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Light as a Chronobiologic Countermeasure for Long-Duration Space Operations

Alexander Samel and Philippa Gander, Editors

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Preface

This report presents the results of an investigation on how circadian rhythms and sleep are influenced by changes in posture and wake-rest schedule. The experiment is part of a series aimed at finding and estimating the power of natural means for avoiding or at least minimizing the impairing and disruptive effects of weightlessness and time shifts on the circadian system and sleep, as well as on performance and behavior.

NASA Ames Research Center, Life Science Division, has a long tradition in investigating and solving problems associated with postural changes induced by microgravity itself or by its simulation in the laboratory. Special emphasis has been placed on the functioning of different physiological systems, e.g., cardiovascular and endocrine functions, musculoskeletal system as well as on the testing and introduction of countermeasures for the prevention of impairment of these systems during simulated and actual space flights. A comprehensive knowledge of human, i.e., behavioral and physiological factors in the aviation environment has been developed by the Aerospace Human Factors Research Division. For several years, the Institute for Aerospace Medicine of the Deutsche Forschungsanstalt für Luft- und Raumfahrt (DLR-German Aerospace Establishment) in Cologne has been involved in the investigation of similar problems associated with the exposure of man to changes in gravity, pressure (underwater medicine), radiation and time structure. These two institutions have cooperated in mutual research activities in the field of aviation and space physiology over the years leading to intensive collaborations, which are continued by the present study.

For this purpose, a research associateship award of the National Research Council on behalf of the National Academy of Science was provided for a scientist of the Institute for Aerospace Medicine over a period of 17 months. The collaboration was further manifested through the provision of technical assistance, physiological devices, biochemical methods and analytical procedures. This report focuses not only on the practical results anticipated in the proposal, but also on the testing of theoretical assumptions and model calculations.

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Summary

Long-duration space missions require adaptation to work-rest schedules which are substantially shifted with respect to earth. Astronauts are expected to work in two-shift operations and the environmental synchronizers (zeitgebers) in a space craft differ significantly from those on earth. A study on circadian rhythms, sleep and performance was conducted by exposing four subjects to 6° head-down tilt bedrest (to simulate the effects of the weightless condition) and imposing a 12-h shift (6 h delay per day for two days). Bright light was tested in a cross-over design as a countermeasure for achieving faster resynchronization and regaining stable conditions for sleep and circadian rhythmicity. Data collection included objective sleep recording, temperature, heart rate, and excretion of hormones and electrolytes as well as performance and responses to questionnaires. Even without a shift in the sleep-wake cycle, the sleep quantity, circadian amplitudes and 24-h means decreased in many functions under bedrest conditions. During the shift days, sleepiness and fatigue increased, and alertness decreased. However, sleep quantity was regained, and resynchronization was completed within seven days after the shift for almost all functions, irrespective of whether light was administered during day-time or night-time hours. The time of day of light exposure surprisingly appeared not to have a discriminatory effect on the resynchronization speed under shift and bedrest conditions. The results indicate that simulated weightlessness alters circadian rhythms and sleep, and that schedule changes induce additional physiological disruption with decreased subjective alertness and increased fatigue. Because of their operational implications, these phenomena deserve additional investigation.

Operational Summary

Introduction

Long-duration space missions require adaptation to work-rest schedules which are substantially shifted with respect to earth. Astronauts are expected to work in two-shift operations, and the zeitgebers in a space craft differ significantly from those on earth. Bright light (>2500 lux) has been proposed to be an effective means to stabilize the circadian system, and to hasten resynchronization after shifts of environmental time.

Methods

The influence of simulated microgravity conditions and artificial environmental synchronizers (zeitgebers) on the circadian system, sleep and performance of four healthy males was studied during two periods of 14 days each. Both parts of the study started with control measurements of the circadian system over a period of three days at home, followed by eleven days of investigations on a variety of circadian functions, sleep and performance in an isolation unit under bedrest conditions with 6° head-down tilt. After laboratory baseline recordings of three days duration, the sleep-wake cycle was delayed by six hours on two consecutive days, resulting in a completely inverted daily routine, compared to the outside world. An exposure to artificial bright light (>3500 lux) (on the two shifting days and the day after) was carried out in a blind, cross-over design. Two subjects were exposed to the light for five hours each day before their expected circadian temperature trough in order to accelerate their circadian response to a delay of the sleep-wake cycle (experimental condition). The other two were exposed to bright light during the maximum of their circadian temperature cycle (control condition). The light exposure procedure was reversed during the second part of the study. The state of the circadian system was assessed by temperature and EKG readings as well as by determination of hormone and electrolyte excretion rates. Sleep duration and quality were estimated by polysomnography and sleep logs. Four different performance tests, as well as several questionnaires, were administered at 3-h intervals during wakefulness while the subjects were in the laboratory.

Results

Circadian rhythms– Disturbances of the circadian system were generated by the change of posture and the shift of the rest-activity cycle. In several body functions, the acute adaptation to the HDT bedrest condition led to a change of daily means (heart rate, sodium, cortisol, epinephrine) and a decrease of circadian amplitudes (heart rate, sodium, potassium, cortisol, norepinephrine). Chronic adjustment to bedrest was seen in very slowly increasing excretion rates for potassium and epinephrine. The delay of environmental zeitgebers mainly affected acrophases and amplitudes. Internal dissociation occurred. Resynchronization was achieved fastest (about three days after the shift) for the rhythms of sodium, norepinephrine, and heart rate. Moderate adaptation speeds (four to six days) were found for epinephrine, temperature and 6-hydroximelatoninsulphate (6-OHMS) rhythms. A complete resynchronization within seven days after the shift was not achieved for the cortisol and potassium rhythms. In general, the calculated resynchronization rates resembled those found in subjects undergoing transmeridian travel. One of the main hypotheses of the investigation, i.e., that exposure to bright light during different times of day would differentially affect

resynchronization rates, was not supported for the majority of body functions. Only 6-OHMS and potassium rhythm resynchronization speeds differed, depending on the time of day of light exposure. The direct influence of light on melatonin secretion was not surprising.

Model calculations showed that the influence of an additional zeitgeber, simulating the experimental and control protocols, resulted in faster adaptation than simply shifting the sleep-wake cycle.

Sleep– The 6° HDT-bedrest condition led to a reduction of total sleep time (TST) by about 5%, i.e., 20 min per sleep period. The proportions for light sleep (non-REM stages 1 and 2), slow-wave-sleep (NREM stages 3 and 4), and paradoxical sleep (REM) were not different from the values for age-matched ambulatory subjects sleeping horizontally. The change of environmental zeitgebers affected sleep quality for at least four days after the shift. The reduction of TST amounted to about 50 min each night when compared with the baseline bedrest condition, and about 70 min when compared to an age-matched ambulatory, horizontally sleeping group of subjects. These sleep periods were characterized by a higher portion of slow-wave-sleep mainly at the expense of NREM stage-2 sleep. The distribution of sleep stages changed in such a way that slow-wave-sleep was enhanced during the first half of sleep, and that awakenings were prolonged during the second half. REM sleep was reduced for at least four days after the shift during the last quarter of sleep. The timing of light exposures had no significant effect on sleep parameters.

Performance tests and questionnaires– Mean reaction times on the performance tests did not show circadian rhythmicity. The longer reaction times observed with the delay of the zeitgebers can be attributed to the effects of the accompanying sleep deprivation, rather than to circadian variation. Reaction times became progressively shorter over each (11 day) phase of the experiment, i.e., the learning process continued throughout the study. We cannot exclude the possibility that this extended learning period and/or adaptation to weightlessness obscured a time-of-day effect. The overall improvement in reaction times across each study phase suggests that the improvement due to learning overcame any adverse effects of the sleep debt accumulated during bedrest. In contrast, the responses to three (out of four) question-naires (i.e., alertness, fatigue, sleepiness) showed a clear circadian oscillation. During the shift condition, the ratings were significantly worse than during baseline and after the shift days, i.e., sleepiness and fatigue scores were enhanced, and subjects felt less alert. The different times

of light exposure did not have significantly different effects on the ratings.

Discussion

The present study represents the first experiment mea-suring circadian functions, objective sleep, and a set of performance tests under the condition of a combination of 6° head-down tilt bedrest and a 12-h shift of environ-mental zeitgebers. The combination was chosen in order to document the impact of shift schedules for space missions under earthbound conditions. We did not address the issues of changes of the musculoskeletal or the cardiovascular systems.

The results of this study indicate that the simulation of weightlessness may have an impact on circadian rhythmic functions. It also affected sleep by reducing the length of the sleep episode. The shift schedule led to a further deterioration of the circadian system and sleep quantity and a changed distribution of sleep stages. These changes were accompanied by impaired alertness and increased fatigue and sleepiness.

The second goal of the study was to test whether light treatment administered at different times of day could be used as a countermeasure for lessening these impairments. Our results do not indicate a time-of-day effect of light treatment. However, simulation results derived from a van der Pol oscillator model indicate that an additional zeitgeber simulating either the experimental or the con-trol protocol causes roughly the same acceleration in resynchronization of the circadian system, by comparison with a simple shift of the sleep-wake cycle. Therefore, our data do not exclude the possibility that treatment with light of at least 3500 lux can be a zeitgeber, but other potential zeitgebers (e.g., social interaction or activity) should also be considered in future investigations.

The present study has several limitations. First, the experiment was conducted with only four subjects, although the cross-over design strengthens the results. Some subtle effects might have been disclosed with a larger number of subjects. Second, polygraphic sleep and the urinary and performance variables were not moni-tored under normal ambulatory conditions with subjects sleeping in the horizontal position. Thus, results had to be compared to results from a different set of subjects and potentially different conditions. This may bias the interpretation of some results of this study.

In spite of these limitations, our results document that the circadian system, sleep and behavior of humans will be affected by space travel. Further investigations should extend the knowledge of the quantity and quality of the changes of these variables associated with long-duration

space operations. They should consider alternative and combined countermeasures for the prevention or at least minimization of the adverse effects of weightlessness and shift schedules.

An experiment of this magnitude cannot be performed without the support of many people. The Human Research Facility (Dee O'Hara) provided an excellent basis for the investigation. The subjects were expertly cared for by the HRF staff under the leadership of Joan Wilderman of Bionetics Corp.

Chapter 1: Overview

ALEXANDER SAMEL, PHILIPPA H. GANDER, and R. CURTIS GRAEBER

Background

Long-duration space missions require adaptation to work-rest conditions which are substantially different from normal routines on earth (ref. 1). First, astronauts are often expected to work in two-shift operations and are therefore forced to follow unusual duty-rest cycles. Second, the environmental time cues that are associated with space missions differ distinctly from those on earth (ref. 2). Third, astronauts are exposed to the reduction or absence of gravity and to a confinement situation that influences many psychophysiological functions (ref. 3). There is good reason to anticipate that these factors will cause alterations in the circadian regulatory system. The resulting instability of circadian state may lead to impairment of sleep quantity and quality (ref. 4), which may cause serious problems for the performance and productivity of the crew when operating during extended missions on the Space Shuttle, Space Station, or on a lunar or mars base.

To date, no systematic investigations of human circadian rhythmicity have been conducted during space operations. In Spacelab simulation studies, however, analyses of the 24-h rhythms of various physiological variables revealed internal dissociation due to shifts of the sleep-wake schedule during the mission (refs. 2 and 5). Changes in circadian parameters were also observed in bedrest studies with controlled, unaltered photoperiods (refs. 6 and 7). The deterioration of daily means and circadian amplitudes can be attributed to the alteration of the activity level, i.e., reduction of masking (refs. 8 and 9), and to the change of posture during bedrest (refs. 10 and 11). Furthermore, in investigations with light exposure similar to the Skylab environment, free-running rhythms were observed (ref. 12). These experimental results indicate that deterioration of circadian rhythmicity can occur even during missions without a change of the normal work-rest schedule. There is limited evidence from space-flight studies with other species that the light-dark cycle may be a less efficient synchronizer in microgravity, and that gravity may influence the period of the circadian pacemaker (refs. 13, 14, and 15).

The prolonged space flights and alterations of the activity-rest cycle planned for the dual-shift crew operations of future space missions would be expected to produce greater disturbances in the circadian system and sleep (refs. 4 and 16). Although the sleep-wake cycle will possibly be shifted prior to launch towards the schedule of the inflight duty-rest cycle, for several body functions the adjustment speed will be slower than for the sleep-wake cycle, thus causing a desynchronization between internal and external time systems (refs. 1 and 17). In space, this desynchronization may be enhanced due to the weak zeitgebers provided by the environment of the Space Shuttle or Space Station. Under these circumstances, the circadian system may never regain the normal steady state observed on earth. Additionally, operational demands may require further changes in work schedules during long duration missions.

Investigations on sleep in space were conducted either as self-reported sleep quality and duration (ref. 16), or as EEG- and EOG-recordings during Skylab missions (ref. 18). In general, these results indicate that disruptive factors generated by mission requirements and environmental influences often led to shortening of the average sleep duration during space flights relative to control periods on earth, as well as to impaired sleep quality. While results from systematic subjective sleep estimations or from polysomnographic recordings during space flights are very scarce, there are no previous data from bedrest studies.

Disturbances of sleep and circadian rhythms can negatively impact human performance. Sleep loss, either acute or chronic, results in diminished performance capability (refs. 19, 20, and 21). Performance, alertness and motivation were observed to depend upon the underlying circadian cycle in subjects during sustained wakefulness (refs. 22 and 23). Fatigue-related decrements in performance are therefore dependent on the amount of sleep as well as on the actual circadian phase (refs. 24 and 25). Consequently, acute phase shifts of the sleep-wake cycle can adversely affect human performance capability through desynchronization as well as the sleep deficit that often accompanies such phase shifts (refs. 20 and 26).

In other fields of chronobiological research, several methods are currently being discussed and tested for the stabilization of circadian rhythms or for an enhancement of resynchronization when the circadian system is disturbed. Exogenous melatonin can accelerate the adaptation speed of several physiological rhythms in the field (ref. 27) and in the laboratory (ref. 28). However, the increase, although significant, is small and very variable among subjects. A more promising technology appears to

be carefully timed exposure to bright light (ref. 29). Czeisler et al. found significant advance shifts after a delay of the sleep-wake cycle by 12 h, when exposing subjects to bright light (>>3500 lux) centered shortly after the temperature minimum for three consecutive cycles, compared with subjects exposed to darkness during their new subjective day (ref. 30). They repeated the protocol of "constant routine" in a study with shift workers, who were either exposed to bright light during their work hours at night and darkness during the day or exposed to ordinary light during night work and no regimen for times off work (ref. 31). Again, they found a considerable shift of the circadian system in the light treated group, but not in the other, along with significant improvements in both alertness and cognitive performance (ref. 31). Because of the different treatments, i.e., the 24-h control of light exposure in the experimental group versus no control of light exposure in control, the results cannot be applied directly to designing light strategies for the prevention or alleviation of jet-lag or maladaptation of shift-workers. The protocol used in these studies is also open to the criticism that the constant routine employed to "unmask" circadian phases is associated with a forced 12-h delay in sleep. Thus the overt temperature minimum may not be the circadian minimum. Because of the lack of data for at least three circadian cycles and therefore lack of knowledge about the transition process, it is pure speculation to explain the results by the use of mathematical models, as done by Czeisler et al. (ref. 30), and Strogatz (ref. 32). In spite of this weakness, the efficacy of light treatment is clearly worth pursuing. Honma et al. (ref. 33) studied the effects of single bright light pulses in two free-running subjects and could only produce phase advances. The most convincing results for the effectiveness of light treatment were presented by Wever et al. (ref. 29). In investigations with several subjects, they could expand the entrainment limits for temperature under free-running conditions from ±2.34 h (sd = 0.17 h) with a light-dark zeitgeber of 300:0.1 lux to about ±6 h when the intensity of illumination during the light phase was increased to 3000 lux (ref. 34). They also found that the temperature rhythm could be entrained by the strong light zeitgeber independently from the sleep-wake cycle.

The main objective of the present investigation was to • assess the response to bright light for various rhythm parameters in subjects who are living under simulated weightless conditions. The alterations of the circadian phase, 24-h mean, daily amplitude, and the length of the period can provide an estimation of the magnitude of desynchronization and dissociation among different body functions (refs. 35 and 36). Such changes in physiological functions, and also in sleep quantity and quality, are

expected to occur under actual conditions, i.e., during space missions (ref. 1).

Methodology

An experiment with 4 healthy, male subjects, aged 35 to 41 (mean ± sd = 38.0 ± 2.6) was performed during two periods each of 14 days duration (fig. 1.1). The subjects were selected on the basis of their willingness to remain alcohol and caffeine-free for 30 days, their availability for confinement to the bedrest facility during 11 consecutive days in each experimental period, results of a physical screening exam, and information obtained from a standardized confidential background questionnaire.

Each part of the study was initiated by three days of home baseline data collection in order to assess the circadian status under normal conditions. A 420g ambulatory physiologic monitoring system (PMS-8, Vitalog Corp., Redwood City, California) was used to record heart rate, nondominant wrist activity and rectal temperature. The subjects recorded information on sleep, diet and mood in a paper-and-pencil log. Additionally, the subjects were encouraged to synchronize to the laboratory daily routine (i.e., by going to bed at about 11 p.m. and getting up at about 7 a.m.), in order to reduce adaptation to laboratory conditions. A dietary restriction of no caffeine or alcohol was imposed throughout the length of each study interval.

Eleven days of each study period were spent in the Human Research Facility (HRF), an isolation unit of the Life Sciences Division at NASA Ames. In order to simulate weightless conditions and minimize the masking of circadian rhythmicity by physical activity, the investigation was performed as a bedrest study with 6° head-down tilt (HDT). Additionally, the environmental time cues were comparable to Space Shuttle or Space Station conditions, i.e., dim light of about 500 lux during the day and less than 20 lux during the sleep periods, and a shifted daily routine. At the beginning of the first 11-day period in the HRF, the subjects were randomly assigned to one of the two four-bed rooms, either as experimental or as control. Subjects were not allowed to raise their head, sit up, or get out of bed for the 10 days of bedrest. Any prone activity (e.g., reading; writing; watching movies but not regular TV; etc.) was acceptable during the study. Video movies were simultaneously presented to the subjects to reduce boredom and sleepiness during the bedrest period. Fluid intake was ad lib, and meals were provided three times a day. Identical meals were provided in the two parts of the study. Snacks were not allowed (except for a restricted selection of fruit upon request) in order to encourage similar meal patterns among subjects. Fluid

intake and output was measured. The lighting in the room was controlled on a 16L:8D cycle (except on time manipulation days) with lights off at 2300 h and on at 0700 h. Although each subject had access to an individual "emergency" bedlight, asleep (i.e., at least resting) and awakening times were controlled by the HRF staff. A thermoneutral horizontal shower was given between 1400 h and 1600 h local time on day 10.

The subjects underwent a complete 12-h time shift to an inverted sleep-wake schedule during the third and fourth day in the laboratory, i.e., days 7 and 8 of the schedule (fig. 1.1), by delaying the sleep period by six hours each day. During both parts of the study, all subjects were exposed to bright light of at least 3500 lux for five hours on the two shift days and the day after. The treatment light bulbs (Medic-Light Inc., Lake Hopatcong, NJ) emit light similar to the visible part of the sun's spectrum. Light intensity could be regulated between 7000 and 10,000 lux. The light banks were positioned on stands beside the bed with an angle of about 30° towards the subject's face. The actual brightness of light ranged between 8000 and 9500 lux when subjects looked directly into the light, and was between 3000 and 3700 when subjects looked to the ceiling. Finally when they looked to the wall on the opposite side of the bed to the light, the intensity was between 600 and 1750 lux, depending on distance and reflectance of the wall. Subjects were instructed to look in the direction of the light. The ordinary room illumination was between 400 and 700 lux and should not greatly affect the circadian system (ref. 37). The experimental group was exposed to bright light on days 7, 8 and 9 at a time coinciding with the declining phase of their individual circadian temperature rhythm (estimated for each individual from his preceding temperature minimum). These light pulses were predicted to enhance the rate of phase delay of the circadian system (ref. 30). The control group was exposed to the same light pulses, but centered on the peak of the temperature rhythm, where they were expected to have little or no phase shifting effect. During days 8 to 14 the sleep-wake cycle was inverted with sleep occurring between 1100 h and 1900 h baseline time. Recovery to the environmental time cues outside the laboratory (Pacific Standard Time) took place on day 14.

The light conditions were reversed between the groups during the second part of the study, thus reflecting a cross-over design. This design was chosen in order to obtain intraindividual responses to different experimental conditions.

For assessing the state and the dynamics of the circadian system, temperature, nondominant wrist activity and EKG were recorded continuously with the Vitalog

PMS-8. In addition, urine samples were collected at regular intervals of three hours during awake times and, if subjects needed to urinate, during sleep periods, for determination of hormone (cortisol, catecholamines, 6-hydroxy-melatoninsulfate) and electrolyte excretion rates (potassium, calcium, and sodium). Mental performance was assessed by cognitive, computerized tests every three hours from 0700 h until 2200 h each day for approximately 30 min each time. Four tests, a reaction time test (ref. 38), logical reasoning (ref. 39), and two memory and search tasks (ref. 40), were included in the test sessions. The amount and quality of sleep was evaluated by polysomnography. This procedure included electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) that was read by Medilog recorders (Oxford Inc.) during the night hours in the HRF. For this purpose, a total of nine electrodes were attached to the head, according to the international 10-20-system (A1, A2, C3, 2 ground-electrodes, 2 EOG-electrodes, and 2 EMG-electrodes).

Interindividual differences necessitated that the study have a cross-over, design, i.e., each subject served as his own control for the placebo, administered at a phase where it was expected to have no phase-shifting effect on the circadian system.

The timing of the experimental (as opposed to placebo) light treatment was designed to force the system to delay. Practical considerations argued for a delay, because subjects can sleep better and more easily after delaying sleep until six hours later than when trying to sleep six hours earlier. The duration (5 h) and intensity (>3500 lux) of the pulses was selected to enable direct comparison with the experiments of Czeisler et al. (ref. 30). The timing of the light treatment in this experiment was also chosen to test theoretical assumptions (refs. 41 and 42) about the mechanism of the action of light on the circadian system (see chapter 2).

Organization of this Report

This technical memorandum is divided into several chapters. This overview (chapter 1) describes the broad background and the methodological approach. In addition, the main goals and a discussion of the literature relevant to this experiment is presented.

Chapter 2 focuses on the temperature rhythm and the description of circadian dynamics by two models. The temperature rhythm is widely used as a marker of the human circadian system, because of its stability in time-free environments and because it is readily monitored continuously.

Because external desynchronization disrupts the stable relationships between rhythms in different body functions, and therefore intensifies jet-lag, chapter 3 deals mainly with internal desynchronization and dissociation, and reveals the influence of external factors on this phenomenon.

Chapter 4 focuses on sleep. Until this experiment, sleep had not been recorded objectively by means of EEG, EOG and EMG in head-down tilt bedrest conditions. The results will address the influence of weightless simulation and shift schedules on sleep quantity and sleep quality.

Finally, the results of the four performance tests as well as of the questionnaires are presented in chapter 5.

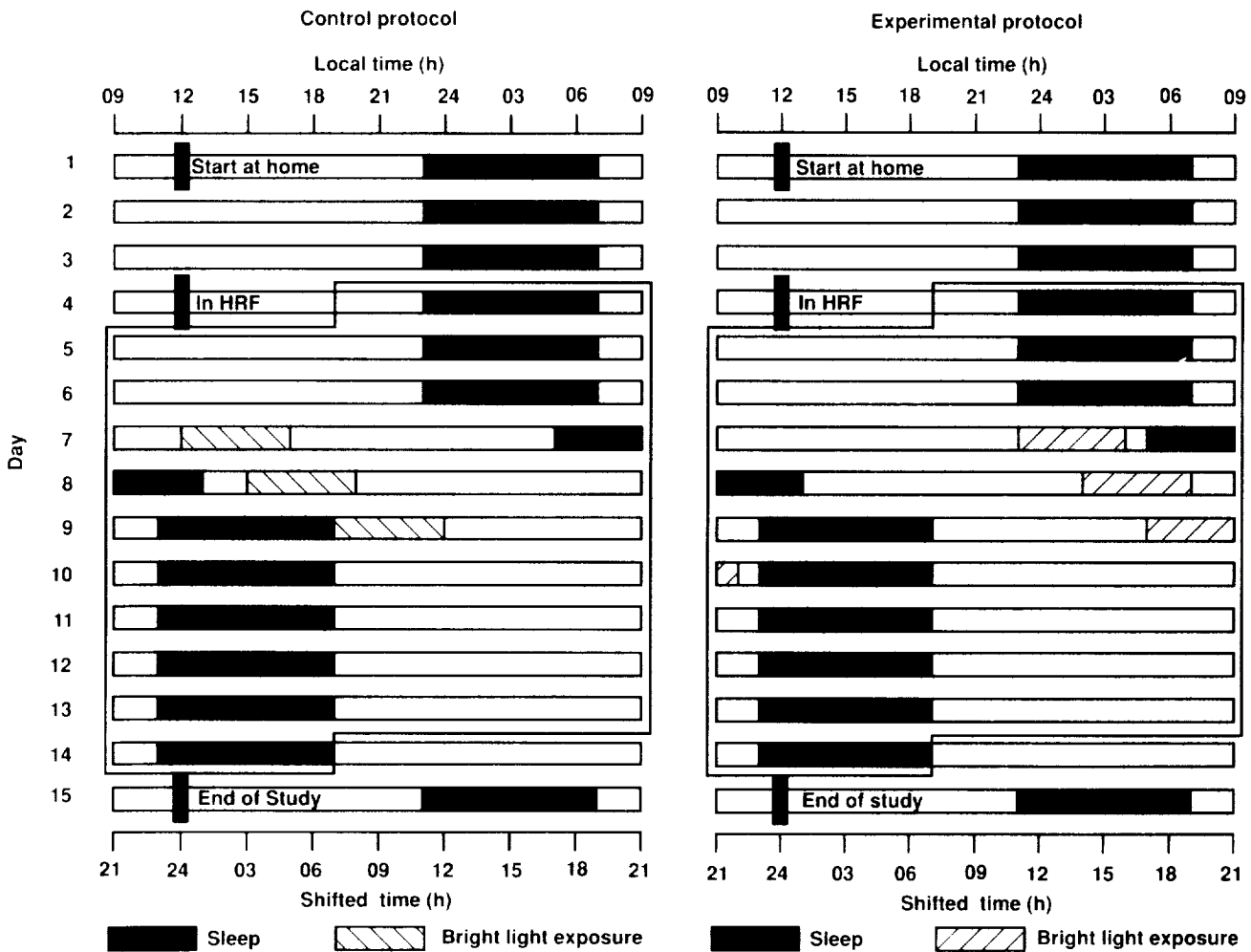Figure 1.1. Overview of the experimental protocol. The study days are indicated from top to bottom, the time of day (either before the shift, top, or after the shift, bottom) is denoted on the x-axis. Shifts of the sleep-wake cycle and theoretical light exposure times were as indicated in the figure. Actual times of light exposure were calculated for each individual from his preceding temperature minimum.

Chapter 2: The Circadian Rhythm of Temperature and Its Modeling

ALEXANDER SAMEL and PHILIPPA H. GANDER

Following the current convention, we used the temperature rhythm as a phase marker of the underlying circadian pacemaker. The estimation of circadian phase is a critical issue, because both animal and human studies suggest that the action of light pulses depends on where in the circadian cycle they occur. The bedrest protocol, used to simulate weightlessness, also limits physical activity. Therefore the masking effect of the rest-activity cycle on the temperature rhythm is minimal in this study, making phase estimation less complex.

A number of descriptive mathematical models for the circadian system have been proposed. Klein and Wegmann (refs. 2 and 43) have derived an exponential model to describe the rate of resynchronization of the circadian system after time-zone shifts. We have tested this model, which is based on data from field studies, against our laboratory data. Van der Pol oscillators have often been used in simulations of the circadian system in time-free environments and after zeitgeber shifts. We therefore also conducted simulation studies using a single Van der Pol oscillator and modifying parameter values to simulate individual differences in our temperature data.

Temperature

Methods

As mentioned before, temperature data (as well as heart rate and non-dominant wrist activity data) were collected in 2-min samples using a Vitalog system. For subsequent analysis, data were averaged over 20-min epochs and smoothed by robust, locally weighted regression (ref. 44). Circadian parameters (daily mean, diurnal amplitude, acrophase and diurnal minimum) were determined for temperature and heart rate by two different methods. In order to locate the temperature minima, the data sets were subjected to multiple complex demodulation (ref. 45). Diurnal means, circadian amplitudes and acrophases were estimated by sinusoidal fit procedures, shifting a 24-h window in 1-h intervals over the entire data set.

Results

Circadian waveforms– The raw data from all subjects and over the entire study periods are displayed in fig-ure 2.1. Data recording started at about 1300 on day 1 and continued for 14 cycles of 24 hours. Each data point represents a 20-min average. Some loss of data occurred, e.g., on days 12 and 13 of subject A during the control phase of the experiment (fig. 2.1(a)), in this case caused by malfunction of the recording system. Usually temperature data loss was due to extracting or damaging the rectal probe.

The individual data sets clearly indicate that the diurnal course of temperature is essentially similar in the two experimental conditions for each subject, although a four-week gap lay between the two recording periods. Obviously, circadian rhythms are characteristic for each individual, and different among subjects, e.g., high daily amplitudes under normal conditions (i.e., baseline days at home) in subjects A and D, in contrast to moderate amplitudes in B and low in subject E. The time of the circadian minimum on day 6 (the last night before the shift) was on average 0500 h (sd = 0.56 h, n = 8). The rhythm altered during the change of the environmental time structure and during the early days of the recovery phase. This behavior seemed to be independent of the time of light exposure.

Circadian timing of light pulses–For the experimental condition, the aim was to provide 5-h light pulses which ended at the time of the circadian minimum. In contrast, for the control condition, the light was timed to cover the circadian maximum. The timing of three pulses could not be determined, because the waveform was too disrupted to identify the adjacent circadian minimum. Two-way ANOVA of the remaining 21 pulses showed that the circadian timing of experimental and control pulses was significantly different (F = 95.01, p ≤ 0.0001) and that the circadian timing of pulses was not significantly different between subjects (F = 0.73, p = 0.5528). Experimental pulses began on average 5.3 h before the minimum, while control pulses began on average 8.9 h after the temperature minimum (fig. 2.2).

Nevertheless, the phase shifts of the temperature rhythm produced by experimental pulses (mean = –2.1 h) and control pulses (mean = –2.3 h) were not significantly different (one-way ANOVA, F = 0.04, p = 0.8474). Considering all the pulses together, the size of the phase shift produced was not significantly correlated with the circadian timing of the pulse (F = 0.01). We cannot exclude the possibility that the effects of the second and

third pulses were unpredictable, because the circadian system had not completely adapted to the first pulse. Therefore, we repeated the analysis for first pulses only. Control and experimental first pulses produced the same average phase shift (−2.5 h, one-way ANOVA, F = 0.00). Similarly, there was no significant correlation between the circadian timing of first pulses and the phase-shift they produced in the temperature rhythm (F = 0.01).

Rates of resynchronization– In both control and experimental conditions, resynchronization to the 12-h environmental time delay was achieved by a clear delay for three subjects (fig. 2.3). However, for subject E, the raw data as well as subsequent analysis did not indicate whether the rhythm shifted by a delay or an advance, although the temperature minima on days 8 and 11 suggest a delay. Significant circadian minima could not be identified for days 9 and 10 (fig. 2.3(d)).

