A Laboratory Comparison of Clockwise and Counter-Clockwise Rapidly Rotating Shift Schedules, Part III: Effects on Core Body Temperature and Neuroendocrine Measures

Albert Boquet
Crystal E. Cruz
Thomas E. Nesthus
Cristy A. Detwiler
William R. Knecht
Kali A. Holcomb

Civil Aerospace Medical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

November 2002

Final Report

U.S. Department
of Transportation

**Federal Aviation
Administration**

N O T I C E

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Technical Report Documentation Page

1. Report No. DOT/FAA/AM-02/20	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle A Laboratory Comparison of Clockwise and Counter-Clockwise Rapidly Rotating Shift Schedules, Part III: Effects on Core Body Temperature and Neuroendocrine Measures		5. Report Date November 2002
		6. Performing Organization Code
7. Author(s) Boquet A, Cruz CE, Nesthus TE, Detwiler CA, Knecht WR, and Holcomb KA		8. Performing Organization Report No.
9. Performing Organization Name and Address FAA Civil Aerospace Medical Institute P.O. Box 25082 Oklahoma City, OK 73125		10. Work Unit No. (TRAIS)
		11. Contract or Grant No.
12. Sponsoring Agency name and Address Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591		13. Type of Report and Period Covered
		14. Sponsoring Agency Code
15. Supplemental Notes This report was performed under Task HRR518.		

16. Abstract

Most researchers suggest that shift rotation in a forward or clockwise direction produces less disruption of circadian rhythms than those that rotate in a backward or counter-clockwise direction. This is based upon extrapolation from quasi-experimental studies of shift-workers and research on the effects of jet lag, which indicate that westward travel results in less disruption of circadian rhythms. The effect of direction of rotation on cortisol, melatonin, and core body temperature was examined in participants randomly assigned to either a clockwise or counter-clockwise shift rotation. Twenty-eight participants worked a day shift (0800-1600) for one week followed by either a clockwise (n=14) or counter-clockwise (n=14) shift rotation for two weeks. Participants wore a flexible rectal temperature sensor for the three weeks of the study and were allowed to remove the sensor for 90 minutes each day. Saliva samples were collected at the end of the baseline week for later assay for melatonin and cortisol, and were time-locked to collection times during the two "shiftwork" weeks. No group differences were found for cortisol for either of the workweeks. The clockwise group, however, had a significantly greater increase in melatonin during the early morning shift, compared with the counter-clockwise group. Finally, the analyses of core body temperature revealed a significantly lower amplitude and a delay of the acrophase for the counter-clockwise group during both weeks of testing. While cortisol levels did not appear to differ based on direction of shift rotation, there were inherent differences with melatonin secretion and core body temperature. Precisely why such differences exist remains unclear but may be similar to that seen with circadian resynchronization during westward travel. Furthermore, it is not clear if these differences would persist in individuals exposed to longer periods of shift rotations.

17. Key Words Shiftwork, Cortisol, Melatonin, Core Body Temperature, Circadian Rhythms, Clockwise, Counter-Clockwise	18. Distribution Statement Document is available to the public through the National Technical Information Service; Springfield, VA 22161		
19. Security Classif. (of this report) Unclassified	20. Security Classif. (of this page) Unclassified	21. No. of Pages 19	22. Price

Form DOT F 1700.7 (8-72)   Reproduction of completed page authorized

ACKNOWLEDGMENTS

A Laboratory Comparison of Clockwise and Counter-Clockwise Rapidly Rotating Shift Schedules, Part III: Effects on Core Body Temperature and Neuroendocrine Measures

Because of the increasing industrialization of our society, shiftwork is necessary in many occupations. Accordingly, much research has been conducted on the effects of shiftwork as it relates to the health and well being of individuals engaged in such activities. The underlying rationale is that shiftwork presents a potential source of strain that may ultimately take a toll on the shiftworker, in any one of a number of ways. For instance, Monk (1988) has postulated a model of shiftwork strain composed of three interactive components: sleep, issues associated with the biological clock, and social/domestic factors. All three components play an interactive role in determining how someone will cope with the demands of shiftwork.

Given the complexity of managing shiftwork and performance in the workforce, the role of management and industry is to try to implement work schedules that will result in the best compromise between productivity and employee satisfaction, health, and performance. Debate in the literature, bolstered in part by a large body of research on jet-lag, has suggested that shift schedules that change in the direction of the circadian cycle (i.e., clockwise or forward rotations), result in less physiological and mental disruption than those that rotate in the opposite direction (Monk, 1986; Barton & Folkard, 1993). If this is true, perhaps these same kinds of schedules might result in less of a physical challenge, thus ameliorating the social and emotional impact of working different shifts. Nevertheless, while jet-lag studies have shown that individuals adapt more quickly to westward travel (Wegman & Klein, 1985), jet travel and shiftwork are only partially analogous. That is, exposure to daylight acts as an exogenous cue thereby promoting adaptation to new time zones as one travels. However, these same daylight cues are often in conflict with shiftwork schedules. Consequently, we are left with little empirical evidence to support the claim that clockwise rotations result in less disruption and therefore, less physical challenge to the shiftworker (Barton & Folkard, 1993).

Addressing the importance of maintaining circadian rhythmicity by shift schedule design, Lavie, Tzischinsky, Epstein, and Zomer, (1992) compared workers on clockwise and counter-clockwise, 5-day-per-shift schedules. They reported better synchronization of sleep-wake cycles and earlier sleep times following midnight shifts for those individuals in the clockwise rotation. In addition, they infer that the quicker adaptation of biological rhythms of the clockwise group may be important in enhancing health outcomes in this group. However, the lack of statistically significant results implies that the authors may have overstated their findings.

In addition, there is evidence that people never fully adapt to shifts other than standard daytime hours (Naitoh, Kelly, & Englund, 1990). Even those individuals on permanent night shifts show poor adjustment in circadian markers and overall sleep quality compared with their day-working cohorts. The reasons for this include, but may not be limited to, reverting to a "normal" schedule on their days off, difficulty sleeping in the daytime, individual differences in ability to adapt to shiftwork, and the mismatch of schedule with exogenous cues such as daylight.

While individuals may never fully adapt to shiftwork physiologically, there is some evidence that at least some individuals tolerate shiftwork better than others (Costa, Lievore, Casaletti, & Gaffuri, 1989). In a study of 24 participants matched for age and work experience, Costa et al. found that those individuals who demonstrated greater flexibility in sleep habits, resistance to drowsiness, and reported lower manifest anxiety had a greater tolerance to shiftwork. Notably, however, these characteristics did not affect the adjustment of oral temperature to night shiftwork, indicating that physiological adaptation likely did not take place.

