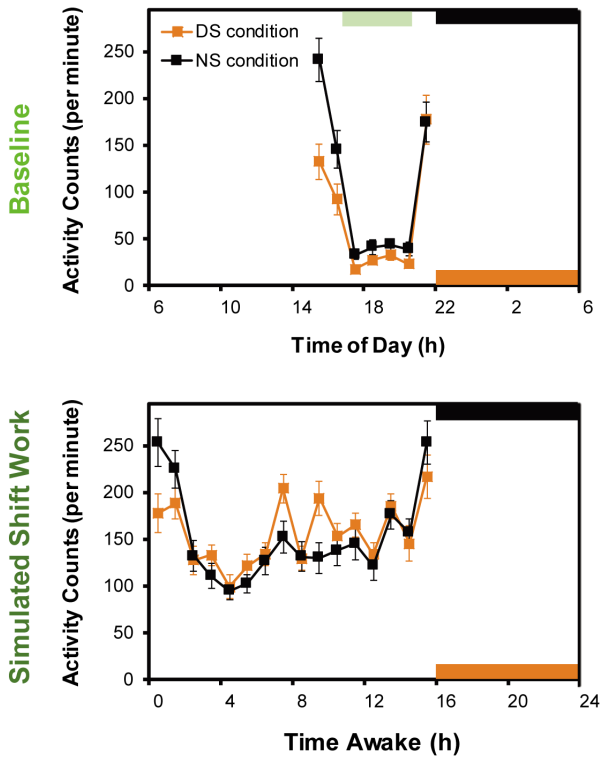

Fig. 5. Mean number of activity counts for the day shift (DS; orange) and night shift (NS; black) conditions at baseline (top) and during simulated shift work (bottom). Squares represent observed means; error bars indicate ± 1 standard error. Orange horizontal bar, scheduled sleep opportunity for the DS condition; black horizontal bar, scheduled sleep opportunity for the NS condition; light green horizontal bar, blood sampling period requiring subjects to be seated most of the time.

of condition by time of day ($F_{83,996}$=1.33, p=0.029). During wakefulness on the third day of simulated shift work, there was likewise no significant main effect of condition ($F_{1,2101}$=0.02, p=0.89). There was, however, a significant main effect of time awake ($F_{191,2101}$=4.43, p<0.001), as well as a significant interaction of condition by time awake ($F_{191,2101}$=1.40, p<0.001). The DS condition exhibited greater activity than the NS condition halfway through the simulated shift day (Fig. 5, bottom panel). Overall, the differences in activity between the two conditions were small.

Mixed-effects ANOVA with average activity counts from wrist actigraphy as a covariate revealed that baseline activity was a significant covariate of both baseline HR ($F_{1,985}$=233.47, p<0.001) and baseline HF-HRV ($F_{1,985}$=25.35, p<0.001). An increase of one activity count was associated with an increase of HR by 0.022 bpm (± 0.001 bpm) and a decrease of HF-HRV by 0.43 ms^2

