THE EFFECTS OF CIRCADIAN RHYTHMS ON VISUAL THRESHOLDS

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Thesis submitted to the Faculty of the Department of Psychology of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Psychology

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November, 1984

Blacksburg, Virginia

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by

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(ABSTRACT)

The possibility that visual thresholds fluctuate in a circadian rhythmic (twenty-four hour) pattern was examined. Subjects were tested at 0400h, 1000h, 1600h and 2200h in a 30 minute dark adaptation procedure using a von Békésy tracking method. Two experiments were performed; the first tested cone functions with a red 1 degree monochromatic test stimulus located ten degrees nasally. There were no sex differences for the ten male and female subjects used in this study. However, there were time of day differences with higher thresholds at 1000h than at any other time.

The second experiment examined both rod and cone thresholds with a heterochromatic test stimulus. Sex differences were found for the 10 male and 10 female subjects. The rods for males were found to exhibit significantly higher thresholds at 1000h than any other time. There were no significant differences for females. The null results for females may be due to an influence of the menstrual cycle on vision. The rod differences for males is thought to occur because of rod outer segment

shedding which also has a circadian rhythm. A circadian rhythm questionnaire was administered to the subjects and it was found that morningness and vigorousness related to visual thresholds.

Acknowledgements

Until I began my thesis, I was typically a night person. However, the thirty or so nights that were spent by sleeping in three hour shifts on various couches in the Psychology Department completely changed my circadian rhythms. I am convinced that I have totally eradicated my own rhythms in order to describe and explain the circadian rhythms of others. This thesis could not have become the success that it did without the help of many great friends.

I would first like to thank my advisor, Al Prestrude, for his total support of me both during my thesis and while I have been in graduate school. He has departed a great deal of wisdom to me which I hope will stick. His excitement for my thesis always made the tough times easier to take. I am forever grateful for his support and knowledge. I would also like to thank Steve Zaccaro and John Casali for their guidance and support during my thesis.

It always amazes me what friends will do for each other. I would like to thank Jim Rudd, Nancy Yeager, Steve Clarke, Jim Nimmer, Fritz Streff, Patti Watkins, and Jil Stoddard for their humor, understanding, and all round good times that we had which helped me make it through the past two years. I would also like to give a special thanks to Ann Talton, who is not only a great friend but actually volunteered to be a subject in my study. And special thanks also goes to Mariann Staba who gave me the moral support to finish my study as well as making my life a happy one.

And finally I wish to thank my mother, Sandra, and my father, Daniel, for both their moral and financial support over these past few years. They listened to the many times that I complained and always stood by my side.

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INTRODUCTION

The nature of the universe is not erratic but rather it is rhythmic. This rhythmicity occurs in many events around us, from the orbiting of the earth around the sun, to the division of cells in the body. Every animal, from single celled organisms to humans, exhibits biologically based rhythms. These rhythms can be defined as a series of biological events that repeat themselves in the same order through time. These events can change their characteristics over time, that is, they can be entrained or synchronized to another rhythmic stimulus with a different phase. A circadian rhythm (circa=about, dies=day) is one that repeats itself about once every 24 hours. Such rhythms include the human wake/sleep cycle, blood pressure and body temperature among others. Ultradian rhythms, on the other hand, are those that last less than 21 hours such as respiration, electrocardiogram, and REM sleep. Infradian rhythms are those that have a period greater than 28 hours such as the female menstrual cycle and norepinephrine excretion (Minors & Waterhouse, 1981).

Rhythms can be characterized by their frequency, period, amplitude, and phase. The frequency is the number of cycles, or completions of a set of events, per unit of time. A period is the time for one cycle to take place. A circadian rhythm is defined as having a period of between 20 and 28 hours. Amplitude refers to the range of a cycle from peak to trough. And the phase of a rhythm refers to the position of the rhythm in time (Minors & Waterhouse, 1981). Rhythms can also be characterized by whether they are endogenous or exogenous. Endogenous rhythms are those that are internally controlled, as in DNA replication. Whereas exogenous rhythms are completely controlled by the environment as in the movement of the ocean tides or the rate of plant growth.

Most animals have free running rhythms that are greater than or less than but not equal to twenty-four hours. Free running rhythms are those that are released from synchronization with environmental rhythms. The free running rhythms for the human are about 25 hours. In most animals, biological events have their own free running rhythms which are entrained or synchronized to the environmental 24 hour light-dark cycle. The entrainment to the environment is controlled by Zeitgebers (German for 'time-givers'). Typically the most powerful Zeitgebers are sunrise and sunset. However, in humans, socialization with other humans can be just as powerful as a Zeitgeber.

The Biological Clock: Where is it?

Every organ and almost every biological variable in the human has an endogenous circadian rhythm. However, the fact that these rhythms can become desynchronized from one another indicates that the search for one biological clock could be futile. Rather, it might be more useful to look for the coordinator or synchronizer of all the rhythms.

As light is an extremely important Zeitgeber in humans, the search for the coordinator of rhythmicity should begin with the eye. Light stimulates the rods and cones in the retina which in turn stimulate the suprachiasmatic nucleus (SCN) of the hypothalamus by several pathways. Ablation of the SCN has been shown to cause either a free running period or an erratic synchronization to a light-dark cycle (Minors & Waterhouse, 1981).

From the SCN information projects to the superior cervical ganglion which contains neurons projecting to the pineal gland with noradrenergic synapses. Noradrenaline acts on the receptors in the pineal gland to stimulate cyclic AMP which causes the production of N-acetyltransferase in the pineal gland. N-acetyltransferase is an enzyme used in changing serotonin (a neurotransmitter) into melatonin (a hormone produced mainly in the pineal gland). Both melatonin and serotonin are necessary for the regulation of sleep. Melatonin has also been found to regulate the activity of the gonads and the bleaching of skin pigment cells. And serotonin is not only a neurotransmitter but also a powerful vasoconstrictor (McFarland, 1981).

Thus, the stimulation of the retina by light causes changes in levels of melatonin and serotonin which regulate sleep, activity of the gonads, bleaching of skin pigment cells, changes in vasoconstriction as well as changes in a neurotransmitter. These changes, in turn, could synchronize other variables in the human by regulating other hormones and neurotransmitters. The pathway described is only one possible way in which the circadian rhythms of the various parts of the human can become synchronized. However, it does illustrate the complex nature of circadian variations in the human.

Variables Exhibiting Rhythms

The impact of circadian rhythms on biological life is remarkably strong. Biological variables such as catecholamine excretion, body temperature, endorphine output, blood pressure, blood eosinophils, plasma-protein, aldolase and 17-OHCS all exhibit marked circadian rhythms. These variables are important for mental and physical performance, altitude tolerance and physical fitness as well as general health (Akerstedt & Froberg, 1976; Davis, Buchsbaum, & Bunney, 1978; Klein, Wegmann & Bruner, 1968).

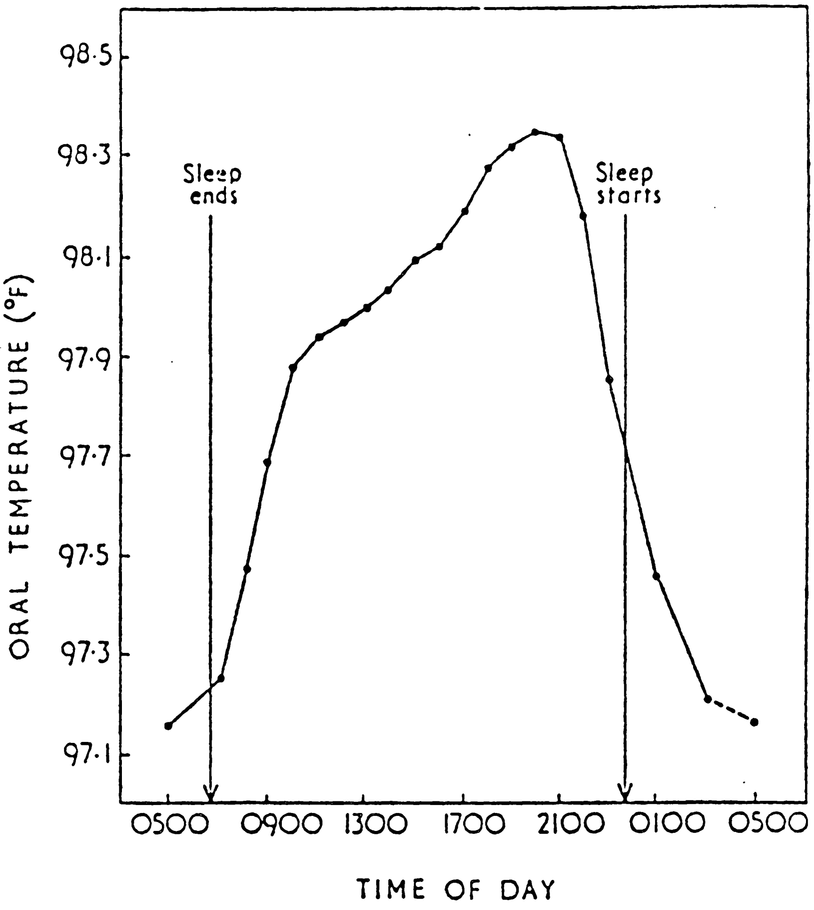
Drugs that are given at one time of the day might have little effect, while if given at another time could be toxic. An example is the drug ouabain which, when given to mice at 8 a.m., 60% died; but when the drug was given at 8 p.m., only 15% died (Moore-Ede, 1975). Antihypertensive drugs, antihistamines, anabolic steroids and many other drugs are more effective in humans at certain times of the day than at others. For example, if an antihistamine is given at 7 a.m., the effect will last around 16 hours. However, if the drug is given at 7 p.m. the antihistamine effect will last only around seven hours (Moore-Ede, 1975).