Perhaps oral temperature, as used in the Costa et al. study isn't the best measure of physiological adaptation to shiftwork. One alternative is to use cortisol as a physiological marker. Cortisol is the principal gluccocorticoid of the adrenocortical hormones with widespread systemic effects. The most notable of these is its ability to markedly stimulate gluccogenesis; hence, its classification as a gluccocorticoid. Cortisol has a relatively static diurnal rhythm, which according to

Munck, Guyre, and Holbrook (1984), has both diurnal and metabolic functions, is controlled by hypothalamic signals, and is regulated via a sensitive negative feedback loop.

What makes cortisol a particularly good marker in studies of circadian disruption is its release during emotional stress. For example, shiftwork tolerance was investigated by Hennig, Kieferdorf, Moritz, Huwe, and Netter (1998) in a sample of 24 night shiftworkers. They found a reversal of the circadian pattern for cortisol in 18 of the 24 workers after the fifth night of shiftwork. The 6 non-adapters demonstrated less sleep, greater variability in their sleep patterns, and higher neuroticism scores. The authors concluded that there are individual differences in shiftwork tolerance as revealed by cortisol secretion.

In a similar study, Hale, Williams, Smith, and Melton (1971) found adrenocortical activity late in the morning shift among a sample of air traffic controllers at O'Hare Airport in Chicago. However, following the night shift, a depression in adrenocortical activity was observed. As a result, the authors suggested that nocturnal wakefulness acted as a stressor, accounting for some of the elevation noted in both control and experimental groups.

The relationship between cortisol levels and shiftwork is particularly apparent in cortisol responses to sleep deprivation, an inherent characteristic of working night shifts. Leproult, Copinschi, Buxton, and Van Cauter (1997) examined male participants in normal sleep schedules, partial sleep deprivation, and total sleep deprivation conditions. Both partial and total sleep deprivation resulted in elevated cortisol levels on the second recovery day following deprivation. This was accompanied by a delay in the recovery of the lull in cortisol secretion, suggesting a disruption of the negative feedback system of the hypothalamic-pituitary-adrenocortial (HPA) axis.

Another commonly used circadian marker is body temperature because of its highly entrained rhythm, regulated by sensitive negative feedback loops in temperature-regulating centers of the hypothalamus (Guyton & Hall, 2000). This highly efficient system has proven a useful circadian marker in studies like that of Reinberg, Vieux, Ghata, Chaumont, and Laporte (1978), who found that low amplitude in the oral temperature rhythm may reflect the ability to adjust to shiftwork. In contrast, Knauth and Harma (1992) found that large amplitude in oral temperature rhythm prior to shiftwork was positively associated with health-related indices of shiftwork tolerance.

Like body temperature, melatonin secretion has proven to be a reliable measure of intrinsic circadian rhythmicity. However, unlike body temperature, melatonin is relatively insensitive to exogenous cues, other than light. As such, it may be a more reliable marker of circadian rhythmicity than body temperature. Produced by the pineal gland following its synthesis from tryptophan, concentrations of melatonin are relatively high at night and at their lowest levels during the day with the rhythm entrained by photic stimuli, exercise, certain drugs, and exogenous melatonin (Guyton & Hall, 2000).

Roden, Koller, Pirich, Vierhapper, and Waldhauser (1993) examined the circadian melatonin profile for permanent night shiftworkers, compared with matched controls. The night shiftworkers as a group displayed no adaptation of circadian profiles for either melatonin or cortisol. All workers scored high in job satisfaction, indicating that high shiftwork tolerance is explained by more than circadian orientation.

The findings of Roden et al. (1993) are opposed to others who found wide variations in melatonin profiles, particularly the onset of melatonin release, among permanent night shiftworkers when compared to day workers (Weibel, Spiegel, Gronfier, Follenius, & Branden-Berger, 1997). Others have found forward shifts in the mean peak of melatonin as well as a lack of an identifiable acrophase during night work among permanent night shiftworkers (Quera-Salva, Defrance, Claustrat, DeLattre, & Guilleminault, 1996).

Melatonin has been shown to phase shift following time zone changes, with the rate of return to its baseline rhythm determined by how quickly the time zones change (Harma, Laitinen, Partinen, & Suvanto, 1993). Following a round trip flight from Helsinki to Los Angeles, the phase delay for melatonin peaked at 5 hours, 59 minutes. Resynchronization of the rhythm had occurred within five days of the flight home, leading the authors to recommend a five-day recovery following numerous, rapid time zone transitions.

Reentrainment of the melatonin rhythm following time zone transitions is dependent on cues in the new time zone and direction of travel. Westward travel results in faster resynchronization of the rhythm as the system entrains to a period longer than a day. This is analogous to a clockwise rotation, which ultimately results in a longer day as the work week progresses. Roach, Fletcher, Rodgers, and Dawson, (2000), followed 14 members of a surveillance patrol on a 13-day routine flight. Their findings indicated that the onset of melatonin phase was delayed in westward flight

with a slower recovery. Eastward flight resulted in a phase advance of melatonin onset with faster subsequent recovery following small acute time zone changes. While these findings are contrary to studies showing faster recovery times and phase advances following westward flight (Graeber, 1988), only melatonin *onset* was measured, thus the overall fit of the curve may have been quite different.

In a study of the adaptation of permanent night shiftworkers, Weibel et al. (1997) measured melatonin and core body temperature. They found that shifting participants to a daytime sleep period resulted in an uncoupling of the melatonin and temperature rhythms, with temperature displaying a homogenous pattern consistent with their usual sleep-wake cycle. Of interest was the observation that adaptation to night shiftwork was highly variable with respect to melatonin. Thus, the two systems are differentially affected by consistent nighttime work.

In a study of the relationship of gender to the adjustment of circadian markers to night shiftwork, Hakola, Harma, and Laitinen (1996) found no changes in salivary melatonin levels following a rotation to consecutive night shifts. While other markers such as cortisol and body temperature exhibited an effect for shift, no differences were noted for gender. Furthermore, no differences due to shift or gender were noted for melatonin.