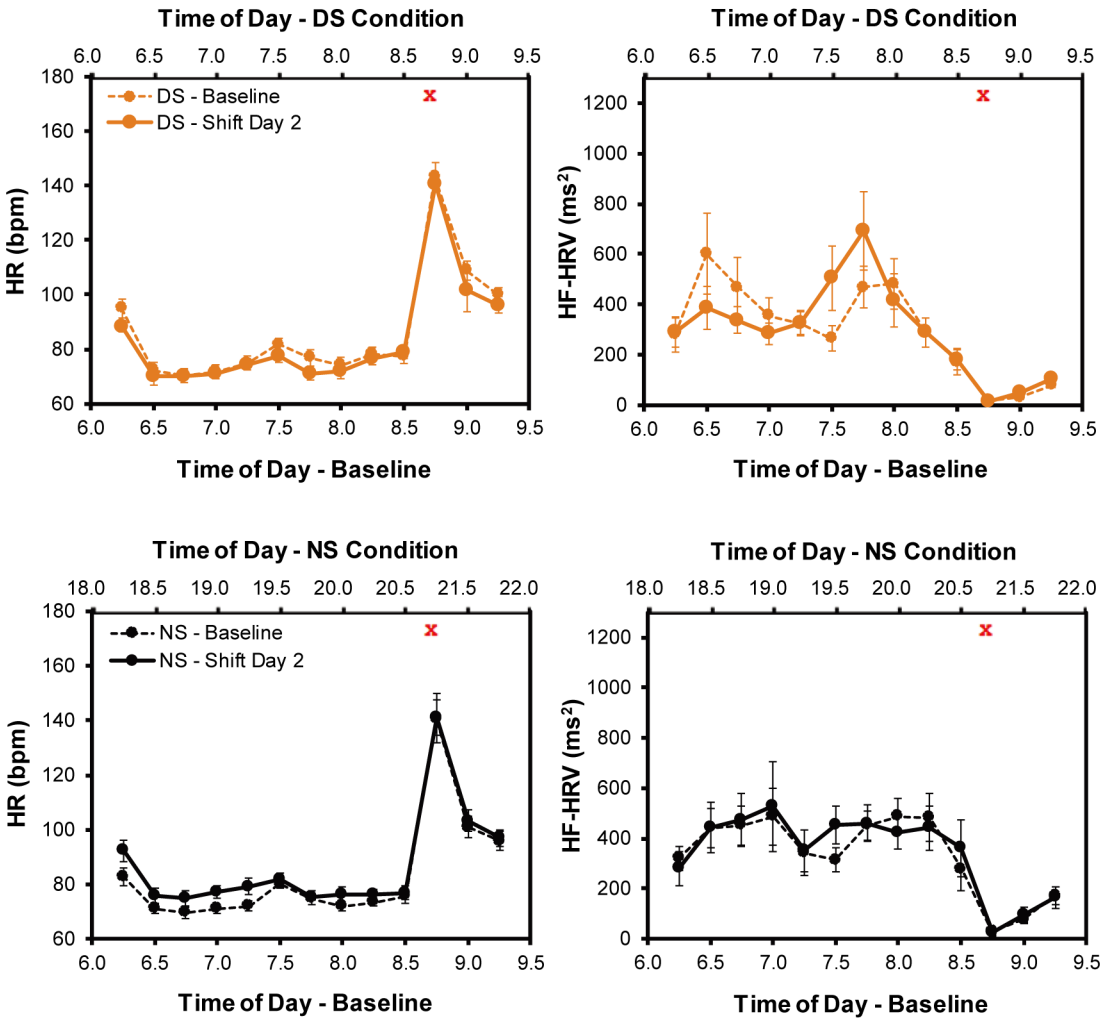

Fig. 6. HR (left panels) and HF-HRV (right panels) for the stepping exercise in the day shift (DS; orange) and night shift (NS; black) conditions, at baseline (dotted lines) and during simulated shift work (solid lines). Data are shown for 150 min (10 data points) before exercise, 15 min (1 data point) during exercise, and 30 min (2 data points) after exercise. Dots represent observed means; error bars indicate ± 1 standard error. Red markings, stepping exercise.

(± 0.09 ms^2). Activity during the third day of simulated shift work was also a significant covariate of both HR ($F_{1,1983}$=399.15, p<0.001) and HF-HRV ($F_{1,1983}$=57.17, p<0.001) during the simulated shift work day. An increase of one activity count was associated with an increase of HR by 0.020 bpm (± 0.001 bpm) and a decrease of HF-HRV by 0.40 ms^2 (± 0.05 ms^2)—very similar to what was found for baseline.

Effects of exercise during simulated shift work on HR and HF-HRV

The study 1 protocol contained two stepping exercise sessions, scheduled at 2 h and 45 min of scheduled wakefulness at baseline and on the second shift day (Fig. 1). The stepping exercise served as a controlled procedure to measure the cardiac autonomic activity response to more intense physical activity. Figure 6 shows HR and HF-HRV pre-exercise, during exercise, and immediately post-exercise at baseline and on the second day of simulated shift work in the DS or NS conditions.

