

Melatonin and cortisol assessment of circadian shifts in astronauts before flight

Whitson PA, Putcha L, Chen Y-M, Baker E. Melatonin and cortisol assessment of circadian shifts in astronauts before flight. J. Pineal Res. 1995;18:141-147. © Munksgaard, 1995

Abstract: Melatonin and cortisol were measured in saliva and urine samples to assess the effectiveness of a 7-day protocol combining bright-light exposure with sleep shifting in eliciting a 12-hr phase-shift delay in eight U.S. Space Shuttle astronauts before launch. Baseline acrophases for 15 control subjects with normal sleep-wake cycles were as follows: cortisol (saliva) at 0700 (0730 in urine); melatonin (saliva) at 0130 (6-hydroxymelatonin sulfate at 0230 in urine). Acrophases of the astronaut group fell within 2.5 hr of these values before the treatment protocols were begun. During the bright-light and sleep-shifting treatments, both absolute melatonin production and melatonin rhythmicity were diminished during the first 3 treatment days; total daily cortisol levels remained constant throughout the treatment. By the fourth to sixth day of the 7-day protocol, seven of the eight crew members showed phase delays in all four measures that fell within 2 hr of the expected 11- to 12-hr shift. Although cortisol and melatonin rhythms each corresponded with the phase shift, the rhythms in these two hormones did not correspond with each other during the transition.

Peggy A. Whitson,¹ Lakshmi Putcha,¹ Yu-Ming Chen,² and Ellen Baker³

¹Medical Sciences Division, and ³Astronaut Office, NASA/Johnson Space Center, Houston TX; and ²KRUG Life Sciences, Houston, TX, USA

Key words: bright light—urine—saliva—light-dark cycle—astronauts—Space Shuttle

Address reprint requests to Peggy A. Whitson, PhD, Medical Sciences Division, Mail Code SD, NASA/Johnson Space Center, Houston, TX 77058, U.S.A.

Received October 10, 1994; accepted December 21, 1994.

NASA's continuing efforts to maximize the use of valuable flight time have included attempts to shift crews' work-rest schedules before flight so as to allow "around-the-clock" operations during missions. People who work in 24-hr operations consistently demonstrate impairments in performance and mood, drowsiness, fatigue, and disturbances in sleep [Akerstedt et al., 1982; Gold et al., 1992; Englund et al., 1985]. The complexity and demanding nature of many space flight tasks dictate that performance decrements that may arise from "shift-change" work be minimized whenever possible.

Many methods are known to alter circadian patterns in humans. For example, modifying the work schedule gradually over time—with corresponding shifts in the sleep period—can improve performance to some extent. However, systematic studies of shift-workers suggest that sleep-shifting alone is of limited value, probably because of mismatching between the body-temperature cycle and the sleep-wake cycle [Reinberg et al., 1984; Czeisler et al., 1990]. Another means of shifting circadian rhythms is by periodic, timed exposures to bright light (7,000-12,000 lux). Czeisler and colleagues demonstrated that bright light can shift cycles of body temperature, cortisol release, and urine output independent of the sleep-wake cycle [Czeisler et al., 1986]. These effects are thought to be due to suppression of melatonin production [Reiter, 1985; Czeisler

et al., 1986; McIntyre et al., 1989]. Other shifting techniques involve the use of pharmacologic agents. Vitamin B12 has been used to phase-advance circadian rhythms in healthy subjects [Honma et al., 1991]. Melatonin has been used effectively in treating jet-lag [Arendt et al., 1986] and blind subjects with disturbed sleep and circadian rhythms [Arendt et al., 1988].

Melatonin production waxes and wanes in synchrony with the light-dark cycle. Its plasma concentration increases sharply at dusk, rises 10- to 50-fold over the course of the night, and is suppressed during the day. Many studies evaluating the organization of human circadian-rhythm systems suggest that plasma melatonin is a good marker of shifts in those rhythms [Lewy et al., 1980; Arendt, 1990; McIntyre et al., 1987; Reiter, 1985]. The melatonin cycle is known to be sensitive to light and darkness. Shanahan and Czeisler [1991] found that the average 24-hr plasma-melatonin waveform maintained a fixed phase relative to that of core body temperature, even when light-dark cycles were masked.