While subjective and behavioral indices of shiftwork adaptation are important, they lack the rigor necessary to objectively evaluate changes or shifts in the circadian rhythm of the individual. Biochemical markers with relatively entrained circadian rhythms such as cortisol and melatonin may provide useful and less subjective measures of changes in diurnal patterns. Temperature has also been used because of its sensitivity to changes in schedule. With this in mind, the current investigation used core body temperature (CBT) to test the effects of a clockwise vs. counter-clockwise shift system on the circadian profile of 28 individuals in a simulated work environment. Salivary cortisol and melatonin were measured during work to assess differences in production due to the different work schedules. According to Aston-Jones, Chen, Zhu, and Oshinsky (2001), CBT, melatonin, and cortisol may share a common biological rhythm generator (the suprachiasmatic nucleas of the hypothalamus).

METHODOLOGY

A 3-week protocol was designed for a laboratory comparison of rapidly rotating clockwise and counterclockwise shifts, where schedule rotation represented the between-groups factor in a mixed-model, repeated measures design. Participants were run in groups of five, with the direction of rotation balanced so the first group was assigned to the clockwise rotation, the second group was assigned to the counter-clockwise rotation, etc. Although the protocol for the experiment was extensive including multiple computer tests, physiological measures, and subjective measures, this paper will focus on the temperature and biochemical measures; therefore, only the procedures related to data collection for these measures will be discussed in detail.

Participants
Demographics and Group Assignment

Thirty people between the ages of 20 and 55 ($M = 41.2$ years) were recruited and screened from the general population to participate in the study. Participants signed the informed consent form approved by the FAA Institutional Review Board and were paid for their participation in the study. Two participants withdrew from the study before completing the protocol. Participants were assigned to either the clockwise rotation condition ($n = 14$) or the counter-clockwise rotation condition ($n = 14$). Group assignments were made in the order that participants were recruited and on the basis of their availability. Participants in the clockwise rotation included 7 males and 7 females, with an average age of 40.6 years ($SD = 9.4$ yrs.). Participants in the counter-clockwise rotation included 5 males and 9 females with an average age of 41.9 years ($SD = 9.0$ yrs.). All participants were non-smokers and light- or non-users of caffeine and alcohol. Additional details regarding the participant sample and their recruitment and selection are reported elsewhere (Cruz, Detwiler, Nesthus, & Boquet, 2002).

Procedures and Apparatus

Participants in the study reported to the laboratory at the Civil Aerospace Medical Institute (CAMI) for 8 hours per day, 5 days per week, for 3 weeks. The first week (Monday-Friday) for both rotation conditions was comprised of day shifts (0800-1600). During this week, participants were trained on computerized tasks and habituated to the laboratory environment and to wearing a physiological monitor. During the next 2

weeks, participants worked one of the shift rotation schedules shown in Table 1, as determined by their group assignment. Note that the clockwise rotation allowed for 24 hours off at each shift rotation and a 48-hour weekend before returning to work again. By comparison, the counter-clockwise rotation allowed only 8 hours off at each shift rotation and an 80-hour weekend before returning to work again.

On the first day of the study, participants were oriented to the laboratory and a detailed daily schedule for the study. Two one-time questionnaires were completed, a Morningness-Eveningness Questionnaire (Horne & Ostberg, 1976) and a biographical questionnaire. In addition, participants were given physiological monitoring devices and daily logbooks, and were trained on their use. Finally, participants were trained on the Multiple Task Performance Battery (MTPB) and the Bakan Vigilance Task. The physiological monitors and all sensors except the chest band were worn 22.5 hours per day to accommodate a 1.5-hour break for showers and leisure activities. The only restriction was that the monitor should not be removed while sleeping or napping. The chest band sensor was only worn while working at the laboratory. The Bakan Vigilance Task was administered at the beginning and end of each workday, and the MTPB was performed 3 times each day. The daily protocol is presented in Table 2.

Table 1
Clockwise and Counter-Clockwise Shift Rotation Schedules

	Clockwise Rotation			Counter-Clockwise Rotation	
Day	Work Hours	Hours Between	Day	Work Hours	Hours Between
Monday	0600-1400	16	Monday	1400-2200	16
Tuesday	0600-1400	24	Tuesday	1400-2200	8
Wednesday	1400-2200	16	Wednesday	0600-1400	16
Thursday	1400-2200	24	Thursday	0600-1400	8
Friday-Sat	2200-0600	48	Thur-Friday	2200-0600	80

Table 2
Daily Experimental Protocol

Time (in hours)		Activity
Start	End	
00:00	00:30	Download & initialize Miniloggers; Subjective ratings; Collect saliva
00:30	01:00	Bakan Session 1
01:00	02:30	MTPB Session 1
02:30	02:45	Subjective ratings; Collect saliva
02:45	03:15	Break
03:15	04:45	MTPB Session 2
04:45	05:00	Subjective ratings; Collect saliva
05:00	05:30	Meal Break
05:30	07:00	MTPB Session 3
07:00	07:30	Bakan Session 2
07:30	08:00	Download & initialize Miniloggers; Subjective ratings; Collect saliva

Participants were instructed to treat their participation in the study as a full-time job, to refrain from drinking alcohol or taking any drugs or medications during the course of the study with the exception of ibuprofen, birth control pills, estrogen replacement, and non-drowsy formula allergy medications such as Claritin™. In addition, participants were instructed not to consume any caffeinated beverages or chocolate and were not allowed to eat bananas during the study because of the potential interference with the radio-immunoassays for cortisol. Diet was not otherwise controlled in the study.

Participants were tested with the Intoxilyzer 9000™ breath alcohol test at the beginning of each workday to ensure compliance with the study protocol. None of the participants tested positive during the study. A final day of testing on Day 22 of the study included a final Bakan test session, check-in of equipment, an exit questionnaire regarding the study experience and a cohesiveness questionnaire. This was done to mitigate an end-of-study effect at the end of the previous week and to allow for data collection on the weekend following the last shiftwork week.

Physiological Monitor

The Series 2000 Minilogger™ ambulatory physiological monitor (MiniMitter™ Co., Inc., 20300 Empire Ave., Bend, OR 97701) was used to measure the inter-beat-interval (IBI) of heart beats, core body temperature, and wrist activity. The monitor was approximately 120 x 65 x 22mm and weighed 125 grams. Sensors included: 1) a Polar™ chest band for measuring IBI, 2) a Yellow Springs Instruments™ (YSI) flexible rectal temperature sensor, and 3) a small wrist activity monitor. All of these channels were direct-wired to the monitor. A sampling rate of once per minute was used for temperature and activity. The chest band, an adjustable elastic band with a rubber section containing electrodes, was placed around the chest. The temperature sensor was a flexible tube approximately 36" long. The wrist activity monitor was approximately 1" square by 0.25" thick and was worn on the non-dominant wrist like a wristwatch.