Complex demodulation analysis and sinusoidal fit procedures resulted in nearly identical estimates of the time course of resynchronization. For the day-time light exposure, the correlation coefficient was r = 0.97 (F = 764.7; p < 0.001; df = 1,46) between complex demodulation minimum and sinusoidal acrophase assessments, and under the light-treatment-at-night condition, the correlation coefficient was r = 0.96 (F = 567.0; p < 0.001; df = 1,47).

Significant differences could be detected for circadian parameters when comparing baseline and shift conditions, on a day-to-day basis independent of the light exposure conditions (one-way ANOVA).

Acrophases were shifted significantly on day 7 when compared to baseline days 1 to 4 (p ≤ 0.05). A further shift took place on the following days, for days 8 to 13 were significantly different when compared to days 1 to 6 (p ≤ 0.005). Acrophases of days 9 to 13 differed significantly from those of days 1 to 8 (p≤ 0.001 for each comparison), exhibiting a further (and final) resynchronization towards the (new) environmental time. These numbers were assessed for both experimental and control conditions, i.e., light exposure during the subjective night and during the subjective day.

Daily means did not differ significantly during the entire experiment, compared to baseline (day 1 to 3 of each phase, fig. 2.4). On the other hand, circadian amplitudes on days 8 to 13 were significantly lower than on days 1 and 2 (p ≤ 0.05) for the light pulses administered during the subjective day (control condition). With light exposure during the night, significant reductions in amplitude with respect to baseline occurred only on days 8 and 9 (fig. 2.4). Additionally, day 9 values were significantly lower than day 4, 5, or 6 (p ≤ 0.05).

No significant differences could be found between the different light exposure conditions. Mean, acrophase and amplitude did not differ, when non-parametric statistics were applied (Wilcoxon matched-pairs signed-ranks test). None of the parameters differed significantly between conditions when one-way ANOVA was used to compare grouped shift days and grouped recovery days (table 2.1), except for daily mean levels which were significantly higher during the recovery days of the control protocol, i.e., when light was offered during the subjective day.

When combining both data sets for investigating changes in circadian rhythmicity imposed by postural alterations, the analysis did not reveal any significantly different values for diurnal level (F = 0.660, p = 0.422) and amplitude (F = 0.989, p = 0.326) nor shifts in acrophase (F = 0.054, p = 0.817). This analysis was conducted with the data from the three baseline days and two days of supine position before the time shift occurred.

Discussion

To discuss these results, it is necessary to take into account the different conditions during different stages of the experimental protocol. Circadian rhythmicity was influenced by a loosely controlled situation during baseline recording at the subjects' home, by comparison with the strictly controlled situation when subjects entered the laboratory. However, since subjects went to bed on arrival in the laboratory, we cannot separate the adaptation to the laboratory and the adaptation to head-down tilt. In the laboratory, the study protocol based on a matched cross-over design is the optimal experimental setup for evaluating the efficiency of light treatment.

Weightlessness simulation– The results of this study did not concur with findings from other investigations (refs. 2, 6, 11, and 17) that the temperature rhythm is affected by the change of posture. However, at home, activities during the day were very variable and sleep times very irregular, and thus the circadian rhythmicity of temperature was considerably masked leading to substantial variations in diurnal acrophases (fig. 2.3). Thus, statistical analyses could not reveal alterations in circadian parameters, although under controlled laboratory conditions, acrophases stabilized. Therefore, the results neither confirm nor refute possible posture-dependent changes in the circadian temperature rhythm which have been found, in some cases, to occur very slowly under simulated weightless conditions (refs. 2 and 11).

Table 2.1. Effect of different light exposure times on circadian parameters of temperature for various combinations of days. Significant differences between conditions are indicated by *, 0.05 > p ≥ 0.01 (matched t-test). NTLE: Night time light exposure; DTLE: Day time light exposure.

Days	Parameter	NTLE Mean ± SD		DTLE Mean ± SD		F-value	p-value	df	Significance
1-3	Mean	36.80	± 0.16	36.75	± 0.20	0.524	0.4842	1,11	ns
	Acrophase	16.3	± 2.3	15.4	± 1.5	1.240	0.2891		ns
	Minimum	3.6	± 2.0	4.3	± 2.0	1.195	0.2977		ns
	Amplitude	0.45	± 0.22	0.48	± 0.11	0.308	0.5899		ns
4-6	Mean	36.75	± 0.19	36.75	± 0.11	0.004	0.9485	1,10	ns
	Acrophase	16.7	± 1.4	16.6	± 1.5	0.183	0.6781		ns
	Minimum	4.4	± 1.2	4.4	± 1.9	0.038	0.8504		ns
	Amplitude	0.45	± 0.14	0.43	± 0.17	0.249	0.6284		ns
7-9	Mean	36.75	± 0.15	36.85	± 0.08	3.355	0.0969	1,10	ns
	Acrophase	21.1	± 3.0	21.1	± 3.3	0.008	0.9316		ns
	Minimum	9.7	± 3.2	9.2	± 3.5	0.537	0.4807		ns
	Amplitude	0.24	± 0.10	0.28	± 0.10	2.209	0.1680		ns
10-14	Mean	36.72	± 0.20	36.83	± 0.13	3.433	0.0814	1,17	ns
	Acrophase	3.2	± 1.4	3.1	± 1.4	0.189	0.6697		ns
	Minimum	15.7	± 1.7	15.2	± 1.2	1.085	0.3190	1,13	ns
	Amplitude	0.33	± 0.17	0.28	± 0.15	3.223	0.0902	1,17	ns
7-14	Mean	36.74	± 0.19	36.84	± 0.11	6.405	0.0171	1,29	*
	Acrophase	0.7	± 3.7	0.8	± 3.6	0.077	0.7834		ns
	Minimum	12.9	± 3.9	12.5	± 3.9	1.560	0.2257	1,23	ns
	Amplitude	0.29	± 0.15	0.27	± 0.13	0.462	0.5023	1,29	ns

Light treatment– Light treatment was expected to influence the diurnal course of temperature, but we could not find a significant difference between day-time and night-time light exposure. The major difference between our results and those of Czeisler et al. (ref. 30) is that in our data significant phase delays were observed with light pulses (either single or accumulated stimuli) given during the subjective day, whereas Czeisler's data implied minimal phase shifts at this time. The existence of a "dead zone" (where the circadian system is insensitive to light) in the middle of the circadian day is characteristic for light phase response curves in all species studied (ref. 46). This leads us to the alternative interpretation that the phase delays we observed in association with the control light pulses were in fact largely due to the delay of the wake-rest schedule. We also cannot exclude the possibility that the regular schedule of activities (meal times, performance tests, etc.) associated with the bedrest conditions and socialization contributed as zeitgebers to our result. The fact that the experimental light stimuli did not produce significantly greater delays than the controls suggests that even light in the delaying region of the phase response curve had a minimal effect.

Our data agree with Czeisler's in that the timing of pulses is critical in the region of the circadian minimum, i.e., a small delay in the timing of the pulse can switch the response from a delay to an advance. In this context, it is interesting to note that Honma et al. (ref. 33) could only produce phase advances with single light pulses under free-running conditions. Although much effort was expended in the estimation of the circadian phase (minimum), the on-line raw temperature data of subjects A and B indicated a faster adjustment after the first light pulse than really took place, as indicated by the off-line analysis. This resulted in the subsequent light pulse being given too late in the circadian cycle and producing a phase advance one day later, as shown in figure 2.3. Thus, in practice, light pulses do not appear to be a robust countermeasure for accelerating phase delays. We do not believe that masking has a major influence on circadian parameters during the bedrest condition. Both the crossover experimental design and the supine position of the subjects after adaptation to 6° HDT minimized the potentially misleading effects of masking.

We were able to show that the temperature rhythm adapts to a shift in the artificial laboratory zeitgebers (i.e., simulated change of the complete rest-activity cycle in combination with light treatment) comparably to the way it adapts after transmeridian flight when travellers or pilots expose themselves to various effective synchronizers (refs. 2 and 43). Thus, under conditions where natural zeitgebers are absent or weak, i.e., in a space craft, or during irregular work schedules, artificial synchronizers can be effective. In this context, we must emphasize that the "rapid" phase shifts found by Czeisler et al. (ref. 30) and mentioned by Strogatz (ref. 32) do not exceed those found by Klein and Wegmann for adaptation to real time-zone shifts (refs. 2 and 43). Thus, the conclusion drawn by Strogatz (ref. 32) "that it is possible to shift the human circadian clock more rapidly than we have ever thought possible" must be corrected. Both authors (refs. 30 and 32) also argue that the temperature rhythm can be reset by bright light via "Type 0" resetting, leading to extinction of the rhythm curve for at least one cycle. From our raw data it is clear that this kind of resetting was exhibited only by subjects A (night-time exposure, i.e., experimental condition) and E (day-time exposure; control condition), i.e., independent of the timing of the light pulse in the circadian cycle. Of course, the possibility remains that the circadian clock was driven to a singularity (stationary state) and then reset, but that the rhythm of temperature did not accurately reflect this process due to the coupling mechanism between the clock and the rhythm. However, in the six remaining cases, the treatment had only minimal effects on temperature rhythm amplitudes, although the resynchronization process was at least as fast. Indeed, only continuous recording of physiological variables (as we did) can answer the question of whether or not light pulses, of the intensity and duration used in Czeisler's experiments and ours, can really cause "Type 0" resetting. It is not sufficient to use mathematical models (topology (ref. 32)) to infer what happens to the circadian system between two discrete observations on either side of the light protocol, as was the case in Czeisler's experiments (ref. 30). Furthermore, the continuous transition of an oscillatory system like the temperature rhythm from one steady state into another can also be described by the more familiar Van der Pol oscillator (ref. 41), as discussed below.

Modeling

Resynchronization rates– The adaptation process of the temperature rhythm can be assumed to have a non-linear, asymptotic course between the old and the new environ-

mental times (ref. 43). The underlying model describes only roughly the response of the circadian system to time-zone transitions. Responses for different body functions varied greatly for individual subjects as well as between subjects.

However, the model can be used to describe formally the differences in dynamic behavior among individuals and body rhythms. The model can be described by the following mathematical equation (ref. 43):

$$R(t) = \Delta f \cdot e^{-\partial t}$$

where

t   time in days

Δf time-zone difference in hours

$R(t)$ residual of time-zone difference in hours after t days

∂ adaptation constant in 1/day

For the estimation of the adaptation constants, we used the technique of least-squares, non-linear regression (ref. 47) between exponential fit and data. In order to assess the quality of the fit, correlation coefficients were calculated for the log-transformed real and fitted curves, separately for the temperature data for each individual and experimental condition.

The adaptation constants presented in table 2.2 were derived from applying non-linear regression to the minimum times of the individual temperature rhythm curves (see table 2.4). Baseline values for the estimation of adaptation constants were obtained from the two bedrest nights prior to the first shift, when the circadian system appeared to be stable. The correlation coefficients of the log-transformed value range from 0.75 to 0.97, thus confirming the exponential process of adaptation between pre- and post-shift steady states of the temperature rhythm. The interindividual differences are large. As already illustrated in figure 2.1, subject D adjusted quickly, resulting in a high ∂-value, whereas subject A needed several days more for resynchronization of the temperature rhythm, as indicated by a low ∂-value. Intraindividual differences are also important. Adaptation was faster for two subjects under light treatment during the subjective night, whereas the other two subjects' rhythms adapted faster under daytime light treatment, as can also be seen from figure 2.1(a). The variations within subjects were smaller than between subjects. The results indicate little, if any, influence of time of day of the light treatment.

Table 2.2. Adaptation constants ∂ for the temperature rhythm after a 12-h transition of the external zeitgebers. DTLE day-time light exposure; NTLE night-time light exposure.

Subject	Condition	∂	SD	F-value	p-value	df	Rate
A	DTLE	0.337	0.034	54.8	0.0018	1,4	0.97
	NTLE	0.169	0.038	46.2	0.0005	1,6	0.94
	Combined	0.213	0.036	137.5	≤0.00005	1,12	0.96
B	DTLE	0.315	0.044	67.7	0.0002	1,6	0.96
	NTLE	0.239	0.049	29.5	0.0016	1,6	0.91
	Combined	0.274	0.035	112.1	≤0.00005	1,14	0.94
D	DTLE	0.433	0.060	7.7	0.0320	1,6	0.75
	NTLE	0.519	0.060	14.1	0.0094	1,6	0.84
	Combined	0.472	0.042	23.1	0.0003	1,14	0.79
E	DTLE	0.242	0.052	34.3	0.0042	1,4	0.95
	NTLE	0.292	0.040	41.2	0.0030	1,4	0.95
	Combined	0.265	0.030	87.4	≤0.00005	1,10	0.95
All	DTLE	0.327	0.025	64.9	≤0.00005	1,26	0.85
	NTLE	0.263	0.030	67.2	≤0.00005	1,28	0.84
	Combined	0.292	0.021	126.8	≤0.00005	1,56	0.83

The mean adaptation constant (∂ = 0.292) was not estimated by simply averaging individual values, but by applying the same analytic procedure (nonlinear regression) to the combined data. With ∂ = 0.347, resynchronization of the circadian system would reduce the remaining gap by 50% within two days, i.e., an initial desynchronization by 12 h would shrink to 6 h after two days and to 3 h after four days. The average value (∂ = 0.292), indicates a slightly slower process of resynchronization under bedrest conditions than during real transmeridian transitions (ref. 43). The phase delays in our study were caused by some combination of (1) the delay in the wake-rest schedule; (2) the treatment by light, independent of time of day; and (3) perhaps the stimulation by the different tasks required by the experimental protocol, which may have led to activation of and activity by the subjects during scheduled wake times. There are no experimental results from humans that indicate that activity can influence the circadian pacemaker, but animal studies suggest such an impact (refs. 48 and 49).

Van der Pol simulations– For a more detailed investigation, the dynamics of the transition of the circadian system were simulated by a Van der Pol oscillator (refs. 41 and 42). This assumes that the circadian pacemaker behaves like a harmonic oscillator. Therefore, the equation used for the determination of relevant dynamic pacemaker variables was as follows:

$$x''(t) + m \cdot [x^2(t) - 1] \cdot x'(t) + w^2 \cdot x(t) = z(t)$$

where

t	time
x(t)	variable, dependent on t
x'(t), x''(t)	1st and 2nd derivates of x(t)
m	stiffness (m > 0), determines the degree of nonlinearity
w	intrinsic oscillator frequency
z(t)	external forces

The equation describes a second-order system of a self-exciting, self-sustaining oscillator the characteristics of which are determined by the stiffness m, the intrinsic period of T = 2p/w, and the (summarized) external force z(t) which corresponds to the zeitgeber in biological processes. We used a rectangular zeitgeber with a light-dark cycle LD = 16:8, except on shift days. On those two days, the period length of the zeitgeber was set to 30 h, the LD cycle was 22:8. In accordance with Gundel and Wegmann (ref. 41) we chose a negative zeitgeber, which is effective during night hours. An additional positive portion of five hours duration was introduced to simulate the bright light pulses.

For each subject, the circadian behavior of the temperature rhythm was simulated, using identical input parameters for the two different conditions (table 2.3), except for the timing of the additional zeitgebers representing light treatment. Zeitgeber strength was assumed to be 50% lower under bedrest conditions (z_{br}) than at home (z_{hom}). Of course, as few parameters as

possible should be manipulated in different simulation trials in order to extract the essence of the oscillation model. These altered parameters reflect the individual adaptation dynamics for each subject. Long oscillator periods, high stiffness values, and increased zeitgeber strength are associated with more rapid phase delays (e.g., subject D). The converse could explain the slow resynchronization of subject A. The main purpose for the use of the oscillator model was to find out whether light exposure at different times of day would affect the resynchronization speed differently. Furthermore, model calculations also served for the estimation of differences in circadian behavior without light treatment, i.e., without additional zeitgebers, but otherwise unaltered variables. The shifts of real temperature minima and simulation calculation minima show that the Van der Pol solutions resemble experimental results qualitatively (tables 2.4 and 2.5), i.e., for the most critical transition days (days 8, 9) slower adjustment for day time light treatment than for night time light treatment could be simulated (subject E) as well as vice versa (subject A). Furthermore, it was possible to simulate deceleration and acceleration of the adaptation rate as was observed for the different shifts on different days for subject D (table 2.5, days 8 to 10). Simulation data also show that light treatment (or any another effective zeitgeber) accelerates the resynchronization process when applied at appropriate times in the circadian cycle. However, adverse effects can appear, as can be shown for subject A, days 8 and 9 (in real data and in simulation data) when light was not applied at the correct time. When appropriate parameter values were selected, no significant differences occurred between the experimental results and the corresponding simulation data (table 2.5). Furthermore, Van der Pol simulations verify that the resynchronization process is significantly slower only under conditions without the application of an additional zeitgeber.

The application of the Van der Pol equation was intended to examine the phase-dependence of light treatment as a zeitgeber on a self-exciting, self-sustaining oscillatory system such as the circadian timing system. A series of solutions of the equation show that the circadian phase response to a shift of zeitgebers and the benefit of an additional synchronizer can be well described by a Van der Pol oscillator. Of course, it is not possible with such a simple model and with no further mathematical manipulations to explain all aspects of real biological data. However, as shown by Gundel and Wegmann (ref. 41), a nearly complete extinction of the rhythm amplitude can be achieved, as postulated by Kronauer (ref. 42) and apparent in subjects A and E under one experimental condition. In addition, Gundel and Wegmann (ref. 41) showed that phase shifts could also occur with only minor changes of amplitude in simulations of Van der Pol equation oscillators. It was not possible to simulate completely the transient phase advances experienced by subjects A and B under nighttime light treatment, but a decrease of the adaption speed (subject A, day 9) was achievable.

Van der Pol equation solutions showed that appropriately timed treatment by additional zeitgebers significantly enhanced resynchronization speeds, notably when the zeitgeber pulse was administered as recommended and investigated by Czeisler et al. (ref. 30). We failed to confirm our hypothesis that shifting the light exposure to track the optimal circadian phase from cycle to cycle would be the most efficient way to enhance resynchronization of the circadian system, except for subject B. His resynchronization was faster on days 9 to 11 under the conditions chosen by us than would have been expected under those recommended by Czeisler. The different light application times (control versus experimental) had only marginally different effects, as verified by the real data as well as by simulation data.

Table 2.3. Constants from Van der Pol oscillator model calculations for different temperature adaptation curves.

Parameter	A	B	D	E
w_f [d^{-1}]	0.9897	0.9796	0.9796	0.9897
T_f [h]	24.25	24.5	24.5	24.25
μ	0.25	0.25	0.5	0.25
z_{hom}	−0.5	−0.5	−0.5	−0.5
z_{br}	−0.25	−0.25	−0.25	−0.25
z_{add}	0.5	0.5	0.5	0.75
D_{ph} [h]	−2.0	−2.0	−2.0	−2.0

Table 2.4. Experimental and simulation results of temperature minimum shifts (all numbers in hours).
Abbreviations:

BSL—Baseline temperature minimum
ED—Experimental day-time light treatment
EN—Experimental night-time light treatment
SD—Simulation of day-time light treatment
SN—Simulation of night-time light treatment
SW—Simulation without light treatment
SC—Simulation with constant time of light treatment, i.e., light treatment was simulated at the same GMT time for three consecutive days at the time of the first pulse in the experimental (night-time) protocol.

	Subject A						Subject B					
DAY	ED	EN	SD	SN	SW	SC	ED	EN	SD	SN	SW	SC
BSL	4.17	6.0	0.0	0.0	0.0	0.0	4.83	5.83	0.0	0.0	0.0	0.0
7	3.67	2.5	1.0	1.0	1.0	1.0	3.33	1.0	2.0	2.0	2.0	2.0
8	4.67	−1.5	3.5	2.5	2.5	2.5	3.67	0.0	4.0	3.5	3.5	3.5
9	8.67	3.17	7.5	3.5	5.5	10.0	5.33	6.33	8.0	9.0	7.0	8.0
10	8.33	5.5	9.0	10.0	7.0	10.0	10.33	10.00	10.0	10.0	8.5	10.0
11	—	8.17	9.5	10.0	8.0	11.0	10.33	9.33	10.0	10.5	9.5	10.0
12	—	9.5	10.0	10.0	9.0	11.0	11.00	9.67	11.0	11.0	10.0	11.0
13	11.33	9.83	10.5	10.5	10.0	11.0	11.67	10.00	11.5	11.5	11.0	11.5
14	——	——	11.0	11.0	11.0	11.0	——	——	12.0	12.0	11.0	12.0

	Subject D						Subject E					
DAY	ED	EN	SD	SN	SW	SC	ED	EN	SD	SN	SW	SC
BSL	5.33	5.33	0.0	0.0	0.0	0.0	4.5	4.5	0.0	0.0	0.0	0.0
7	2.83	3.83	2.0	2.5	2.0	2.5	0.33	2.0	1.5	2.0	1.5	1.5
8	7.0	8.83	6.0	6.0	4.5	6.5	3.67	4.0	5.0	5.5	3.0	6.5
9	11.17	10.17	8.5	9.5	7.5	9.0	—	—	9.5	11.0	6.0	10.5
10	9.83	10.83	10.5	10.0	9.0	10.5	—	—	10.5	11.5	7.5	11.5
11	10.17	10.00	11.5	10.5	10.0	11.5	7.67	10.67	11.5	11.5	8.5	11.5
12	9.83	10.17	11.5	11.5	10.5	11.5	11.00	9.67	11.5	11.5	9.5	11.5
13	10.50	10.50	11.5	11.5	11.5	11.5	11.00	11.33	11.5	11.5	10.5	11.5
14	——	——	12.0	12.0	11.5	12.0	——	——	12.0	12.0	11.0	12.0

Table 2.5. Comparison of shifts in the times of temperature minima under various conditions for the three most critical shift days 8, 9, and 10. (Wilcoxon matched-pairs signed-ranks test: ns, $p > 0.05$; *, $0.05 \geq p > 0.01$; **, $p \leq 0.01$.) Significances are indicated for the comparison with SW. All other comparisons were insignificant. Abbreviations are as in table 2.4.

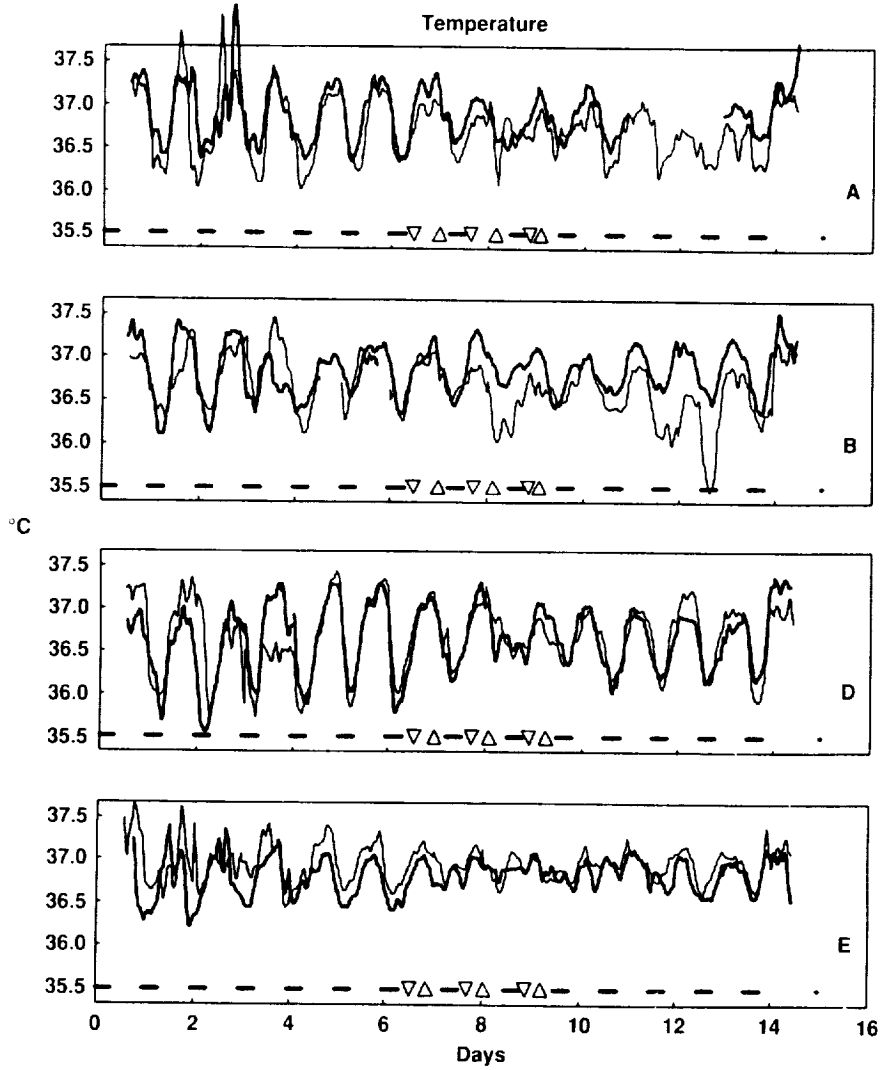|	Mean ± SD	Significance
ED (data control)	7.3 ± 2.8	*
EN (data experimental)	5.6 ± 4.3	ns
SD (simulated control)	7.2 ± 2.5	**
SN (simulated experiment)	7.0 ± 3.1	ns
SW (simulated without light zeitgeber)	5.8 ± 2.3	−
SC (simulated with fixed light zeitgeber)	7.7 ± 2.8	**

Figure 2.1. Raw temperature data of each subject with control (thick lines) and experimental (thin lines) conditions overlayed. Degrees C are denoted on the y-axis, number of days on the x-axis. Black bars indicate sleep periods, upright triangles indicate light exposure at night (experimental condition), inverted triangles indicate daytime light exposure (control condition). Capital letters on the right side refer to individuals.
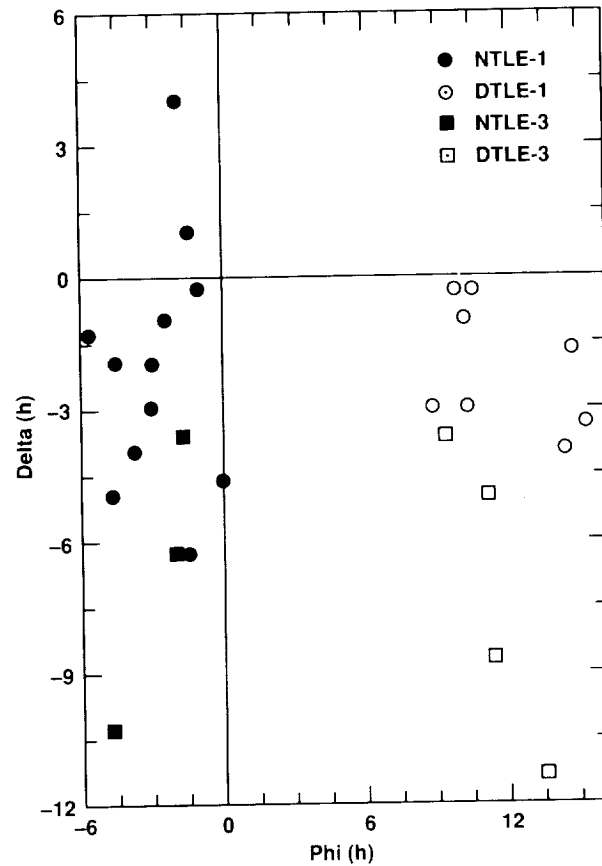
Temperature phase response curve

Delta (h)

6 —
● NTLE-1
⊙ DTLE-1
3 —
■ NTLE-3
□ DTLE-3

0

-3

-6

-9

-12

-6   0   6   12

Phi (h)

Figure 2.2. Phase responses of the temperature rhythm. The phase-shift DELTA is measured between the circadian minima before and after the light pulse. The phase of the light stimulus (PHI) is measured from the preceeding temperature minimum to the midpoint of the pulse. A pulse occurring at the time 0 is centered on the temperature minimum. DTLE-1 indicates responses to individual light pulses in the control condition. DTLE-3 indicates the total phase shift for each subject after all three pulses have been completed in the control condition. NTLE-1 and NTLE-3 indicate the corresponding responses in the experimental light condition.