Cortisol, a glucocorticoid hormone, is secreted by the adrenal cortex in response to adrenocorticotrophic hormone (ACTH). Numerous studies have examined the circadian aspects of ACTH secretion and its effect on cortisol secretion [Angeli, 1974]. Although sleeping and eating can temporarily affect cortisol levels, the normal cortisol

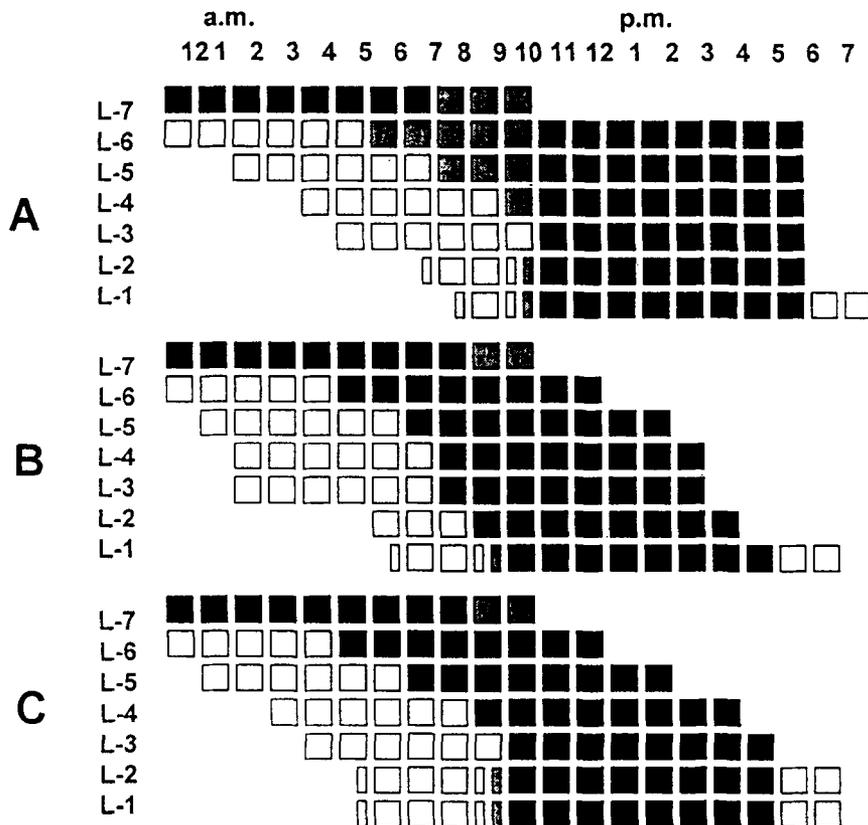


Fig. 1. Circadian-shifting protocols (A, B, and C) followed by astronauts (N=8) before launch. Open squares indicate exposure to bright light (>7,000 lux); hatched squares indicate darkness, dim light, or dark goggles; filled squares indicate sleeping period. L-n is the number of days before launch.

rhythm seems to persist in the presence of short-term manipulation of the sleep-wake cycle. The possibility that core body temperature and cortisol secretion could be controlled by a single pacemaker [Wever, 1979] is an example of the complex interrelationships among physiological and biochemical rhythms. Other endocrine factors such as catecholamines in the urine are good indicators of circadian shifting in humans as well [Reiter, 1988].

Assessments of endocrine rhythms typically rely on measuring hormone levels in blood or plasma. However, saliva or urine samples can be an effective, noninvasive alternative to multiple blood draws. Cortisol concentrations in saliva reportedly correlate well with free cortisol in plasma [Meulenberg and Hofman, 1990]. Correlations also have been noted between serum and saliva melatonin [McIntyre et al., 1987]. Most of the melatonin excreted in urine is the metabolite 6-hydroxymelatonin sulfate, which also correlates well with plasma melatonin [Nowak et al., 1987].

In order to assess the effectiveness of a 12-hr phase shift attempted over a 7-day period, we monitored melatonin and cortisol levels in a group of astronauts before space flights. The progress of the phase shift was followed daily by periodic measurements of melatonin and cortisol in saliva and urine samples during the treatment period.

Materials and methods

Sample collection

All procedures involving human subjects were approved by the Johnson Space Center Human Research Policy and Procedures Committee. The test group consisted of eight Space Shuttle astronauts (five male, three female) from three missions. Subjects provided saliva and urine samples during a 48-hr period before the bright-light-plus-sleep-shift protocol and again during each day of the 7-day treatment protocol. Saliva samples were collected approximately every 2 hr while subjects were awake using Salivettes (Sarsted, Inc., Newton, NC). Urine samples were collected as individual PRN voids. Compliance with the treatment protocols was neither monitored nor recorded. All saliva and urine samples were processed for storage at -40°C until analysis. All samples from each individual were assayed for melatonin or cortisol on the same day to minimize analytical variation.