Temperature Data

The temperature data were reduced for each participant by computing 1-hour averages for each 24-hour epoch. All analyses consisted of the final 72 hours of the 3 weeks (Baseline, Shiftwork Week 1, and Shiftwork Week 2). To control for the effects of exogenous factors, a custom platform in EXCEL (Solver) was designed to fit a cosine function to the averaged temperature data. The function was fit to the raw data curve by minimizing the sum-of-squares (ΣSS) representing the sum of point-wise squared differences between the values of the data and the curve being fit to each individual data point. In those few cases where the program failed to fit the curve (i.e. left a large residual ΣSS), Solver allowed for visual identification of the mismatch. The amplitude, phase, and period were then manually adjusted and Solver re-run to obtain an acceptable solution. A more detailed description can be found in Appendix A.

Salivary Cortisol and Melatonin

Saliva Collection Procedure. Saliva samples were collected to obtain measurements of cortisol and melatonin concentrations. Pre-labeled plastic collection vials with cotton rolls were used to collect the saliva samples. Participants were instructed to rinse their mouth with cold water 15 minutes before collecting saliva samples and to refrain from eating, drinking, or chewing gum for 1 hour prior to saliva collection. Participants placed a cotton roll in their mouth and held it between their cheek and gums for up to 3 minutes, then spit any additional saliva into the collection vial and pushed the cotton roll into the vial with their tongue to avoid touching it with their fingers. After capping the vial, samples were placed directly into a freezer at home or at the laboratory. Samples were transported from home using Styrofoam boxes and freezer packs. The samples were stored at -20^{0}C for long-term storage.

A 24-hour baseline was collected from Thursday to Friday at the end of week 1 (Days 4-5). Participants were given pre-labeled salivary collection vials and instructed to collect their saliva at 0845, 1045, 1400, 1645, 1845, 2200, 0045, 0245, and 0600. They were given watches programmed with these collection times so that an auditory alarm would remind them to collect the sample. The baseline collection times were chosen to correspond to the laboratory collection times during the shiftwork weeks (Table 3).

Assay Procedure. Saliva samples were analyzed for cortisol and melatonin by radioimmunoassay. A Beckman Gamma 5500 counter was used for the radioactive counting of all samples. The saliva samples were thawed at room temperature for approximately 30 minutes. Each sample was placed in a collection tube and centrifuged for 4 minutes at 3000 g. Detailed procedures for cortisol and melatonin assays are provided in Appendix B.

Table 3
Saliva Sampling Schedule for Shiftwork Weeks

	Clockwise Rotation					Counter-Clockwise Rotation			
Shift[1]	Collection Times				Shift	Collection Times			
EM1	0600	0845	1045	1400	A1	1400	1645	1845	2200
EM2	0600	0845	1045	1400	A2	1400	1645	1845	2200
A1	1400	1645	1845	2200	EM1	0600	0845	1045	1400
A2	1400	1645	1845	2200	EM2	0600	0845	1045	1400
M	2200	0045	0245	0600	M	2200	0045	0245	0600

[1] EM1=First Early Morning Shift
EM2=Second Early Morning Shift
A1 = First Afternoon Shift

A2 = Second Afternoon Shift
M = Midnight Shift

Design and Data Analysis

Data analyses were conducted utilizing SPSS 10.0 for Windows. The majority of analyses utilized the General Linear Model for Repeated Measures procedure with the Greenhouse and Geisser (1959) correction for degrees of freedom. Significant main effects were followed with Tukey's Honestly Significant Difference (HSD) tests to compare means. Interactions were followed with appropriate simple effects analyses. Alpha was set at .05.

The temperature data were analyzed using a 2 (rotation condition) x 3 (week) mixed model design. A second analysis consisted of a mixed model ANCOVA using the baseline data as the covariate to control for the effects of baseline differences on responses to shiftwork.

Two separate analyses were conducted for both cortisol and melatonin. The initial analyses consisted of a manipulation check in which the raw scores were assessed by a series of 3 (week) x 4 (sample) x 2 (rotation condition) mixed-model ANOVAs, where week and sample were the within-subjects factors, and rotation condition was the between-subjects factor. For this analysis, the baseline samples were compared with their respective, time-locked workweek samples for the appropriate day (e.g., the first early morning shift samples of weeks 1 and 2 compared with the baseline early-morning samples, etc.).

The second set of analyses consisted of comparing the delta change scores computed by subtracting the baseline values from the values obtained during the two shiftwork weeks. To assess individual responses to the tasks, a series of 2 (week) x 2 (day) x 4 (sample) x 2 (rotation condition) mixed-model ANOVAs were separately employed for the morning and afternoon shifts. Since there was only one midnight shift in both of the workweeks, a 2 (week) x 4 (sample) x 2 (rotation condition) model was employed.

RESULTS

Core Body Temperature

The results of the ANCOVA for the measures of core body temperature revealed significant main effects for rotation condition for both Amplitude, $F(1, 21) = 14.62$, $p < .001$ and Acrophase[1], $F(1, 21) = 4.43$, $p < .05$. Amplitude for the counter-clockwise rotation ($M = 0.3°$ C) was significantly lower than the amplitude for the clockwise rotation ($M = 0.5°$ C). In addition, an 84-minute delay of the acrophase was found for the counter-clockwise condition ($M = 1808$h) relative to the clockwise condition ($M = 1644$h).

To illustrate these findings, Figure 1 presents the average temperature values and the cosine, best-fitting curves for the 72-hr epochs of interest for the clockwise and counter-clockwise rotations. Apparent in the illustrations is an attenuation of the amplitude of the curves and an 84-minute delay in the acrophase in the counter-clockwise rotation, compared with the clockwise rotation.