The Shape of Circadian Rhythms

The most scrutinized circadian rhythm is the body temperature rhythm. It has been thoroughly studied for two reasons. First, it is an easy measure to obtain. Secondly, and most importantly, many other rhythms resemble the temperature curve and even follow it when the organism is entrained to a different time schedule (Colquhoun, 1971).

Insert Figure 1 about here

Figure 1 depicts the mean body temperature curve for 70 naval men. The measurements were made every hour during the waking period and every two hours during sleep. The peak reading was 98.35° F at 2000h (i.e. 8 p.m.) and the low was 97.16° F at 0500h (i.e. 5 a.m.). There was a



Circadian rhythm of oral temperature in a group of 70 young men.

FIGURE 1

(From Colquhoun, 1971)

rise in temperature from 0500h to 1000h and a continuing, but slower rise, until 2000h. Subsequently, there was a drastic linear drop in temperature.

Performance on many tasks also seems to follow the temperature curve. For example, simple reaction time, vigilance, numerical calculations, time estimation and card sorting all show improvement from morning to evening and then performance deteriorates late at night (Blake, 1967a; Buck, 1977; Mullin & Corcoran, 1977). Short and long term memory, operant conditioning and even depression and mania vary in the same rhythmic pattern as temperature with more depression, mania, etc. occurring at higher temperatures (Elsmore and Hursh, 1982; Folkard, 1982; Wehr, 1982).

Practical Aspects of Circadian Rhythm Research

Humans, more than any other animal, have been able to control their environment. Night can be turned into day with the discovery of fire and electricity. This environmental control has led to the use of shiftwork in industry. Today, many factories are producing twenty-four hours a day. However, there is serious concern about the effects of shiftwork on employees' health, attitudes, productivity, performance and turnover. Many studies have found that employees working on night shifts have less sleep, more interrupted sleep, more stomach and eating disorders, more accidents and less productivity than other shifts (Akerstedt & Torsvall, 1978; Koller, Kundi & Cervinka, 1978; Smith, Colligan & Tasto, 1982). However, these problems can be significantly reduced by applying circadian rhythm principles to shift work rotation.

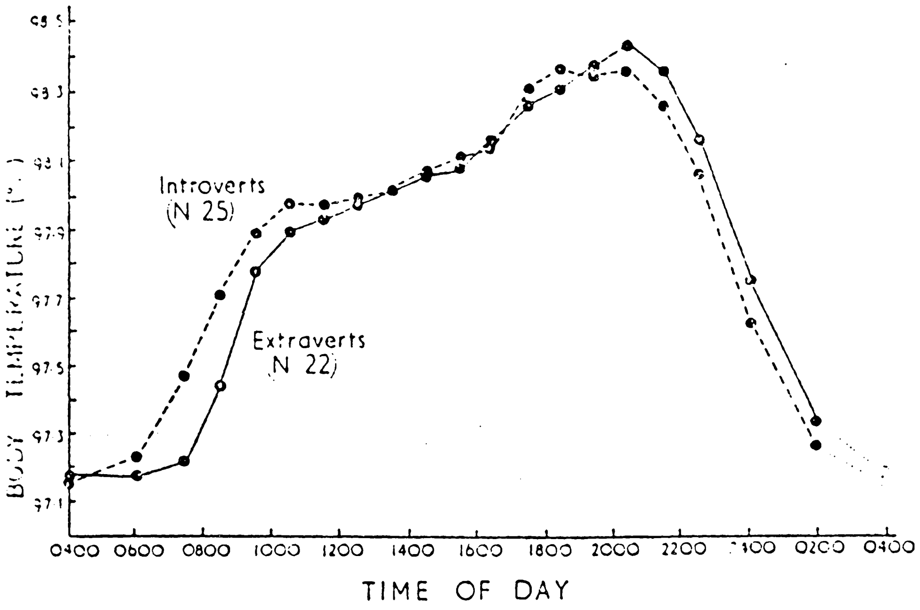
For example, in rotating shifts, one can either use a phase advance or a phase delay system (i.e. lengthening or shortening the psychological day). A phase advance is a re-entrainment or re-synchronization from a day of, for example, 24 hours to one of 20 hours or of moving from a night shift to a day shift. A phase delay, on the other hand, is a re-entrainment from a day of 24 hours to one of 28 hours as in going from a night shift to a morning shift. Since human's free running rhythms are 25 hours rather than 24 hours, it becomes easier to phase delay the worker (i.e. lengthen the day as in moving from a night shift to a morning shift). This was demonstrated most clearly by Czeisler, Moore-Ede & Coleman (1982). They were able to significantly and dramatically improve not only worker satisfaction, health and turnover but also significantly improved potash production at the Great Salt Lake Minerals and Chemicals Corporation. The previous shift system provided for a weekly rotating phase advance schedule. However, by introducing a phase delay rotating system with 21 day rotations, Czeisler *et al.*, were able to gain the improvements described above.

Another area of particular importance is that of group differences for circadian rhythms. It is quite possible that the "morning lark and night owl" syndrome may indeed be true. Blake (1967b) examined the body temperature curve for introverts and extroverts. Introversion and extroversion were determined by the Heron Personality Inventory. Figure 2 shows that the temperatures of the introvert group rose more rapidly in the early morning and fell earlier at night than did the extrovert group. As was seen earlier, the temperature rhythm can be an indication of performance with high performance relating to high temperatures and low performance relating to low temperatures (Blake, 1967b).

Insert Figure 2 about here

Hildebrandt and Stratman (1979) selected three morning and three evening-type nurses using the Horne-Ostberg questionnaire and compared their circadian rhythms for temperature, heart rate, and subjective ratings of vigilance. After one night shift they found that the morning-type nurses had poorer circadian rhythms for body temperature and heart rate than did the evening-types. The rhythms for the evening-types became flatter while the rhythms for the morning-types had an increase in amplitude.

The relationship between the amplitude of circadian rhythms and the ease of entrainment was studied by Reinberg, Vieux, Ghata, Chaumont & La Porte (1978). Low amplitudes of circadian rhythms were thought to be associated with rapid phase shifts. Reinberg *et al.* used 20 shiftworkers in oil



Body-temperature rhythms of introverts and extraverts.

FIGURE 2

(From Blake, 1967b)

refineries as subjects. Self measurements of oral temperature, grip strength, and peak expiratory flow were taken and urine was collected for analysis. The experiment lasted eight weeks after which mean amplitudes and phase shifts were calculated for each variable. A significant negative correlation between phase shift and rhythm amplitude was found for oral temperature, peak expiratory flow and urinary 17-OHCS. Thus, individuals exhibiting low amplitudes for these variables may be better suited for shift work than those who exhibit high amplitudes.

Circadian Rhythm Questionnaires

The idea of identifying individuals who would adapt easily to shiftwork was extended by Folkard, Monk & Lobban (1979). They developed a paper and pencil test to identify individuals whose circadian rhythms adjusted more easily to shiftwork. The test contained nineteen questions answered using a 10 cm visual analog rating scale (VAS). Forty-eight full and part time nurses were used as subjects to validate the questionnaire. The test-retest reliability correlation coefficient was 0.60. A factor analysis revealed three higher order factors termed Factor R (rigid sleep type vs. flexible sleep type), Factor V (vigorous vs. languid type) and Factor M (morning vs. evening types).