17
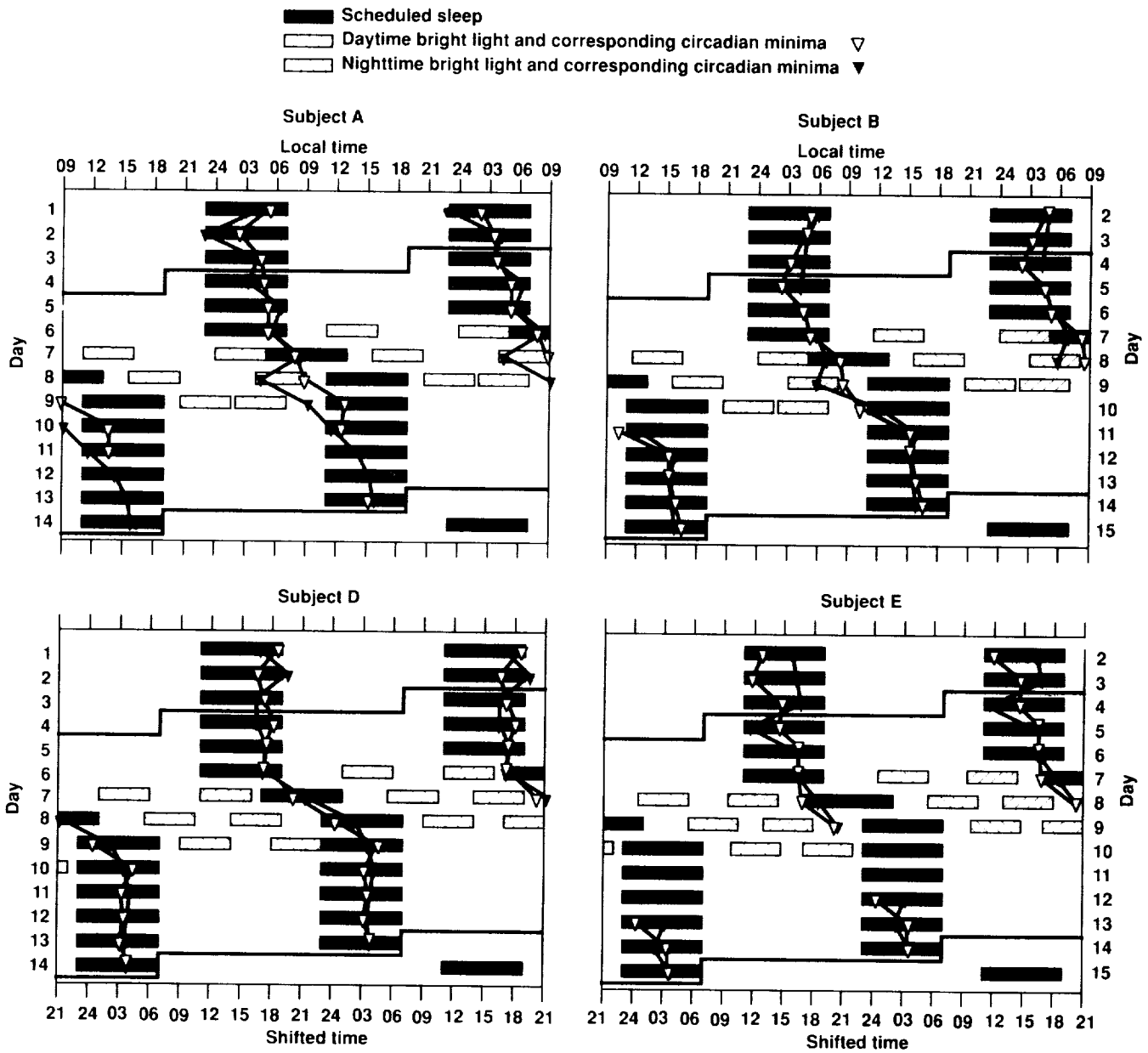
Figure 2.3.   Temperature adjustment curves for each individual for the two conditions superimposed. The data on the left side of each figure have been replicated on the right side, but moved up one day. This "double plotting" presents days 1 and 2, 2 and 3, etc. across the page, as well as days 1,2,3, etc. down the page.
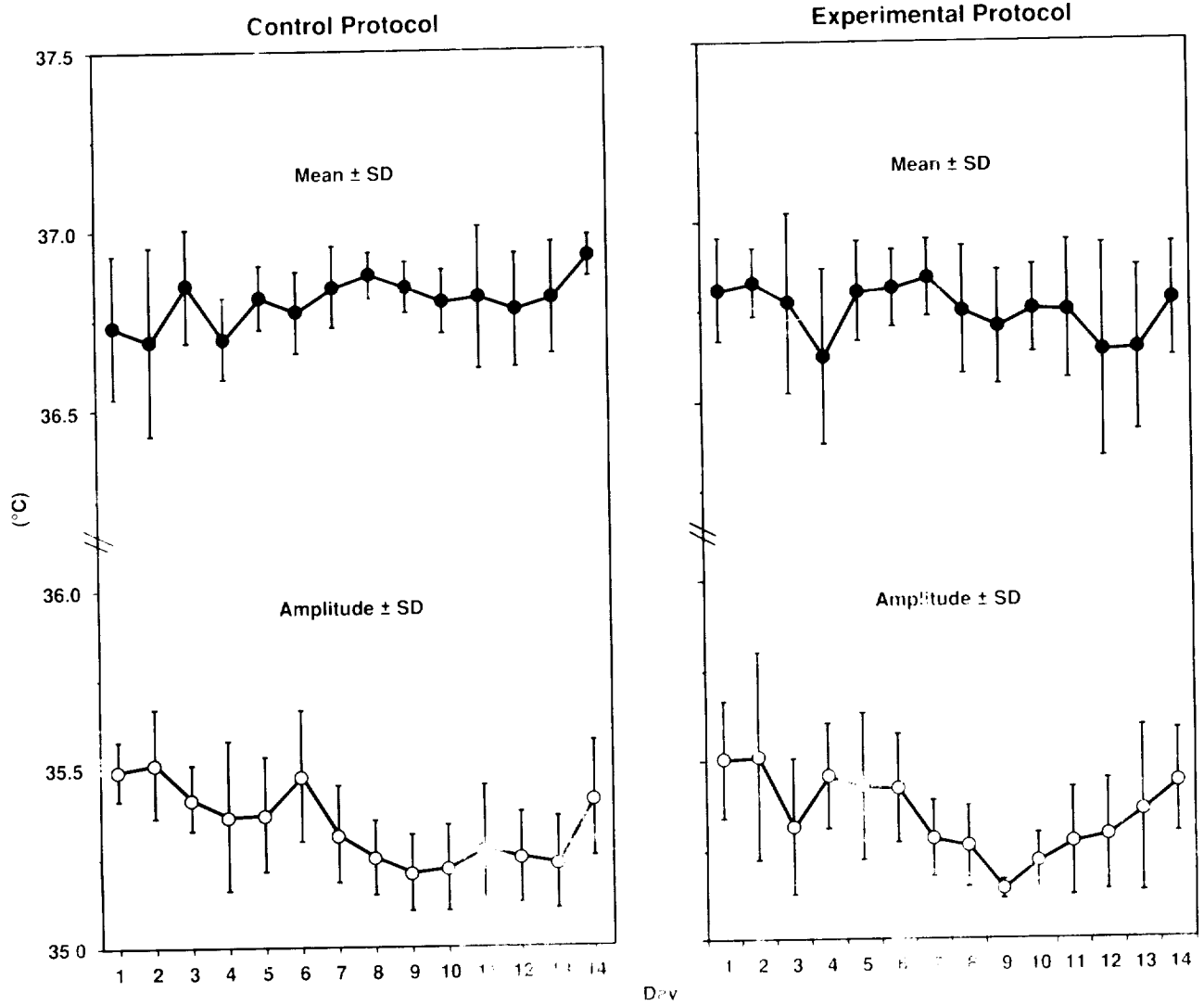
Figure 2.4. Diurnal means and circadian amplitudes for the two conditions. Control protocol refers to day time light exposure (DTLE), experimental protocol to night time light exposure (NTLE).

Chapter 3: Desynchronization and Dissociation

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Under unaltered 24-h external zeitgebers, the circadian system is in a stable state, i.e., rhythms in all body functions have a nearly fixed phase relationship to each other and to the sleep-wake cycle. A shift of environmental time is associated with desynchronization between the body clock and the external time. As a result of this desynchronization, the new zeitgebers trigger the circadian system to resynchronize into a new stable condition. The transition between these two nearly steady states is usually characterized by reduced circadian amplitudes and a break down of the phase relationships between rhythms in different body functions as they adapt at different rates (refs. 36, 43, and 50). Thus, during the adaptation process, internal dissociation appears for several days. There is evidence, at least for thermoregulation, that homeostatic regulation may be compromised until internal synchrony is regained (ref. 14).

To investigate the phenomenon of rhythm dissociation under conditions simulating the effects of weightlessness, we examined rhythms in urinary excretion of six hormones and electrolytes, as well as the rhythms of heart rate and temperature. The urinary variables were only monitored during the head-down tilt (HDT) period. Therefore we cannot describe the transition from ambulatory to simulated weightless conditions. However, this has been thoroughly documented in other studies (refs. 3, 6, 7, 10, 17, and 51). During the adaptation period (first 48 h) fluids shift towards the head, prompting a diuresis with fluid loss mainly from the extracellular compartment. This is accompanied by increased sodium excretion (natriuresis) and associated changes in other hormones and electrolytes. The effects of these physiological changes on circadian rhythms are seen mainly in changes in mean levels and amplitudes, rather than in phase. The major adaptation is completed within 48 h and subsequent changes are relatively small and gradual. This subsequent adaptation becomes important, however, in long-duration missions and experiments (ref. 51).

Methods

Temperature and heart rate were recorded in 2-min samples on Vitalog recorders. Urine was collected in regular 3-h intervals during the wake-time, and during the night, if the subjects urinated.

After assessment of the volume, four aliquots of each sample were refrigerated or frozen, respectively, for the subsequent determination of concentrations of cortisol, epinephrine and norepinephrine, 6-hydroximelatoninsulphate, as well as sodium and potassium. Cortisol, sodium and potassium concentrations were measured at NASA Ames, Life Science Division. The remaining aliquots were kept frozen on dry ice and shipped to the DLR-Institute for Aerospace Medicine in Cologne, Germany, for subsequent analysis.

Cortisol and 6-hydroximelatoninsulphate concentrations were determined by radioimmunoassay procedures. Unconjugated catecholamines were assessed by a simple sample clean-up procedure using a weak cation exchange column, followed by a High Pressure Liquid Chromatograph (HPLC) separation and determination of the catecholamines via electrochemical detection. Sodium and potassium concentrations were calculated by means of an ion-specific electrode.

Circadian parameters (diurnal mean, amplitude and acrophase) were estimated for each condition, subject and rhythm, by sinusoidal fit procedures, shifting a 24-h window in 3-h intervals over the entire data set. It should be noted that it is necessary to assume that the rate of excretion is constant throughout the time interval between samples. This assumption is incorrect, at least for the first sample after sleep, and reduces the precision with which the acrophase can be estimated. However, our results do not differ significantly from those of comparable studies in which urine samples were collected at regular 3-h intervals throughout the 24-h cycle (refs. 17, 28, 43, and 52).

The rhythm parameters were investigated for significant changes due to body position (supine vs. erect) and different bright light conditions (night time vs. day time exposure). When significant differences between light treatment conditions were not found, we treated the results independently of the study phase, i.e., we assumed the number of subjects to be eight in order to increase the statistical probability for detecting differences due to light-independent influences. Additionally, this procedure makes the analysis conservative.

Results

Because of the enormous amount of data, only the raw data for 6-hydroximelatoninsulphate (6-OHMS) and potassium excretion rates are shown (figs. 3.1 and 3.2). These were the only two rhythms which showed consistent differences between the control and experimental light treatments. These functions also exhibited opposite circadian behavior before the time displacement, i.e., whereas potassium was excreted mainly during day-time, 6-OHMS values peaked during night-time. During and after the zeitgeber delay, potassium excretion was inverted for several days due to the 12-h shift and the inertia of its circadian rhythmicity, while the 6-OHMS rhythm adapted faster to the changed sleep-wake cycle (fig. 3.2), particularly when subjects were treated with light during subjective night hours.

Shift of the Wake-Rest Schedule

Acrophases– Independently of light exposure conditions, the 12-h phase delay in environmental time produced significant delays in the acrophases of all body functions when compared with baseline values (figs. 3.3-3.7). (For phase estimates, baseline days were usually the three days preceding the first time shift). However, the start of the resynchronization varied among body functions. For example, the results from the one-way ANOVA of the circadian phases of the sodium excretion rhythm is presented in table 3.1. Clearly, resynchronization started on day 7. The acrophase was delayed significantly on each successive day from day 7 to day 9. Thereafter it remained stable, i.e., it was resynchronized by day 9, within the limits detectable by this technique. A similar response, with significant acrophase delays beginning on day 7 and ending on day 9, was observed for the rhythms of heart rate, epinephrine, and norepinephrine. In contrast, the rhythms in the other urinary variables adapted to the delayed wake-rest cycle more slowly. Significant acrophase delays were not found until day 8 for 6-OHMS, day 9 for potassium, and day 10 for cortisol. The resynchronization was also slower, as indicated by the fact that the acrophases did not delay significantly from day to day. Rather, it took two cycles to accumulate a statistically significant delay (i.e., day 12 was different from day 10, but not from day 11). Yet the 6-OHMS rhythm had almost completely resynchronized by the end of the bedrest period (day 13, table 3.2). However, the other two rhythms, i.e., of potassium and cortisol excretion, needed more days and were not completely adapted to the shifted time at the end of the bedrest period. From table 3.2, it can be seen that the circadian rhythms of heart rate, sodium and the catecholamines had adjusted within four days as judged by the return of the acrophase to its

normal relationship to the sleep-wake cycle, within the limits of baseline variability, i.e., between 0.5 and 1.5 h (table 3.2, days 4 to 6). As mentioned before, the rhythms of 6-OHMS (6 days), potassium (>7 days), and cortisol (>7 days) needed much more time for resynchronization.

Sodium, potassium, and epinephrine excretion acrophases on day 14 were significantly different from all other acrophases. These rhythms were already responding to the 12-hour shift back to the baseline time on the last day of each study phase.

Internal dissociation is demonstrated very clearly when the time courses of the acrophases for different body functions are compared. As illustrative examples, figures 3.8. and 3.9. each compare the shifts of the acrophases of two different rhythms from their baseline positions on day 6 (the day before the schedule delay). The data for both light treatments have been combined. The potassium rhythm showed significant differences between light treatment conditions (as demonstrated later), and therefore the standard deviation increased during resynchronization days. Nevertheless, its rate of adaptation was significantly slower than that of sodium (fig. 3.8). Similarly, the adaptation of the rhythm of cortisol excretion was significantly slower than the adaptation of the rhythm of epinephrine excretion (fig. 3.9).

To obtain a more general view, adaptation constants α were estimated for the rhythm of each body function. Following the exponential model (refs. 2 and 43) mentioned in chapter 2, ∂ was assessed using the technique of least-squares, nonlinear regression (ref. 47). Although the different constants (table 3.3) reveal the differences in resynchronization rates among variables,

Table 3.1. Comparison of daily sodium acrophases (light treatment conditions combined, 1-way ANOVA: F = 100.53; df = 10,73; – p ≤ 0.001).

Day	Mean ± SD	Significance of shift
4	14.0 ± 1.8	
5	15.5 ± 1.0	
6	15.7 ± 0.7	
7	18.8 ± 1.3	–
8	0.8 ± 2.3	–
9	4.7 ± 2.8	–
10	3.2 ± 1.9	
11	3.6 ± 1.0	
12	4.1 ± 1.2	
13	3.6 ± 1.0	
14	6.9 ± 2.3	–

Table 3.2. Acrophases of different circadian functions under control and experimental conditions.

Day	Sodium		Potassium		Melatoninsulphate	
	Control	Experiment	Control	Experiment	Control	Experiment
4	14.5 ± 1.0	15.1 ± 1.2	13.5 ± 1.2	11.5 ± 2.2	4.2 ± 1.2	3.6 ± 0.7
5	15.5 ± 1.4	15.5 ± 0.6	11.1 ± 1.7	12.0 ± 1.8	4.3 ± 1.7	3.8 ± 1.4
6	15.5 ± 0.5	15.8 ± 0.9	12.5 ± 1.0	12.2 ± 1.3	2.9 ± 1.7	3.2 ± 0.4
7	18.7 ± 1.7	19.0 ± 1.3	13.0 ± 2.0*	13.8 ± 2.8	2.8 ± 6.2	----†
8	1.8 ± 2.7	23.8 ± 1.5	12.9 ± 0.9*	15.3 ± 2.3	6.0 ± 1.0*	8.7 ± 2.2
9	5.2 ± 3.5	4.4 ± 2.7	15.3 ± 2.7*	16.9 ± 1.7	6.8 ± 1.6*	10.7 ± 2.8
10	3.7 ± 0.4	2.7 ± 2.7	20.6 ± 4.5*	18.1 ± 1.7	10.1 ± 2.2*	12.1 ± 2.1
11	3.7 ± 0.9	3.6 ± 1.2	18.4 ± 3.3*	18.8 ± 2.4	12.5 ± 2.3*	13.7 ± 0.8
12	3.9 ± 1.4	4.2 ± 1.1	20.0 ± 3.9*	22.6 ± 2.3	14.0 ± 1.2*	14.1 ± 1.2
13	3.2 ± 1.0	3.9 ± 0.9	21.9 ± 3.7*	23.5 ± 1.5	14.5 ± 1.1*	15.4 ± 0.7
14	7.0 ± 3.1	6.7 ± 1.9	3.1 ± 3.2	2.1 ± 1.4	16.1 ± 1.1	16.2 ± 0.8

Day	Cortisol		Epinephrine		Norepinephrine	
	Control	Experiment	Control	Experiment	Control	Experiment
4	9.2 ± 1.7	9.2 ± 1.4	13.5 ± 1.2	13.6 ± 1.8	14.0 ± 1.6	15.3 ± 0.8
5	10.4 ± 1.5	9.7 ± 2.2	13.4 ± 1.1	14.8 ± 1.1	9.2 ± 0.5	11.6 ± 2.2
6	10.9 ± 1.4	10.4 ± 1.1	14.0 ± 1.0	14.6 ± 1.0	10.1 ± 3.8	11.9 ± 2.4
7	9.7 ± 0.8	11.3 ± 2.2	17.1 ± 1.0	18.1 ± 0.5	12.9 ± 6.1	23.2 ± 0.5
8	9.1 ± 1.1	9.1 ± 1.3	18.2 ± 0.9	20.1 ± 3.0	17.2 ± 2.5	17.0 ± 2.3
9	11.2 ± 3.1	12.2 ± 4.2	0.8 ± 1.1	23.6 ± 2.0	1.1 ± 3.3	22.6 ± 4.4
10	15.5 ± 2.8	15.3 ± 4.6	0.1 ± 1.2	23.7 ± 0.5	1.9 ± 1.6	22.6 ± 2.4
11	16.7 ± 3.3	19.0 ± 1.4	23.8 ± 1.2	23.5 ± 1.3	23.4 ± 1.4	21.6 ± 2.0
12	18.7 ± 0.9	20.4 ± 1.5	0.1 ± 2.7	0.9 ± 0.7	21.2 ± 2.3	20.8 ± 0.3
13	20.1 ± 2.3	21.3 ± 1.6	23.2 ± 2.9	0.8 ± 0.9	22.3 ± 2.4	23.0 ± 2.9
14	20.6 ± 1.7	21.4 ± 1.6	2.4 ± 2.8	3.0 ± 1.2	4.0 ± 2.7	3.6 ± 1.4

Notes: 1. Significant differences between conditions are indicated by *, $0.05 > p \geq 0.01$ (Wilcoxon matched-pairs signed-ranks test).
2. †No number because of insignificant rhythm in three subjects.
3. For potassium, there was a significant difference between conditions for days 7-13 combined.
4. For 6-OHMS, there was a significant difference between conditions for days 8-9 combined, and for days 10-13 combined.

the dynamic process and the discrepancies between body functions can be more impressively illustrated by the theoretical curves derived from our experimental data (fig. 3.10). This approach, however, ignores individual differences. A fast resynchronization process is implied by the course of the acrophases for sodium, norepinephrine, and heart rate rhythm. Epinephrine, temperature, and 6-OHMS rhythms need more time for adjustment. Finally, potassium and cortisol rhythms exhibit the highest inertia to the zeitgeber shift.

Amplitudes– Preshift data for the urinary variables were taken from day 6 to allow for the physiological adaptation to weightlessness, as mentioned previously. For heart rate and temperature, days 5 and 6 were combined for baseline. Preshift amplitudes were compared with values during the shift (days 7-9 combined) and post-shift (days 11-13 combined). Significant differences (1-way ANOVA) could be detected for heart rate ($p = 0.0016$ pre to shift; $p = 0.0087$ post to shift) and temperature ($p < 0.001$ pre to shift; $p = 0.0030$ pre to post), and for sodium ($p < 0.001$ for every other condition to shift days), 6-hydroximelatoninsulphate ($p < 0.001$ pre to shift; $p = 0.0014$ post to shift) and epinephrine excretion ($p = 0.0247$ post to shift). In all these functions, the circadian amplitude was lowest during shift days as defined above (figs. 3.11-3.14). Amplitudes of potassium

Table 3.3. Adaptation constants ∂ and t (t =$1/\partial$) of all measured body rhythms after a 12 hour transition of the external zeitgebers.

Function	∂	SD	t
Sodium	0.667	0.073	1.50
Norepinephrine	0.523	0.064	1.91
Heart Rate	0.442	0.036	2.26
Epinephrine	0.343	0.024	2.92
Temperature	0.292	0.021	3.42
6-Hydroximelatoninsulphate	0.284	0.027	3.52
Potassium	0.182	0.023	5.49
Cortisol	0.140	0.018	7.14

and norepinephrine excretion rhythms did not change (figs. 3.15 and 3.16), whereas cortisol (fig. 3.17) showed a higher amplitude on shift days (significant between shift and post-shift days; p = 0.0448).

Means– Daily mean levels of potassium excretion were significantly affected (but see results and discussions below) showing higher values during and after the shift (p = 0.0054 pre to shift; p = 0.0024 pre to post). The daily mean level of 6-OHMS excretion also declined (p = 0.0011 pre to shift; p = 0.0213 pre to post). Cortisol excretion had enhanced levels during the shift-days, compared to post-shift (p = 0.0062), and norepinephrine exhibited reduced levels after the time displacement, compared with pre-shift days (p = 0.0158).

Light Treatment

Due to the small number of subjects, it was not possible to perform an ANOVA by subjects and by days for experimental versus control light treatments. Therefore, at least two days of the resynchronization period were included in each ANOVA in order to detect any significant differences. Thus, N denotes the product of subjects and days in the following (excluding days when no significant rhythm could be detected). Significant differences should not occur during baseline, and in almost all functions and circadian parameters they did not, except for heart rate acrophases on days 4 to 6 (table 3.4 and fig. 3.3).

Acrophases– During and after treatment days, acrophases were significantly different for day-time and night-time exposure for potassium (fig. 3.4) on days 8 to 13 (p = 0.0480, N = 23) and for 6-hydroximelatoninsulphate (fig. 3.5) on days 7 to 9 (p = 0.0156, N = 8) and 10 to 13 (p < 0.001, N = 16), respectively. However, all other rhythms resynchronized without any significant differences in speed between control and experimental conditions (table 3.2 and figs. 3.3-3.7).

Amplitudes– Circadian amplitudes of norepinephrine and 6-OHMS were significantly enhanced under experimental light treatment during the following periods: Norepinephrine on days 6 to 9 (p = 0.0156, N = 7) and 6-OHMS on days 10 to 13 (p = 0.0356, N = 16). Again, the other body functions did not exhibit a significant difference in their circadian amplitudes between the different light treatments.

Means– Under night-time light exposure, diurnal means of epinephrine excretion (p = 0.001, N = 11, fig. 3.14) and heart rate (table 3.4, fig. 3.11) increased significantly during the three treatment days. On days 10 to 13, more 6-OHMS was excreted by the night-time treated group (p < 0.001, N = 16, fig. 3.13). No significant differences in daily means could be observed in the other body functions (figs. 3.12, 3.15, 3.16, and 3.17).

Simulated Weightlessness Condition

Whereas temperature and heart rate were recorded during pre-bedrest days, all hormones and electrolytes were collected only during the bedrest periods (except for the last day).

Heart rate revealed significant alterations due to postural changes (table 3.4(b)). When comparing the data of three days at home with the days before the shift of the environmental time cues during bedrest, diurnal means decreased significantly by about 10 bpm during bedrest, and circadian amplitudes also decreased significantly, i.e., by 6 bpm or nearly 45% on average (fig. 3.11). In contrast, acrophases did not change due to postural alterations.

For the estimation of posture-dependent changes of diurnal means and circadian amplitudes in excretion rates, we assumed that the acute adaptation period from erect into 6° head-down tilt (HDT) or supine position lasted at most two days (defined as "adapt" in table 3.5). Because of the influence of time shifts on these parameters, we

Table 3.4.a. Effect of different light exposure times on circadian parameters of heart rate for various combinations of days. Significant differences between conditions are indicated by: *, $0.05 > p \geq 0.01$; **, $0.01 > p \geq 0.001$; ***, $p < 0.001$ (matched t-test). NTLE: Night-time light exposure; DTLE: Day-time light exposure.

Days	Parameter	NTLE Mean ± SD	DTLE Mean ± SD	F-value	p-value	df	Significance
1-3	Mean	76.9 ± 5.4	75.1 ± 6.5	0.541	0.4766	1,11	ns
	Acrophase	15.8 ± 3.5	14.8 ± 1.4	1.141	0.3084		ns
	Amplitude	12.2 ± 4.7	15.7 ± 8.0	1.514	0.2441		ns
4-6	Mean	66.6 ± 4.5	65.5 ± 6.7	0.228	0.6433	1,10	ns
	Acrophase	16.0 ± 2.7	13.5 ± 1.4	7.890	0.0185		*
	Amplitude	7.6 ± 3.7	7.9 ± 3.5	0.021	0.8876		ns
7-9	Mean	67.2 ± 3.0	65.1 ± 3.7	13.370	0.0038	1,11	**
	Acrophase	22.0 ± 3.4	21.2 ± 3.4	2.235	0.1630		ns
	Amplitude	4.0 ± 1.0	4.2 ± 1.2	0.336	0.5736		ns
10-13	Mean	65.3 ± 3.0	65.4 ± 3.4	0.022	0.8855	1,11	ns
	Acrophase	1.6 ± 1.1	1.9 ± 1.5	0.257	0.6199		ns
	Amplitude	5.5 ± 1.8	5.1 ± 1.7	0.389	0.5421		ns
7-13	Mean	66.1 ± 3.1	65.2 ± 3.5	3.752	0.0633	1,27	ns
	Acrophase	0.1 ± 2.9	23.9 ± 3.4	0.328	0.5717		ns
	Amplitude	4.9 ± 1.6	4.7 ± 1.7	0.105	0.7480		ns

Table 3.4.b. Effect of weightlessness simulation (postural change) on circadian parameters of heart rate. Significances as above. Erect: Home recording days; Supine: Bedrest recordings before time shift.

	Erect	Supine	F-value	p-value	df	Significance
Mean	76.0 ± 6.0	65.9 ± 5.3	37.050	0.0000	1,47	***
Acrophase	15.3 ± 2.6	14.7 ± 2.4	0.613	0.4378	1,46	ns
Amplitude	13.9 ± 6.7	7.6 ± 3.5	17.020	0.0002	1,47	***

used only days 6 and 13 (figs. 3.11-3.17) for the calculation of "supine" data; finally, "post" data were derived from the last day (day 14) of each study phase. It should be noted that the subjects were adapting to both the upright posture and the 12-h phase change in environmental time on this day. Thus, we incorporated only 40 circadian cycles for the assessment of significant differences between posture conditions. The results of the statistical analyses are presented in table 3.5.

The results indicate that the bedrest condition produced major changes in nearly all excretion rates. The steepest decrease could be observed in daily sodium levels; more than 40% reduction between the adaptation and supine condition, and more than 45% between supine and post-bedrest (table 3.5). Potassium excretion declined significantly during the adaptation period (fig. 3.15, days 4 and 5, $p \leq 0.001$, N = 8), but the changes between conditions were minimal. As for hormones, the excretion of

cortisol was significantly lower during supine and post-bedrest conditions compared to adaptation, whereas in 6-OHMS, the daily level decreased after bedrest, but not during HDT. As for the catecholamines, epinephrine was excreted in significantly greater quantities after the adaptation period (table 3.5, fig. 3.14). Note that "supine" refers to data from days 6 and 13 (and not in between). Norepinephrine was excreted at a significantly higher rate during recovery from bedrest.

Circadian amplitudes of excretion rates exhibited a similar course to diurnal means, i.e., when the daily level dropped, usually the amplitude did also, and vice versa (figs. 3.11-3.17). No change could be observed for 6-OHMS when comparing the three conditions, whereas for all other body functions under study, significant differences occurred between at least two conditions, and often between all three conditions (i.e., "adapt," supine, "post"; table 3.5).

Table 3.5. Means, standard deviations, and t-test results (one-way ANOVA) for daily means and amplitudes dependent upon posture. Significance levels (*: $0.05 \geq p \geq 0.01$, **: $p < 0.01$; ***: $p < 0.001$).

Body Function	Daily Means Adapt (mean ± SD)	p	Supine (mean ± SD)	p (adapt)	p (supine)	Post (mean ± SD)
Sodium [μmol/min]	126.0 ± 25.2	***	73.8 ± 7.3	***	***	39.7 ± 9.8
Potassium [μmol/min]	35.4 ± 7.5	ns	37.7 ± 4.3	*	ns	42.1 ± 4.8
Na/K-Ratio	4.09 ± 0.62	***	2.01 ± 0.31	***	***	0.99 ± 0.22
Cortisol [ng/min]	3.65 ± 0.84	*	2.96 ± 0.83	**	ns	2.69 ± 0.75
6 OHMS [ng/min]	16.6 ± 4.6	ns	17.4 ± 4.2	ns	*	13.4 ± 2.9
Epinephrine [ng/min]	2.35 ± 0.63	*	3.15 ± 1.01	***	***	5.15 ± 1.26
Norepinephrine [ng/min]	19.3 ± 8.0	ns	16.9 ± 6.1	**	***	30.3 ± 9.1

Body Function	Circadian Amplitudes Adapt (mean ± SD)	p	Supine (mean ± SD)	p (adapt)	p (supine)	Post (mean ± SD)
Sodium [μmol/min]	72.6±25.9	**	50.2 ± 11.4	***	***	18.2 ± 14.8
Potassium [μmol/min]	18.0 ± 5.9	*	14.3 ± 4.2	**	ns	11.7 ± 4.8
Na/K-Ratio	2.27 ± 1.00	***	1.08 ± 0.33	***	*	0.41 ± 0.23
Cortisol [ng/min]	2.69 ± 0.84	***	1.65 ± 0.81	**	ns	1.61 ± 0.50
6-OHMS [ng/min]	14.3 ± 5.1	ns	15.1 ± 3.9	ns	ns	14.0±1.9
Epinephrine [ng/min]	1.85 ± 0.63	ns	2.14 ± 0.53	*	ns	2.38 ± 0.41
Norepinephrine [ng/min]	7.00 ± 5.6	**	3.2 ± 1.2	ns	***	9.5±2.5

A continuous change due to bedrest could not be observed, except for the diurnal level of two body functions. When correlating ongoing time (consecutive 24-h periods) with excretion rates (days 4, 5, and 14 were excluded from the analysis due to acute adaptation to bedrest), a weak but significant positive correlation could be found for potassium ($r = 0.32$; $p = 0.0107$; $F = 6.930$) and for epinephrine excretion ($r = 0.28$; $p = 0.0264$; $F = 5.179$). Amplitudes did not show a trend over HDT days, except that temperature exhibited a weak, significant negative correlation ($r = -0.33$; $p = 0.0050$; $F = 8.424$).

Discussion

This experiment was designed to examine the effects of light treatment at different phases of the circadian cycle on the rates of adaptation of different rhythms to a 12-h delay of the environmental time. The unshifted bedrest period served as baseline and the supine position provided nearly unmasked conditions due to inactivity. The adaptation of the urinary variables from ambulatory conditions to head-down tilt was not monitored in this study. However, from similar investigations (refs. 10 and 17) as well as from the literature (refs. 2, 7, and 51) we already know many of the responses of various body functions to the HDT-position.

Shifted Time Structure

Our results clearly reveal the process of internal dissociation. Whereas some body functions could adjust their rhythms within three days, others needed at least seven days for complete resynchronization. Similar results have been reported from jet-lag investigations in the field (refs. 2, 6, 36, and 43) or the laboratory (ref. 28). Different entrainment limits for specific functions have also been found in experiments in time isolation (refs. 35 and 50). It is possible that the range of entrainment limits is associated with the range of resynchronization speeds, i.e., a body function with a rhythm with broader entrainment limits might adjust faster to a shift of zeitgebers than a rhythm with narrower entrainment limits.

The average adaptation constants found for the rhythms in each body function also show the divergence of the resynchronization process. The rank order of resynchronization speeds in this experiment resembles the rank order estimated under completely different experimental conditions (refs. 36 and 53). In this context, the

convention of using the temperature rhythm as a circadian phase marker becomes questionable. The unmasked temperature rhythm shows an intermediate speed of resynchronization. If it accurately reflects the time course of the circadian pacemaker, it becomes necessary to explain how some functions can resynchronize faster, and others slower, than the circadian pacemaker. The four rhythms which resynchronize faster than temperature (sodium, norepinephrine, epinephrine, heart rate) may all be strongly influenced by the wake-rest schedule, in which case masking may have accelerated the apparent rate of adaptation. It must then be asked why potassium and cortisol resynchronized more slowly than temperature. It is interesting to note that, in desynchronized free-running subjects, the rhythms of temperature, potassium and cortisol have a common period which is different from that of the sleep-wake cycle (ref. 54).