Bright-light and sleep-shifting protocols

The timing of the sleep shifts and bright-light exposures is illustrated in Figure 1. These protocols were developed by Drs. K. Stewart and C. Eastman of Rush University College of Medicine under contract to NASA. Subjects were instructed either to sleep in the daytime (1000 to 1800)

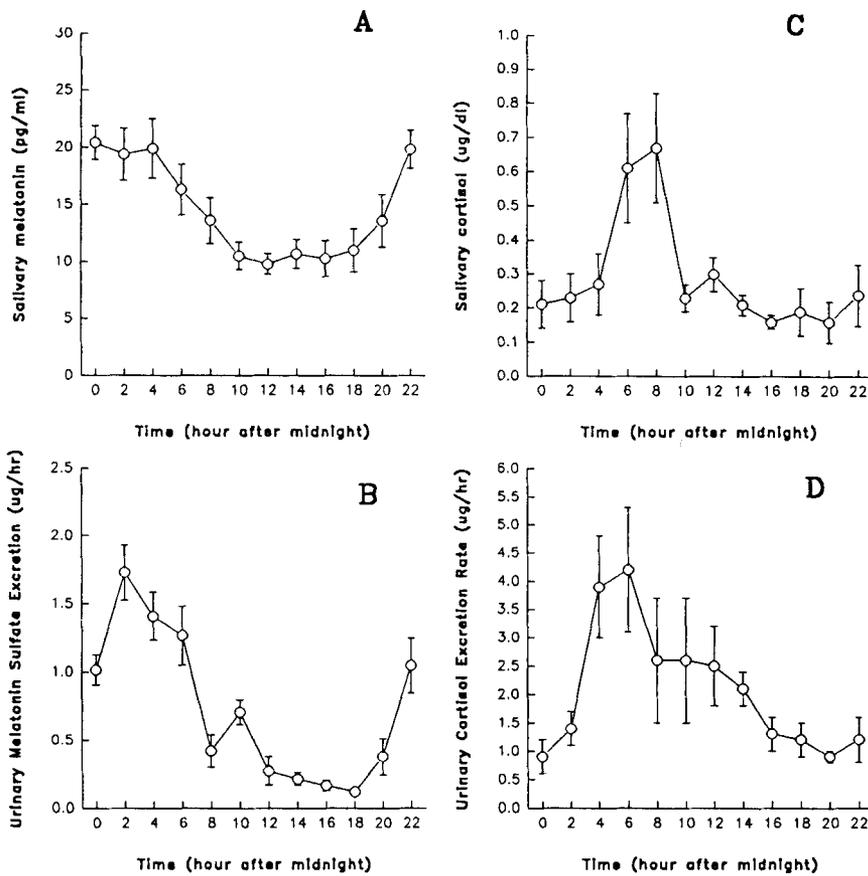


Fig. 2. Melatonin and cortisol rhythms in untreated control subjects. Panels A and B reflect melatonin or melatonin sulfate concentrations in saliva or urine, respectively. Cortisol rhythms are shown in Panel C (saliva) and Panel D (urine). Points are means, and error bars SEM, for 15 subjects (14 in Panel A).

(Protocol A, N=3) or to go to bed progressively later and sleep later over a 6-day period (Protocols B and C) (N=3 and N=2, respectively). All subjects were exposed to bright light (7,000-12,000 lux) for 2-6 hr daily before their scheduled sleep periods. Light was delivered via special ceiling lights, designed by Dr. C. A. Czeisler of Harvard Medical School, installed in the crew quarters at the Johnson and Kennedy Space Centers. Subjects following Protocol A were instructed to wear dark goggles (or remain in a dimly lit area) for several hours before bedtime so that their light-dark cycles were the same as those for subjects following Protocols B and C.

Sample Analyses

Melatonin. Melatonin was measured in saliva samples by direct radioimmunoassay as described by Fraser et al. [1983]. The antibody was purchased from Stockgrand (Surrey, UK), the melatonin from Sigma (St. Louis, MO). The [125]-I melatonin tracer was from Amersham (Arlington Heights, IL). Individual urine samples were assayed for 6-hydroxymelatonin sulfate (MTS) by direct radioimmunoassay (Stockgrand) as described by Aldhous and Arendt [1988].