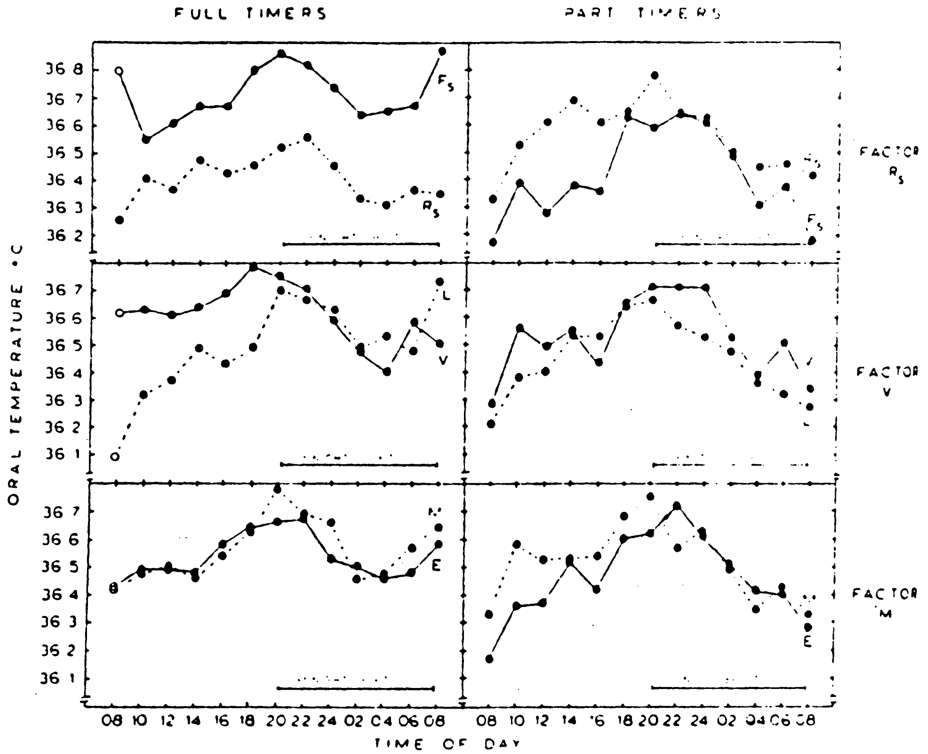
High scorers on Factor R claimed an inability to sleep at unusual times and a preference for regular meal times.

Low scorers (flexible types) didn't mind sleeping at unusual times or irregular meal hours. High scorers on Factor V claimed to have low levels of drowsiness after reduced sleep while low scorers (languid types) said they felt very drowsy with reduced sleep and found it hard to overcome this drowsiness. High scorers of Factor M claimed to prefer working at normal times of the day and found it easy to get up early in the morning. Those that scored low (evening types) claimed to find it hard to wake up early and could work at odd times.

Figure 3 depicts the temperature curves, split between part-time and full time nurses and the three factors. There was little difference in the part-time nurses' temperatures on the factors. However, there were significant differences for the full time nurses. The highly rigid nurses had much lower temperatures than the more flexible nurses. And the highly vigorous nurses had initially higher temperature curves than the languid types. These temperature differences indicated faster entrainment to night shifts for 'flexible' and 'vigorous' nurses. There were no differences on Factor M.

Insert Figure 3 about here

Significant positive correlations were found between Factor R and the relative amplitudes of the circadian rhythms of water, sodium, and chloride in the urine. On



Mean temperature curves for the high and low scorers (based on a median split) on each factor shown separately for the full and part-timers. The open circles (○) indicate that less than 75% of the subgroup contributed to the point.

FIGURE 3

(from Folkard, Monk & Lobban, 1979)

subjective ratings of alertness, the 'evening' nurses showed less of a decrease than did the 'morning' nurses. Flexible type nurses also claimed to have slept significantly 'better' and to have been disturbed less by noise than the 'rigid' types. It is interesting that the morningness scale was not very predictive of adjustment. Rather, factors relating to sleeping habits and drowsiness were most effective in differentiating adjustment.

This questionnaire is a giant step toward understanding circadian rhythms. Continuing research is needed, however, especially in the area of the senses. If the senses (vision, audition, tactile, olfaction, etc.) also vary in a circadian fashion, the implications are enormous. For example, one would want watchmen in the army to be on duty when their senses were most acute. And one would want sonar operators to be working at their circadian auditory peak. NASA keeps their astronauts working in shifts under the very unusual circumstance of 90 minute days. Thus, the astronauts no longer have the usual day-night Zeitgebers to create a circadian rhythm. Yet, they must be very sensitive to auditory alarms, smells and computer-displayed visual information which allow for the life-sustaining operation of the space ship. Submarine personnel also operate under nonexistent or rather artificial light/dark cycles. Therefore, many individuals working in shifts or under artificial or nonexistent light dark schedules, have jobs which require the maximum acuity of the various senses.

Without this maximum acuity, performance and health can deteriorate and accidents occur. A sobering fact which demonstrates the need for this research is that the Three Mile Island nuclear accident occurred at 4 a.m. after the workers had just rotated onto a different shift schedule; one which circadian rhythm researchers would have avoided (Refer to Figure 1 for example. This particular time will appear again in the review and in the data of the present study) (Rovner, 1983).

The present study examined certain aspects of the visual senses and their 24 hour variation. Very little research has been performed in this area. However, the few studies that have been done in the area of the senses, do reveal some definite trends. The following is a discussion of the research that has already been done in this area and the trends that have been found.

Literature Review

Rogers and Vilkin (1970) studied pain thresholds using electrical current as the stimulus. They found that the detection threshold for electrical current was significantly higher in the morning than in the evening, and that pain thresholds were also significantly higher in the morning than the evening. Unfortunately, Rogers and Vilkin based their study on measurements taken at only two times during the day so the results must be taken with caution and the actual shape of the cutaneous pain rhythm is undefined.

Davis, Buchsbaum and Bunney (1978) also used electrical current as a pain stimulus in determining circadian pain sensitivity. They conducted two experiments. The first simply confirmed that pain thresholds are higher in the morning than in the afternoon. The second experiment required subjects to be injected with naloxone, a narcotic antagonist which reverses the effect of endorphins and increases pain sensitivity. It was found that, with the injection of naloxone, subjects' pain thresholds were lower in the morning and higher in the evening than it had been in the previous experiment. They explained their results by suggesting that endorphins not only play a role in pain perception, but also vary in a circadian fashion. However, again readings of pain threshold were taken at only two times during a 24 hour period so no information about the shape of the rhythm can be discerned.

Procacci, Corte, Zoppi and Maresca (1974) used radiant heat to determine the thresholds for thermal pain sensitivity. Readings were taken at four times throughout the day. They confirmed the results of Davis, Buchsbaum & Bunney (1978) and Rogers & Vilkin (1978) that pain thresholds are highest in the morning (at 0800h) and lowest in the evening (at 1800h). However, the four data points suggest a curve that begins at 0000h rises to reach a peak at 0800h, then falls through a point at 1200h that is equal to the 0000h point, continues to fall reaching a low point at 1800h and then rises again until 0000h.

The studies just mentioned all found a circadian rhythm for pain thresholds. And all the studies were consistent in finding a high threshold for pain in the morning falling off to a low threshold in the evening.

Few experiments on the circadian variation of audition have been performed. No experiment has even ever tested the circadian variation of audition thresholds. Delay, Smith & Isaac (1978) did perform an experiment using squirrel monkeys to determine the effects of illumination on auditory thresholds. They found that the thresholds were lower in the light than in the dark. Delay*et al.* interpreted this as indicating that arousal via the reticular formation was higher in the light and thus thresholds for detection of an auditory signal were lower. Thus, there might be an interaction of illumination with auditory thresholds.

The visual sense has, at least to some degree, been studied in relation to circadian rhythms. Besharse, Hollyfield & Rayborn (1977) found that the rate of shedding of rod outer segments in tadpoles under constant illumination, or in diurnal conditions of 12 or 2 hours of light per day, is significantly increased compared to that of animals in darkness. They also found that bursts of renewal activity occur in response to light. Thus when exposed to light, rod outer segments shed. Bassinger, Hoffman and Matthes (1976) also found that rod outer segments in the frog shed in response to light. They, however, found that those segments which did shed became primed to shed before the onset of light, suggesting a possible circadian rhythm for rod outer segment shedding. The shedding of rod outer segments at the onset of light is a very adaptive response. Rods typically are used less in the day and thus the best time for shedding would be daytime.

Young (1978) found that cones also shed their membranes. And this shedding occurs at the onset of darkness. Again, this response is very adaptive as cones are not used at typical night time levels of illumination. Young (1978) suggested that circadian rhythms are entrained by light and the first step in this entrainment is the reception of light by the rods and cones. Thus, according to Young, "The visual cells pick up the beat from the oscillation of light in the environment, then transmit that rhythm along the neural pathways of the visual system, from which it is distributed throughout the body by a variety of neuroendocrine networks." However, Young has not really shown that a circadian variation exists for rod or cone receptors. He has only shown that they shed in response to light or darkness.

Jacklet (1969) did find a circadian rhythm in the activity of an isolated eye of the *Aplysia.* The eye of the *Aplysia* was kept in total darkness while optic nerve impulses were recorded. It was found that peak activity occurred around dawn and decreased throughout the day. The eye was also found to have a free running period of about 27 hours.