The shift of the time structure had a great impact on circadian amplitudes. For those functions which regained their preshift amplitudes, these changes can be attributed exclusively to the zeitgeber shift, and not to slow adaptation to HDT. Significant amplitude disruption was generally associated with faster phase adaptation. Thus, the amplitudes of heart rate and sodium, 6-OH-elatoninsulphate and epinephrine excretion rhythms were severely disrupted, but only for a short transition period (3 days), whereas temperature needed more time for readjustment of phase and amplitude. The interference of postural alteration with time displacement may have led to a prolonged amplitude decline in the temperature rhythm, since both are known to affect the circadian system (refs. 2, 7, 10, and 11). The rhythms of potassium and norepinephrine excretion exhibited no change in amplitudes, while cortisol showed higher amplitudes during the shift. There are several factors which may have contributed to the different amplitude responses of the different rhythms. First, in some cases there may have been an ongoing adaptation to the HDT condition. Second, the different physiological subsystems represented by the overt rhythms may respond differently to the influence of the circadian pacemaker during the resynchronization period. Third, light treatment, independent of time of day, may have masked the amplitude in some functions (refs. 9 and 37).

Changes of the 24-h mean levels are not unexpected during the delays of the wake-rest schedule, i.e., when periods of longer wakefulness within a 24-h cycle change the level (ref. 55). Mean level changes on other days, as observed for potassium, 6-OHMS and cortisol excretion rates and for heart rate, may be attributable to the same factors as mentioned above for the amplitude changes.

Light Treatment

The 6-OHMS rhythm was the only variable which showed a consistent time-of-day dependence in its response to light pulses. All three circadian parameters, i.e., acrophase, amplitude, and diurnal mean of this body function were significantly different between the two light exposure conditions. In all other functions investigated, at most one parameter showed a significantly different response depending on exposure time. Daily levels of heart rate and epinephrine, and amplitudes of norepinephrine, and potassium excretion acrophases exhibited time-of-day dependent responses to light pulses.

The impact of light on the secretion of melatonin and consequently on the excretion rate of 6-OH-melatoninsulphate is expected due to the inhibitory effect of bright light on melatonin synthesis (ref. 37). Thus, it is not surprising that the acrophase shifted faster under the effect of secretion depression by bright light exposure during early night hours than during day time when only minimal secretion occurs. Diurnal means and circadian amplitudes on the days after light treatment were significantly greater after the night-time light exposure, leading to a significantly faster recuperation of the rhythmicity of melatonin. The bright light therapy seems to be as effective as melatonin treatment on the rhythm of this body function when compared with other studies (refs. 27, 28, and 34). However, the outcome of this study on 6-OH-melatoninsulphate excretion can also be interpreted in a different and more complex way. As can be seen from the raw data and the statistical analyses: (1) there were no differences between the experimental and control conditions for amplitudes and daily means during treatment days; (2) melatonin secretion under both conditions dropped significantly from normal values (pre-shift days) by about 50% as soon as the shift went beyond the entrainment limits during the treatment days. Thus, independent of the light treatment, the melatonin secretion rhythm was apparently suppressed by the lengthening of the wake-rest schedule beyond the entrainment limits (ref. 34), and the faster resynchronization under light treatment at night may have been due to the greater effective strength of this zeitgeber on the rhythm course of melatonin.

The differences in acrophase shifts between conditions were most divergent on the first day after the shift. Three days after the time displacement, the delay produced by the night-time treatment was only 1.5 h greater on average than that produced by the day-time light treatment. Similar results were obtained for potassium excretion rhythms, whereas the acrophases of all other body functions did not differ between conditions.

The significantly higher diurnal levels of heart rate and epinephrine during night-time light exposure can be explained by the treatment per se, which can be anticipated to be more stressful for subjects when sleep deprived than under normal conditions.

The differences between light treatment conditions in the responses to the time displacements are rarely significant, and when they occur, they are not very dramatic. Thus, although some functions showed faster resynchronization with night-time light exposure, the circadian system as a whole apparently did not.

Simulated Weightlessness

The simulated microgravity environment led to disturbances of the circadian system. Although the interpretation of the results of this study regarding excretion rates is often difficult due to the lack of data prior to the bedrest condition, conclusions can be drawn from the remaining data and from comparisons with the results of similar experiments.

Whereas temperature did not exhibit any alterations of rhythmicity due to postural changes (but see discussion in chapter 2), heart rate amplitudes and levels were affected. The main causes for the lowered values are the change in posture associated with a changed demand on cardiac performance due to reduced and differently distributed body fluids (refs. 3, 51, and 56) and inactivity (refs. 17 and 37). This early adaptation to immobilization and fluid distribution towards the upper part of the body can be also seen in the high excretion rate in sodium accompanied by lower excretion of potassium. The stimulation of cardiopulmonary receptors and of aortic and carotid baroreceptors resulted in an inhibition of the sympathetic nervous system and the antidiuretic hormone (ADH), eventually leading to a high Na/K-ratio (table 3.5). The rate of sodium loss peaks during the first day of HDT due to a diuresis and natriuresis (ref. 51), producing a negative balance of fluid and sodium. Increased excretion of potassium began to become significant towards the end of the 10 days 6° HDT. Thus, the Na/K-ratio increased, but a net loss of fluid and electrolytes was found which could lead to a substantial deficit in long duration bedrest studies or space operations when not treated with countermeasures.

The decreasing excretion of cortisol is inconsistent with findings that often cortisol or 17-hydroxicorticosteroids increased during bedrest studies (refs. 17 and 51). We believe that the acceptance of the bedrest situation by an individual can influence the secretion and excretion of these stress hormones. Furthermore, we measured only the urinary output of adrenal breakdown products as an

indicator of gland secretion, thus possibly obscuring the real response (ref. 51).

The reduced excretion of catecholamines during the bedrest condition ("adapt" and "supine" in table 3.5) in relation to the post-HDT situation revealed a lowered sympathoadrenal activity. The decrease of heart rate during HDT apparently reflected enhanced parasympathetic activity (refs. 10, 56, and 57).

When the acute and, in some body functions major, adaptation to the HDT position was complete, after about two days, the unmasked circadian rhythmicity was revealed. This was indicated by the smooth transitions between wakefulness and sleep and the absence of distinct peaks in the raw data (temperature, heart rate). Furthermore, the diurnal course of excretion rates (before the disturbance by the shift, and after resynchronization) revealed a very individual, but regular pattern (e.g., fig. 3.1, subject B). The unmasking of a rhythm was usually accompanied by a decrease in the circadian amplitude such that levels during the lower part of a diurnal curve did not change, whereas levels during the upper part were reduced.

In this context, we must emphasize that the constant routine protocol as chosen by Czeisler et al. (refs. 30 and 58), may obscure the circadian time course. Since sleep deprivation can cause high mental and physical loads, the consequent activation can lead to significant masking of an investigated rhythm, and only a careful within-subjects investigation can reduce systematic errors. Of course, the same argument can be made for the masking ability of sleep itself (ref. 8), and for the adaptation process when using HDT as an "unmasking method." Therefore we chose the final day before the time shift and the final day before leaving the supine position as reference for most comparisons, assuming that for most body functions, these days were least disturbed by the HDT or time-shift condition. In fact, the concept of completely "unmasking" a circadian rhythm may well be erroneous, if any output rhythms, e.g., sleep-wake, feedback on the circadian pacemaker(s).

Conclusions

We were able to show that the disturbances of the circadian system were profound. They were generated by (1) the change of posture, and (2) the shift of zeitgebers. They were manifested in a desynchronization of the circadian system from environmental time, and in an internal dissociation among body functions.

Means as well as amplitudes were affected by the HDT, mainly due to acute adaptation to bedrest, but also due to a chronic adjustment. Both rhythm parameters

were also affected by the time displacement. The bedrest condition appeared to have only minor effects on acrophases, whereas the delay of the sleep-wake cycle affected acrophases most. The treatment by light might have improved circadian adjustment, however, for most functions there were no discernible differences between experimental and control protocols, either for resynchronization speeds or for the recuperation of amplitudes. This implies that any acceleration of adaptation caused by the light pulses was largely independent of the time in the circadian cycle at which light was administered. We can not exclude a minor effect, since two body functions, one of which is very strongly masked by light exposure, exhibited significant differences between experimental and control conditions. Furthermore, statistical analysis from a limited number of subjects (four under the two different time-of-day light exposure conditions) might obscure minor, but significant differences.
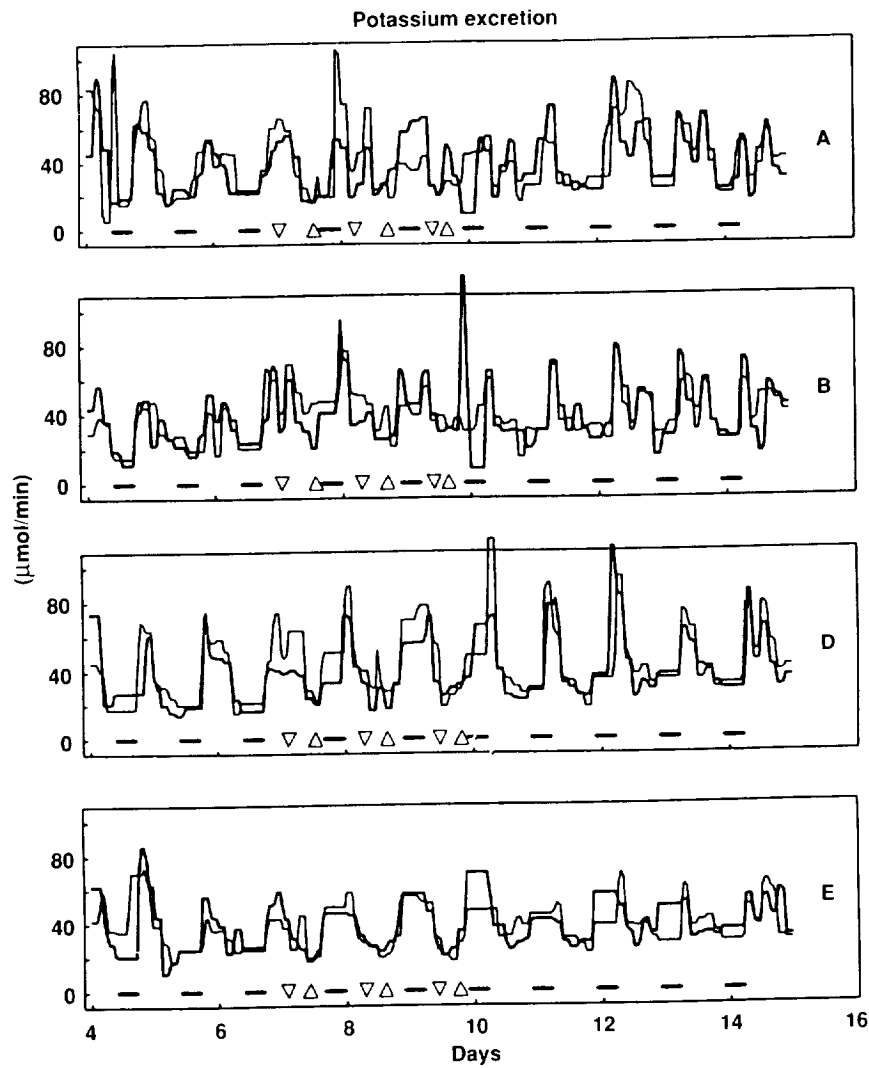
Figure 3.1. Potassium excretion raw data for each subject under control (thick line) and experimental (thin line) conditions. Rates in μmol/min are indicated on the y-axis. Black bars indicate sleep periods, upright triangles indicate light exposures at night, inverted triangles indicate daytime light exposures. Capital letters on the right side refer to individuals.
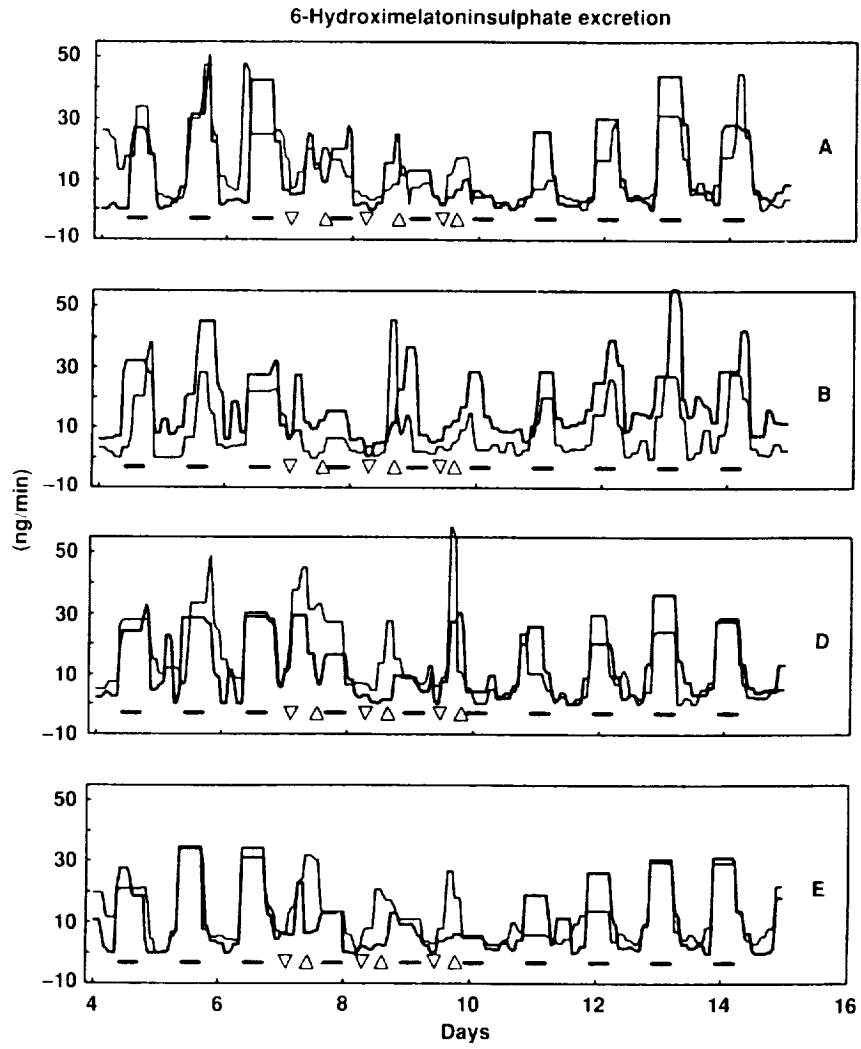
Figure 3.2.   Raw data of 6-hydroximelatoninsulphate. Symbols and abbreviations as in figure 3.1.

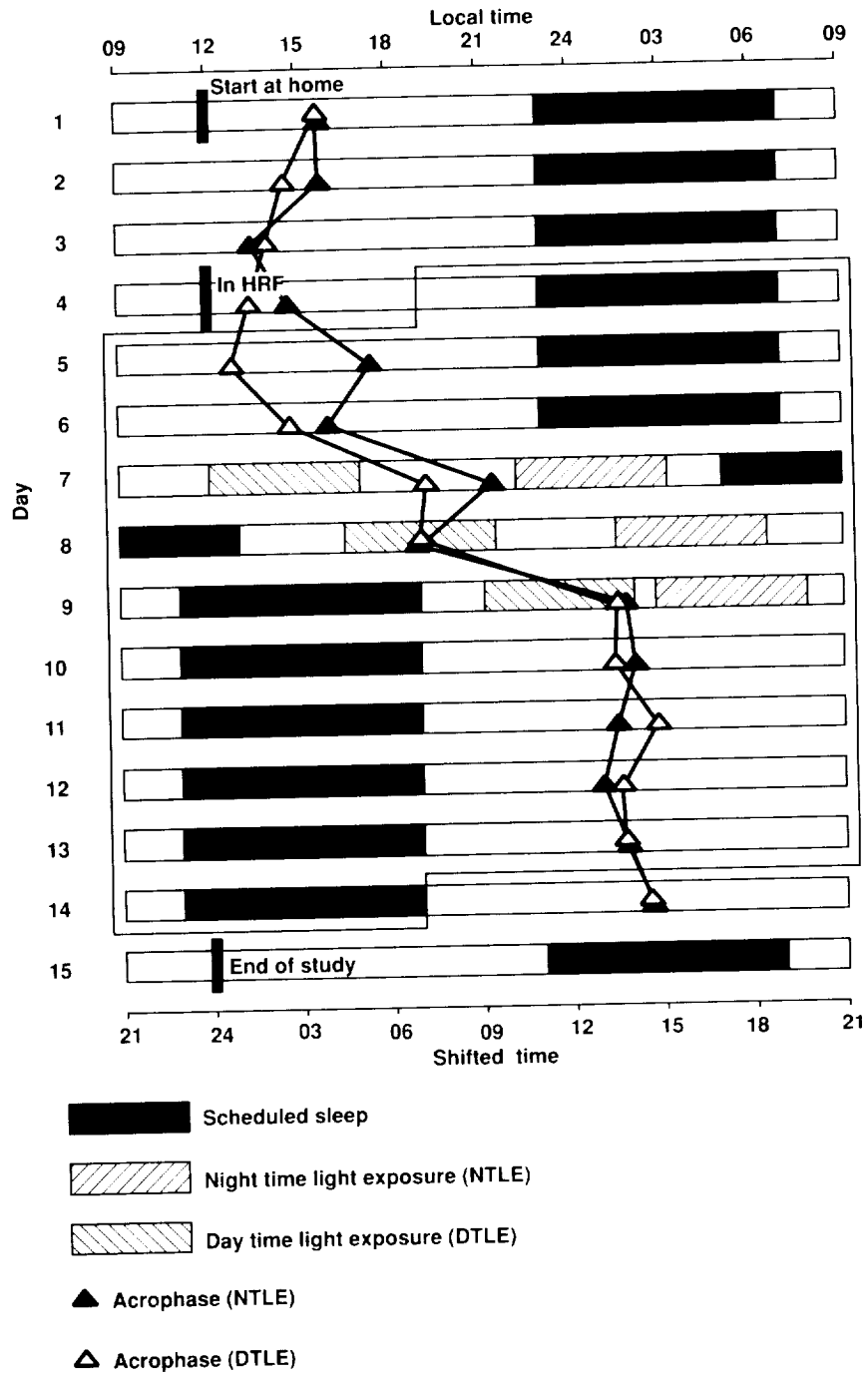# Heart rate: shift of acrophases

Figure 3.3. Heart rate acrophases under control and experimental conditions, averaged for all subjects.
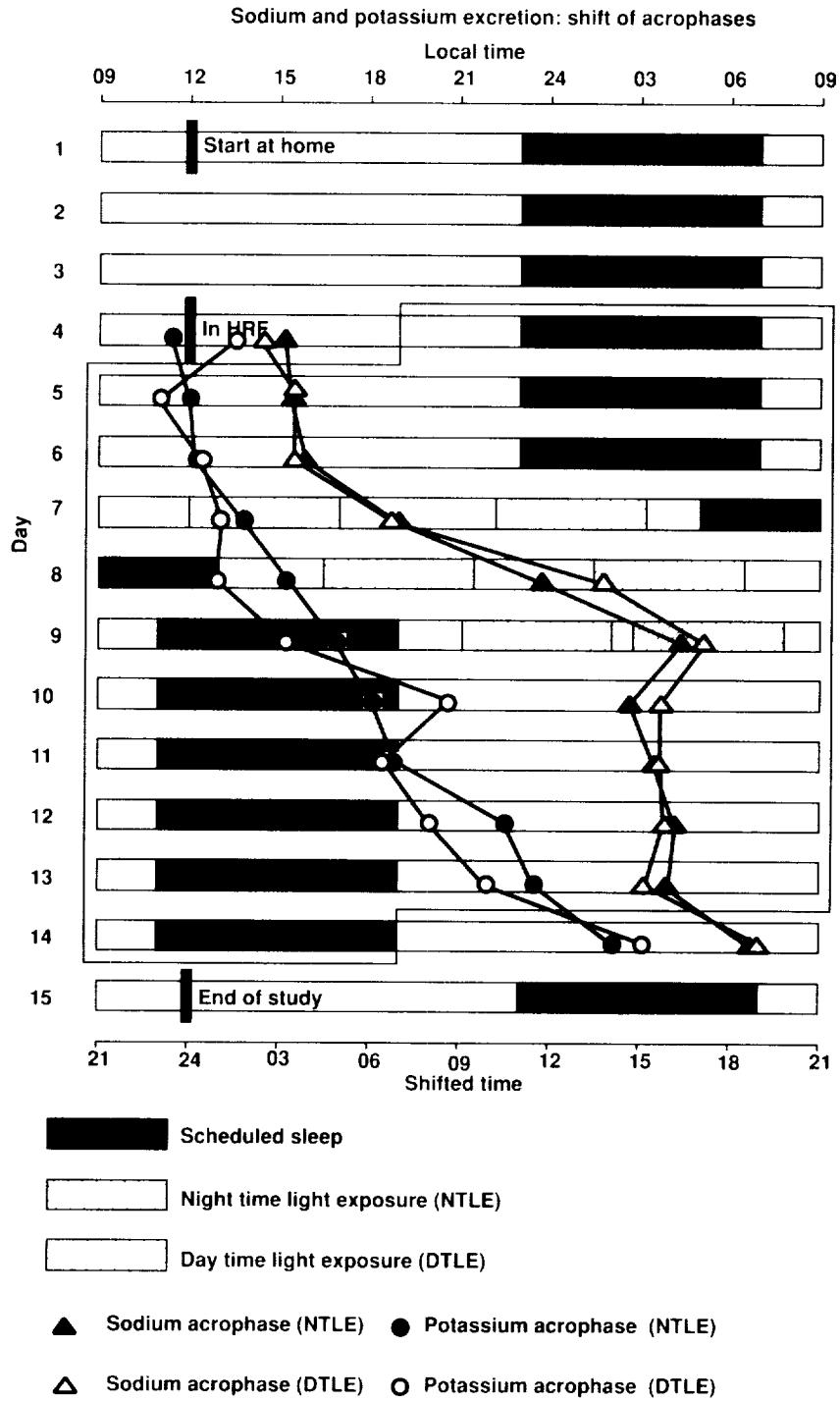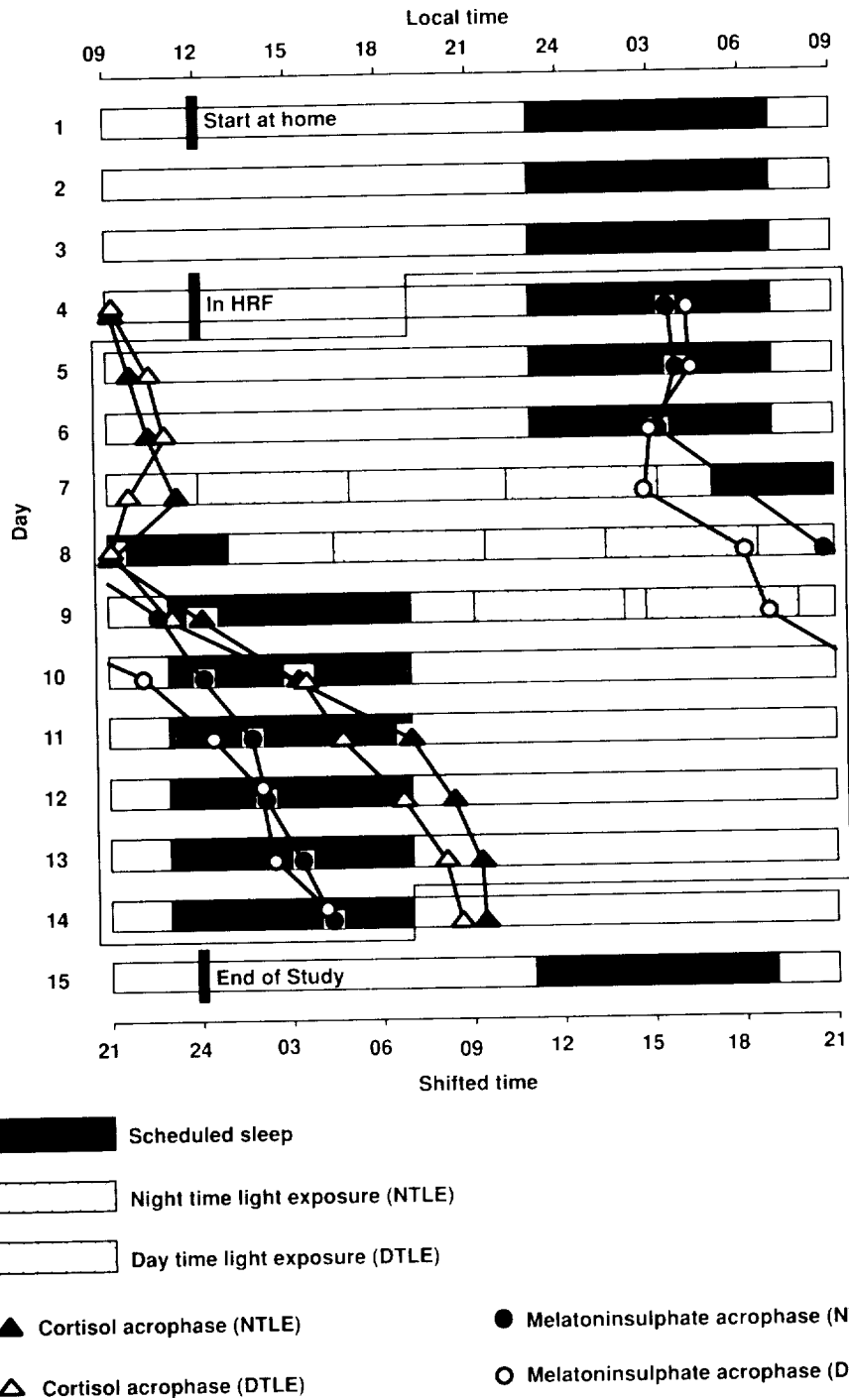
Figure 3.4. Sodium and potassium excretion acrophases under control and experimental conditions, averaged for all subjects.
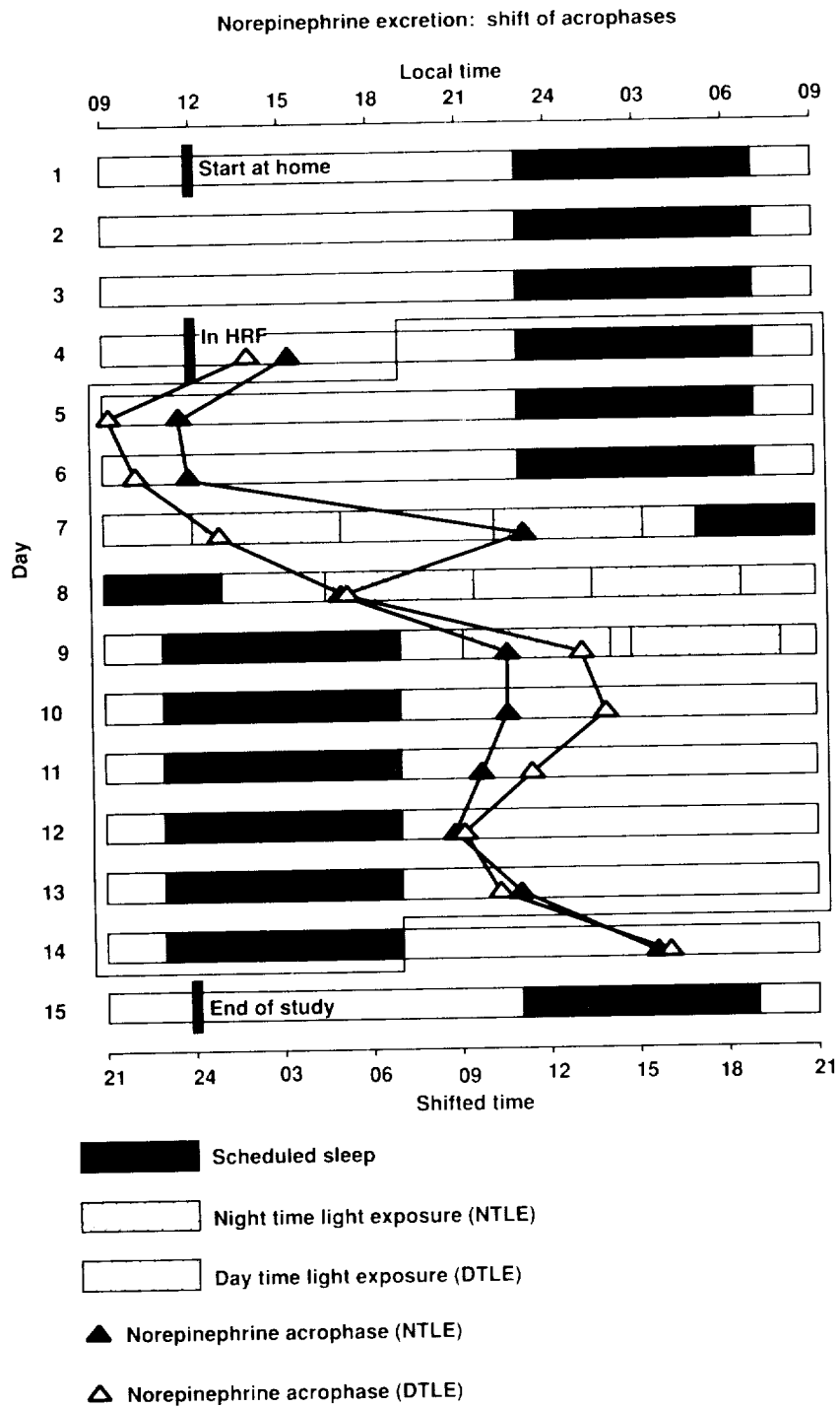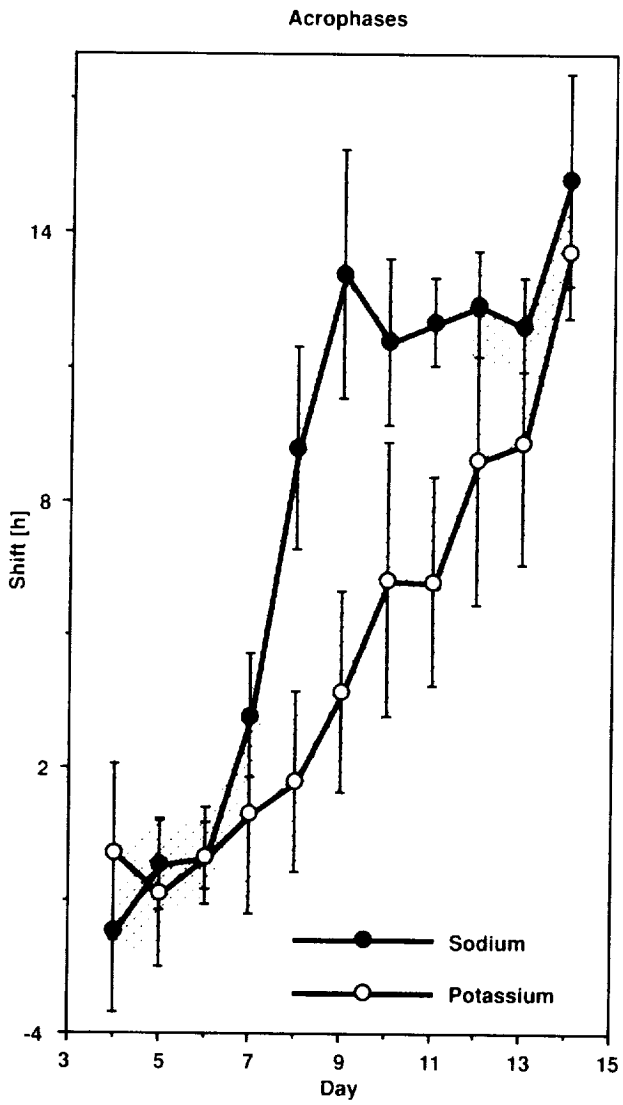
Figure 3.5. *Cortisol and 6-hydroximelatoninsulphate excretion acrophases under control and experimental conditions, averaged for all subjects.*