Cortisol. Cortisol was assayed in saliva samples as described by Chen et al. [1992]. Free cortisol in urine was

extracted using methylene chloride (1:5 urine:methylene chloride) before radioimmunoassay (Clinical Assays, Incstar, Stillwater, MN).

Data analysis

Cosinor [Nelson et al., 1979] and cross-correlation [Markowitz et al., 1988] methods were used to analyze the raw data with respect to time. Cosinor analysis was based on least-squares fit of the cosine function to a series of observations. This technique allowed characterization of the mesor (the 24-hr time-series mean), acrophase (peak time, referenced to local midnight) and amplitude (half of the peak-to-trough variability). Phase shifts (in hours) were calculated both for each day and for the entire period by subtracting the acrophase of the final profile from the acrophase of the initial profile. (A negative number reflects a phase delay and a positive number a phase advance.) Cortisol and melatonin profiles for the test group were also compared by cross-correlation with those of 15 control subjects with normal sleep-wake cycles.

Results

Control subjects

Normal melatonin rhythms were confirmed in 14 of the 15

Table 1. Acrophases and phase delays calculated before and after a 7-day bright-light/sleep-shifting protocol in 8 subjects*

Sleep-shift protocol	Subject ID	Melatonin (saliva)			Melatonin sulfate (urine)			Cortisol (saliva)			Cortisol (urine)		
		Before trtmt	After trtmt	Phase delay	Before trtmt	After trtmt	Phase delay	Before trtmt	After trtmt	Phase delay	Before trtmt	After trtmt	Phase delay
A	1	2.8	17.3	13.5	3.3	18.9	15.6	6.1	17.8	11.7	8.5	21.8	13.3
A	2	5.2	16.8	11.6	3.5	15.8	12.3	6.8	19.7	12.9	8.0	23.0	15.0
A	3	4.3	17.8	13.5	3.1	16.9	13.8	7.1	21.3	14.2	7.5	21.0	13.5
B	4	3.9	15.4	10.4	4.7	13.2	8.5	6.5	18.9	12.5	8.3	16.3	8.0
B	5	5.8	—	—	2.1	13.3	11.2	5.8	18.4	10.1	7.6	14.9	7.3
B	6	6.8	—	—	3.6	13.6	10.0	7.5	16.7	9.2	8.2	15.4	7.2
C	7	4.5	15.0	10.5	4.2	14.4	10.2	7.4	14.5	7.1	9.8	18.3	8.5
C	8	—	—	—	2.1	5.3	3.2	6.3	19.2	12.9	7.6	12.5	4.9

*Acrophases are hours after midnight; phase delays are hours; — means missing measurement. Acrophases were calculated from RIAs for cortisol (in saliva and urine samples) and from melatonin (in saliva) and melatonin sulfate (in urine) (see text).

control subjects; melatonin concentrations in the saliva of the 15th subject did not change over the course of the day. Melatonin and melatonin sulfate concentrations in saliva and urine, respectively, were low during the day, began to increase at 1800, peaked after midnight, and began to decrease at dawn (Fig. 2, panels A and B). Cosinor analysis indicated that the melatonin acrophase was 0130 (saliva) and the melatonin-sulfate acrophase was 0230 (urine).

Cortisol concentrations in the control subjects followed a classic diurnal pattern (Fig. 2, panels C and D), with concentrations lowest at 2000, remaining low until an abrupt peak around 0600, and decreasing thereafter. Cosinor analysis indicated cortisol acrophases of 0700 (saliva) and 0730 (urine). Individual variations in cortisol concentration were greater than those for melatonin.

Test subjects

The cortisol and melatonin acrophases in the test group were within 2.5 hr of those for the control group (Table 1). During the first 4 days of bright-light exposure, melatonin production was suppressed (Fig. 3, panel B), but cortisol did not change significantly (Fig. 3, panel A). The melatonin rhythm (as a function of time of day) diminished during this period, but the cortisol rhythm was preserved. By the end of the 7-day treatment period, the peak melatonin concentrations were the same as they had been before the treatment, and they appeared within 2 hr of the expected 12-hr phase delay. Figure 4 shows the pattern of melatonin sulfate in the urine of one subject who completed Protocol A.