There were no differences between conditions in pre-exercise HR ($F_{1,124}$=0.08, p=0.78) on the second day of simulated shift work. Mixed-effects ANOVA of exercise reactivity in HR, as a function of condition (DS vs. NS) and session (baseline day vs. second simulated shift work day), showed no significant effects of condition ($F_{1,7}$<0.01, p=0.97), session ($F_{1,7}$<0.01, p=0.97), or their interaction ($F_{1,7}$=0.51, p=0.50). Similarly, there were no differences between conditions in pre-exercise HF-HRV ($F_{1,124}$=0.08, p=0.78). Mixed-effects ANOVA of exercise reactivity in

HF-HRV also showed no significant effects of condition ($F_{1,7}$=0.42, p=0.54), session ($F_{1,7}$=2.11, p=0.19), or their interaction ($F_{1,7}$=1.15, p=0.32).

Combined effects of endogenous circadian rhythm, sleep/wake state, and physical activity on HR and HF-HRV

In study 2, which involved laboratory-based shift work schedules simulating real-world, around-the-clock Naval operations (Fig. 2), we set out to assess the combined and interacting effects of the circadian pacemaker, sleep/wake state, and physical activity on HR and HF-HRV. Figure 7 shows mean HR and HF-HRV for each watch section across time of day, from 12:00 on the second day until 12:00 on the third day.

Mixed-effects ANOVA of HR, as a function of time of day and state (scheduled sleep versus wake vs. simulated watchstanding), with watch section as a covariate, showed a significant main effect of time of day ($F_{287,3173}$=4.20, p<0.001) and a significant main effect of state ($F_{2,3173}$=639.63, p<0.001). There was also a significant interaction of time of day by state ($F_{358,3173}$=2.83, p<0.001). There was no significant effect of watch section as a covariate ($F_{3,3173}$=1.98, p=0.12).

Mixed-effects ANOVA of HF-HRV showed no significant main effect of time of day ($F_{287,3173}$=1.10, p=0.12). There was, however, a significant main effect of state ($F_{2,3173}$=85.01, p<0.001) and a significant interaction of time of day by state ($F_{358,3173}$=1.29, p<0.001). There was no significant effect of watch section as a covariate ($F_{3,3173}$=2.16, p=0.090).

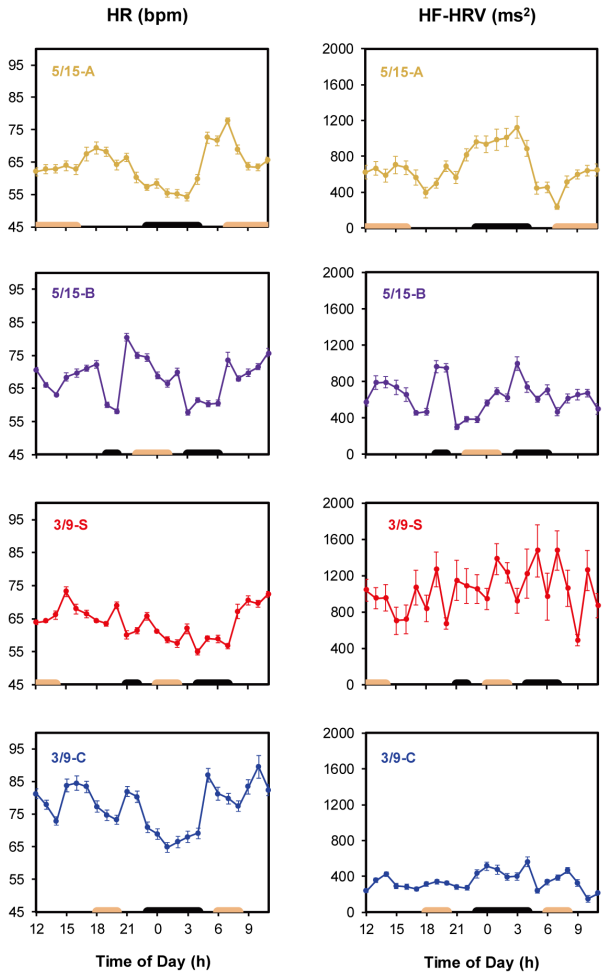

Fig. 7. HR (left panels) and HF-HRV (right panels) for the 5/15-A (yellow), 5/15-B (purple), 3/9-S (red), and 3/9-C (blue) watch sections. Dots represent observed means; error bars indicate ± 1 standard error. Black horizontal bars, scheduled sleep opportunities; orange horizontal bars, scheduled watchstanding.