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[1] Acrophase refers to the time of the peak of the temperature rhythm for a specified period.
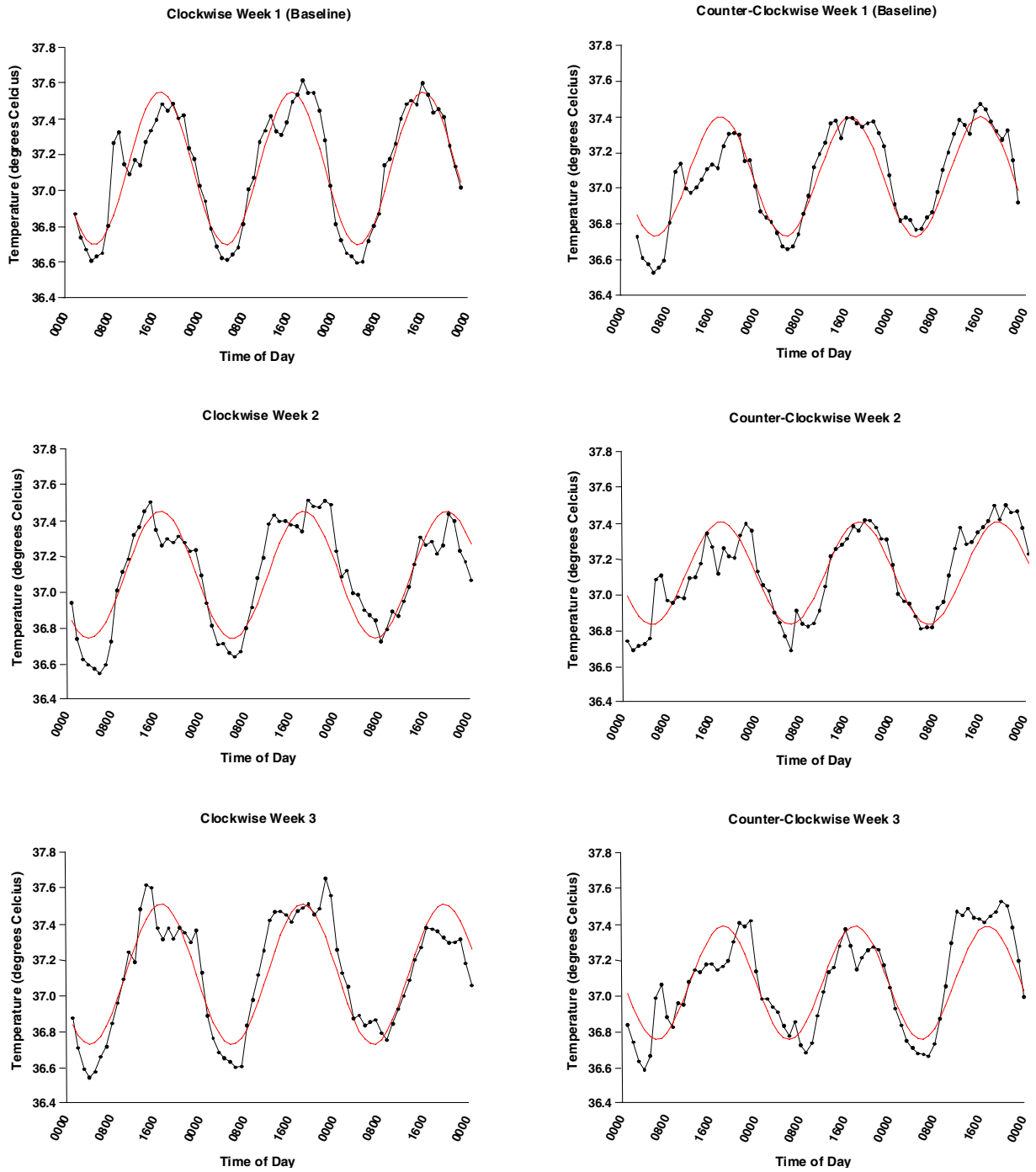
Figure 1. Temperature Data and Cosine Functions for Clockwise and Counter-Clockwise Rotation Conditions for All Work Weeks

Cortisol

Raw Score Analysis

Sample means for the early morning shifts (EM1 and EM2), afternoon shifts (A1 and A2) and the midnight shift (M) are presented in Figure 2. The analysis of the early morning shift data yielded significant main effects for sample for both EM1, $F(3, 78) = 40.68$, $p < .05$ and EM2, $F(3, 78) = 43.05$, $p < .05$. Tukey post hoc comparisons revealed that Sample 1 (0600 h) was significantly higher than Samples 2 (0845 h), 3 (1045 h), or 4 (1400 h) for both early-morning shifts.

Similarly, significant main effects for sample were found for A1, $F(3, 78) = 41.83$, $p < .001$, and A2, $F(3, 78) = 54.78$, $p < .001$. Tukey post hoc comparisons indicated that for A1, samples 1 (1400 h) and 2 (1645 h) were significantly higher than samples 3 (1845 h) and 4 (2200 h), but not different from each other. For A2, sample 1 (1400 h) was significantly higher than samples 2 (1645 h), 3 (1845 h), and 4 (2200 h). In addition, sample 2 (1645 h) was significantly higher than sample 4 (2200 h).

The omnibus test for the midnight shift resulted in a significant week by sample interaction, $F(6, 150) = 3.09$, $p < .05$. Simple effects analyses of sample at each level of week revealed significance for all three weeks, $F(4, 100) = 89.78$, $p < .001$, $F(4, 100) = 86.25$, $p < .001$, and $F(4, 100) = 64.17$, $p < .001$, respectively. The post hoc comparisons of the mean differences for sample at each level of week revealed that sample 4 (0600 h) was significantly higher at each week than samples 1 (2200 h), 2 (0045 h), and 3 (0245 h). Figure 3 reveals that cortisol levels for the 0600 h sample during baseline were higher than the two shiftwork weeks. The least significant difference (LSD) between the baseline week and the first week of shiftwork was statistically significant.