Epinephrine excretion: shift of acrophases

Local time

09 12 15 18 21 24 03 06 09

1 Start at home

2

3

4 In HRF

5

6

7

8

9

10

11

12

13

14

15 End of study

Day

21 24 03 06 09 12 15 18 21
Shifted time

Scheduled sleep

Night time light exposure (NTLE)

Day time light exposure (DTLE)

▲ Epinephrine acrophase (NTLE)

△ Epinephrine acrophase (DTLE)
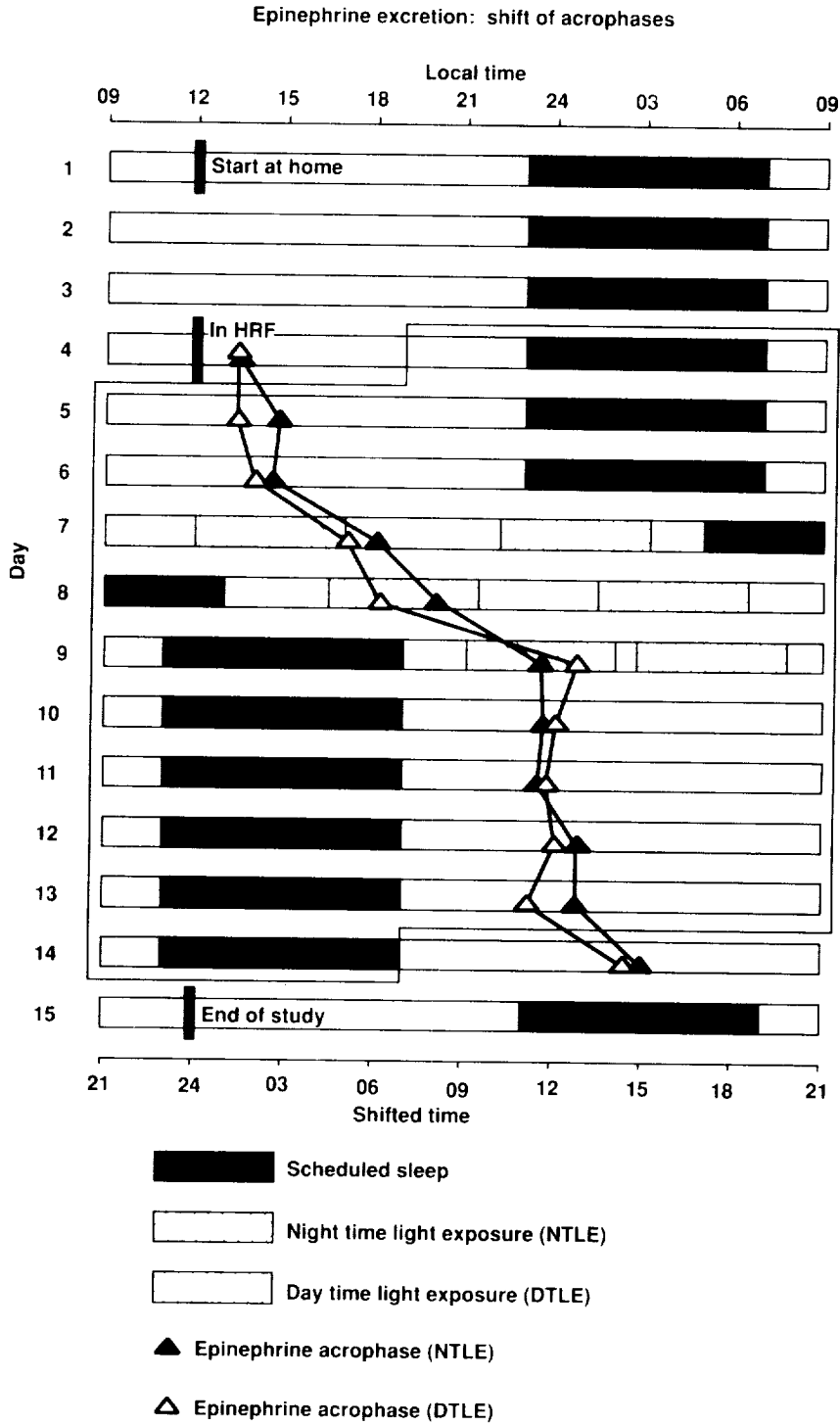
Figure 3.6.   Epinephrine excretion acrophases under control and experimental conditions, averaged for all subjects.

Norepinephrine excretion: shift of acrophases

Local time

Figure 3.7. *Norepinephrine excretion acrophases under control and experimental conditions, averaged for all subjects.*

Legend:

- ▮ Scheduled sleep
- ▭ Night time light exposure (NTLE)
- ▭ Day time light exposure (DTLE)
- ▲ Norepinephrine acrophase (NTLE)
- △ Norepinephrine acrophase (DTLE)

Axis labels: Local time (09, 12, 15, 18, 21, 24, 03, 06, 09); Shifted time (21, 24, 03, 06, 09, 12, 15, 18, 21); Day (1–15). Day 1 "Start at home", Day 4 "In HRF", Day 15 "End of study".

Figure 3.8.   Shifts of sodium and potassium excretion
acrophases (mean ± SD) relative to day 6.
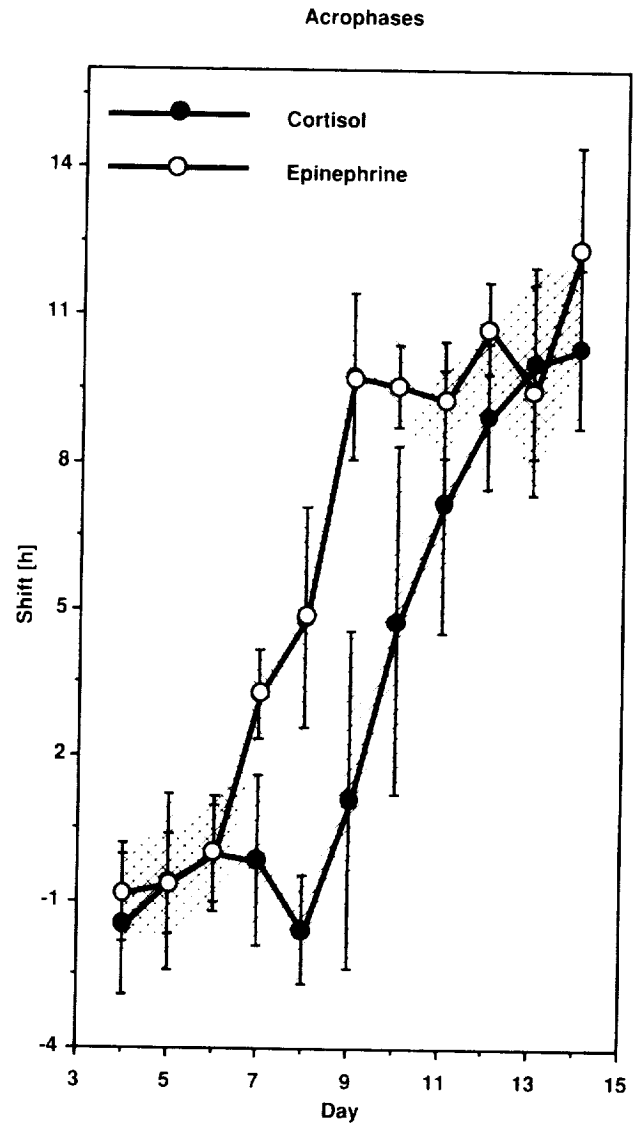
Figure 3.9.   Shifts of epinephrine and cortisol excretion
acrophases (mean ± SD) relative to day 6.
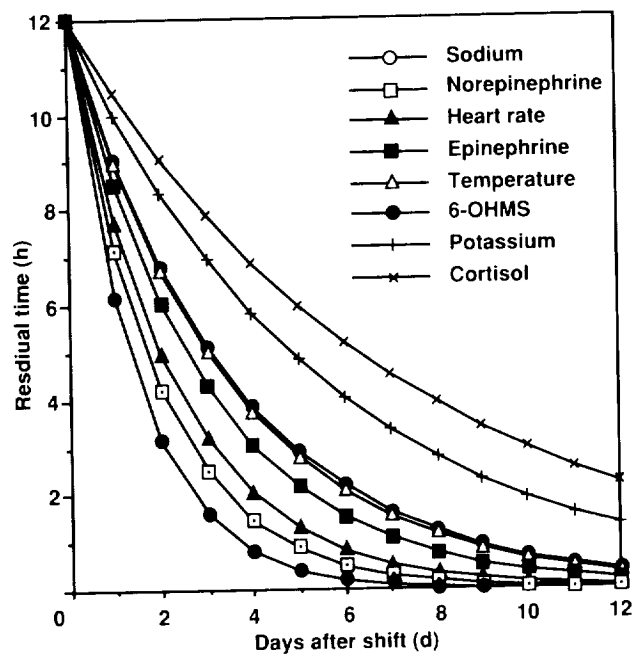
Figure 3.10. Exponential fits to the resynchronization course of various body functions, according to the method of Klein and Wegmann (refs. 37 and 70). Shading corresponds to post-shift bedrest days.
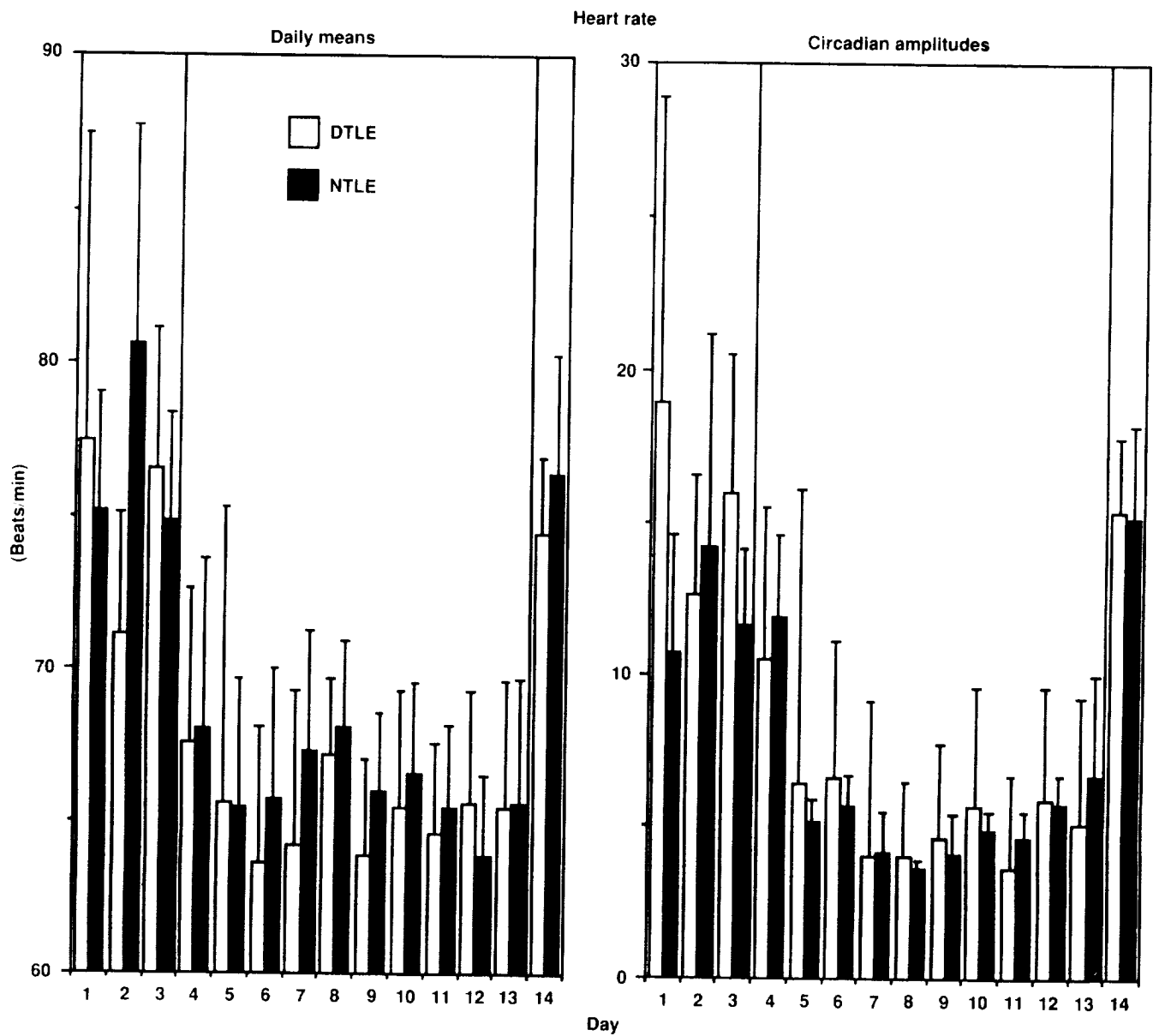
Figure 3.11. Daily means and circadian amplitudes of heart rate under control and experimental conditions, averaged for all subjects.
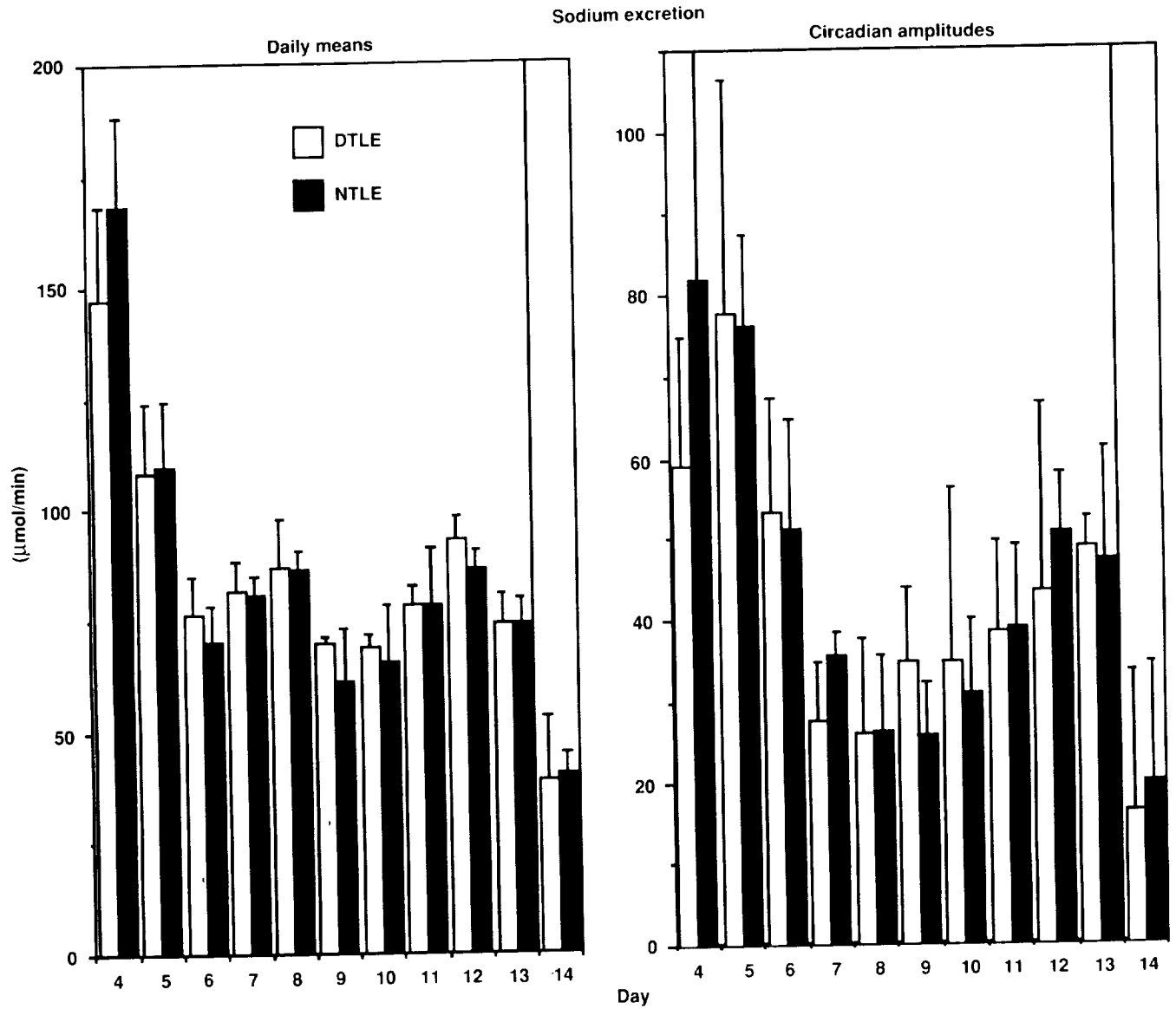
Figure 3.12. Daily means and circadian amplitudes of sodium excretion under control and experimental conditions, averaged for all subjects.
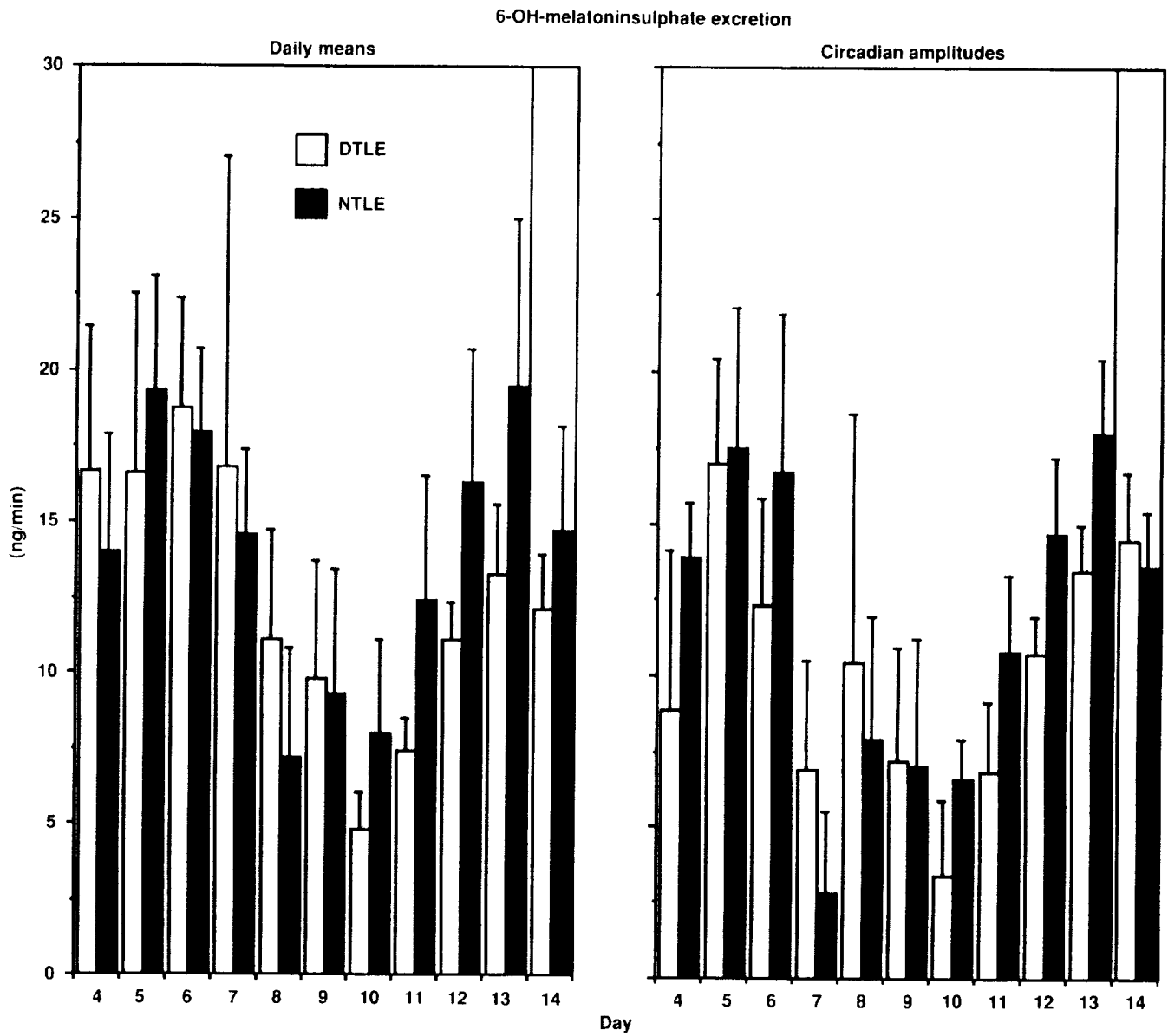
6-OH-melatoninsulphate excretion

Figure 3.13. Daily means and circadian amplitudes of 6-hydroxymelatonin excretion under control and experimental conditions, averaged for all subjects.
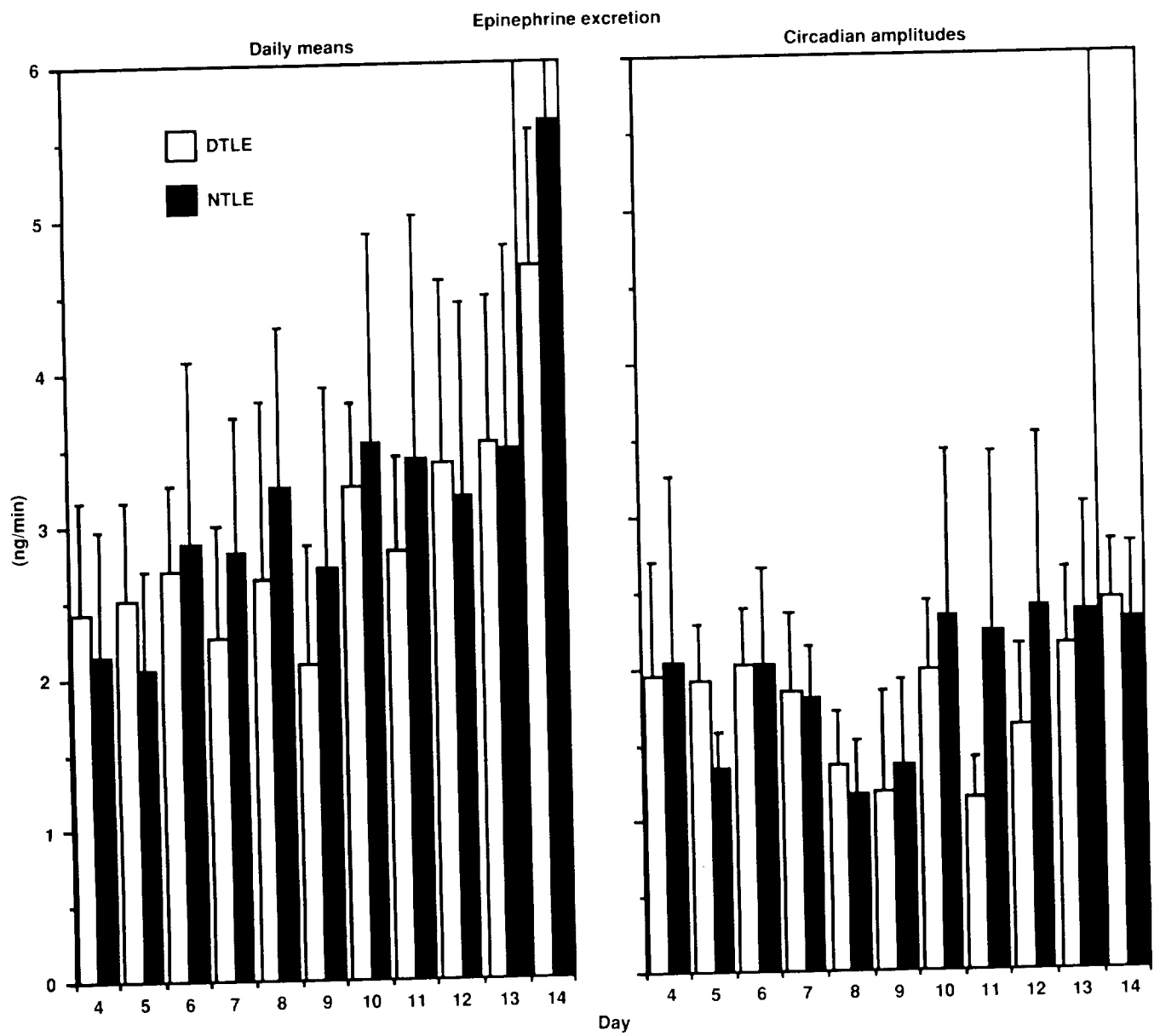
Figure 3.14. Daily means and circadian amplitudes of epinephrine excretion under control and experimental conditions, averaged for all subjects.
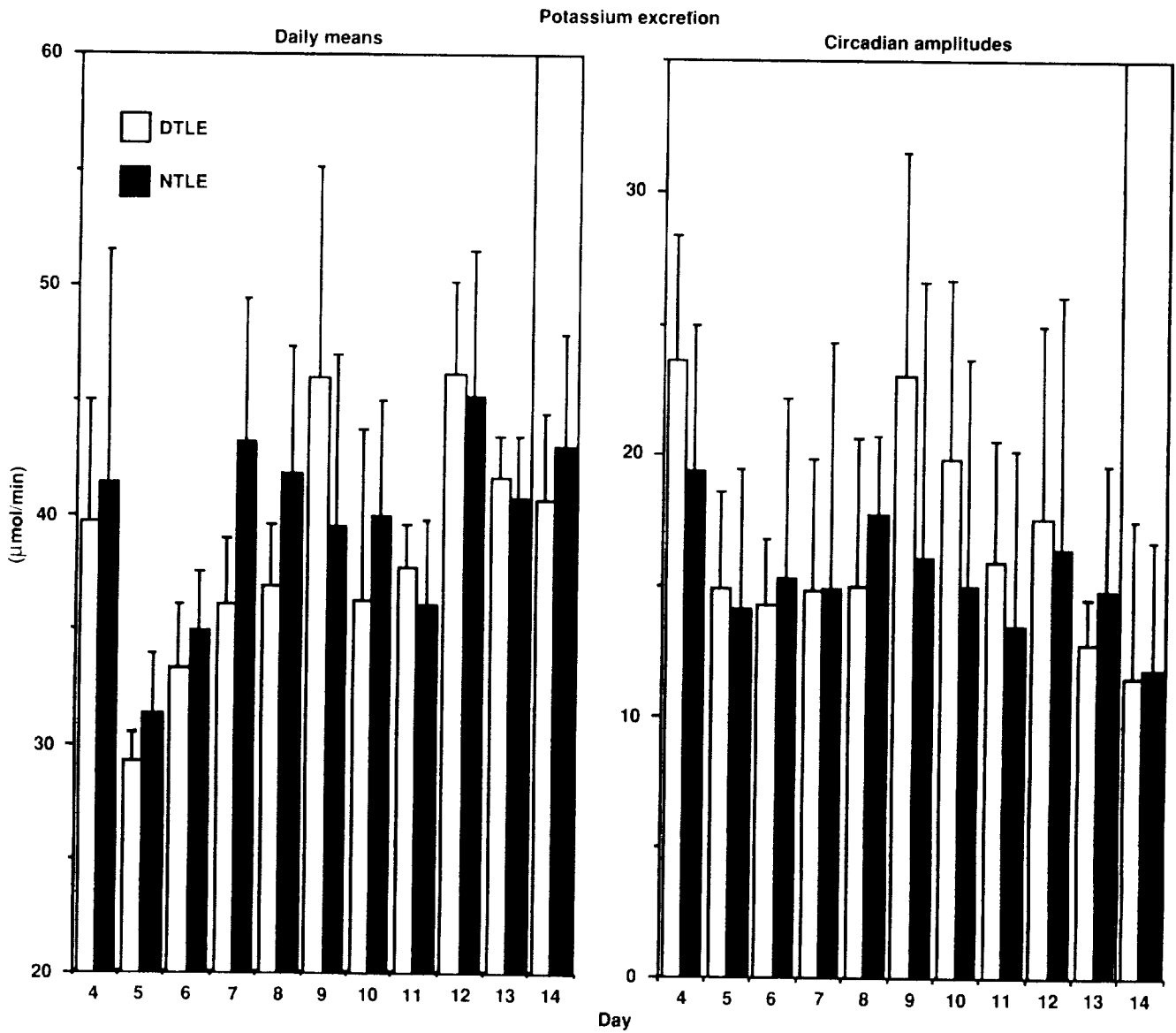
Figure 3.15. Daily means and circadian amplitudes of potassium excretion under control and experimental conditions, averaged for all subjects.
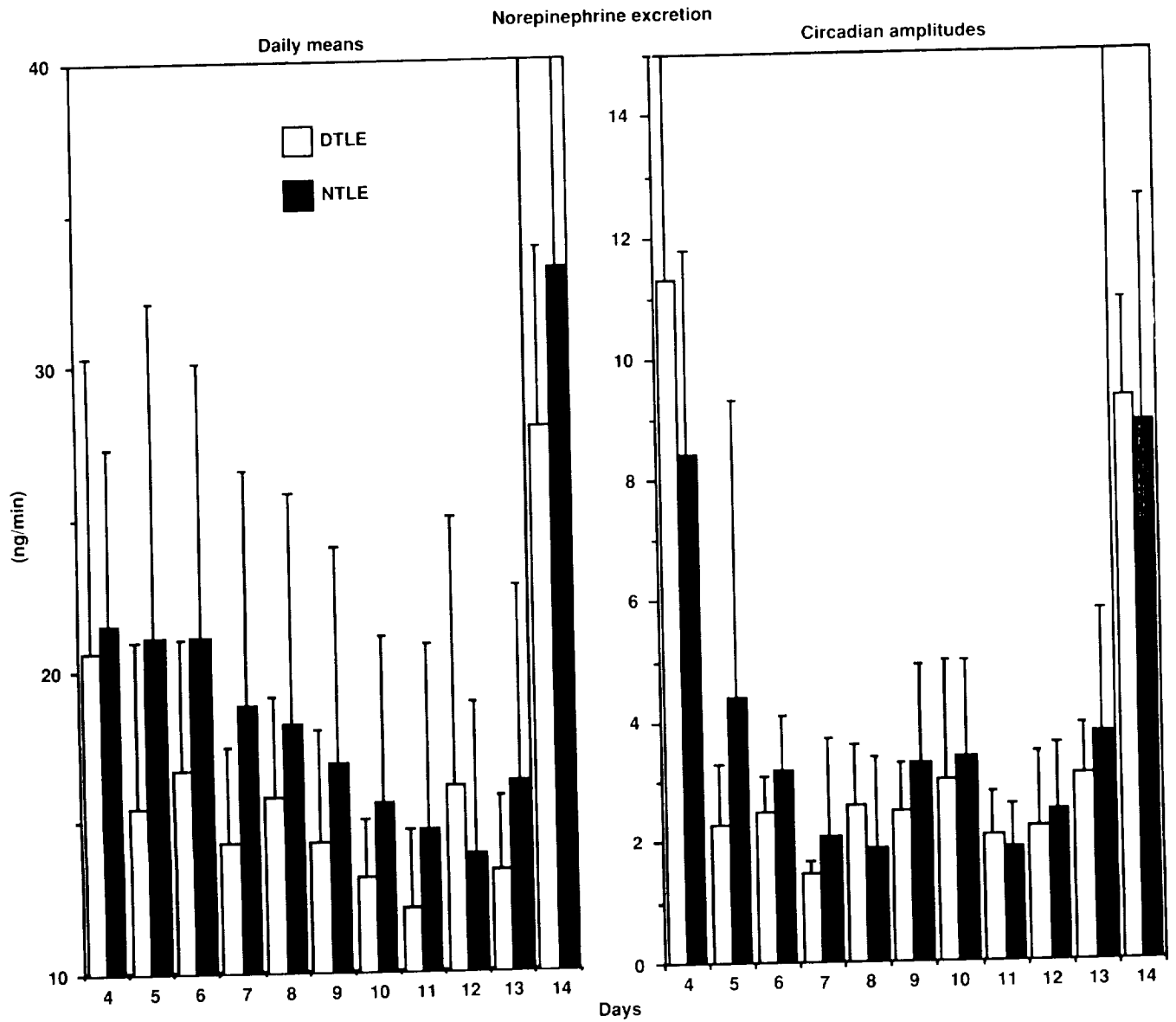
Figure 3.16. Daily means and circadian amplitudes of norepinephrine excretion under control and experimental conditions, averaged for all subjects.
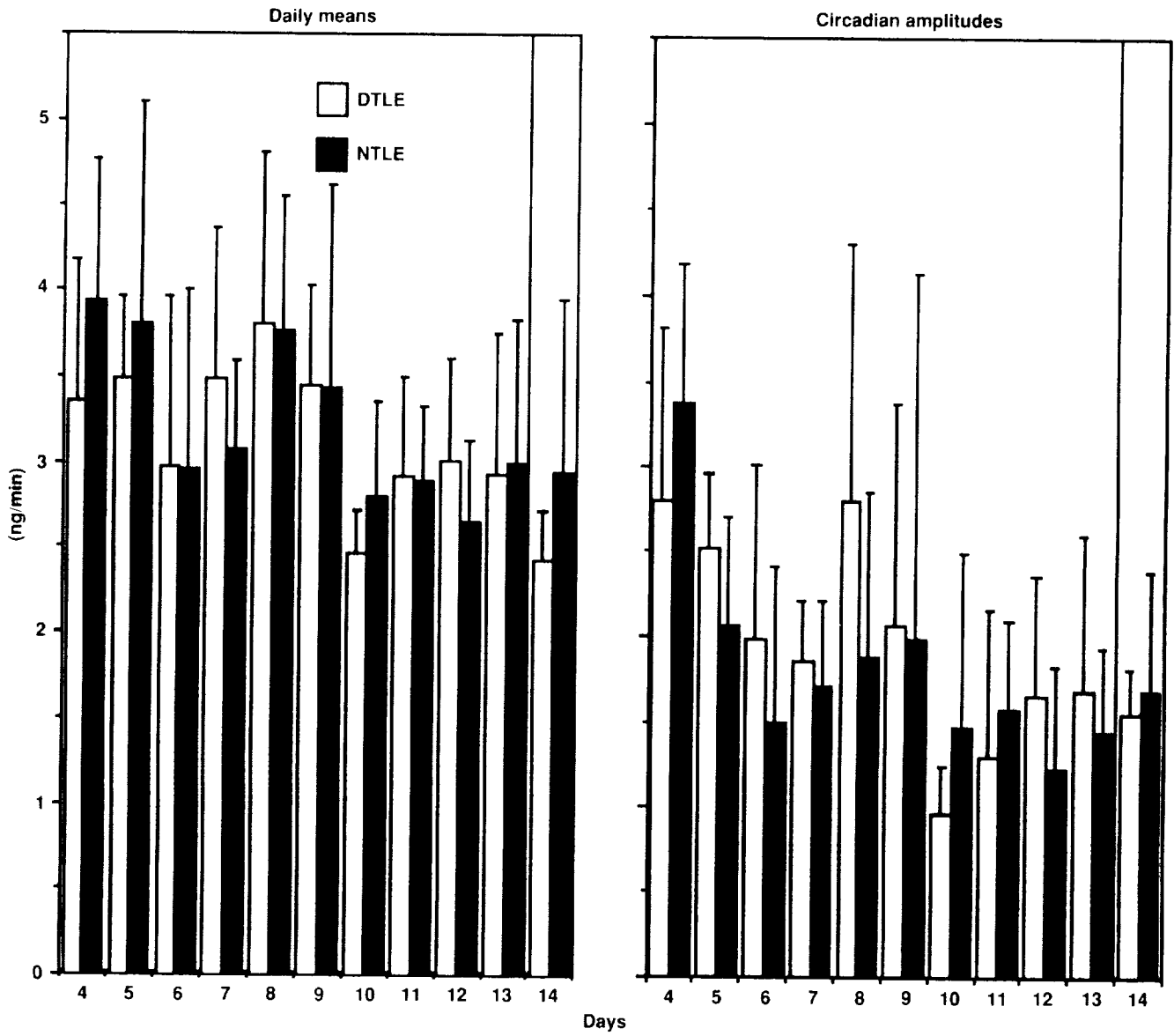
Figure 3.17. Daily means and circadian amplitudes of cortisol excretion under control and experimental conditions, averaged for all subjects.