Cortisol and melatonin acrophases for the test subjects during the entire 7-day treatment period are shown in Figure 3 (panels C and D). The melatonin acrophase of the three subjects who followed Protocol A changed from 0300–0400 to 1500–2300 after only 3 days of treatment. The more gradual change in melatonin acrophase of the

subjects who followed Protocols B (N=3) or C (N=2) paralleled the more gradual changes in their sleep-wake times (Fig. 1). Cortisol acrophases were similar to melatonin acrophases except for one subject in the Protocol C group. With this one exception, all subjects achieved the expected circadian shift within the 7-day treatment period. Phase delays were 11–15 hr for subjects using Protocol A, and 7–12 hr for those using Protocols B or C (Table 1).

Discussion

Bright-light exposures have been used to treat sleep disorders associated with transmeridian travel, delayed sleep-phase syndrome, disrupted sleep in shift workers, and other sleep disorders [Lewy et al., 1987; Czeisler et al., 1986, 1991; Rosenthal et al., 1990]. Bright light is known to suppress melatonin production in humans [Lewy et al., 1980; Reiter, 1985; McIntyre et al., 1989], probably via its effects on the suprachiasmatic nucleus (SCN) [Adler et al., 1992]. The timing of bright-light exposure is critical for setting the magnitude and direction of circadian-rhythm shifts [Czeisler et al., 1989; Minors et al., 1991].

Shanahan and Czeisler [1991] found melatonin rhythms and plasma cortisol to be closely related to the body-temperature cycle both before and after bright-light-induced phase shifting. More recently, however, McIntyre et al. [1992] demonstrated a significant suppression of melatonin in subjects exposed to light from midnight to 0300, with no significant effect on plasma cortisol. Touitou et al. [1992] also showed that plasma melatonin concentration decreased after bright-light exposure, even if mean or peak plasma cortisol concentration did not change. Our results also indicate that cortisol rhythms do not necessarily reflect melatonin rhythms, at least early in the phase-shifting period. Although no blood samples were collected for this study, the cortisol and melatonin patterns in saliva and urine samples in our subjects suggest

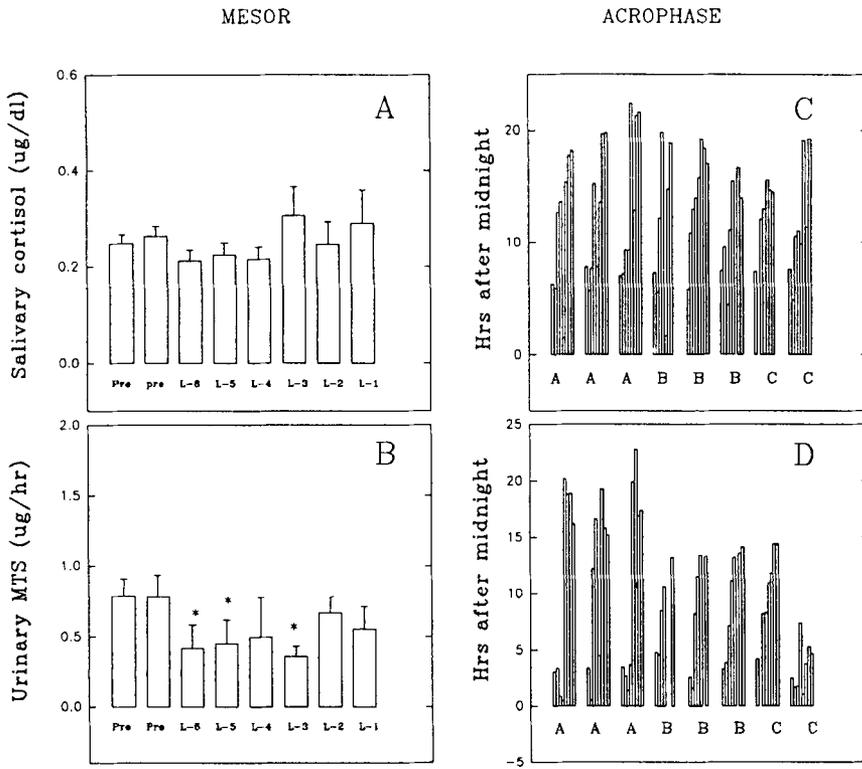


Fig. 3. Panels A and B illustrate the mean mesors calculated from cortisol (saliva) and melatonin sulfate (urine) from eight astronauts during baseline tests (pre-) and treatment days (L-6 to L-1). Error bars are SEM; asterisks denote significant difference relative to baseline values ($P < 0.05$). Panels C and D illustrate the acrophase profiles of cortisol and melatonin sulfate, respectively, during the entire treatment period. Each group of bars represents an individual subject; the protocol followed is denoted on the X axis. The latter two panels illustrate the individual differences between crew members.