Rosenwasser, Raibert, Terman & Terman (1979) conducted an experiment on rats which probably offers the best evidence to date for visual circadian rhythmicity. Rosenwasser *et al.* trained rats, in a dark cage, to observe a screen upon which a dim spot of light was projected during brief trials initiated by the rat. The visual stimuli were presented only half the time. The rats were rewarded with electrical brain stimulation if they pressed a key when the light was presented or following a no response trial when the light was not presented. They found that visual sensitivity, as measured by the d' statistic, definitely followed a circadian rhythm with strong free running peaks at about 24.4 hours. Rosenwasser *et al.*, interpreted their findings as suggesting that visual rhythms are endogenous and could be an important influence in entraining other biological rhythms by light-dark cycles.

Fowlkes (1983) kept a lizard in complete darkness and introduced light stimuli every hour. Fowlkes monitored electrical changes in the retina and determined that the lizard is maximally sensitive to light around 1100h and least sensitive around nightfall when they are asleep.

And finally, Powers, Bassi & Rosen (1982) tested human subjects' scotopic (rod) visual thresholds in a dark adaptation experiment. They tested two subjects between noon and 2 pm and midnight and 2 am in a task that involved detecting a brief, peripheral 508nm light after an hour of dark adaptation. The method of constant stimuli was used for the experiment. Powers *et al* found that one subject was consistently more sensitive than the other but that both were more sensitive during the day than night by 0.33 and 0.35 log units of attenuation respectively. However, only two subjects were used in this experiment and therefore the generalizability of these results are questionable. Also, only two testing periods were used. Thus, there are not enough data points to describe the visual threshold rhythm. And finally, only scotopic thresholds were tested. It is quite possible that photopic (cone) thresholds have a different circadian rhythm, especially if the rhythm is mediated by rod and cone outer segment shedding respectively.

The Experiment

It has been shown that light cycles are major Zeitgebers mediated by optic connections through several pathways. Thus, vision appears to play a major role in the synchronization of circadian rhythms in man. However, little is known about how vision, in humans, varies throughout the day. And even less is known about how visual thresholds vary throughout the day. This thesis was an extension of the Powers *et al.* study in which scotopic visual thresholds were examined for a circadian rhythm.

Six changes from that study were made, however. First, the Powers *et al.* study examined only scotopic thresholds, the following thesis examined both scotopic and photopic thresholds. Second, a much larger subject size was employed to aid in the generalizability of the study. Third, an analysis was employed to determine if there were any sex differences. Fourth, Folkard, Monk & Lobban's (1979) questionnaire was given to all subjects to determine if any of the scales related to visual thresholds. Fifth, subjects were tested at four times of the day: 1000h, 1600h 2200h, and 0400h instead of two. And finally, a procedure similar to the methods of adjustment which provides a continuous record was used instead of the method of constant stimuli which provides periodic assessments.

Photopic and scotopic thresholds were examined in two different ways. First, a dark adaptation procedure was used. But unlike the Powers *et al.* (1982) study which first dark adapted the subjects for one hour before testing, this study used a traditional dark adaptation procedure. In dark adaptation, individuals' visual sensitivity in a darkened room increases with time up to a point beyond which the increase becomes negligible. Typically, in a dark adaptation experiment, the subject is initially exposed to a bright light to bleach all the photopigments in the eye. Then the light is turned off and the threshold for a small spot of light is measured. In this study, a method similar to the method of adjustment was used to determine thresholds. Basically, the subject continuously adjusts the intensity of a small spot of light between the points where it can just barely be detected and then not detected. A graph of these adjustments can be made by plotting time of dark adaptation on the abscissa and log attenuation on the ordinate. The log attenuation is a measure, in log units, of the intensity of the test stimulus. The numerically higher the log attenuation is, the lower the threshold is (i.e. the more sensitive the eye to light). The graph is known as a dark adaptation curve and consists of two characteristic declines. The first rapid decline is due to the dark adaptation of the cones (photopic threshold) and lasts for five to fifteen minutes. The second slower decline is due to the dark adaptation of the rods (scotopic threshold) and lasts for another twenty minutes.

The dark adaptation procedure was well-suited for this experiment. Circadian rhythms are dynamic in nature and the dark adaptation procedure allows for dynamic measurement of these rhythms over a half-hour period. Thus, a much more accurate picture of the circadian variation of visual thresholds can be determined. Also, both scotopic and photopic thresholds can be determined.

The second method for determining photopic and scotopic thresholds was by performing two separate studies using different wavelengths of light for the test stimulus. The first used a long wavelength light source as the test stimulus (to which the rods are relatively insensitive) while the other used a heterochromatic wavelength light to which both the rods and cones are sensitive.

The Hypotheses

Three hypotheses were made concerning this study. First, it was thought that rods and cones would have different circadian rhythm peaks. Since Powers *et al.* (1982) found that rods were most sensitive at noon and least sensitive at midnight, it was hypothesized for this study, that 1000h would produce the lowest thresholds (i.e. subjects would be most sensitive at 1000h) for rods while 0400h would produce the highest thresholds (i.e. subjects would be least sensitive at these times). It was also thought that the thresholds for 1600h and 2200h would lie somewhere in between the 0400h and 1000h testings. This hypothesis was also supported from pilot data obtained on two subjects for this study and the fact that most circadian rhythms have a peak around 1000h and a minimum around 0400h. No specific hypothesis was made concerning the photopic curves except that it was expected that they would have different peaks from the scotopic curves.

Second, it was hypothesized that there would be sex differences for the visual threshold circadian rhythms. Thus, males and females were thought to have different thresholds. Both males and females were still thought to have peak rod thresholds at 1000h with troughs at 0400h but it was thought that the thresholds for the two sexes would be different. This hypothesis was not based on empirical research, but rather on the possibility that the female menstrual cycle may influence daily rhythms.

And third, it was thought that a questionnaire, similar to the one developed by Folkard *et al.* (1979) would relate to visual circadian rhythms. This would be invaluable as a tool for differentiating between individuals for shift work as well as providing a possible direction for future research. No specific hypothesis was made concerning the exact relationship of the questionnaire to the visual circadian thresholds. However, this study was able to act as an independent validation of the questionnaire to a type of circadian rhythms that had not been tested by Folkard *et al.*

METHOD

Experiment 1

Subjects

Six male and four female undergraduate students, ranging in age from 19 to 21 years, were used for this study. All of the subjects were volunteers from undergraduate psychology courses at Virginia Polytechnic Institute and State University and received credit toward their final grade in the classes. None of the subjects was told of the specific hypotheses of the study. All subjects filled out a consent form (see Appendix B) and were told that they could discontinue their participation at any time.

Apparatus

The observers sat in a completely light-tight, sound-attenuated experimental room, 4 feet wide by 4 feet long by 8 feet high. The subjects sat in an adjustable chair and placed their chin in an adjustable head and chin rest. Attached to the head and chin rest was a blinder (a blackened piece of round metal) which was placed in front of the subject's left eye to preclude use of that eye during the experiment.

A modified Marietta adaptometer was used to both preadapt and test the subjects' visual thresholds. A schematic diagram of the adaptometer is depicted in Figure 4.

Insert Figure 4 about here

Two light sources were contained in the adaptometer. The first, I1, provided a 1 degree test stimulus and was a long wavelength gas discharge glow tube, 7920253, made by Metcom, Inc., USA. The second light source, I2, provided the bleaching (preadaptation) light and was a 60 watt tungsten filament bulb. The test stimulus was brought to a focus at the observer's eye by two lenses, L1 and L2, while the size of the test stimulus was controlled by an artificial pupil P, with an aperature of $3mm^2$. The artificial pupil, a piece of black metal with a hole drilled in it, was required to control for fluctuations in the subjects' own pupil size during the course of the experiment. A beam splitter, B, is used to place the bleaching light in the optical path of the observer's eye. A third lens, L3, is used to focus the bleaching light at the beam splitter. H is a heat absorbing light. And O is the eye of the observer. The test stimulus was located at ten degrees nasal to the fovea by cross hairs illuminated with red light. The intensity of the cross hairs could be adjusted by the observer. An optical wedge, W, which is a set of counter rotating variable neutral density filters, was used by the subjects to adjust the