Cortisol Delta Change Score Analysis

There were no significant effects on either the early morning or the afternoon shifts for the change score analyses for cortisol. Analysis of the change scores for the midnight shift, however, revealed a significant main effect for sample, $F(3,78) = 4.12$, $p < .05$. Tukey
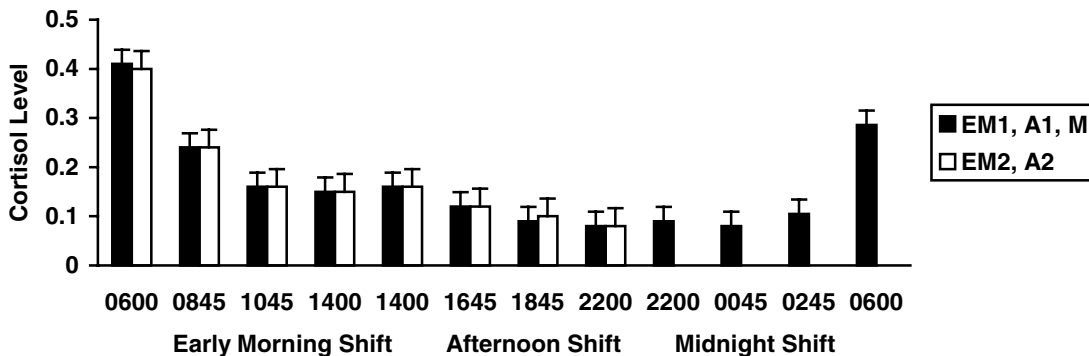

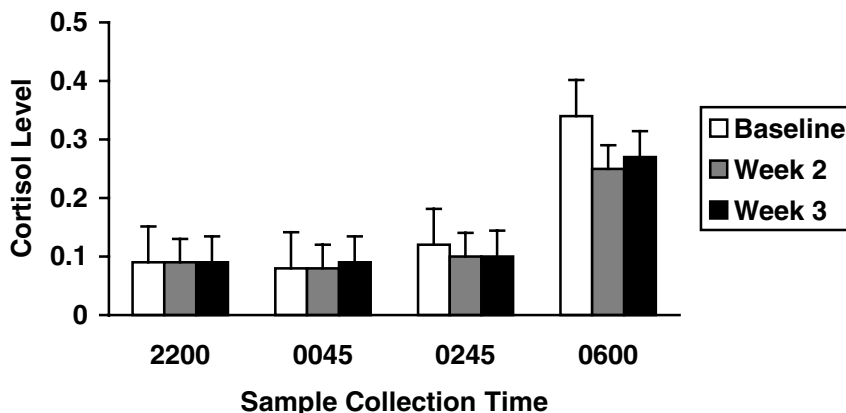**Figure 2.** Cortisol Levels for Each Shift by Sample Time

Figure 3. Cortisol Levels for Midnight Shift (Sample by Week Interaction)

post hoc comparisons indicated that there was a significantly greater change from baseline for the 6:00 a.m. sample, when compared with the three earlier samples (Figure 4).

Melatonin

Raw Score Analysis

Sample means for the (HPA)early morning shifts (EM1 and EM2), afternoon shifts (A1 and A2), and the midnight shift (M) are presented in Figure 5. The analysis of the early morning shift data revealed a main effect for sample for EM1, $F(3, 72) = 49.77$, $p < .001$ and EM2, $F(3, 72) = 28.09$, $p < .001$. Tukey post hoc comparisons of the mean differences indicated that sample 1 (0600 h) was significantly higher

than samples 2 (0845 h), 3 (1045 h), or 4 (1400 h) for both shifts. A main effect for rotation condition was also present for EM1, $F(1, 24) = 7.21$, $p < .05$, indicating that the clockwise group had significantly higher melatonin levels across all three weeks compared with the counter-clockwise group (Figure 6).

Analysis of melatonin levels for the afternoon and midnight shifts revealed week by sample interactions for A1, $F(6, 144) = 6.31$, $p < .01$; A2, $F(6, 144) = 11.52$, $p < .001$; and M, $F(6, 144) = 10.81$, $p < .001$ (Figure 7). The results of simple effects analyses for sample at each level of week are presented in Table 4. Tukey post hoc comparisons revealed that for both afternoon shifts, sample 4 (2200 h) was significantly higher than samples 1 (1400 h), 2 (1645 h), or 3 (1845
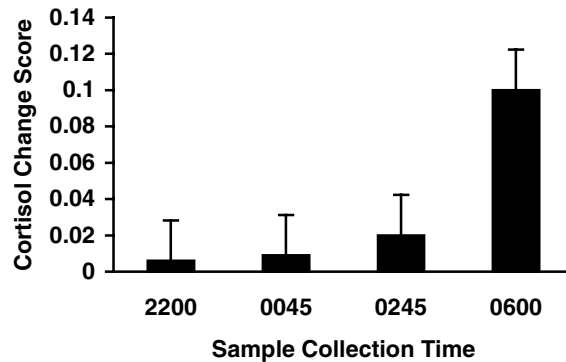

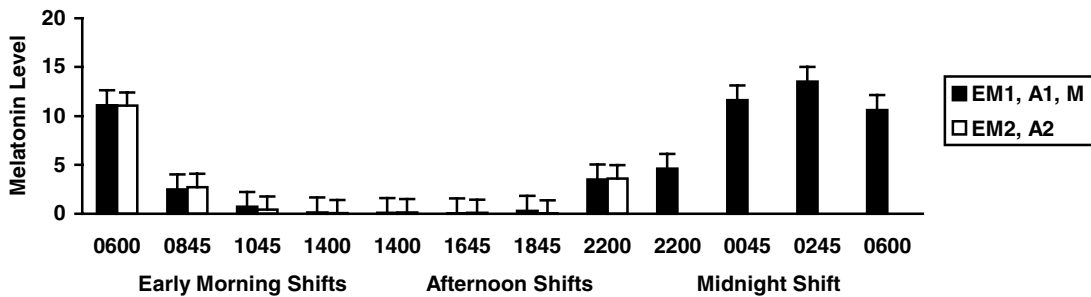**Figure 4.** Cortisol Change Score for Midnight Shift Samples

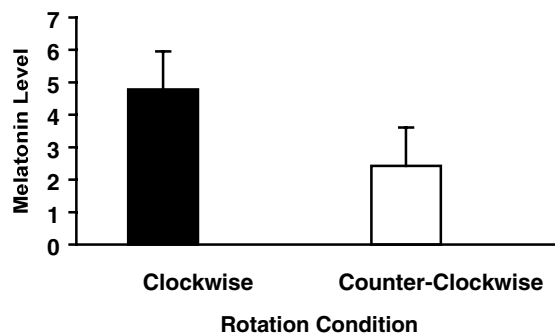**Figure 5.** Melatonin Levels for Each Shift by Sample Time

Figure 6. Melatonin Levels for EM1 by Rotation Condition

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h). For the midnight shift, post hoc analyses revealed that during the baseline week, samples 2 (0045 h), 3 (0245 h), and 4 (0600 h) were significantly higher than sample 1 (2200 h) and that sample 3 (0245 h) was also higher than samples 2 (0045 h) and 4 (0600 h). During week 2, samples 2 (0045 h) and 3 (0245 h) were significantly higher than samples 1 (2200 h) and 4 (0600 h). Finally, during week 3, samples 2 (0045 h), 3 (0245 h), and 4 (0600 h) were significantly higher than sample 1 (2200 h).