that this technique can be effective for assessing cycles of these hormones. The 30- to 60-min lags in acrophase for saliva and urine concentrations probably reflect hepatic-metabolism and renal excretion times for cortisol and melatonin, respectively.

We conclude that the combination of bright light and

sleep shifting was reasonably effective (within 2 hr of the anticipated phase delay) in shifting the circadian cycle by nearly 12 hr over a 7-day period. Our results also indicate that the melatonin rhythm was suppressed during the first days of treatment, but the cortisol rhythm was not. Czeisler et al. [1986] found bright-light-induced phase shifting to

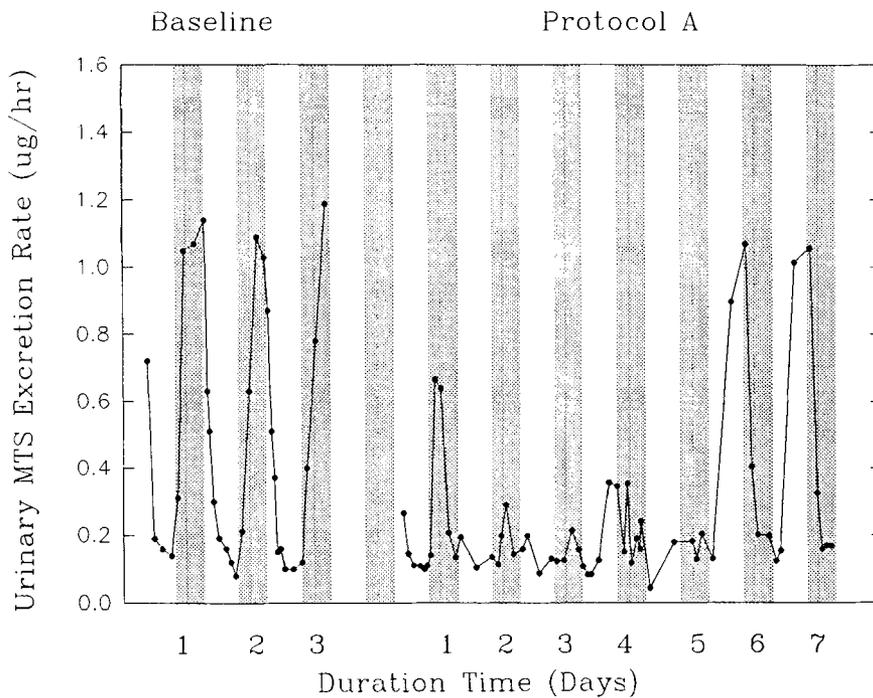


Fig. 4. Melatonin sulfate rhythm in urine samples from one subject in the Protocol A group during the baseline and treatment periods. The shaded areas indicate the dark portion of the light-dark cycle, irrespective of the treatment protocol.

be independent of the sleep-wake cycle. However, the shifting times in our protocol corresponded more closely to the sleep-wake cycle than to the dark-light cycle. Further study of the independent contributions of these variables is needed to elucidate the mechanisms involved, i.e., whether melatonin suppression is a *prerequisite* for light-induced phase shifts.

Body temperature is used extensively to monitor the rate and extent of adjustments in circadian time structures and is known to be affected by bright-light treatment and other rhythm-shifting protocols [Reinberg et al. 1979; Wever, 1979; Graeber, 1988]. Future studies will include measurement of body-temperature cycles as well as assessments of cognitive performance and alertness over time, as cognitive and physical performance tend to be optimal around the circadian crest of the body-temperature rhythm and lowest near its trough. In addition, rest-activity cycles, normally adapted to light-dark cycles [Monk et al., 1984], and absolute illumination levels will need to be recorded systematically during Space Shuttle flights.