Chapter 4: Sleep

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Objective sleep quality and quantity has not yet been measured under bedrest conditions. Although polysomnography was conducted under space conditions (ref. 18), the results reflected the disruptive influence of mission requirements on sleep more than the effect of mission requirements on sleep more than the effect of weightlessness *per se*. Because bedrest combined with 6° head-down tilt (6° HDT) has been shown to produce a good simulation of the effects of weightlessness on many body systems (ref. 59), we anticipate that sleep should be affected comparably by these conditions as it would be by microgravity without mission constraints.

In the laboratory, sleep is recorded primarily for clinical purposes (sleep disorders), and not for the estimation of sleep parameter changes due to changes of environmental time. In the field, however, sleep duration and efficiency has been assessed in order to estimate the effects of transmeridian flights on sleep quality (refs. 60 and 61).

Methods

Sleep was polygraphically recorded by means of a flexible, compact and transportable system. This system was a modified 8-channel analog recorder (Medilog 9000, Oxford Instruments Inc.) with leads for recording one EEG signal (C3-A2), two EOG signals (ROC-A2, LOC-A1), and one EMG signal (two chin-electrodes), thus using four out of eight channels. Recordings were made for the scheduled sleep periods (fig. 1.1), i.e., the times between lights off and lights on. This was between 2300 h and 0700 h (before the time shift), 0500 h and 1300 h (during the first shift night), and 1100 h and 1900 h (after the shift), with respect to local time (Pacific Standard Time). A low luminance lamp (62 lux) enabled the subjects to use light in case of an emergency during sleep periods. The remaining part of the sleep-wake cycle, although spent in bed, was not monitored polygraphically. Subjects were requested not to nap and every effort was made by the investigators, nursing staff, and other subjects to ensure that they complied.

The signals replayed from the analog recordings were scored for the estimation of sleep stages according to Rechtschaffen and Kales (ref. 62). A normal night of sleep is characterized by cyclic alternations between two different types of sleep: non-REM (NREM) sleep, which includes 4 stages, and REM or paradoxical sleep. NREM stages 1 and 2 are sleep stages dominated by fast brain

waves and usually occur at the beginning of a sleep cycle. The percentage of NREM stages 1 and 2 in each NREM/REM cycle increases as sleep continues. NREM stages 3 and 4 represent slow-wave sleep and are associated with 'deep' sleep. They occur predominantly during the first half of sleep. NREM stage-1 sleep combined with rapid eye movements (REM) characterizes the occurrence of dreams (ref. 28 and 63). In addition, several other sleep parameters were calculated from the records. Time in bed (TIB), was defined as the time between lights off and lights on. Total sleep time (TST) was defined as the amount of time spent in NREM stages 1 to 4 and REM, i.e., TIB minus wake periods. Sleep efficiency was defined as the quotient of TST and TIB. The number of awakenings, and latencies to each of the five different stages of sleep, were also noted.

The first two nights of the first phase of the study were assumed to be adaptation nights and were not included in the analyses. The remaining nights of sleep before the time shift were combined as baseline. To assess the effects of postural changes (horizontal versus 6° HDT), these baseline data were compared with data from a normal healthy male group of similar age (ref. 63). They also served as a baseline for determining the effects on sleep of the delay of the wake-rest cycle. The effects on sleep of the experimental and control light protocols were also compared.

The statistical methods (one-way or multiple ANOVA) for examining differences between conditions are the same as those used in the analyses of the other rhythms.

Results

The total number of sleep records was 80. Due to the low quality of some sleep recordings, only those where sleep parameters (e.g., latencies, stages) could be scored unambiguously were included in the analyses. This, together with the exclusion of the adaptation nights (as defined above), reduced the number of nights of sleep included in the analyses to forty-three (table 4.1).

Total Sleep Time and Sleep Stages

Total sleep times and the percentages of time spent in the various sleep stages were not significantly different

Table 4.1. Sleep records used in the analyses. The first two nights of the first study phase were considered to be adaptation nights and were therefore excluded from the analyses. Numbers in parentheses indicate the number of nights of data included for each subject.

| Baseline | A(1), B(1), D(1), E(2) | |
	Control	Experimental
Shift	A(2), B(2), E(1)	A(2), B(1), E(2)
Resynchronization	A(2), B(3), D(3), E(2)	A(1), B(3), D(2), E(2)
Final	A(1), B(2), D(2)	A(1), B(2), D(1), E(1)

between the two light conditions (two-way ANOVA, days by light treatment). The days were then grouped as in previous rhythm analyses, i.e., baseline days preceding the shift (indicated in figures and tables as B); shift days (no. 7 and no. 8); resynchronization days (no. 9, no. 10, and no. 11); and the final two days of each part of the study (no. 12 and no. 13). No significant differences could be detected between light treatment conditions, even for the most critical nights of the shift and resynchronization period. Indeed, two-way ANOVA (grouped days by light treatment) revealed significant differences between the different groups of days in the percentage of sleep time spent in NREM stage 2 ($F = 3.17$, $p = 0.0364$) and NREM stage 4 ($F = 6.87$, $p < 0.001$), but no differences between experimental and control light conditions.

The baseline values of several sleep parameters in our subjects under 6° head-down tilt bedrest conditions were more than one standard deviation from the means for a comparable age-matched group of ambulatory males sleeping in the horizontal position (ref. 63) (table 4.1). Total sleep time (TST) was 22 min shorter on average for the group under study than for the subjects from the study of Williams et al. (ref. 63). The sleep efficiency, i.e., the relation between the time from lights off to lights on (defined as time in bed TIB) and total sleep time (TST) was 13% lower. The percentage of sleep time spent in NREM stage-1 was significantly longer under head-down tilt conditions. In contrast, NREM stage-4 sleep tended to be shorter during baseline head-down tilt (table 4.2).

The shift of the environmental time structure led to a significant decrease in TST of about 45 min during each of the following three nights. On day 13, TST again dropped significantly compared to baseline values. Consequently, sleep efficiency was also reduced significantly on days 7, 9, and 13 (fig. 4.1). NREM stage-2 sleep showed the greatest reduction with the decrease in total sleep time. The percentage dropped significantly (about 20%, or 10% of TST) on the two days following the shift (fig. 4.2). In contrast, NREM stage-4 sleep increased from 3% of TST on baseline days to 11% of TST on average during the

three consecutive nights after the shift, declining thereafter to baseline values (table 4.2). Slow-wave-sleep (SWS) was significantly enhanced during the shift (20.0% of TST, $p < 0.001$) and resynchronization period (16.0% of TST, $p < 0.05$) when compared to baseline (11.0% of TST). In addition, during the shift, SWS was significantly longer than during resynchronization ($p < 0.05$) and during the final period (14.2% of TST, $p < 0.01$).

Sleep Latencies and Wake Times During Sleep

The latencies to the first occurrence of all sleep stages, i.e., latency to 10 min of persistent sleep (LPS), and the latencies to the NREM sleep stages 1 to 4 (LS1, LS2, LS3, LS4) as well as to paradoxical or REM-sleep (LREM), did not differ significantly between control and experimental conditions, i.e., timing of light exposure (two-way ANOVA, light condition by days, $p > 0.05$). The head-down tilt bedrest condition increased the latency to persistent sleep (LPS) and decreased the latency to the first REM episode, when compared to ambulatory subjects sleeping in the horizontal position (analysis is based on one standard deviation; table 4.3).

A day-by-day comparison revealed no significant changes in sleep latencies in response to the wake/rest cycle delay, although a trend to shorter LPS was observed for the second and third days after the shift, i.e., days 8 and 9 (table 4.3). However, when combining days (baseline, shift, resynchronization, and final), subjects needed more time to reach NREM stage-1 sleep during the resynchronization and final period than during the shift nights ($p < 0.05$). NREM stage-3 sleep latencies were prolonged during the final days when compared to baseline.

The number of awakenings decreased significantly ($p < 0.05$) on days 8 and 12, and early awakenings were prolonged on day 9 ($p < 0.01$). The overall sum of the duration of awakenings was significantly ($p < 0.05$) higher on days 7, 9 and 13 (comparable to the results of changes in sleep efficiency).

Table 4.2: Comparison of baseline sleep parameters with those from 30-39 year old males [Williams et al., ref. 79] and with shift and post-shift day recordings. %SPT (sleep period time) values given in (ref. 79) were transformed to percent TST; significant differences between baseline values for the present study and subsequent days are indicated by * p ≤ 0.05, ** p ≤ 0.01.

DAY	TIB (min)	TST (min)	S1 (Percent TST)	S2 (Percent TST)	S3 (Percent TST)	S4 (Percent TST)	REM (Percent TST)
Williams	434.6 ± 20.5	421.5 ± 21.9	5.8 ± 3.5	57.7 ± 7.4	5.8 ± 1.5	6.9 ± 5.3	23.8 ± 3.9
Baseline	478.4 ± 3.6	399.0 ± 15.6	10.1 ± 1.7	54.2 ± 5.1	8.1 ± 3.3	2.9 ± 2.5	24.8 ± 2.8
7	466.8 ± 22.0	344.0 ± 42.4**	11.2 ± 2.7	44.4 ± 7.8*	6.9 ± 1.3	11.3 ± 3.6**	26.2 ± 4.4
8	463.0 ± 28.2	358.0 ± 42.3*	8.0 ± 2.4	45.3 ± 5.4*	9.5 ± 0.6	12.4 ± 3.7**	24.7 ± 4.1
9	477.9 ± 3.7	354.6 ± 41.6*	9.3 ± 2.1	49.8 ± 7.5	7.8 ± 4.0	9.8 ± 6.6**	23.3 ± 3.9
10	479.5 ± 0.8	375.3 ± 9.8	9.1 ± 3.6	49.5 ± 4.4	9.9 ± 2.3	6.0 ± 2.3	25.5 ± 4.3
11	469.8 ± 13.5	370.1 ± 16.3	10.5 ± 1.3	49.5 ± 4.2	7.8 ± 1.9	6.6 ± 2.4	25.6 ± 4.7
12	480.0 ± 0.0	397.8 ± 11.8	10.1 ± 2.4	49.4 ± 2.8	7.9 ± 1.8	4.7 ± 2.5	27.9 ± 3.7
13	478.0 ± 4.4	352.5 ± 39.3*	11.5 ± 5.2	48.0 ± 6.7	7.3 ± 1.3	8.5 ± 2.6*	24.7 ± 4.8

Table 4.3. Comparison of baseline sleep latencies with those from 30-39 year old males [Williams et al., ref. 79], and from shift and post-shift day recordings. Significant differences from baseline values are indicated by *: p ≤ 0.05, **: p ≤ 0.01.

DAY	LPS[a] (min)	LS1[b] (min)	LS2[c] (min)	LS3[d] (min)	LS4[e] (min)	LREM[f] (min)
Williams	5.8 ± 3.9	0.0 ± 3.9	6.8 ± 3.3	37.5 ± 33.0	42.4 ± 28.8	85.4 ± 30.1
Baseline	32.7 ± 23.8	-4.1 ± 6.4	-1.2 ± 5.3	28.6 ± 16.0	71.4 ± 62.4	35.1 ± 23.4
7	25.0 ± 17.9	-2.2 ± 9.7	0.4 ± 10.7	24.6 ± 6.4	28.8 ± 7.1	28.3 ± 32.3
8	10.8 ± 16.6	14.0 ± 22.9*	5.2 ± 14.4	22.8 ± 28.9	29.9 ± 29.0	28.2 ± 22.4
9	10.6 ± 9.5	-1.7 ± 3.6	0.4 ± 3.6	16.8 ± 10.5	48.9 ± 43.0	34.5 ± 31.6
10	20.1 ± 15.4	-6.1 ± 8.7	-3.0 ± 8.4	19.5 ± 15.3	23.9 ± 16.4	46.3 ± 40.4
11	22.2 ± 23.0	-4.2 ± 4.3	-2.1 ± 3.9	9.4 ± 6.9*	18.7 ± 5.1	51.7 ± 14.3
12	24.7 ± 16.4	-7.5 ± 7.0	-4.8 ± 6.1	11.2 ± 3.6	54.2 ± 79.2	41.1 ± 33.8
13	47.2 ± 42.4	-4.1 ± 7.7	-1.7 ± 7.7	12.5 ± 5.4	38.6 ± 49.4	59.6 ± 8.9

[a]LPS: Latency to onset of 10 min of persistent sleep (counted from lights off).
[b]LS1: Latency to stage 1 sleep (counted from onset of persistent sleep).
[c]LS2: Latency to stage 2 sleep (counted from onset of persistent sleep).
[d]LS3: Latency to stage 3 sleep (counted from onset of persistent sleep).
[e]LS4: Latency to stage 4 sleep (counted from onset of persistent sleep).
[f]LREM: Latency to REM sleep (counted from onset of persistent sleep).

Sleep Distribution

In order to detect alterations in the distribution of sleep stages within the sleep episodes, the time from lights off to lights on (TIB) was considered in quarters, and each quarter scored for the percentage of time spent in slow wave sleep (SWS), paradoxical sleep (REM) and awake.

Slow-wave-sleep, i.e., NREM stages 3 and 4, changed significantly on some days when compared to baseline (fig. 4.3). During the second quarter of sleep, SWS was prolonged on days 7 and 13 (p < 0.05). SWS was also extended on day 8 during the third and fourth quarter when compared to all other days (p < 0.05), and was shorter during the second quarter when compared with days 7, 11 and 13. Shift days had significantly (p < 0.05) more SWS in the second half of sleep than resynchronization days, and more SWS in the last quarter of sleep than final days.

Paradoxical sleep was significantly shorter during the fourth quarter of sleep on days 7, 8, 9 and 10 when compared with the corresponding baseline portion of sleep, but did not change during the first three quarters.

During the last quarter, the duration of awakenings was significantly greater on days 7 and 9 ($p < 0.01$) as well as on days 11 and 13 ($p < 0.05$) when compared to baseline (fig. 4.4). Consequently, the prolongation of wake times during the fourth quarter was detected also in the combination of days, i.e., the shift and resynchronization periods differed significantly ($p < 0.01$) from the baseline period.

Discussion

The most striking difference between our subjects in head-down tilt and ambulatory age-matched subjects sleeping in the horizontal position is the shorter total sleep time observed in head-down tilt. This result is in agreement with the outcome of sleep investigations in space (ref. 4 and 18), although the reduction in average sleep duration under space conditions was even greater, i.e., 0.9 h when compared to earthbound controls. Sleep in space may have been shortened not only because of changes in gravity, but also because of operational requirements. Thus, it can be argued that the controlled condition of 6° HDT bedrest may provide more convincing evidence for alterations in sleep induced by changes of the gravity vector than can be derived from relatively uncontrolled measurements during space missions. In any case, our results confirm that the simulation of weightlessness by 6° HDT is an effective tool for examining space-related issues in sleep.

The large difference in sleep efficiency between our subjects (during baseline) and the subjects of Williams et al. (ref. 63) may be misleading, because our subjects were restricted to eight hours of trying to sleep during lights out. Thus, the time in bed reflects the requirements of the study protocol, rather than the willingness or ability of the subjects to sleep. However, the reduction in total sleep time occurred independently of this limitation and may reflect changes in sleep quantity caused by the bedrest condition.

Sleep was altered by the delay in the wake-rest cycle. A reduction of total sleep time was accompanied by an increase in NREM stage-4 sleep and a large decrease in NREM stage-2 sleep. The increase in slow-wave-sleep

(SWS) during the shift and the resynchronization period can be attributed to the forced six hour delay in sleep on the two shift days, and to the incomplete adaptation to the changed wake-rest cycle during the resynchronization period. The increase of SWS associated with sleep deprivation is very well known and is attributed to the homeostatic process in sleep (refs. 64-66). The significant changes in sleep parameters on the last night of HDT were probably attributable to the anticipation of getting up and leaving the study, and should not be interpreted further.

The prolongation of SWS mainly occurred on the shift and resynchronization days during the second quarter of sleep. Slow-wave sleep was not suppressed by a rebound of paradoxical sleep during the resynchronization nights as was observed after real time-zone transitions (ref. 60 and 61). This may indicate an incomplete recovery from sleep deprivation during the shift period. During the shift and resynchronization days, more frequent and longer awakenings were observed during the fourth quarter of the sleep episode, probably due to incomplete adaptation of key circadian rhythms, e.g., temperature, cortisol, and catecholamines.

We can deduce from our results that a time shift of 12 h conducted under bedrest conditions led to an adaptation process for sleep which lasted at least four days. Due to the restricted number of subjects and the low number of high quality EEG recordings, some subtle effects may have gone undetected. We cannot conclude from our data how long the adjustment of sleep to the altered activity-rest cycle will really last. We can only present a lower limit and a suggestion on the adaptation time on average. The trends for some variables appear to indicate a transition time of two additional days after the minimum estimate of four days. However, the assessment of four to six days for adjustment lies well within the range of resynchronization speeds for many body functions with circadian behavior, as was shown in chapter 3.

The timing of light did not affect the quantity or quality of sleep. Czeisler et al. (ref. 58) have suggested that a bright light cycle can override weak coupling between the circadian system and the sleep-wake cycle. However, our results do not indicate that this coupling is weak, because some EEG variables (SWS, NREM stage 2) and awakenings appeared to resynchronize comparably to other circadian functions.
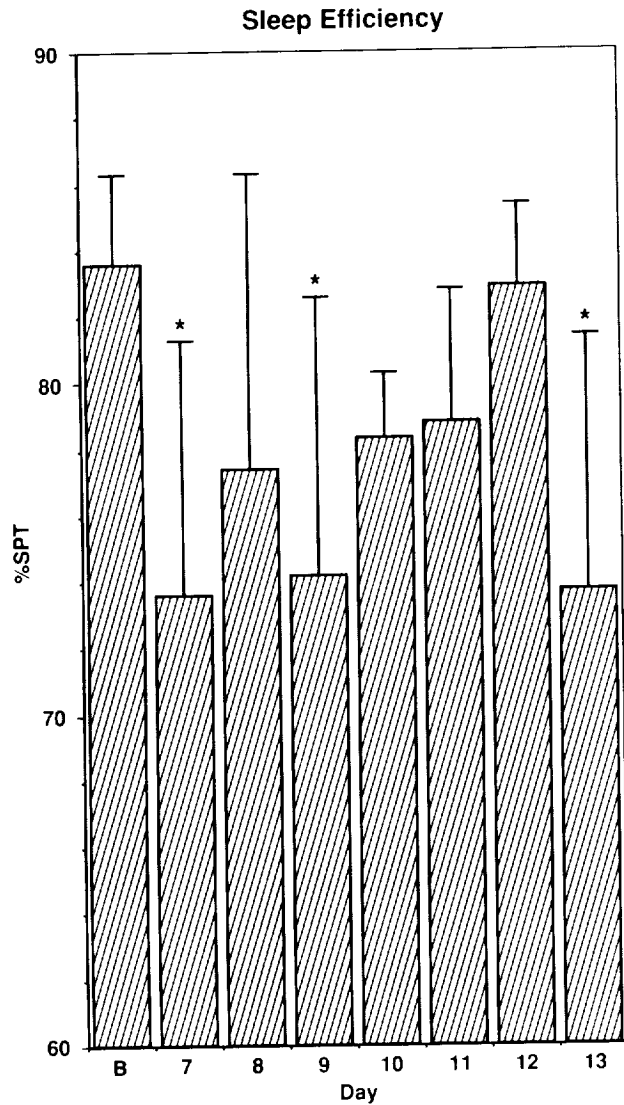
Sleep Efficiency

Figure 4.1. Mean sleep efficiency (percentage of the sleep period time (SPT) that the subjects slept). SPT is defined as the time-in-bed (TIB) minus the wake times before the first persistent sleep and after the last persistent sleep. The sleep on day 7 is the first sleep after the environmental time shift. Significant differences from baseline (B) are indicated by *, $p < 0.05$.
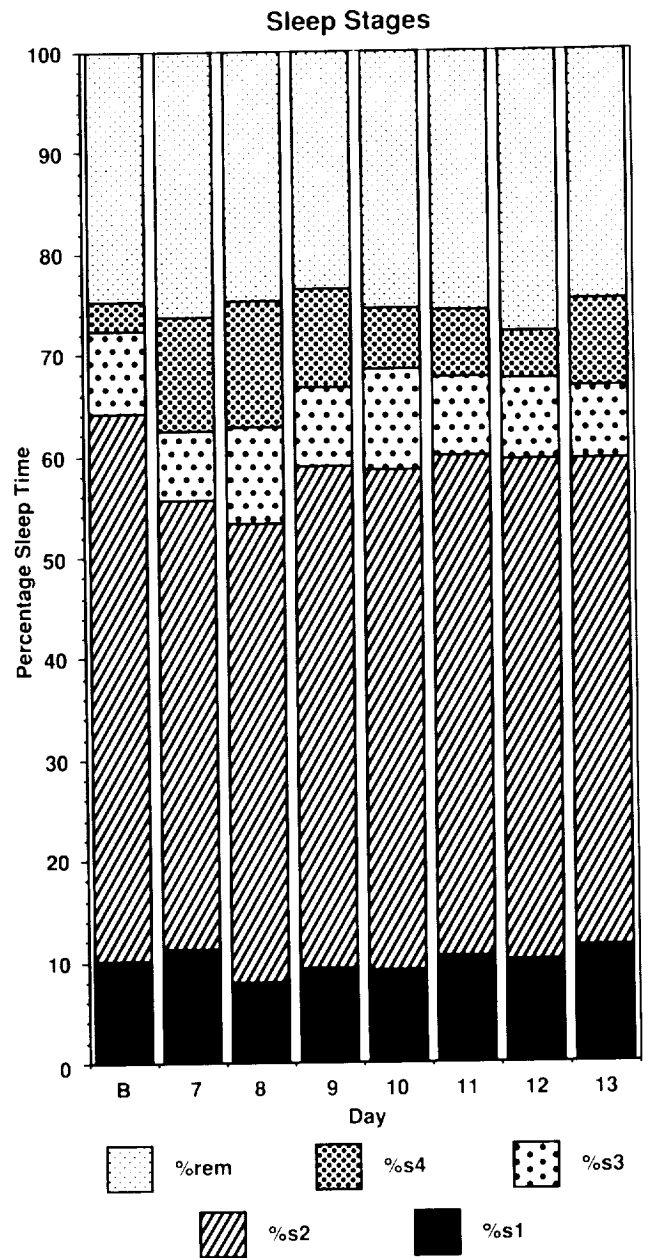
Sleep Stages

Figure 4.2. Mean percentage of total sleep time (TST) spent in each of the four NREM sleep stages and REM sleep, during baseline and after the environmental time shift.
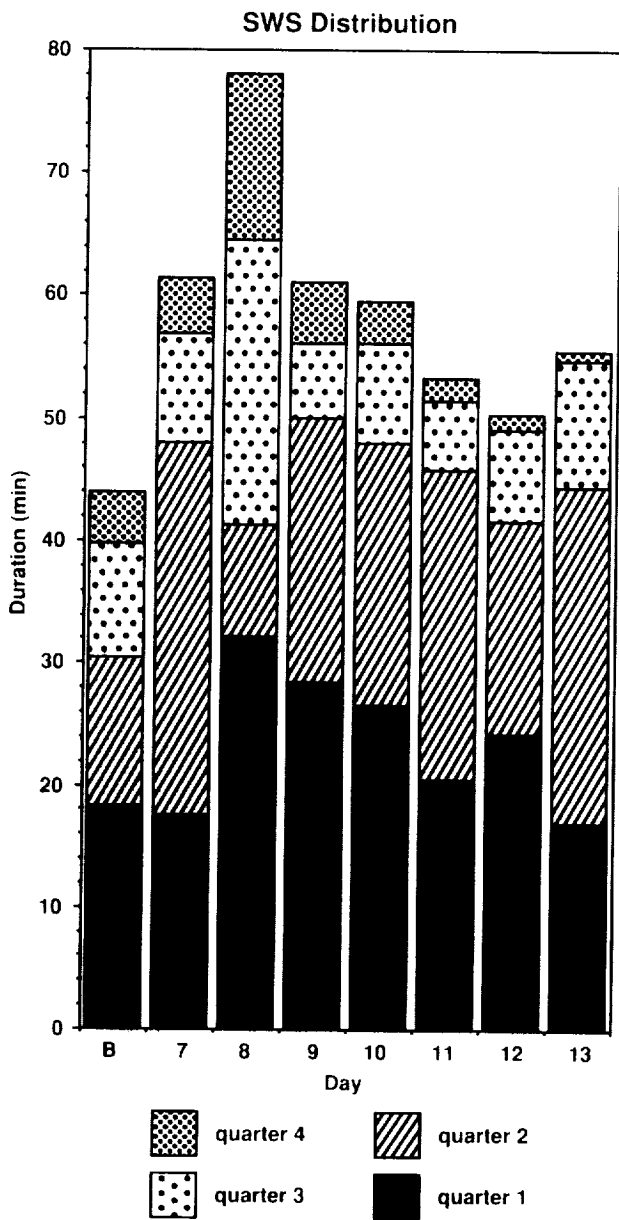
Figure 4.3. Mean slow-wave-sleep duration for each of the four sleep quarters (i.e., quarters of TIB).
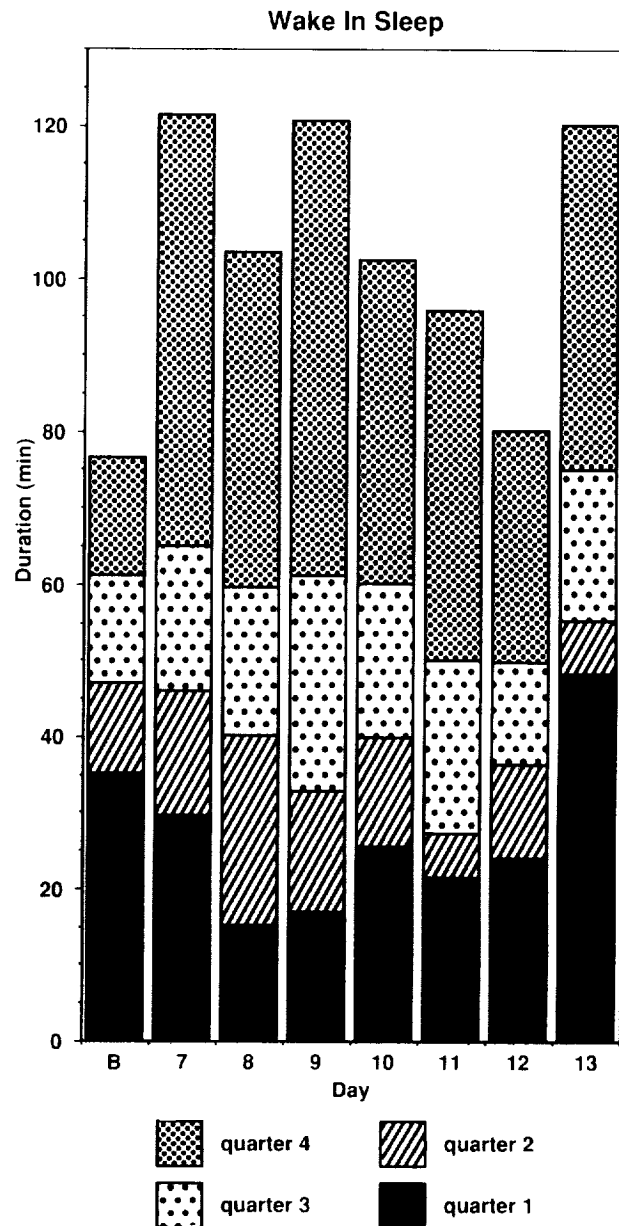
Figure 4.4. Average times spent awake during the four quarters of time in bed. The first quarter includes latency to persistent sleep, the last quarter early awakenings.