Melatonin Delta Change Score Analysis

There were no significant effects for the melatonin change scores for the early morning shifts. Analysis of the afternoon shifts yielded a significant main effect for sample, F (3, 72) = 11.68, $p < .05$. Post hoc comparison of the means indicated that the change for sample 4 (2200) was significantly greater than samples 1 (1400 h), 2 (1645 h), and 3 (1845 h; Figure 8). There was also a significant effect for sample on the midnight shift, F (3, 72) = 31.08, $p < 05$. The analysis of the mean differences revealed a significantly greater change for sample 3 (0245 h) compared with all other samples, in addition to a greater change for sample 4 (0600 h) compared with Samples 1 (2200 h) and 2 (0045 h; Figure 8).

DISCUSSION

The current investigation compared individuals working clockwise and counter-clockwise rapidly rotating shifts using three biological markers: core body temperature, salivary cortisol, and salivary melatonin. The intention was to determine if either or both of these shift schedules would elicit changes in the circadian rhythm of temperature or disrupt the secretion patterns of melatonin and cortisol.

The results of the temperature analysis indicate that the circadian profile was affected as a function of shift schedule rotation. The delay in the acrophase noted in the counter-clockwise group of 84 minutes, while unexpected in an advancing system, might be explained as a function of the schedule. In the past, the counter-clockwise 2-2-1 schedule has been viewed as a multiply advancing system (Della Rocco & Cruz, 1995), with one advance in the rotation from the afternoon to the early morning shift and a second advance from the early morning to the midnight shift. A closer examination of the properties of the schedule, however, reveals that the schedule may be better conceptualized as a mixed system, with one advance from the afternoon to the early morning shift and one delayed day exhibited in the early morning to the
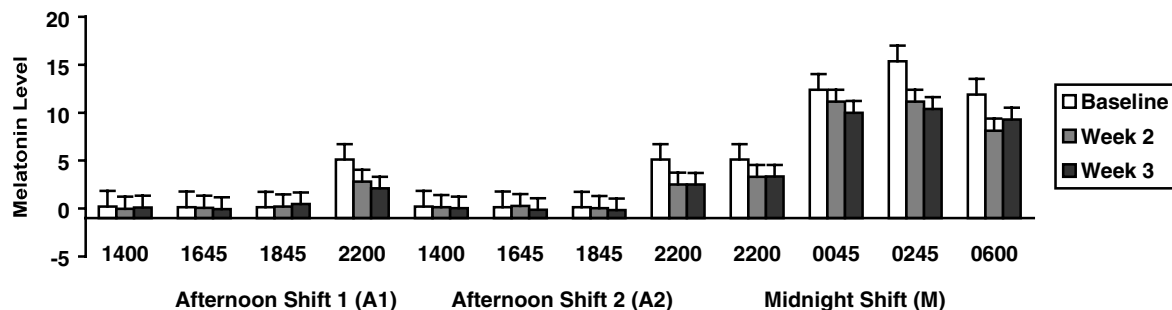

Figure 7. Melatonin Levels for Afternoon and Midnight Shifts by Week and Sample Time

Table 4
Simple Effects Analysis of Week by Sample Interaction for Melatonin for Afternoon and Midnight Shifts

	Statistics for A1			Statistics for A2			Statistics for M		
Effect	df	F	p-value	df	F	p-value	df	F	p-value
Sample at Week 1	4, 96	10.01	.001	4, 96	37.72	.001	4, 96	10.81	.001
Sample at Week 2	4, 96	6.04	.001	4, 96	32.67	.001	4, 96	4.48	.01
Sample at Week 3	4, 96	3.67	.008	4, 96	30.64	.001	4, 96	3.67	.001

midnight shift. That is, individuals work the morning shift, get 8 hours off then return to work later that evening for a second 8-hour shift in a 24-hour period. This prolonged day in the system is more akin to a delaying system or westward travel. For the counter-clockwise group, the early morning shift on the last day, followed by the quick turn to the midnight shift, may have been enough to delay the acrophase in temperature. Such a finding is possible, irrespective of the nap noted in the counter-clockwise group prior to the midnight shift (Cruz, Detwiler, Nesthus, & Boquet, 2002).

The finding that amplitude was attenuated in the counter-clockwise rotating group is interesting in that other research supports the idea that amplitude may be related to individual adaptation to shiftwork (Czeisler, Moore-Ede, & Coleman, 1982). However, the results of this research are equivocal, with some individuals reporting greater adaptability among those individuals with attenuated amplitude and others reporting that greater amplitude is indicative of increased shiftwork hardiness (Czeisler et al., 1982). Therefore, the reduced amplitude in temperature in the counter-clockwise group could indicate more or less adaptability to shiftwork.

With respect to HPA activity, the results of the cortisol analyses indicated that increased levels of cortisol at the beginning of the early morning shifts (0600h) and at the end of the midnight shifts (0600h) can be expected, given the normal early-morning dump of cortisol consistent with its diurnal pattern (Guyton & Hall, 2000). Notably, however, the lack of significant effects for the change score analysis of cortisol during the early morning and afternoon shifts indicates that the individual response to the shifts and the demands of the work environment did not differentially affect the two groups. Thus, the change from baseline for both groups was similar, with no differences noted between the rotation conditions. This is a somewhat unexpected finding, given the current thinking in the field that would suggest that the clockwise rotating group would show lower levels of cortisol.

The raw score analyses of the melatonin data indicate a pattern similar to that of cortisol with the exception of the first early morning shift. Specifically, melatonin secretion followed a pattern consistent with its normal circadian rhythm for both rotation conditions. The initial early morning shift, however, revealed a significant effect for rotation condition with the clockwise group displaying significantly higher levels compared with the counter-clockwise group. This difference appears to result at least in part from higher baseline levels for the clockwise group, which may be driving the differences noted during the subsequent two work weeks.

The results of the raw score analyses are further supported by the observation that no differences were noted between the rotation conditions in the change score analysis. The greater changes noted for the later samples are consistent with diurnally mediated changes in the system. Thus, no differences were noted in the responses of either the HPA axis or the melatonin system with respect to shift rotation.