Acknowledgments

The authors appreciate the efforts of Eden Fields, M. Libby Troell, and J. Vernell Fesperman of KRUG Life Sciences for the RIA of cortisol in urine. We are grateful also for the contributions of Drs. Richard Jennings and Russell Reiter and Ms. Christine Wogan to this report. This study was funded by NASA as part of the Extended-Duration Orbiter Medical Project (DSO 484).

Literature cited

- ADLER, J.S., D.F. KRIPKE, R.T. LOVING, S.L. BERGA (1992) Peripheral vision suppression of melatonin. *J. Pineal Res.* 12:49–52.
- AKERSTEDT, T., L. TORSVALL, M. GILLBERG (1982) Sleepiness and shift work: field studies. *Sleep* 5:S95–S106.
- ALDHOUS, M.E., J. ARENDT (1988) Radioimmunoassay for 6-sulphatoxymelatonin in urine using an iodinated tracer. *Ann. Clin. Biochem.* 25:298–303.
- ANGELI, A. (1974) Circadian ACTH-adrenal rhythm in man. *Chronobiologia* 1(Suppl. 1):253–270.
- ARENDT, J. (1990) Melatonin and rhythmic functions in mammals: Therapeutic and commercial potential. In: *Annual Review of Chronopharmacology*. A. Reinberg, M.H. Smolensky, G. Labrecque, eds. Pergamon Press, London, Vol. 6, pp. 137–153.
- ARENDT, J., M. ALDHOUS, V. MARKS (1986) Alleviation of jet-lag by melatonin: preliminary results of controlled double blind trial. *Br. Med. J. (Clin. Res. Ed.)* 292:1170.
- ARENDT, J., M. ALDHOUS, J. WRIGHT (1988) Synchronisation of a disturbed sleep-wake cycle in a blind man by melatonin treatment (letter). *Lancet* i:772–773.
- CHEN, Y.M., N.M. CINTRON, P.A. WHITSON (1992) Long-term storage of salivary cortisol samples at room temperature. *Clin. Chem.* 38:304.
- CZEISLER, C.A., J.S. ALLAN, S.H. STROGATZ, J.M. RONDA, R. SANCHEZ, C.D. RIOS, W.O. FREITAG, G.S. RICHARDSON, R.E. KRONAUER (1986) Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 233:667–671.
- CZEISLER, C.A., A.J. CHIASERA, J.F. DUFFY (1991) Research on sleep, circadian rhythms and aging: applications to manned space flight. *Exp. Gerontol. (England)* 26:217–232.
- CZEISLER, C.A., M.P. JOHNSON, J.P. DUFFY, E.N. BROWN, J.M. RONDA, R.E. KRONAUER (1990) Exposure to bright light and darkness to treat physiologic maladaptation to night work. *N. Engl. J. Med.* 322:1253–1259.
- CZEISLER, C.A., R.E. KRONAUER, J.S. ALLAN, J.F. DUFFY, M.E. JEWETT, E.N. BROWN, J.M. RONDA (1989) Bright light induction of strong (Type O) resetting of the human circadian pacemaker. *Science* 244:1328–1333.
- ENGLUND, C.E., D.H. RYMAN, P. NAITOH, J.A. HODGDON (1985) Cognitive performance during successive sustained physical work episodes. *Behav. Res. Methods Instru. Computers* 17:75–85.
- FRASER, S., P. COWEN, M. FRANKLIN, C. FRANEY, J. ARENDT (1983) Direct radioimmunoassay for melatonin in plasma (letter). *Clin. Chem.* 29:396–397.
- GOLD, D.R., S. ROGACZ, N. BOCK, T.D. TOSTESON, T.M. BAUM, F.E. SPEIZER, C.A. CZEISLER (1992) Rotating shift work, sleep, and accidents related to sleepiness in hospital nurses. *Am. J. Public Health* 82:1011–1014.
- GRAEBER, R.C. (1988) Aircrew fatigue and circadian rhythmicity. In: *Human Factors in Aviation*. E.L. Wiener, D.C. Nagel, eds. Academic Press, New York, pp. 305–344.
- HONMA, K., S. HONMA, M. KOHSAKA, N. MORITA, N. FUKUDA (1991) Does methylcobalamin (vitamin B12) increase the light sensitivity of human circadian clock? *Jpn. J. Psychiatry Neurol.* 45:171–172.
- LEWY, A.J., T.A. WEHR, F.K. GOODWIN, D.A. NEWSOM, S.P. MARKEY (1980) Light suppresses melatonin secretion in humans. *Science* 210:1267–1269.
- LEWY, A.J., R.L. SACK, L.S. MILLER, T.M. HOBAN (1987) Antidepressant and circadian phase-shifting effects of light. *Science* 235:352–354.
- MARKOWITZ, M.E., S. ARNAUD, J.F. ROSEN, M. THORPY, S. LAXIMINARAYAN (1988) Temporal interrelationships between the circadian rhythms of serum parathyroid hormone and calcium concentrations. *J. Clin. Endocrinol. Metab.* 67:1068–1073.
- MCINTYRE, I.M., T.R. NORMAN, G.D. BURROWS, S.M. ARMSTRONG (1987) Melatonin rhythm in human plasma and saliva. *J. Pineal Res.* 4:177–183.
- MCINTYRE, I.M., T.R. NORMAN, G.D. BURROWS, S.M. ARMSTRONG (1989) Human melatonin suppression by light is intensity dependent. *J. Pineal Res.* 6:149–156.
- MCINTYRE, I.M., T.R. NORMAN, G.D. BURROWS, S.M. ARMSTRONG (1992) Melatonin, cortisol and prolactin response to acute nocturnal light exposure in healthy volunteers. *Psychoneuroendocrinology* 17:243–248.
- MEULENBERG, P.M., J.A. HOFMAN (1990) Differences between concentrations of salivary cortisol and cortisone and of free cortisol and cortisone in plasma during pregnancy and postpartum. *Clin. Chem.* 36:70–75.
- MINORS, D.S., J.M. WATERHOUSE, A. WIRZ-JUSTICE (1991) A human phase-response curve to light. *Neurosci. Lett.* 133:36–40.
- MONK, T.H., E.D. WEISMAN, J.E. FOOKSON, M.L. MOLINE (1984) Circadian rhythms in human performance efficiency under free running conditions. *Chronobiologia* 11:343–354.
- NELSON, M., Y.L. TONG, J.K. LEE, F. HALBERG (1979) Methods for cosinor-rhythmometry. *Chronobiologia* 6:305–323.
- NOWAK, R., I.C. McMILLEN, J. REDMAN, R.V. SHORT (1987) The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulfate excretion rates:

- two noninvasive techniques for monitoring human circadian rhythmicity. *Clin. Endocrinol. (Oxford)* 27:445-452.
- REINBERG, A., C. MIGRAINE, M. APFELBAUM, L. BRIGANT, J. GHATA, N. VIEUX, A. LAPORTE (1979) Circadian and ultradian rhythms in the feeding and nutrient intakes of oil-refinery operators with shift work every 3-4 days. *Diabete Metab.* 5:33-41.
- REINBERG, A., P. ANDLAUER, J. DEPRINS, W. MALBECQ, N. VIEUX, P. BOURDELEAU (1984) Desynchronization of the oral temperature circadian rhythm and intolerance to shift work. *Nature* 308:272-274.
- REITER, R.J. (1985) Action spectra, dose-response relationships and temporal aspects of light's effects on the pineal gland. *Ann. NY Acad. Sci.* 453:215-230.
- REITER, R.J., J.M. GUERRERO, M. PUIG-DOMINGO, C. SANTANA (1988) Type II iodothyronine deiodination: Nyctohemeral rhythms in the cerebral cortex, the pineal gland, and the Harderian glands. In: *Advances in the Biosciences*. W. Reitveld, ed. Pergamon Press, London, Vol. 73, pp. 123136.
- ROSENTHAL, N.E., J.R. JOSEPH-VANDERPOOL, A.A. LEVENDOSKY, S.H. JOHNSTON, R. ALLEN, K.A. KELLY, E. SOUETRE, P.M. SCHULTZ, K.E. STARZ (1990) Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep* 13:354-361.
- SHANAHAN, T.L., C.A. CZEISLER (1991) Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core temperature in men. *J. Clin. Endocrinol. Metab.* 73:227-235.
- TOUITOU, Y., O. BENOIT, J. FORET, A. AGUIRRE, A. BOGDAN, M. CLODORE, C. TOUITOU (1992) Effects of a two-hour early awakening and of bright light exposure on plasma patterns of cortisol, melatonin, prolactin and testosterone in man. *Acta Endocrinol. (Copenh.)* 126:201-205.
- WEVER, A.R. (1979) *The Circadian System of Man: Results of Experiments under Temporal Isolation*. Springer-Verlag, Berlin.