Discussion

As has been demonstrated previously, cardiac autonomic activity is dynamically influenced by endogenous circadian rhythmicity[18–21] and by the timing of sleep[19, 23], as well as by waking physical activity, exercise, and posture[22, 24–26]. However, how these factors combine and interact under conditions of shift work, when there is misalignment between endogenous circadian rhythmicity and the timing of sleep and wakefulness, is not well documented. We investigated this issue in two laboratory-based, simulated shift work studies of HR and HRV, with study 1 (Fig. 1) dissociating the effects of endogenous circadian rhythmicity, sleep, waking physical activity, and exercise, and study 2 (Fig. 2) illustrating their interactions.

The constant routine procedure in study 1 permitted an assessment of the effect of simulated shift work on the endogenous circadian rhythm in HR and HF-HRV, while eliminating confounds known to influence cardiac autonomic activity, including sleep, food intake, physical activity, and posture. In line with previous field research that showed a lack of adaptation in circadian timing, even among permanent night shift workers[37], we found that the timing of endogenous circadian markers (melatonin and cortisol) after exposure to three days of simulated shift work remained *similar* for the DS and NS conditions[36]. The HR and HF-HRV data collected under constant routine showed significant 24-h rhythmicity that was congruent with the well-established circadian rhythm in cardiac autonomic activity[18–21, 38], and also *similar* for the DS and NS conditions (Fig. 3). It follows that the HR and HF-HRV rhythms were not produced by the preceding shift schedule, but rather reflected endogenous rhythmicity

driven by the circadian pacemaker.

The simulated shift days in study 1 permitted an assessment of the effects of wakefulness versus sleep, and temporal displacement of sleep, on HR and HF-HRV. During wakefulness on the third simulated shift day, the NS condition exhibited somewhat lower HR and higher HF-HRV than the DS condition, whereas there were no such differences between conditions at baseline (Fig. 4). This is consistent with a previous study that measured HR in simulated shift schedules, which also found lower waking HR in a night shift schedule as compared to a day shift schedule[39]. The negligible shifting of endogenous circadian timing in the NS condition indicates that this modulation may be influenced by the circadian rhythmicity of autonomic activity itself, potentially via projections from the SCN to the paraventricular nucleus (PVN) of the hypothalamus[40]. Also, during nighttime hours, activity in the nuclei of the ascending arousal system, including the locus coeruleus, is reduced, and is further inhibited by the VLPO nucleus of the hypothalamus[8]. Inhibition of the locus coeruleus, the major noradrenergic center of the brain, leads to disinhibition of parasympathetic nuclei[41], consistent with our findings of lower HR and higher HF-HRV during nighttime wakefulness in the NS condition.

The magnitude of changes in HR and HF-HRV over waking time was considerable (Fig. 4), especially when compared to the amplitude of the endogenous circadian rhythm (Fig. 3). This implies that there was substantial modulation of cardiac autonomic activity by other factors, such as physical activity and posture. Our actigraphy findings indicate that the systematic fluctuations in HR and HF-HRV across waking time (Fig. 4) could be largely explained by systematic variations in activity levels (Fig. 5). The relatively minor difference in activity levels between the DS and NS conditions halfway through the simulated shift day (Fig. 5, bottom), which is also reflected in HR (Fig. 4, bottom left), may be related to the opposite effect of the circadian pacemaker for the two conditions. That is, during this time in the simulated shift work protocol, the circadian pacemaker would have exerted a drive for wakefulness (and potentially more physical activity) in the DS condition and a drive for sleep (and potentially more sedentary behavior) in the NS condition. It should be noted, however, that in both conditions the overall level of physical activity, and the effect thereof on cardiac autonomic activity, was modest. This is corroborated by the results of the stepping exercise (Fig. 6), which increased HR and decreased HF-HRV regardless of condition to a much greater extent than the variations seen in response to regular waking physical activity (Fig. 4).