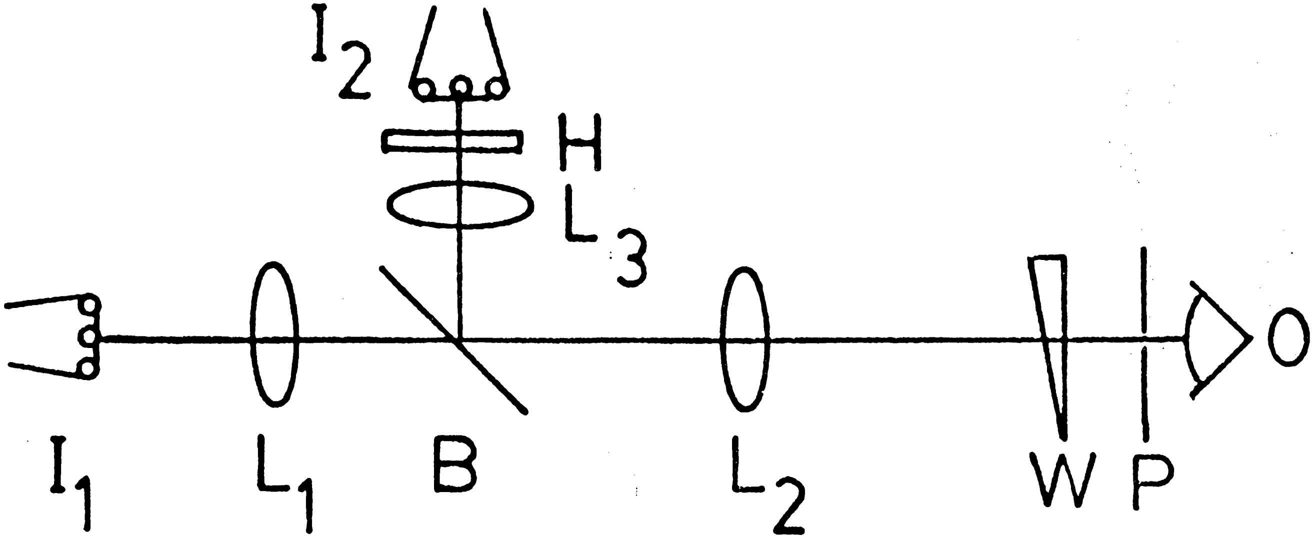


Diagram of Adaptometer

FIGURE 4

intensity of the test stimulus.

The optical wedge was connected into a Laboratory Data Control chart recorder, model 3101-01-01. The test stimulus was driven at 1.0 hertz (at 50% duty cycle) by a Lafayette Flicker Fusion device, model 12025. Two Gra Lab timers controlled the timing of the bleaching light, test stimulus, and chart recorder. A radio, playing music of the subjects' choice, was also connected to the timers. It was thought that the radio would help reduce some of the boredom of the task and attenuate the outside noises.

Procedure

After completing a consent form, subjects were asked to fill out a questionnaire developed by Folkard *et al.* (1979). This questionnaire consists of 19 questions and three factors. The three factors are Factor Morning, Factor Vigorous and Factor Rigidity. Subjects were also requested to answer questions regarding their usual wake up, bed and mealtimes as well as how much sleep they had received before the experiment and whether or not they wore corrective lenses of any type. Females were also asked a series of questions regarding their menstrual cycle (e.g. When did your period last begin?, How long does your period usually last?). A sample questionnaire is contained in Appendix A.

The subjects were requested not to take any drugs, including caffeine, twenty-four hours before the experiment and to get their usual amount of sleep prior to the beginning of the experiment. Subjects were also asked not to eat for two hours prior to each test session. These precautions were taken because it is possible that drugs, digestion of food, and lack of sleep may influence a person's circadian rhythms (Colquhoun, 1971). Questionnaires verified that the subjects had complied.

Each subject was tested at four different times of the day: 0400h (i.e. 4 am), 1000h, 1600h, and 2200h. These times were thought to offer the best chance of finding threshold differences and allow an accurate mapping of the rhythms. Because of possible fatigue effects, the subjects were tested on two consecutive days. On the first day, the subjects were tested at 1000h, 1600h and 2200h. On the second day the subjects were asked to go to bed by 2230h and get up by 0330h to arrive for the 0400h testing.

When the subjects arrived for the first testing, they filled out the questionnaires, and were then asked to sit in the dark adaptation room for a training session. This training session lasted about ten minutes and involved an explanation about the subject's task followed by a two minute trial run. A scenario of the explanation given to the subjects is in Appendix C.

Each subject preadapted the right eye for three minutes. The combination of intensity and duration was sufficient to bleach 99+ % of the visual photopigments (Rushton & Powell, 1972). During dark adaptation the subjects were required to adjust the optical wedge between the points where they could just detect and then not detect the test stimulus with the right eye. This is known as a von Békésy (1947) tracking procedure and is similar to the psychophysical method of adjustment. Initially, the optical wedge was set to its minimum filtering capacity allowing for maximum light penetration so that subjects, in their preadaptation state, were able to detect the light stimulus. Immediately following the preadaptation phase, the subjects were to position their heads so as to be comfortably placed in the head and chin rest and move the blinder in front of the left eye. The subjects were told to stare at the cross-hair (which located the test stimulus at 10° nasal to the fovea) and out of the corner of their eye detect the test stimulus. The subjects were told that they could adjust the brightness of the cross hair any time they felt that it would aid in detection of the test stimulus. As soon as they were comfortably established they were to begin the tracking procedure, adjusting the optical wedge continuously for thirty minutes.

During the training session the subjects were given a test run for two or three minutes, without the bleaching light, after which they were given feedback as to how fast they should be tracking (i.e. they should always be adjusting the wedge) and how much they should track (how far they should adjust the adaptometer). This feedback was given because some individuals were under the impression that they must be very sure that they detect or not detect the test stimulus. The limits between which a light is detected and then is not detected are very small and those individuals that make sweeping tracking motions are probably being far too cautious in their adjustments. This caution gives rise to uninterpretable results because it becomes difficult to determine any differences in thresholds when there are changes of one or two log units due to the subject being overly cautious. Thus, the training session was usually able to iron out any problems that might have occurred because of poor tracking performance. It was also possible to clear up any points that were confusing to the subject before the actual test sessions began.

Two undergraduate research assistants were employed to help run the subjects. Both assistants were trained by the author and were checked on several runs to make sure that they knew what to do and how to do it. All 1000h runs, with the training session, were performed by the author to make sure that all of the subjects received the same orientation.

Subjects were only run from Monday at 1000h thru Friday at 0400h. It was thought that subjects would not adhere to many of the restrictions if they were required to participate over the weekend. There were two exceptions to this. Two subjects, for various reasons had to be run over a weekend, however, strict instructions were given to them about not taking drugs of any kind or staying up late. When asked if they complied with the instructions both indicated that they had.

Experiment 2

Subjects

Ten male and ten female undergraduate and graduate students, ranging in age from 19 to 26 years, were used in this study. The undergraduates were volunteers from undergraduate psychology courses at Virginia Polytechnic Institute and State University and received credit toward their final grade in the classes. The graduate students were volunteers and received only the satisfaction of helping a friend out. Again, none of the subjects was told of the specific hypotheses of the study. As in experiment 1, all subjects filled out a consent form and were told that they could discontinue their participation at any time.

Apparatus

The experimental apparatus used in this study was the same as for experiment 1 with one exception. The long wavelength gas discharge tube, used as the test stimulus in experiment 1, was replaced with a heterochromatic gas discharge tube, model XL670/R1131C, made by the English Electric Valve Company (similar to the Sylvania R1131C tube). This heterochromatic gas tube produced wavelengths of light that both the rods and cones could detect.

Procedure

The procedures used in this experiment were the same as in experiment 1.

RESULTS

Experiment 1 - Cone Thresholds

Graphs obtained for two typical subjects (of the first experiment) are depicted in Figure 5. These graphs clearly show that differences were obtained for the experiments. The direction and extent of these differences are discussed in these next sections.