Chapter 5: Performance and Subjective Assessments

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Methods

Performance Tests

Cognitive and reaction time testing were carried out using four Macintosh SE-based tests. Two memory and search tasks (ref. 40) were used to examine the ability to recognize two (MAST2) or six letters (MAST6) in a series of other letters. A logical reasoning task (ref. 39) and a simple reaction time test, similar to that used by Dinges and Powell (ref. 38), were also administered to the subjects. All tasks were computer generated and presented separately for each individual on a CRT screen.

The MAST is a visual search task that employs different memory load levels through manipulation of the size of the target subset. A series of 20 different capital letters was presented on the screen. Above the row of 20 letters, a second set of two or six letters was shown simultaneously. The letters changed with each of the 16 trials per session for both MAST2 and MAST6, which were presented consecutively. Following the presentation of each subset, the subject had to decide whether all of the letters in the top row were present in any order in the lower series. The subjects responded by pressing a mouse after shifting the pointer to a "yes" or "no" statement.

The logical reasoning test of Baddeley (ref. 39) was implemented on a Macintosh SE. This task presents a series of relational statements like "A follows B," followed by a pair of letters, either "AB" or "BA." The subject must decide whether the statement describing the pair of letters is true or false, and indicate his decision by pressing the mouse button after shifting the pointer to "true" or "false." The series in one session consisted of 32 different statements, either positive "B precedes A," negative "A does not follow B," active "A precedes B" or passive "A is not followed by B" (ref. 67).

Reaction Time Test

The simple visual reaction time test (SVRT) consisted of a series of stimuli at randomized time-intervals lasting in total ten minutes for each session (ref. 38). A "timer-window" counting msec in 16 2/3 msec intervals was presented on the screen simultaneously with a "stop" sign which appeared below the timer window (ref. 68). The response required from the subject was to press the button of the computer mouse to stop the timer from counting, as quickly as possible after the timer started

counting. The timer was reset immediately before each trial. The times between trials were randomized, and were between one and nine seconds.

Questionnaires

The investigation used a questionnaire which contained four different types of questions (fig. 5.1). The upper part was derived from a subjective fatigue checkcard developed by Pearson and Byars (ref. 69) to document subjectively rated fatigue levels in military and civilian aviation (refs. 25 and 70). The middle part of the sheet contained the Stanford Sleepiness Scale (SSS) (ref. 71), while two 100-mm lines serving for the assessment of tension and alertness levels were presented in the lower part.

Procedure

Performance tests, SVRT and questionnaires were administered to the subjects every three hours during wakefulness, starting just after the end of the sleep period at 0700 study time.

In addition to reaction time, the number of incorrect answers was stored for each of the three performance tests; thus the correctness of responses was also analyzed. A detailed analysis of the simple visual reaction time test (SVRT), investigating circadian behavior and the influences of shift and light conditions in more detail, was performed by Hackett (ref. 68).

Practice effects were anticipated in all tests. Therefore, the reaction times were first investigated for practice effects by using a linear trend analyses (ref. 47) (a logarithmic detrending technique was also used, which resulted in similar regression coefficients and did not lead to different results for the various experimental conditions). The detrended data were analyzed with regard to differences between subjects, between times of day, and between light conditions using one- and two-way ANOVA and nonparametric statistics (ref. 47).

Each answer on the fatigue checklist was scored by 2 when checked "better than," 1 when checked "same as," and 0 for "worse than." The scores from the ten questions were summed to give an overall score between 0 and 20 with high scores reflecting low fatigue levels. The operational significance of these scores is discussed by

Samn and Pirelli (ref. 70). The scores derived from the fatigue checklist, from the SSS and from the two 100-mm scales were treated by one-way and two-way ANOVA as well as by nonparametric tests for occurrence of a significant circadian pattern, and of differences between subjects, light conditions and shift conditions.

The days in each study phase were grouped as follows.

	Session numbers	Time period (local time)
Baseline	1-18	Day 1, 1600 h - day 4, 1300 h
Light exposure	19-37	Day 4, 1600 h - day 6, 2200 h
Resynchronization	38-55	Day 7, 0700 h - day 9, 2200 h
Final	56-67	Day 10, 0700 h - day 11, 2200 h

The times here refer to the internal time structure imposed in the bedrest facility, i.e., every morning lights went on at 0700 h study time. On non-shift days, lights went out at 2300 h, while on the two shift days, lights out was at 0500 h. Lights went on again 8 h later, which was then redefined as 0700 h.

Results

Cognitive Performance

The raw reaction time data for the three cognitive tasks and the simple visual reaction time test are presented in figures 5.2-5.5. Because of the cross-over design, subjects A and B performed the first 67 repetitions of each test during the night time light exposure (NTLE) condition, whereas D and E performed them during day time light exposure (DTLE). The latter two subjects did not perform the first seven sessions of MAST2 and MAST6 correctly. Therefore, the results of these sessions were discarded from further analysis. The differences among subjects in coping with the performance tests are obvious. All subjects revealed a practice effect, but the amount varied (table 5.1). For example, subject A started with a mean reaction time of about 9500 msec for a trial of MAST6, whereas subject B needed about 15,500 msec for the same task at the beginning. At the end of the second phase, subject A conducted the test in 9350 msec on average, subject B in 9750 msec. A stable condition was not achieved during the first phase of the study, i.e., 67 sessions with 16 trials (MAST2, MAST6) or even 32 trials (LOGRES) per session did not succeed in overcoming learning effects. Subjects also needed the second

phase to become more acquainted with the performance tests. This trend can also be observed in the averaged time-of-day values, when grouping was performed as mentioned above (fig. 5.6). For all three tests, the reaction time decreased continuously from the baseline to the final period, except for the shift days, when values often remained at high levels (e.g., MAST6). Visual inspection additionally indicated no distinct circadian variation, except for MAST6 during the resynchronization period.

Because of the practice effect, the reaction times were detrended. As mentioned in the Methods section, linear detrending and logarithmic detrending did not show any significant differences; for comparison, regression coefficients are presented for the two techniques in table 5.1(b) (MAST6).

In the detrended data (fig. 5.7), significant differences (on a $p \leq 0.05$ level, two-way ANOVA) between subjects, time of day, and light condition did not occur during baseline (session numbers 1 to 18) for the three cognitive performance tests. During the following experimental period (fig. 5.7), i.e., shift or light exposure days, the reaction times did not exhibit any time-of-day effect in MAST2, whereas in MAST6 subjects reacted significantly more slowly at 0100 h LT compared with 1600 h. In the LOGRES-test, reaction time was significantly longer at 2200 h LT compared with 0700, 1300 h and 1600 h, and at 0400 h compared with 0700 h, 1300 h, and 1600 h. No differences from other day times could be found for 0400 h (MAST6) and for 0100 h (LOGRES), respectively. During the resynchronization period, a time-of-day effect also occurred in MAST6 and LOGRES, but not in MAST2. For MAST6, at 1300 h, reaction time was significantly shorter than at 0700 h and 2200 h. The 1600 h values also were smaller than those at 0700 h and 2200 h. In LOGRES, the effect was less pronounced, with only the 2200 h reaction time being significantly longer than that at 1000 h. During the final experimental period, no significant circadian course could be found, as on the baseline days.

Focusing on the conditions of light exposure and time shift procedure, the detrended data did not reveal significant differences between DTLE and NTLE in MAST2 (T = 1363, p = 0.6064, N = 76; Wilcoxon matched-pairs signed-ranks test), and in MAST6 (T = 1303, p = 0.4075, N = 76). In LOGRES however, significant differences were found for the shift period (T = 1045, p = 0.0305, N = 76). Although on average in LOGRES the reactions were faster by about 300 msec under DTLE-conditions than under NTLE-conditions, the analysis broken down for each subject revealed that only one subject contributed to this result by having a highly significant difference in reaction times between conditions. During the

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Table 5.1. Trend analysis of training effects for different performance tests. Significant coefficients for linear regressions are indicated by: *$0.05 > p \geq 0.01$; **$0.01 > p \geq 0.001$; ***$p < 0.001$. NTLE: Night time light exposure; DTLE: Day time light exposure. (coeff.-coefficient, lin.-linear, log.-logarithmic)

Subj.	Phase	MAST2					MAST6					
		r-coeff.	p-value	F-value	N	Effect	r-lin.	r-log	p-value	F-value	N	Effect
A	I (NTLE)	–0.35	0.0033	9.338	67	**	–0.29	–0.30	0.0179	5.906	67	*
	II (DTLE)	–0.42	0.0004	13.811	67	***	–0.51	–0.51	0.0000	22.749	67	***
B	I (NTLE)	–0.72	0.0000	71.317	67	***	–0.78	–0.79	0.0000	104.218	67	***
	II (DTLE)	–0.57	0.0000	31.721	67	***	–0.58	–0.58	0.0000	33.117	67	***
D	I (DTLE)	–0.59	0.0000	31.120	60	***	–0.45	–0.50	0.0003	14.949	60	***
	II (NTLE)	–0.43	0.0000	14.939	67	***	–0.51	–0.52	0.0000	22.504	67	***
E	I (DTLE)	–0.13	0.3072	1.061	60	ns	–0.37	–0.31	0.0036	9.214	60	**
	II (NTLE)	–0.27	0.0284	5.023	67	*	–0.48	–0.49	0.0000	19.167	67	***

Subj.	Phase	Logical reasoning					Simple visual reaction time				
		r-coeff.	p-value	F-value	N	Effect	r-coeff.	p-value	F-value	N	Effect
A	I (NTLE)	–0.37	0.0018	10.608	67	**	–0.52	0.0000	24.672	67	***
	II (DTLE)	+0.01	0.9101	0.013	67	ns	–0.59	0.0000	35.477	67	***
B	I (NTLE)	–0.73	0.0000	74.471	67	***	–0.73	0.0000	72.032	67	***
	II (DTLE)	–0.58	0.0000	33.559	67	***	–0.52	0.0000	24.519	67	***
D	I (DTLE)	–0.22	0.0759	3.253	67	ns	–0.10	0.9855	0.003	67	ns
	II (NTLE)	–0.45	0.0001	16.392	67	***	–0.31	0.1020	7.012	67	*
E	I (DTLE)	–0.11	0.3738	0.802	67	ns	–0.26	0.0347	4.667	67	*
	II (NTLE)	–0.53	0.0000	25.432	67	***	–0.16	0.1968	1.701	67	ns

resynchronization and the final period, there were also no differences between the two light conditions for the three performance tests.

Data for both light conditions were again combined and investigated by one-way ANOVA for significant changes attributable to the 12 h wake/sleep schedule delay, using the grouping of test sessions noted in the methods section. For MAST2 and LOGRES, the overall comparison did not reveal significant differences (MAST2: $F = 0.0537$, $p = 0.9836$; LOGRES: $F = 2.0717$, $p = 0.103$). However, reaction times on the logical reasoning test were faster on average (Dt = 192 msec) during shift days than during resynchronization days. There were no differences from baseline or final test sessions. In MAST6, subjects reacted more slowly during shift days than during baseline days (Dt = 492 msec) and final days (Dt = 433 msec). For all conditions, the statistical values were $F = 2.819$, $p = 0.0385$.

In addition to the reaction time, the correctness of responses can provide information on the impact of the protocol on performance. The percentage of correct answers broken down for different experimental periods is presented in table 5.2. Conducting one-way ANOVA

on the data, the incorrect responses of MAST2 increased significantly when comparing the baseline and the final period ($F = 2.435$, $p = 0.0072$). All other results, considering the three different performance tests and experimental periods, did not show any significant difference.

Table 5.2. Percentage of correct responses on the performance tests for various experimental periods.

Period	MAST2	MAST6	LOGRES
Baseline	98.6 %	99.0 %	98.5 %
Shift (light)	98.0 %	98.8 %	97.9 %
Resynchronization	98.0 %	99.0 %	98.3 %
Final	97.1 %	98.6 %	98.5 %

The two light conditions did not significantly differ in the number of correct (or incorrect) answers in the MAST2 and MAST6 trials. During light exposure days, there were also no significant differences in the correctness of responses in LOGRES. However, in the following period (resynchronization), the number of incorrect

answers was significantly elevated (p = 0.0252) during the DTLE-condition.

Questionnaires

The responses to three out of four questions revealed a circadian course, i.e., fatigue (fig. 5.8), sleepiness (fig. 5.9), and alertness (fig. 5.10), whereas tension did not show a time-of-day effect (table 5.3). During baseline, subjects felt significantly less fatigued at 1000 h, 1300 h, and 1600 h compared with 0700 h, and more fatigued at 2200 h, compared to all other recorded times of day. They also rated themselves as less sleepy during the entire day than in the morning (0700 h) and evening (2200 h). Alertness ratings corresponded to SSS-scorings, i.e., the ratings at 1000 h, 1300 h, 1600 h, and 1900 h were significantly different from the 0700 h and 2200 h ratings (fig. 5.10). On days with light treatment and the shift, the daily course of fatigue, sleepiness and alertness shifted to early times, resulting in scores not different from each other at 0700 h, 1000 h, 1300 h, 1600 h, and 1900 h, but alertness decreased, and sleepiness as well as fatigue increased significantly at 2200 h and 0100 h, when compared with prior times of day. At 0400, ratings were so low (fig. 5.10) that the scores were significantly different from all other times-of-day scores. During the "resynchronization" period, sleepiness and fatigue ratings of the last-of-day questionnaire (2200 h) were significantly lower than at all other times of day, whereas no difference could be observed between the other scores. Alertness ratings closely resembled baseline behavior, i.e., the 1000 h, 1300 h, 1600 h, and 1900 h

scorings were significantly higher than the ratings at 0700 h and 2200 h, except for the relation between the 1900 h and the 2200 h value. Finally, the results of the questionnaires on the last two days of each phase of the study exhibited a time-of-day effect in a slightly different way for sleepiness and fatigue ratings, i.e., fatigue was significantly reduced at 1000 h and 1300 h and enhanced at 2200 h, compared to 0700 h, 1600 h, and 1900 h. Sleepiness increased at 2200 h, and was minimal at 1300 h. In contrast, the ratings of alertness showed complete recuperation after the time shift. The daily course of ratings peaked at 1300 h, and the values at 0700 h and 2200 h were significantly lower than the others.

The different light exposure conditions produced significant differences in some ratings (table 5.4). Whereas sleepiness did not change between light conditions in all experimental situations, tension was higher in the daytime light treated group during baseline and light exposure days, and fatigue was increased during the final days. The latter corresponded with the alertness ratings which were lower in the DTLE group. When considering only the extended night periods (0100 h and 0400 h), ratings (N = 32) were not better under light exposure than under normal room illumination.

Irrespective of the light exposure, the different shift conditions influenced the ratings significantly. When comparing scores for the four situations, subjects responded very quickly to the changes. Fatigue increased significantly during shift days, compared with resynchronization and final days (p ≤ 0.0013), and decreased during the final days (table 5.3), when compared with

Table 5.3. Mean levels and time-of-day effects in ratings for various experimental periods. Significant differences are indicated by : *0.05 > p ≥ 0.01; **0.01 > p ≥ 0.001; *** p < 0.001.

Period	Fatigue					Stanford Sleepiness Scale (SSS)				
	Mean ± SD	F-value	p-value	N	Effect	Mean ± SD	F-value	p-value	N	Effect
Baseline	11.0 ± 2.3	9.684	0.0000	5,146	***	3.23 ± 0.75	6.461	0.0000	5,146	***
Light (shift)	10.4 ± 2.9	23.240	0.0000	7,144	***	3.21 ± 1.00	4.477	0.0000	7,144	***
Resynchronization	11.4 ± 2.3	8.736	0.0000	5,138	***	2.90 ± 0.88	2.833	0.0181	5,138	*
Final	12.5 ± 2.9	6.790	0.0000	5,98	***	2.56 ± 0.85	2.789	0.0213	5,98	*

Period	Tension					Alertness				
	Mean ± SD	F-value	p-value	N	Effect	Mean ± SD	F-value	p-value	N	Effect
Baseline	31.1 ± 13.2	0.546	0.7410	5,146	ns	50.4 ± 16.1	8.682	0.0000	5,146	***
Light (shift)	25.5 ± 12.6	0.365	0.9211	7,144	ns	49.7 ± 19.7	11.734	0.0000	7,144	***
Resynchronization	24.4 ± 14.0	0.576	0.7183	5,138	ns	58.6 ± 20.9	4.595	0.0007	5,138	***
Final	23.7 ± 12.0	0.408	0.8425	5,98	ns	60.8 ± 19.9	3.472	0.0062	5,98	**

Table 5.4. Ratings for various experimental periods and DTLE and NTLE conditions. Significant differences are indicated by *, 0.05 > p ≥ 0.01; **, 0.01 > p ≥ 0.001; ***, p < 0.001 (Wilcoxon matched-pairs singed-ranks test). DTLE: daytime light exposure, i.e., control condition; NTLE: nighttime light exposure, i.e., experimental condition.

Fatigue

Period	NTLE Mean ± SD	DTLE Mean ± SD	T-value	p-value	N	Effect
Baseline	11.0 ± 2.3	10.9 ± 2.3	812.0	0.5764	76	ns
Light (shift)	10.4 ± 2.9	10.4 ± 2.9	1011.5	0.6864	76	ns
Resynchronization	11.6 ± 2.1	11.2 ± 2.4	693.0	0.0949	72	ns
Final	13.0 ± 3.1	12.1 ± 2.8	340.5	0.0445	52	*

Stanford Sleepiness Scale (SSS)

Period	NTLE Mean ± SD	DTLE Mean ± SD	T-value	p-value	N	Effect
Baseline	3.22 ± 0.74	3.24 ± 0.76	325.5	0.8981	76	ns
Light (shift)	3.26 ± 0.87	3.16 ± 1.16	461.5	0.2453	76	ns
Resynchronization	2.88 ± 0.87	2.92 ± 0.88	251.0	0.7945	72	ns
Final	2.48 ± 0.83	2.63 ± 0.86	164.0	0.2038	52	ns

Tension

Period	NTLE Mean ± SD	DTLE Mean ± SD	T-value	p-value	N	Effect
Baseline	29.0 ± 12.8	33.1 ± 13.3	910.5	0.0101	76	*
Light (shift)	24.3 ± 13.1	26.7 ± 12.1	787.5	0.0120	76	*
Resynchronization	23.1 ± 11.1	25.7 ± 16.4	985.5	0.2513	72	ns
Final	24.1 ± 11.7	23.4 ± 12.5	661.0	0.9850	52	ns

Alertness

Period	NTLE Mean ± SD	DTLE Mean ± SD	T-value	p-value	N	Effect
Baseline	51.4 ± 16.4	49.3 ± 15.8	1172.5	0.1325	76	ns
Light (shift)	49.7 ± 18.7	49.8 ± 20.8	1324.0	0.7322	76	ns
Resynchronization	62.1 ± 19.2	55.1 ± 22.0	701.5	0.0015	72	**
Final	64.3 ± 18.6	57.2 ± 20.7	339.5	0.0040	52	**

baseline and final period (p < 0.001). The effect on sleepiness was even more pronounced. Except for the relationship between the baseline and the shift (or light exposure) period, all other comparisons were significantly different (p < 0.002). Peak scores (highest sleepiness) were found during the baseline period, then a gradual decrease occurred during the shift days. Scores were further reduced during resynchronization days, and sleepiness was rated lowest during the final days of each study phase (table 5.3). The mean values of tension ratings were significantly higher during baseline, compared with the other study periods (p < 0.001). No differences could be found between the other periods. Alertness was significantly (p < 0.001) lower during

baseline and shift days in relation to the other two study periods. Within the first two and within the final two experimental periods, subjects felt similarly alert (table 5.3.(d)).

Discussion

Performance

The most remarkable result is the duration of training effects on the reaction time during the entire two study phases. Usually, subjects started with a very long reaction time which decreased towards the end of a phase.

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Since the reaction times of the first sessions of phase II were higher than in the final session of phase I, the data could not be combined for detrending, but had to be separated. This resulted in different slopes of the regression for each subject and phase. The differences in reaction times and slopes between subjects suggest different occupational experience and extraordinary motivation in one subject who reduced his reaction time by about 40% (MAST6) in competition with his room-mate. Nevertheless, the persisting learning effects seemed to have confounded the anticipated changes due to bedrest, shift and light conditions. This finding resembles the results of a study in which melatonin was assessed as a counter-measure for circadian disruption after phase shifts (ref. 28).

The comparison of detrending techniques (linear regression of the raw data versus linear regression of the log data, i.e., exponential regression of the raw data) did not show any difference in slope for the MAST6 results. However, various parts of the series of test sessions can contribute in a different way to the similar result of the different regression methods. Our subsequent statistical results of MAST6 reaction times, either detrended by linear or exponential functions, exhibited similar, nearly identical significances. To this extent, our results seem to be reliable and robust, although we cannot exclude that more sophisticated detrending methods would possibly produce more significant results, but could also lead to more artificial results.

Within the limits of the above mentioned methodological constraints, the results of this study did not indicate a robust circadian variation, either in reaction times or in correctness of response, for any of the cognitive performance measures. Examining only the final group of sessions of the second study-phase, when contamination by learning effects would be lowest, no circadian variations could be found in the three performance tests. However, a time-of-day effect occurred during the shift days in MAST6 and logical reasoning. These reductions of performance capability at certain times of day can be explained by the influence of sleep deprivation as well as by circadian changes. Our findings tend to support results derived from a bedrest study investigating theophilline effects (ref. 67), and contrast with findings presented by Folkard et al. on a study of rapidly rotating shift schedules (ref. 40). We cannot, however, categorically exclude circadian influences. Furthermore, we do not exclude the possibility that, besides possible effects of the detrending techniques, differences in the mode of application of the tests (computer-based versus paper-and-pencil tests) as well as different laboratory conditions could have influenced the different results. Our performance measurements were also restricted to the waking phase of the imposed wake/sleep schedule, because priority was given to the recording of sleep EEG, which required undisturbed sleep. Thus, it was not possible to measure performance during the expected lowest part of the circadian curve. The prolongation of reaction times during the days following the shift of environmental time also argues for a circadian influence, as has been reported in other experiments with similar measuring techniques (ref. 2), because the subjects should by that time have overcome the sleep deprivation caused by the shift. The performance decrement occurred primarily in reaction times, not in correctness of answers. We assume that the subjects were motivated to respond correctly rather than rapidly under the more difficult conditions.

Differences between light conditions could not be observed for reaction times, as was found in cross-over design studies using theophylline (ref. 67) and melatonin (ref. 28) when similar performance tests were applied. We do not exclude minor but significant effects, when applying the tests to a large number of subjects for a large number of test sessions. However, if the effects were too subtle to be noted in the present study, it is unlikely that they would be sufficiently robust to be of practical importance. The same conclusion can be drawn for the results of the correctness of responses, although a small significant difference was found for logical reasoning responses during the resynchronization period, depending on the preceding lighting condition.

Questionnaires

In contrast to the performance tests, most subjective ratings showed a clear circadian variation, although analyses also had to deal with a lack of data during night hours. We could not find a time-of-day effect in tension. As expected, ratings peaked for vigilance, i.e., most alert, least sleepy and fatigued, during the late morning hours, when scored under baseline conditions, and again during the final days. Under shift conditions, the curves exhibited even more marked variations which can be interpreted as circadian behavior. Morning ratings were elevated due to the abrupt delay of the sleep-wake cycle and the inertia of the circadian rhythms to follow that shift, and late evening scores dropped due to both the declining circadian phase, emphasized by desynchronization, and to sleep deprivation. Similar behavior was reported from a field study of subjects who were desynchronized and sleep deprived by a similar number of hours (ref. 25). Thus, our findings may be relevant to a more general population of operators, who have to perform under shift work conditions. The circadian course was reestablished rapidly, as the results from the resynchronization period showed.

Baseline scores unexpectedly showed significantly impaired alertness, and enhanced sleepiness and fatigue, when compared with resynchronization and the final period. We interpret these results as being caused by the changes in position and the associated effects on the cardiovascular and endocrinological systems, which probably also affected the overall subjective impressions of mental capability. Impairment continued during the shift of the sleep-wake cycle, but ratings improved significantly during resynchronization. This suggests an ongoing process of adaptation to the bedrest condition superimposed on the adjustment to the new zeitgebers. Following this interpretation, the adaptation to bedrest seems to have been largely completed by the final period.

This behavior was found to occur in both light conditions, but was significantly more pronounced in fatigue and alertness ratings for the night-time light treatment than for the day-time treatment. Significant differences in tension ratings between light conditions occurred only twice, during the baseline and shift periods, and should therefore not be overinterpreted.

Two conclusions concerning performance can be drawn from this study: (1) the performance measures used did not show robust circadian rhythmicity, and (2) the timing of light treatment did not significantly alter the adaptation of the performance variables to the 12 h wake/rest schedule delay. This is consistent with the findings from the physiological data.

NAME:_____   DATE _____
                           TIME _____

INSTRUCTIONS: Make one, and only one (x) for each of the ten items. Think carefully about how you feel right now.

STATEMENT		BETTER THAN	SAME AS	WORSE THAN
1)	very lively			
2)	extremely tired			
3)	quite fresh			
4)	slightly fatigued			
5)	extremely peppy			
6)	somewhat fresh			
7)	worn out			
8)	very refreshed			
9)	fairly well fatigued			
10)	ready to drop			

(1) Choose **ONE** statement that best describes how you feel right now:

[ ] feeling active and vital; alert; wide awake.
[ ] functioning at a high level, but not at peak.
[ ] relaxed; not at full alertness; responsive.
[ ] a little foggy; not at peak; let down.
[ ] fogginess; losing interest in staying awake; slowed down
[ ] sleepiness; prefer to be lying down.
[ ] almost in reverie; sleep onset soon; hard to stay awake.