The overall impact of sleep on cardiac autonomic activity was about the same for the DS and NS conditions after correcting for baseline differences (Fig. 4), and similar to what has been found in previous work[39, 42]. However, the temporal dynamics of HR and HF-HRV across the sleep period were not the same between the two conditions. The stages of sleep are distributed differently in daytime sleep compared to nighttime sleep[43–45], and there are well-studied relationships between different sleep stages and cardiac autonomic activity[46, 47]. While beyond the scope of this paper, the different dynamics of HR and HF-HRV during the sleep period could thus be due to differences in sleep architecture between nighttime and daytime sleep. It is also possible that the effect of sleep on cardiac autonomic activity is modulated by endogenous circadian rhythmicity directly.

Our findings in study 1 indicate that behavioral factors, such as sleep, posture, and physical activity, may dominate temporal changes in cardiac autonomic activity relative to endogenous circadian rhythmicity. This was confirmed in study 2, which allowed for an integrated view of the cardiac autonomic activity modulators that were dissociated in study 1. In study 2, we found that regardless of the time of day, HR was lowest and HF-HRV was highest during scheduled sleep, and vice versa, during scheduled wakefulness (Fig. 7). The magnitude of change between scheduled sleep and scheduled wakefulness was larger than the magnitude of change between sedentary simulated watchstanding periods and other waking periods. Thus, in the laboratory-based, simulated shift schedules of study 2, sleep was the dominant driver of HR and HRV.

Limitations

Our studies have some limitations that warrant consideration. Subjects were healthy young adults, free from clinically significant medical conditions including sleep disorders such as obstructive sleep apnea (OSA). OSA, which typically increases in severity during daytime sleep[48], modifies cardiac autonomic activity[49]. As such, our results do not generalize to shift workers with OSA. Additionally, caffeine use was not permitted in our studies, though caffeine is commonly consumed by real-world shift workers[50] as a fatigue countermeasure. We did not address whether or how stimulants or other medications modulate cardiac autonomic activity in shift work settings. Eating habits—or even a single meal—can also alter HR and HRV[51]. Our studies were not designed to assess the impact of meal timing or meal composition on cardiac au-

tonomic activity, and any such effects are therefore intertwined with other factors modulating HR and HRV (especially in comparisons between waking and sleep). Further, we did not study the effects of chronic exposure to shift work, which over time may trigger allostatic mechanisms that modify autonomic activity to maintain homeostasis in the face of the recurring stress of shift work[52]. This could ultimately result in allostatic overload, potentially leading to cardiovascular disease[53].

Conclusion

Our simulated shift work studies investigated the separate and combined effects of various factors affecting cardiac autonomic activity. We found that sleep, physical activity, and exercise are powerful modulators of HR and HRV, whereas the effect of endogenous circadian rhythmicity is comparatively small. In the published literature, some field studies measuring cardiac autonomic activity showed a decrease in parasympathetic activity and/or an increase in sympathetic activity during night shift work[15, 16, 54, 55]. Other studies found the opposite[13, 17] or no difference between day and night shift schedules[14, 56]. These field studies, however, did not systematically account for the effects of activity, posture, stress, workload, use of stimulants such as caffeine, and/or presence of medical conditions affecting autonomic activity. In field research, these effects may be intertwined and act as confounds, which may explain some of the discrepancies in the published literature. Without measuring and controlling or accounting for such factors, therefore, the results of field studies of cardiac autonomic activity must be interpreted carefully—and conclusions regarding cardiovascular disease or risk based on measures of cardiac autonomic activity should be drawn with caution.

Acknowledgements

We thank the staff of the Sleep and Performance Research Center at Washington State University for their assistance in data collection. Study 1 was partially supported by start-up funds from the College of Pharmacy and Pharmaceutical Sciences at Washington State University (Gaddameedhi). Study 2 was supported by Naval Postgraduate School award N62271-13-M-1228 (Van Dongen). Data analysis was supported by Congressionally Directed Medical Research Program award W81XWH-16-1-0319.

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