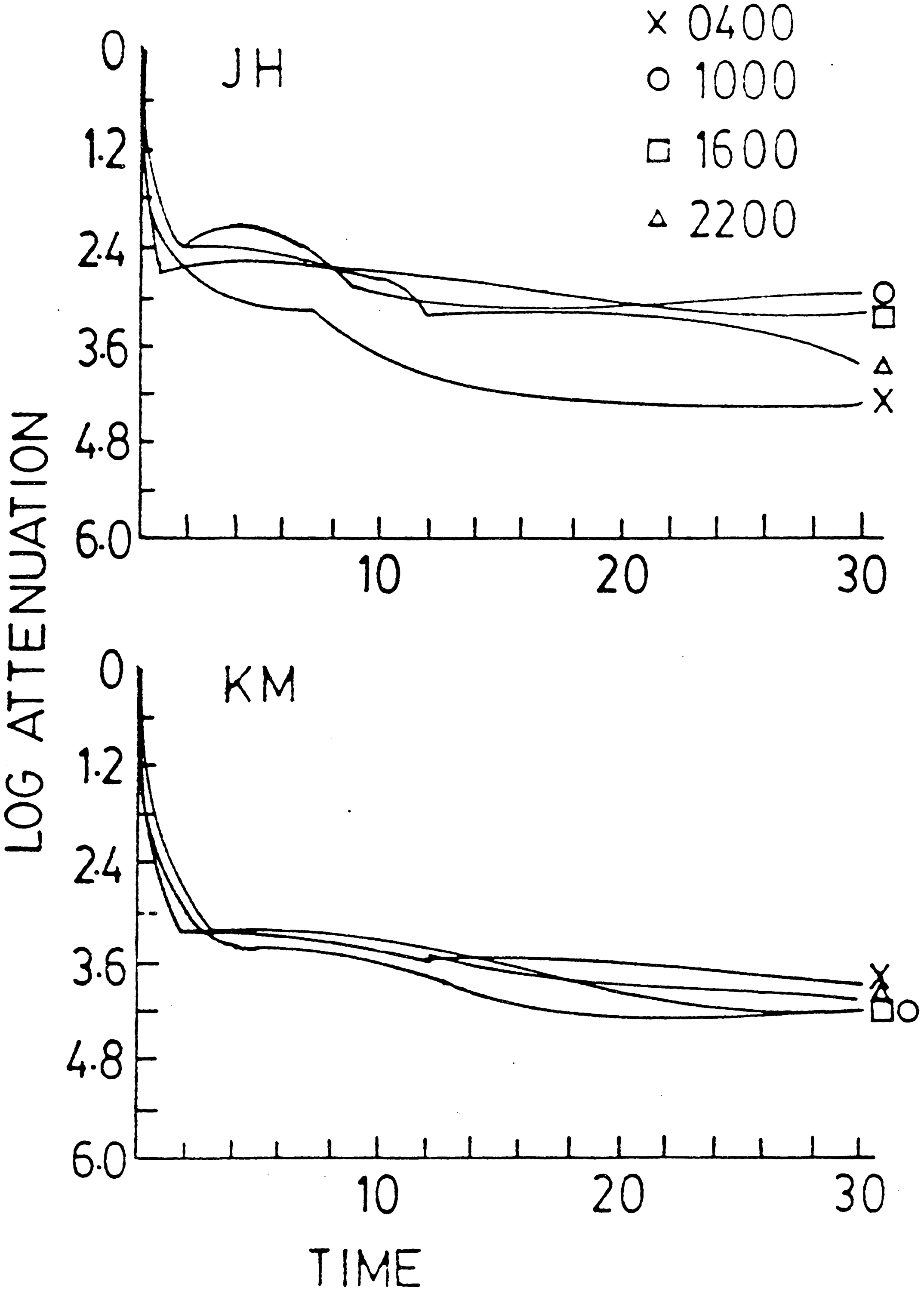
The continuous curves from each of the graphs, obtained during the experiment, were transformed into thirty discrete points for the analyses. This was performed because all of the planned analyses required discrete data points rather than a continuous curve. The transformation involved determining the exact log attenuation for each of the thirty minutes of the experiment. Generally, it was a simple matter of counting over on the X axis of the graph paper to a particular minute and then counting down on the Y axis to determine the log attenuation of that minute. From this, a data set was developed for each individual which contained the time of the testing session, the time within the experiment (1 - 30 minutes) and the corresponding log attenuation. Three means of the log attenuations were calculated then for each graph. These means were 1) the first eleven minutes of the curve; 2) the last nineteen minutes of the curve; and 3) the means for the whole curve.

These means were subsequently used in several types of Analysis of Variance (ANOVA) designs. The three means were calculated so that an analysis of the rods and cones could be performed separately. It was assumed that the rod/cone break would occur within the first 11 minutes. Thus, the curve for the first 11 minutes of experiment 2 could be assumed to be due to the effects of the cones while the last 19 minutes would be due to the rods. A combination of both curves would indicate the function of both rods and cones.

Insert Figure 5 about here

Sex Differences

The first analysis performed was a two factor Analysis of Variance (ANOVA) with repeated measures on one factor to determine if there were any sex differences. The repeated measures factor was the time of day while the independent groups was the sex of the subject. The dependent variable was the mean log attenuations for the three time periods: 1) first 11 minutes, 2) last 19 minutes and 3) the whole curve means. The ANOVA table for the first 11 minute means is located in Appendix C. The ANOVA tables for the last 19 minutes and whole curve means are similar to this table. The ANOVA for the first 11 minutes of the curve revealed no significant differences on sex, F (1, 24) = 0.00, p > .05, as well as for the last nineteen minutes and the whole curve with F (1, 24) = 0.00, p >.05 and F (1, 24) = 0.00, p >.05



Dark Adaptation Curves
Experiment 2

FIGURE 5

respectively. There were also no significant interactions between sex and the time of day on any of the three parts of the curve with F (3, 24) = 1.75, p >.05; F (3, 24) = 2.21, p > .05; and F (3, 24) = 0.10, p > .05 for the first 11 minutes, the last 19 minutes, the whole curve means respectively. Given these results, it was felt that the data for both of the sexes could safely be combined for the remainder of the analyses.

Time of Day Effects

Three single factor repeated measures ANOVA's were performed next to determine if there were main effects for time of day. Three were performed because the limited number of subjects would create extremily small cell sizes in an ANOVA design of any bigger proportions. The ANOVA revealed significant time of day effects for the first 11 minutes, F (3, 27) = 7.68, p < .05, as well as for the whole curve, F (3, 27) = 6.44, p < .05, but not for the last nineteen minutes, F (3, 27) = 1.29, p > .05. The ANOVA table for the first 11 minute means is located in Appendix C. Again, the last 19 minute and whole curve ANOVA tables are similar to this table. A Student-Newman-Keuls multimean analysis, with 27 df, was performed to determine where the differences lay. Table 1 indicates that for both the first 11 minutes and the whole curves, log attenuation means for 1600h, 2200h, and 0400h are all considered statistically the same but all are significantly different and show larger log attenuations than at 1000h. The first 11 minute and whole curve means are graphically depicted in Figure 6.

Insert Table 1 about here

Insert Figure 6 about here

A four factor independent groups ANOVA was performed on the last 19 minutes by treating each minute as a different subject and using the time of day as one of the five factors. This type of ANOVA can only be performed by assuming that each minute is independent of every other minute. It must be argued that a person's vision at any one moment is not influenced by any previous moment. If this can be accepted then the following analysis is correct.

The four factors were sex, subject with the effects of sex taken out, time of day, time in each session, plus the interaction of subject with time of day. Sex again was found to be not significant F (1, 690) = 0.06, p > .05. However, there were significant time of day effects, F (3, 690) = 16.92, p < .05. This ANOVA table is located in Appendix C. A Duncan's multimean test was performed and indicated that, at the .05 significance level, the lowest threshold occured at 2200h (*m* = 3.14 log attenuation) and the highest at 1000h (*m* = 3.00). The mean log attenuations for all four time periods are presented in Table 2.

Table 1

First 11 Minute Mean Log Attenuations

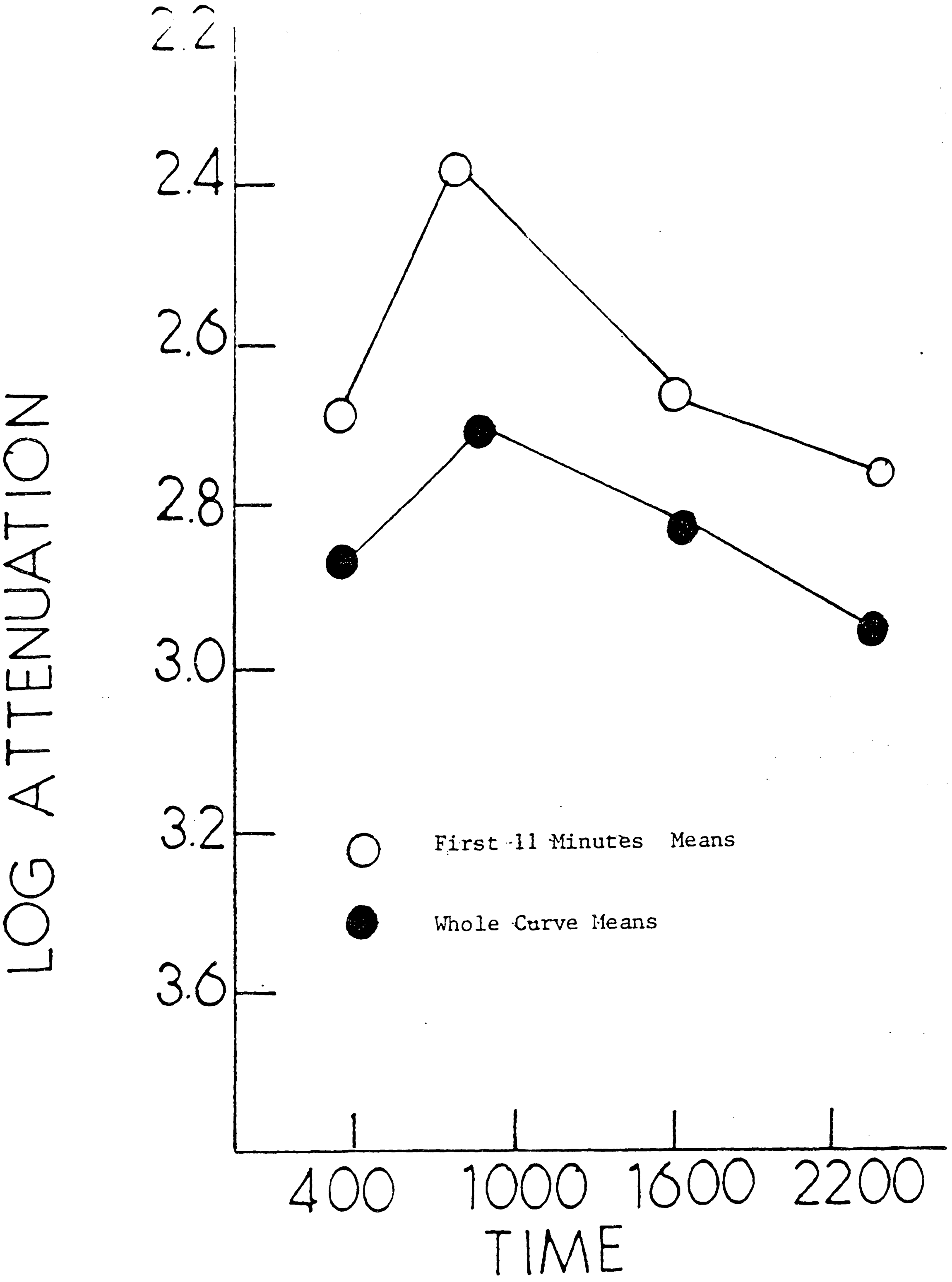
SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	2.39
B	1600h	2.62
B	0400h	2.64
B	2200h	2.76