While few differences were noted with respect to rotation condition, the preliminary nature of the current investigation limits the conclusions to be drawn in a number of ways. First, the participants were only observed for two weeks of shiftwork; thus, the long-term effects of these two shift systems are not addressed here. Second, out of convenience to the participants, only one physiological marker (temperature) was assessed
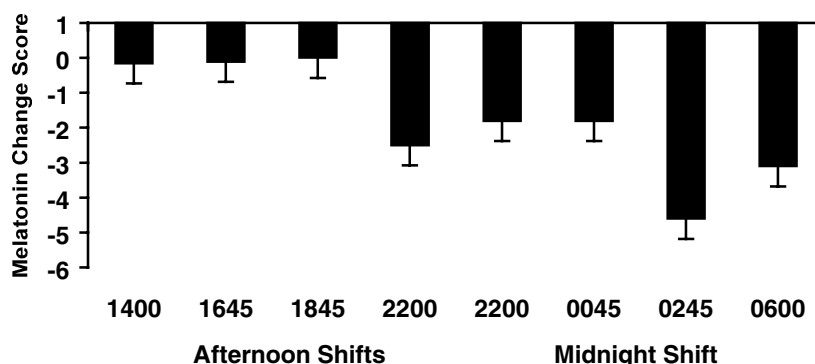

Figure 8. Melatonin Change Scores for Afternoon and Midnight Shifts

for changes in circadian pattern. While melatonin and cortisol were assessed during the work periods, they were not assessed for changes in overall pattern or for recovery following the workweeks. With this in mind, there may have been some differences that were not observed, based upon the current protocol.

As the third paper in a series investigating the response of individuals to clockwise and counter-clockwise, rapidly rotating shift schedules, the findings here reflect minimal differences in biological markers with respect to the rotation conditions, which is in line with the findings in the preceding papers (Cruz et al., 2002; Cruz, Boquet, Detwiler, & Nesthus, 2002). The differences noted for the temperature rhythm between the rotation conditions, while significant, do not necessarily argue against the counter-clockwise rotation on a short-term basis. The absence of significant effects noted for the response of the HPA axis and the melatonin system indicate that these two common markers for shiftwork tolerance were relatively undisturbed other than for expected diurnal variations.

In summary, the results from this study support the assertion by Turek (1986) that clockwise and counter-clockwise shift rotations would similarly perturb circadian rhythms. In fact, the results from all the measures of this study reveal very few significant differences between the clockwise and counter-clock-wise, rapidly rotating shift schedules investigated. Taken together, these results indicate that counter-clockwise schedules currently in use by U.S. air traffic controllers would not likely be improved simply by reversing the direction of rotation. Future research should seek to replicate and extend the findings of this study by investigating longer shiftwork exposure and looking more specifically at the entire circadian rhythm for neuroendocrine measures.

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APPENDIX A

   Custom Cosine Function Platform. In order to analyze the temperature data, a brief routine was written in Microsoft *Excel© '97* (1997) using the *Solver* function that allowed a cosine function to be fit to the raw temperature data. *Solver* enables the user to specify minimization of a specified target cell's value by automatic manipulation of the values in other specified parameter cells. In this case, Solver's net effect was to minimize a sum-of-squares (ΣSS) representing the sum of point-wise squared differences between the values of the data and the curve being fit to each individual data point. To accomplish this, Solver uses a Generalized Reduced Gradient (GRG2) nonlinear optimization algorithm developed by Lasdon and Warren (*Excel '97* Help Files). Conceptually, there are a number of ways of accomplishing this same type of result (Press, Flannery, Teukolsky, & Vetterling, 1988). Most methods are subject to occasional minimization failure. This means that ΣSS can get stuck in a local, rather than a global, minimum. In cases such as these when Solver failed to converge, leaving a large residual, this was visually identified by the mismatch of the fitting curve and the raw data curve. In the few cases where this occurred, the amplitude, phase, and the period were manually adjusted so that they were well within the attractor basin of the global solution on the subsequent re-run of Solver.

   One final theoretical problem with this approach was the typical one associated with trying to fit any periodic function to data sets. In theory, the identical degree of fit is possible with an inverted function having negative amplitude and being half a period out of phase. To address this problem, the fitting process produced nothing but positive values for amplitude. For phase, the true value was assumed to be the one whose absolute value lay closest to zero.

APPENDIX B
Cortisol and Melatonin Assay Procedures

A. Assay for Melatonin

1. Incubation buffer was distributed as described into each of the individual polystyrene tubes. The NSB (blank) tubes received 500 µL, and the MB (maximum binding) tubes received 400 µL. The standard tubes, labeled as A through F, received 400 µL of the corresponding standard solution. The samples (400 µL) and the controls (400 µL) were distributed to their corresponding tubes.

2. 100 µL of the melatonin antibody was delivered to all except the NSB and T (total activity) tubes.

3. 100 µL of the ^{125}I-melatonin tracer was added to all tubes, and all were vortexed. The T tubes were set aside at this point, since they required no further processing until the final step.

4. All tubes were incubated for 20 hours at 4°C in the refrigerator.

5. 100 µL of the second antibody suspension, continuously being stirred during this step, was added to all assay tubes (except T tubes). The assay tubes were maintained at 4°C during this process.

6. The tubes, with the added second antibody, were incubated for 15 minutes at 4°C. Then, 1 mL of cold, distilled water was added to all the tubes (except the T tubes).

7. The tubes were centrifuged for 2 minutes at 2000 x g and 4°C. The supernatants were aspirated and discarded (except the T tubes), and the precipitates were retained for counting.

8. Finally, the tubes were subjected to counting for 2 minutes each in the Gamma 5500 counter.

B. Assay for Cortisol

1. Each standard, A through F, was diluted 1-in-10 with double-distilled water. The standard tubes, coated with antibody and labeled A through F, received 200 µL of the corresponding standard. The NSB (blank) tube was uncoated and received 200 µL of the A standard.

2. 200 µL of each saliva sample was pipetted into antibody coated tubes. This was done in duplicate for each sample.

3. 1.0 mL of ^{125}I-cortisol was pipetted into every tube and each tube was vortexed for mixing. Two uncoated tubes, labeled T, received 1.0 mL of ^{125}I-cortisol for obtaining a total radioactive count.

4. The tubes were incubated for 3 hours. After incubation, all tubes, except T tubes, were decanted for 2 to 3 minutes.

5. All tubes were then counted for 1 minute using the Beckman Gamma 5500™ counter.