(2) 100 mm linear scales (make a vertical mark on the line)

a) how relaxed or tense do you feel right now?
VERY _____VERY
RELAXED   TENSE

b) how drowsy or alert do you feel right now?
VERY _____VERY
DROWSY   ALERT

Figure 5.1. Questionnaire for Fatigue Checklist, Stanford Sleepiness Scale, 10-cm analog scale for tension, and 10-cm analog scale for alertness ratings
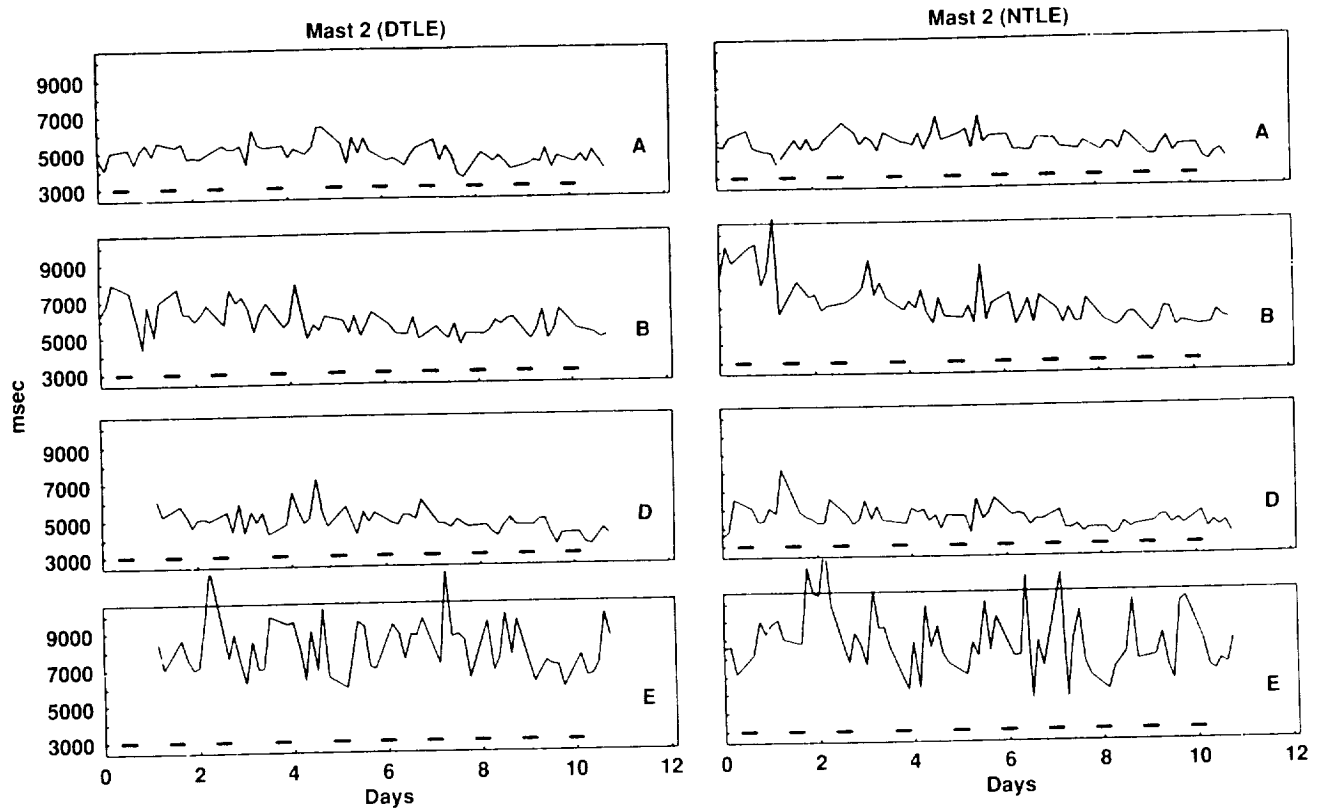
Figure 5.2. Raw data (mean reaction times) for each subject on the memory-and-search task with two letters (MAST2). Letters on the right-hand side of each plot identify the subjects. DTLE-daytime light exposure; NTLE-nighttime light exposure.
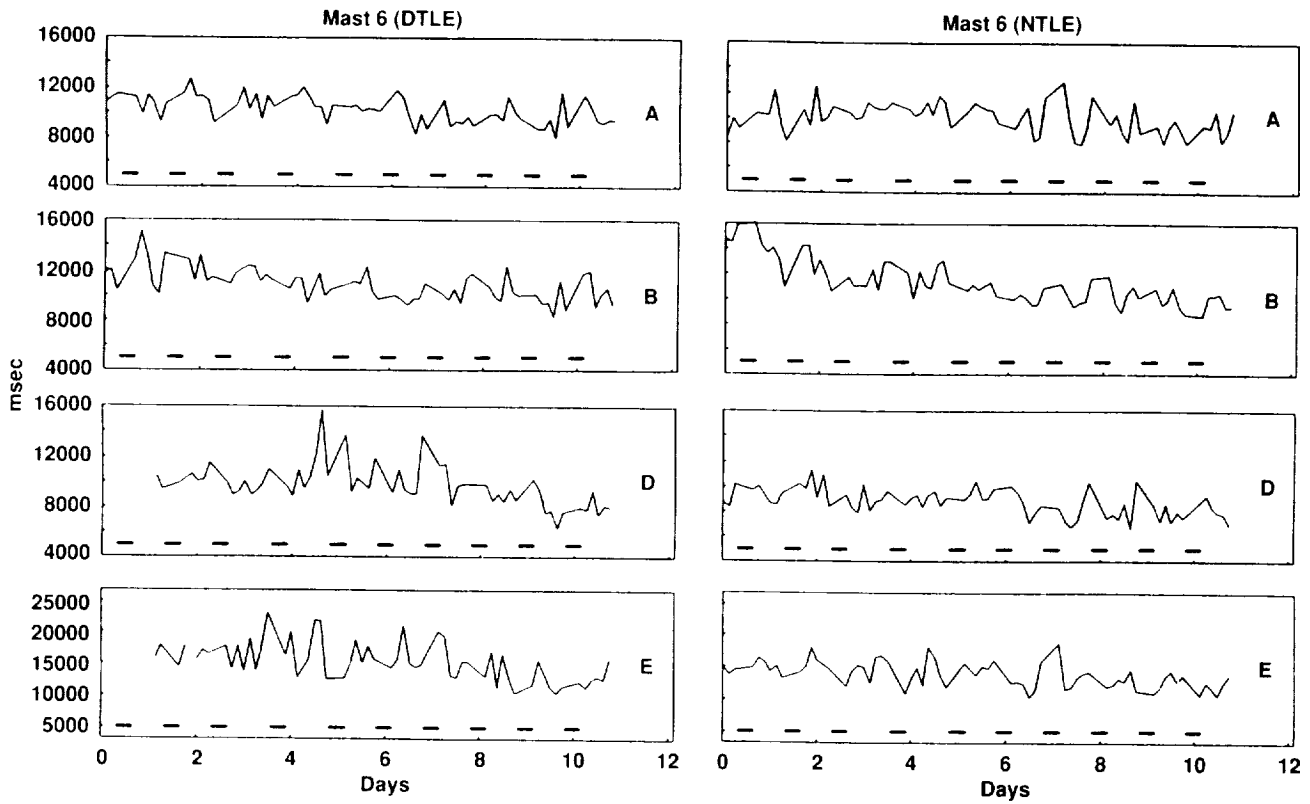
Figure 5.3. Raw data (mean reaction times) for each subject on the memory-and-search task with six letters (MAST6). DTLE-daytime light exposure; NTLE-nighttime light exposure.
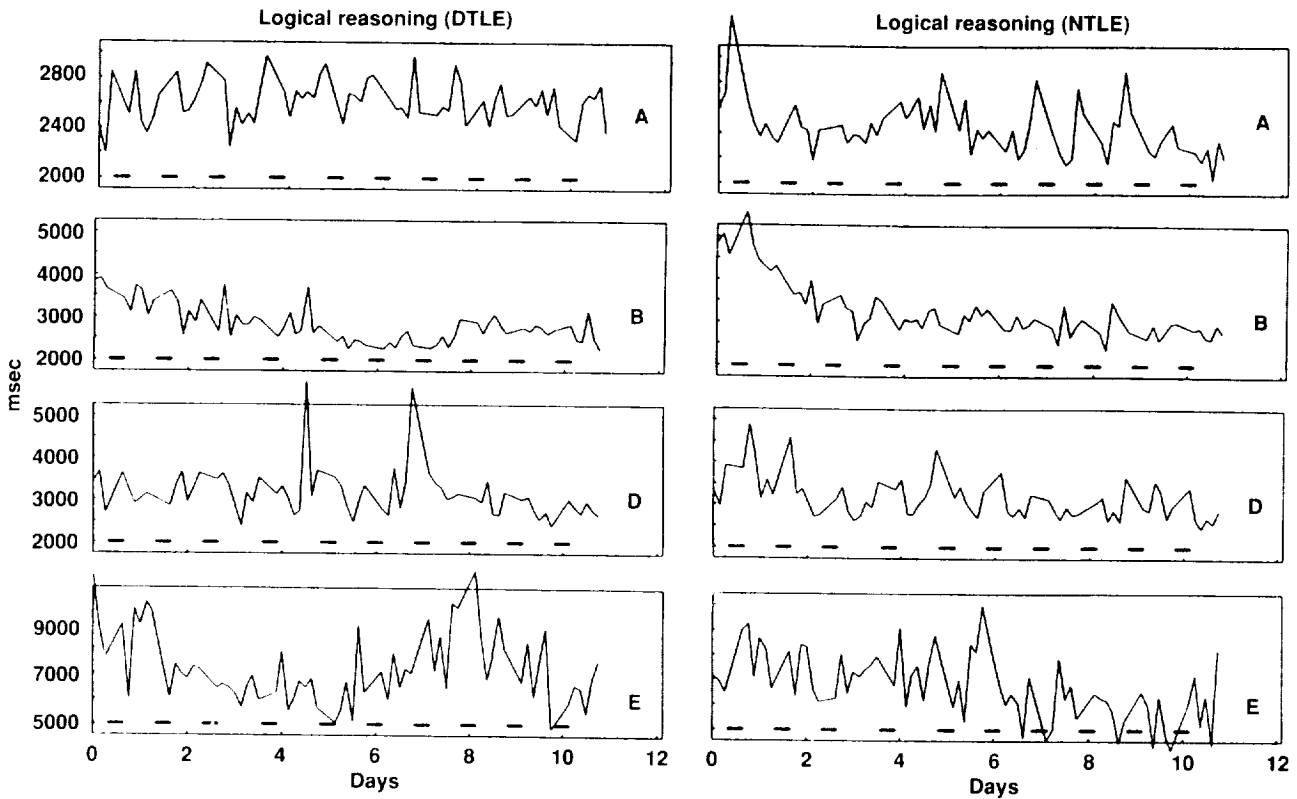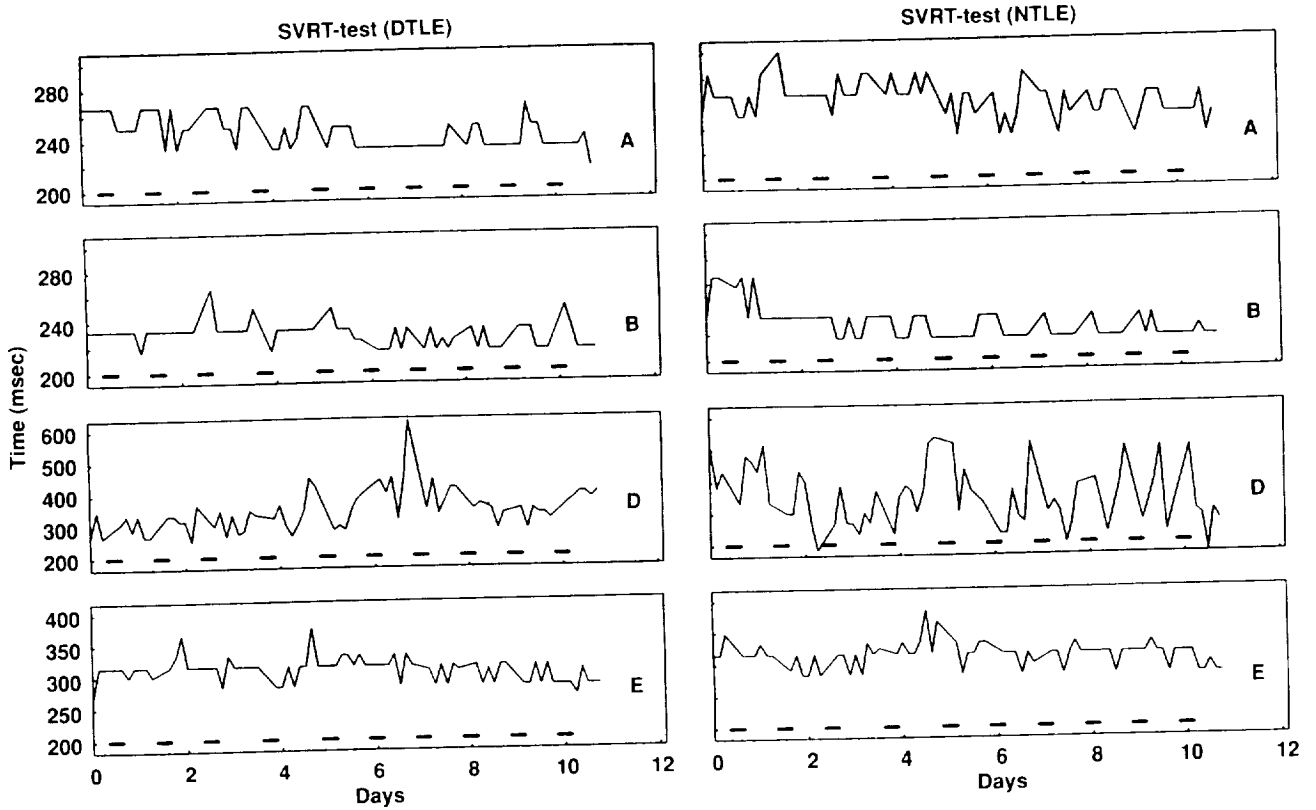

Figure 5.4. Raw data (mean reaction times) for each subject on the logical reasoning task (LOGRES).

Figure 5.5. Raw data (median reaction times) for each subject on the simple visual reaction time test (SVRT). DTLE-daytime light exposure; NTLE-nighttime light exposure.
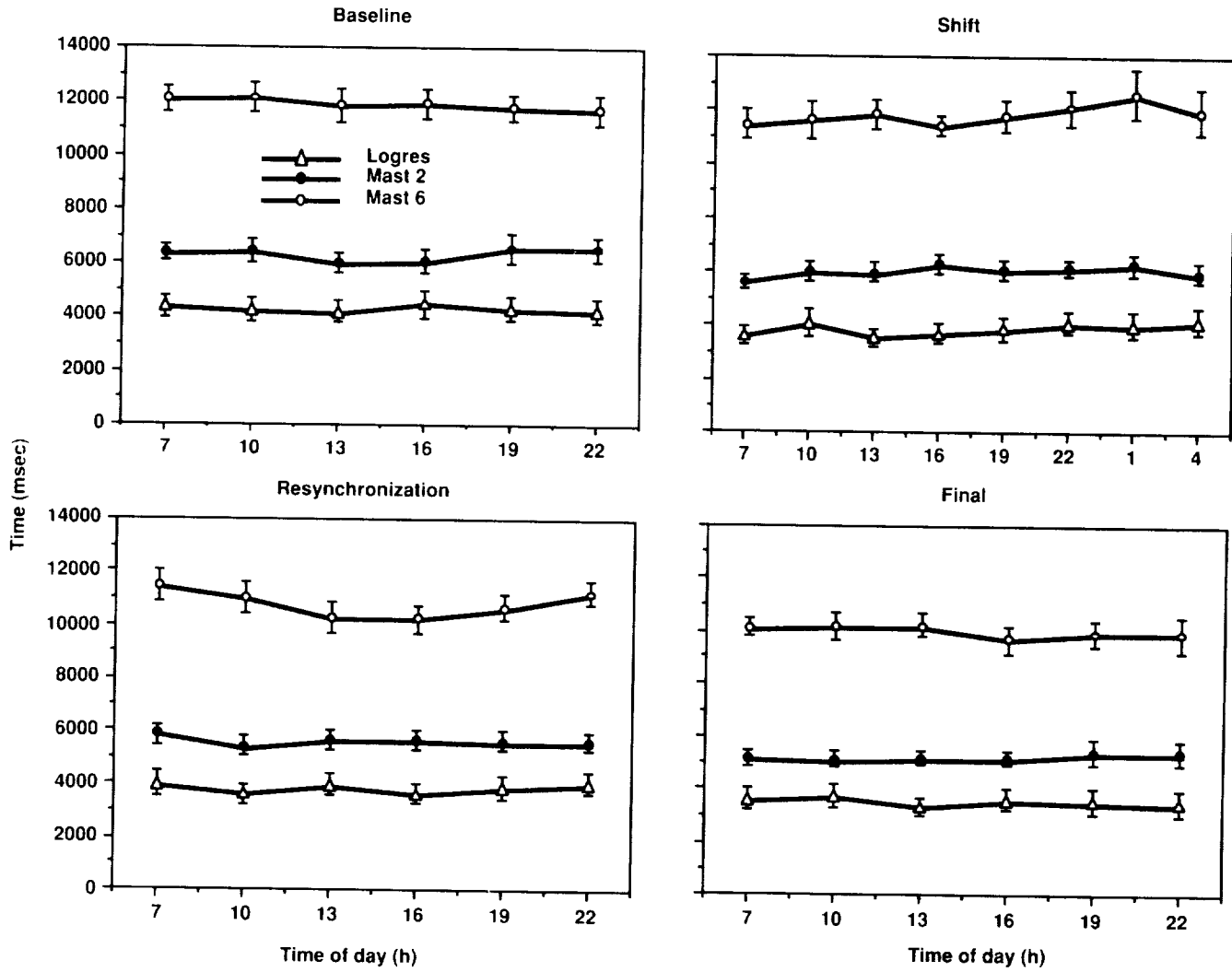
Figure 5.6. Reaction times (mean ± SE) on the MAST2, MAST6, and LOGRES tests at different times of day for four different study periods (with the two light conditions combined): (a) baseline, (b) shift (light), (c) resynchronization, (d) final (definitions presented in text).
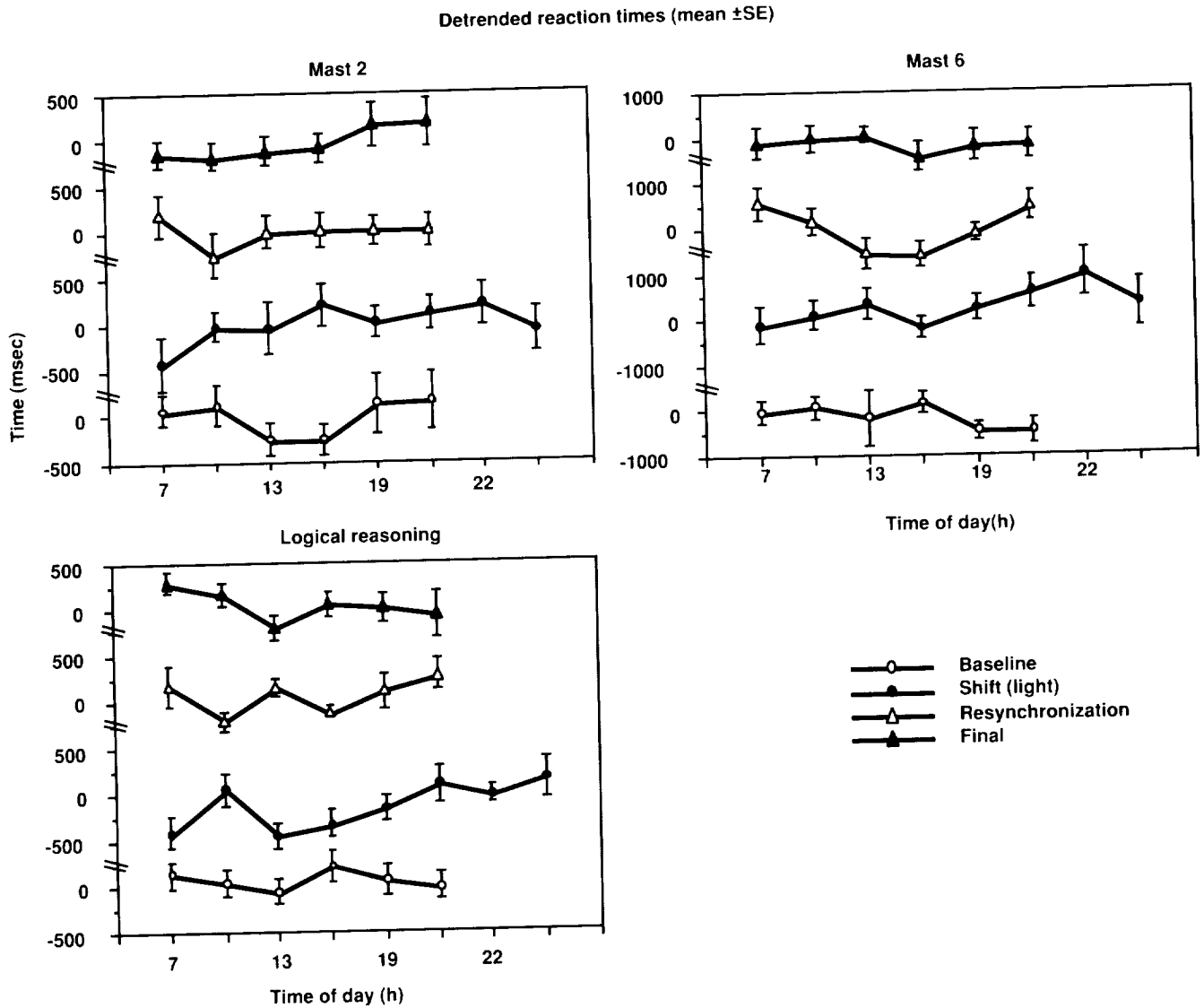
Detrended reaction times (mean ±SE)

Figure 5.7.   Detrended reaction times (deviation from the mean ± SE) dependent on time of day (x-axis): (a) MAST2, (b) MAST6, (c) LOGRES. Different study periods are in successive order from top to bottom.
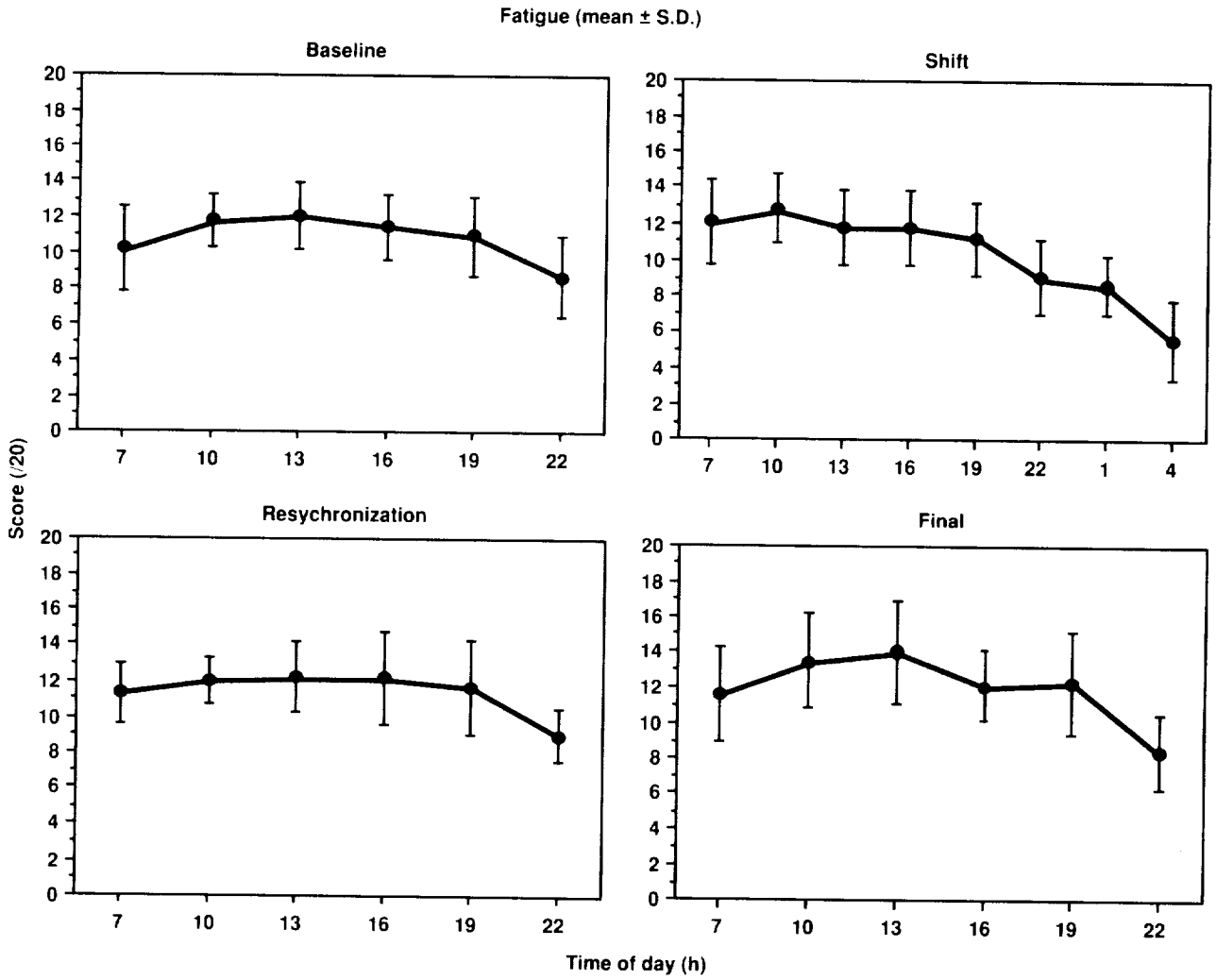
Fatigue (mean ± S.D.)

Figure 5.8.   Scores (mean ± SD) on the fatigue questionnaire, from 0 (most) to 20 (least), as a function of time of day, for each of the four study periods. Control and experimental data are combined.
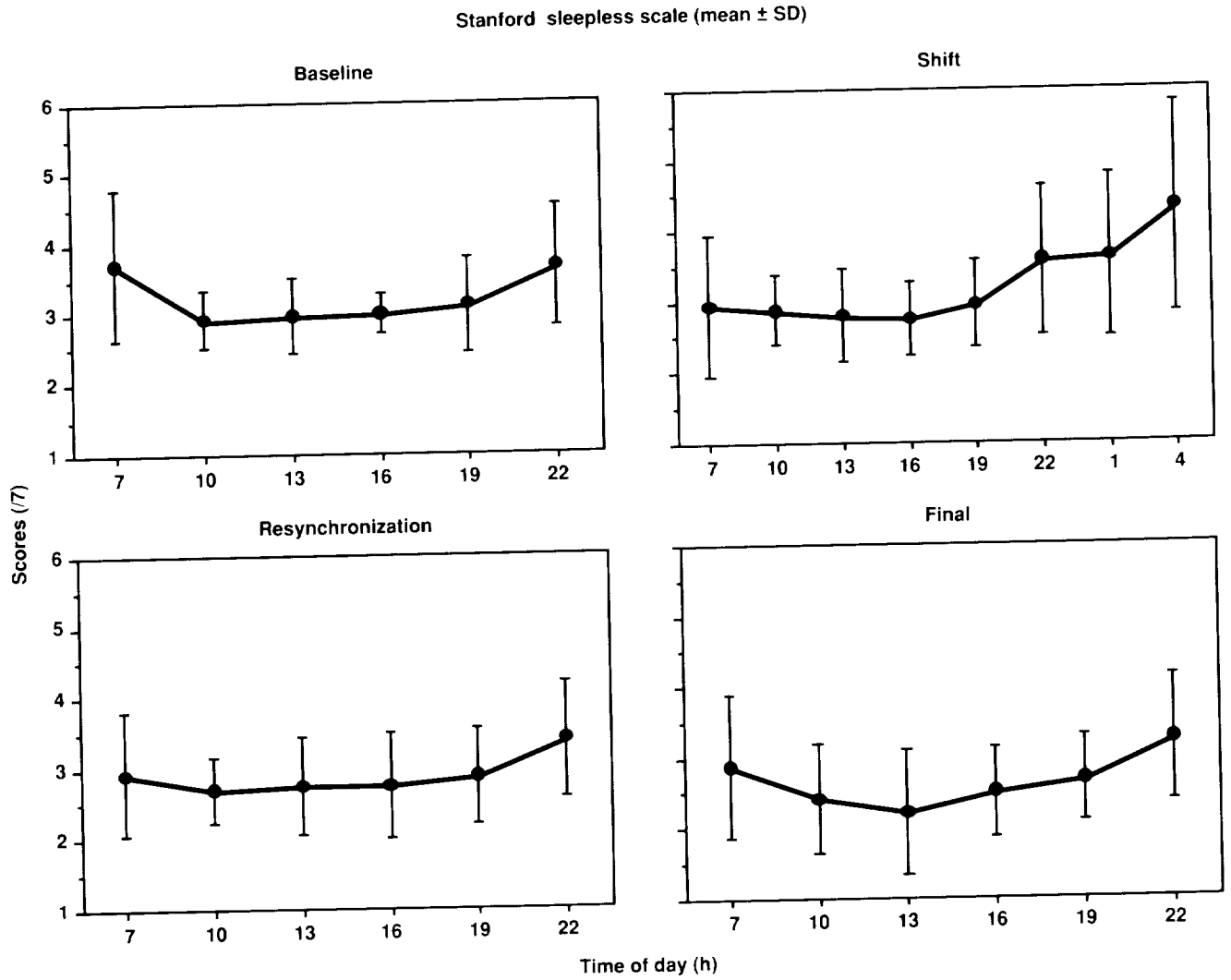
Figure 5.9. Scores (mean ± SD) on the Stanford Sleepiness questionnaire, from 1 (least) to 7 (most), as a function of time of day, for each of the four study periods. Control and experimental data are combined.
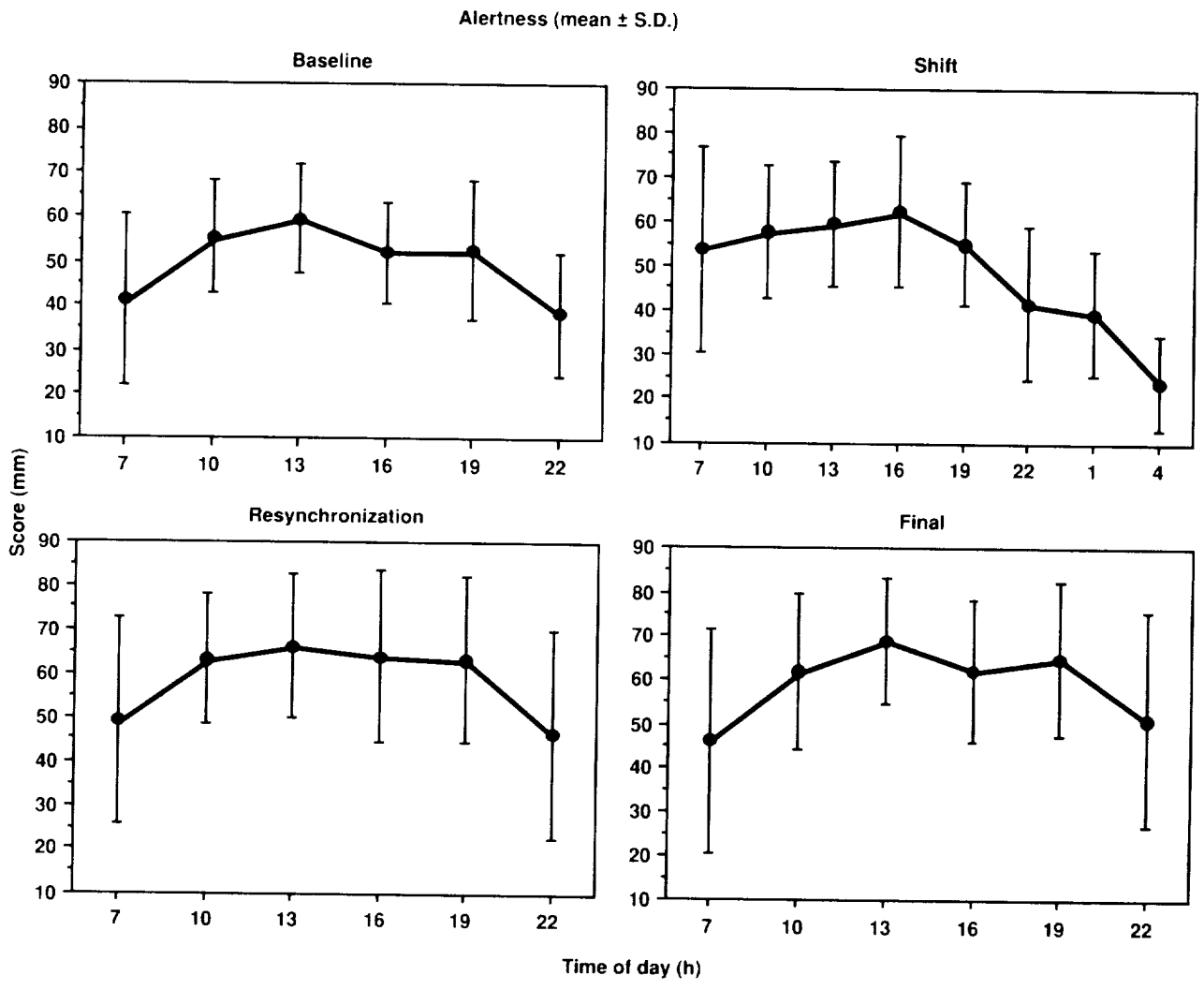
Figure 5.10. Scores (mean ± SD) on the alertness rating scale, from 0 (least) to 100 (most), as a function of time of day, for each of the four study periods. Control and experimental data are combined.

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13. ABSTRACT *(Maximum 200 words)*

Long-duration space missions require adaptation to work-rest schedules which are substantially shifted with respect to earth. Astronauts are expected to work in two-shift operations and the environmental synchronizers (zeitgebers) in a space craft differ significantly from those on earth. A study on circadian rhythms, sleep and performance was conducted by exposing four subjects to 6° head-down tilt bedrest (to simulate the effects of the weightless condition) and imposing a 12-h shift (6 h delay per day for two days). Bright light was tested in a cross-over design as a countermeasure for achieving faster resynchronization and regaining stable conditions for sleep and circadian rhythmicity. Data collection included objective sleep recording, temperature, heart rate, and excretion of hormones and electrolytes as well as performance and responses to questionnaires. Even without a shift in the sleep-wake cycle, the sleep quantity, circadian amplitudes and 24-h means decreased in many functions under bedrest conditions. During the shift days, sleepiness and fatigue increased, and alertness decreased. However, sleep quantity was regained, and resynchronization was completed within seven days after the shift for almost all functions, irrespective of whether light was administered during day-time or night-time hours. The time of day of light exposure surprisingly appeared not to have a discriminatory effect on the resynchronization speed under shift and bedrest conditions. The results indicate that simulated weightlessness alters circadian rhythms and sleep, and that schedule changes induce additional physiological disruption with decreased subjective alertness and increased fatigue. Because of their operational implications, these phenomena deserve additional investigation.

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