Last 19 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.00
A	1600h	3.05
A	0400h	3.05
A	2200h	3.14

Whole Curve Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	2.69
B	1600h	2.83
B	0400h	2.84
B	2200h	2.95



Experiment 1

FIGURE 6

Table 2

Last 19 Minute Mean Log Attenuations

Duncan Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.00
B	1600h	3.04
B	0400h	3.05
C	2200h	3.14

Insert Table 2 about here

Significant differences were also found for the subjects with sex removed factor, F (8, 690) = 179.06, p < .05 and for the interaction between the subjects and the time of day, F (27, 690), p < .05. And finally, significant differences were also found for the time within each session factor, F (18, 690), p < .05. Again, refer to Appendix C for the ANOVA table. A Duncan's multi-mean test indicated that there were eight significantly different groupings of time for the nineteen minutes. Generally, the times were grouped in almost an exact sequential order from the 12th minute of the experiment to the 30th minute. The exact groupings for the times within each session are presented in Table 3.

Insert Table 3 about here

Experiment 2 - Rod and Cone Thresholds

Sex Differences

The data for this experiment were transformed in the same way as in experiment 1. Again, a two factor ANOVA (one between and one within ANOVA) was performed to determine if there were any sex differences on the mean log attenuations for the three time periods 1) first 11 minutes, 2) the last

Table 3

Times Within Each Session

Last 19 Minute Mean Log Attenuations

Duncan Multi-mean Comparison	Time in Minutes	Mean Log Attenuation
A	28	3.25
A	27	3.23
A	29	3.23
A	30	3.23
A B	26	3.22
A B	25	3.21
A B	24	3.17
A B	23	3.17
C B	22	3.12
C D	20	3.08
C D E	21	3.05
D E	19	3.02
F E	18	2.97
F G	17	2.91
F G H	16	2.90
G H	15	2.87
G H	12	2.85
G H	13	2.84
H	14	2.81

19 minutes, and 3) the whole curve means. Sex was used as the between variable while time of day was used as the within variable and mean log attenuation was the dependent variable. There were no sex differences for the first 11 minutes, F (1, 18) = 3.41, $p > .05$, or for the last 19 minutes, F (1, 18) = 3.96, $p > .05$. However, sex was significant for the whole curve means, F (1, 18) = 4.34, $p < .05$, with the males recording a mean log attenuation of 3.53 units and the females a mean of 3.30 units. The ANOVA table for the first 11 minutes is located in Appendix C. The ANOVA tables for both the last 19 minutes and whole curves are similar to this table. Because there was a significant sex difference for the whole curve means it was decided to analyze the data for the males and females separately for the rest of the analyses.

Time of Day Effects

Males

In order to test the hypothesis of time of day effects, single factor repeated measures ANOVA's were performed on the three time periods for each testing session. The dependent variable was the mean log attenuation while the independent variable was the time of day. There were no time of day effects for the first 11 minutes of the curve, F (3, 26) = 2.49, $p > .05$, or for the last 19 minutes, F (3,

26) = 2.15, p > .05. However, there were significant differences for the whole curve means, F (3, 26) = 3.23, p < .05. (Please refer to Appendix C for the ANOVA table for the first 11 minutes. The ANOVA tables for both the last 19 minutes and whole curve means are similar to this table). A Student-Newman-Keuls multi-mean procedure was performed to determine where the differences lay. It was determined that the 0400h mean log attenuation (*m* = 3.67) was significantly numerically higher (i.e. more sensitive) than the 1000h mean (*m* = 3.41). All of the means for all three parts of the curves are presented in Table 4. The whole curve means for both males and females are graphically represented in Figure 7.

Insert Table 4 about here

Insert Figure 7 about here

Again, a three factor independent groups ANOVA was performed using the log attenuations of each minute as the dependent variable. As was stated earlier, this analysis can only be considered correct if it can be assumed that each minute of the task is independent of every other minute. The three factors were the time of day, the subject and the time in the session plus the interaction between the subject and the condition. The ANOVA table is located in Appendix C. The time of day factor was significant, F (3,

Table 4 - Males

First 11 Minute Mean Log Attenuations

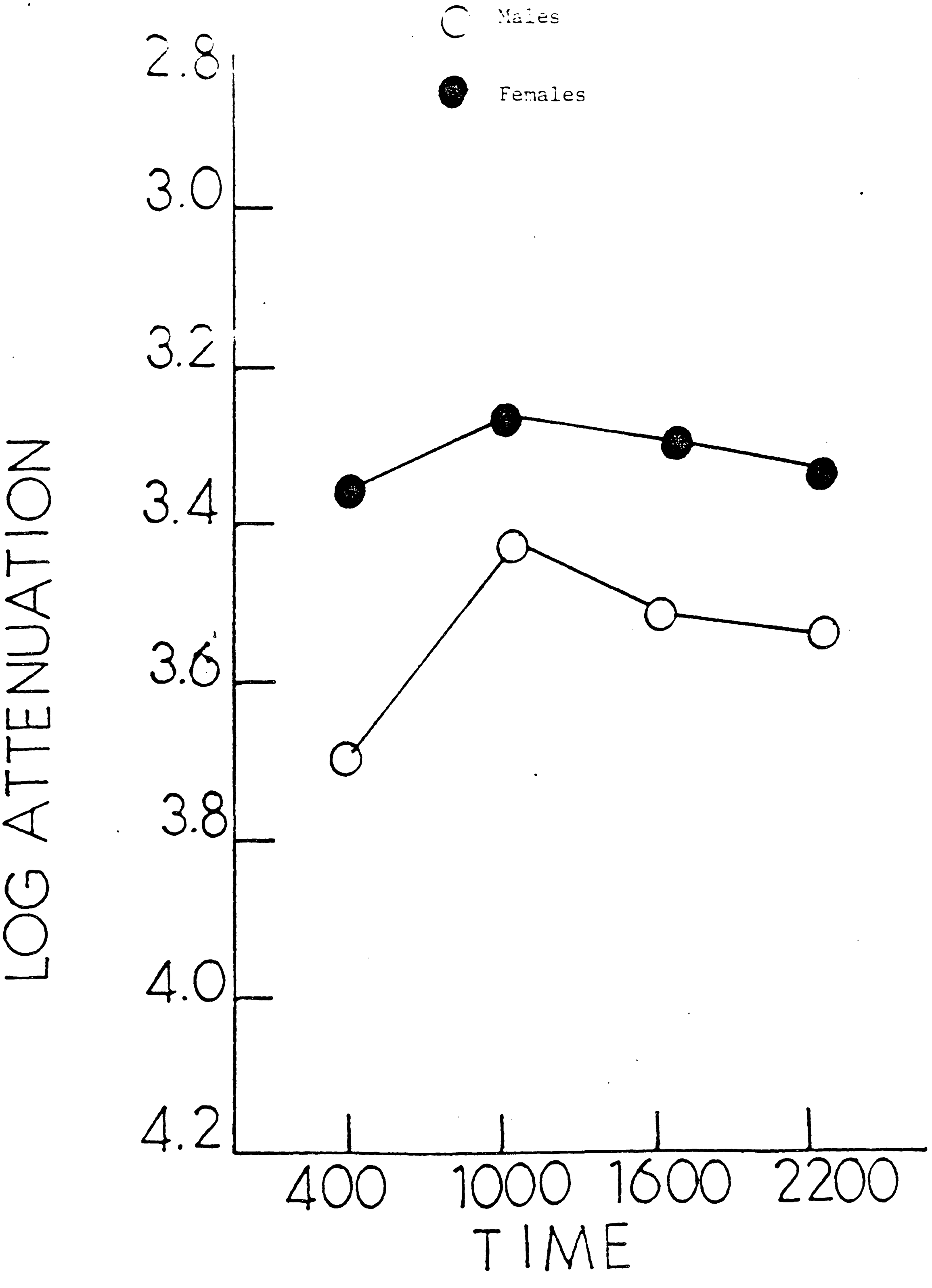
SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.06
A	1600h	3.15
A	2200h	3.22
A	0400h	3.30

Last 19 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.75
A	2200h	3.85
A	1600h	3.87
A	0400h	4.04

Whole Curve Mean Log Attenuations

SNK Multi-mean Comparison		Time	Mean Log Attenuation
	A	1000h	3.41
B	A	1600h	3.51
B	A	2200h	3.54
B		0400h	3.67



Whole Curve Mean Log Ateenuations

FIGURE 7

673) = 47.66, p < .05, and a Duncan's multi-mean analysis was performed to find where the differences were. The mean log attenuation for the 0400h testing was significantly numerically higher (i.e. more sensitive) (*m* = 4.05) than for the 1000h testing (*m* = 3.76) and both were different from the 1600h and 2200h testings which had similar means (*m* = 3.88 and 3.87 for the 1600h and 2200h testings respectively). The results of the multi-mean analysis appear in Table 5.

Insert Table 5 about here

The subjects were also significantly different from each other, F (9, 673) = 243.52, p < .05, along with the interaction of the subjects by time of day, F (26, 673) = 36.71, p < .05. And finally, the times within the experiment were also different from each other, F (18, 673) = 48.06, p < .05. A Duncan's multi-mean analysis on the times within the experiment found 11 different groups for the 19 minutes in this part of the experiment. Again, as in experiment 1, the groupings were in an almost exact sequential order from a low at the 12th minute to a high log attenuation at the 30th minute. The groupings are placed in Table 6.

Table 5 - Males

Last 19 Minute Mean Log Attenuations

Duncan Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.76
B	2200h	3.86
B	1600h	3.88
C	0400h	4.05

Table 6

Times Within Each Session

Last 19 Minute Mean Log Attenuations

Duncan Multi-mean Comparison			Time in Minutes	Mean Log Attenuation
A			30	4.17
A	B		28	4.14
A	B	C	29	4.13
D	B	C	26	4.07
D	E	C	27	4.04
D	E	C	25	4.04
D	E		24	4.03
D	E	F	23	4.02
G	E	F	22	3.95
G		F	21	3.93
G			20	3.90
G	H		19	3.86
I	H		18	3.81
I	H		17	3.79
I			16	3.75
	J		15	3.64
	J		14	3.58
	J		13	3.57
		K	12	3.45

Insert Table 6 about here

Females

The hypothesis of time of day effects was tested with single factor repeated measures ANOVAs using the mean log attenuations for 1) the first 11 minutes, 2) the last 19 minutes, and 3) the whole curve means as the dependent variable. The independent variable was the time of day. The results for the first 11 minutes were not significant, F (3, 27) = 1.03, $p > .05$, as were the results for the last nineteen minutes, F (3, 27) = 0.23, $p > .05$. And finally, the whole curve means were also not significant, F (3, 27) = 0.62, $p > .05$. Again, refer to Appendix C for the ANOVA table for the first 11 minutes. The means for each of these analyses are placed in Table 7.

Insert Table 7 about here

A three factor independent groups ANOVA, like the one employed for the males, was performed next. Again, each minute of each test session was treated as a separate subject and the log attenuation at each of the nineteen minutes was used as the dependent variable. The independent variables were the actual subject, the time of day, and the time of the test session. The interaction between the subject and the time of day was also used. The results indicate that there was no time of day effect, F (3, 672)

Table 7 - Females

First 11 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1600h	2.90
A	2200h	3.01
A	1000h	3.02
A	0400h	3.11

Last 19 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	2200h	3.56
A	1000h	3.59
A	1600h	3.61
A	0400h	3.65

Whole Curve Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1600h	3.26
A	2200h	3.29
A	1000h	3.31
A	0400h	3.38

= 0.91, p > .05. However, there was a significant difference for the subjects, F (9, 672) = 86.58, p < .05, and for the subjects by time of day interaction, F (26, 672) = 12.54, p < .05. And finally, there was a significant difference for the time during the test session, F (18, 672) = 14.09, p < .05. The ANOVA table is located in Appendix C. The 19 minutes of the testing session fell into 10 significantly different groups which were again almost sequentially ordered from the 12th minute to the 19th minute. The groupings for the means are located in Table 8.

Insert Table 8 about here

Because there were no time of day effects for the females, it was thought that something else might be influencing their rhythms. Inspection of individual subject's graphs did show what appeared to be differences in the log attenuations for the different times of day. However, apparently these individual differences were lost when combined. It was thought possible that the female menstrual cycle might influence the visual circadian rhythm. Thus, a two-factor ANOVA was performed to test this hypothesis. The female subjects were categorized as to whether they were in the first fourteen days of their cycle or the last fourteen days. This categorization was the between groups variable while the time of day was the within groups variable. Again, the three parts of the graph 1) the

Table 8 - Females

Times Within Each Session

Last 19 Minute Mean Log Attenuations

Duncan Multi-mean Comparison					Time in Minutes	Mean Log Attenuation
A					26	3.82
A	B				30	3.74
A	B	C			29	3.72
A	B	C	D		24	3.70
E	B	C	D		27	3.69
E	B	C	D		28	3.69
E	B	C	D		25	3.69
E	B	C	D		23	3.64
E	B	C	D		22	3.64
E	B	C	D	F	21	3.62
E	G	C	D	F	20	3.60
E	G		D	F	19	3.58
E	G			F	18	3.57
	G		H	F	16	3.51
	G		H	F	17	3.49
	G		H		15	3.48
	J			I	14	3.40
	J	J		I	13	3.34
		J			12	3.28

first 11 minutes, 2) the last 19 minutes and 3) the whole curve means were analyzed separately. In this analysis, the females from the first study were combined with females from the second study. The ANOVA for the first 11 minutes was not significant, F (1, 12) = 0.69, p > .05, nor was the ANOVA significant for the last 19 minutes, F (1, 12) = 2.19, p > .05. And finally, the ANOVA for the whole curve means was also not significant, F (1, 12) = 1.56, p > .05. The ANOVA table for the first 11 minute means is placed in Appendix C. Again, the last 19 minute and whole curve ANOVA tables are similar to the first 11 minute ANOVA table. The means are presented in Table 9.

Insert Table 9 about here

Questionnaire Data

The circadian rhythms questionnaire that was given to the subjects was scored on three factors 1) Factor Rigidity, 2) Factor Vigorous and 3) Factor Morning. The questionnaire was comprised of a total of nineteen questions of which eight were used for the Factor Rigidity scale, five questions for the Factor Vigorous scale, and six questions for the Factor Morning scale. The questionnaires were scored by adding up the numbers circled for each question within a factor. A median split was performed on these scores to categorize the subjects as to whether they were

Table 9 - Females
Median Split on Day in Cycle

First 11 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	2.81
A	1600h	2.83
A	2200h	2.94
A	0400h	3.00

Last 19 Minute Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	2200h	3.44
A	0400h	3.45
A	1000h	3.45
A	1600h	3.45

Whole Curve Mean Log Attenuations

SNK Multi-mean Comparison	Time	Mean Log Attenuation
A	1000h	3.13
A	1600h	3.14
A	2200h	3.19
A	0400h	3.22

high or low on a factor. This categorization allowed for performing two factor ANOVA's (one between and one within ANOVA's) on the three different parts of the curves: 1) the first 11 minutes, 2) the last 19 minutes and 3) the whole curve means for each of the three factors of the questionnaire.

Questionnaire Analysis - Experiment 1

Given that no sex differences were found for the mean log attenuations for this experiment, it was decided to analyze the questionnaires by combining the data for both males and females. For the present, only the ANOVA's examining the whole curve means have been analyzed for this experiment. The dependent variable for the ANOVA's was the mean log attenuation, while the within independent variable was the time of day and the between independent variable was the median split (whether high or low on a factor) of either Factor Vigorous, Factor Rigidity or Factor Morning.

The ANOVA for the Factor Rigidity scale showed no main effects, $F(1, 8) = 0.00$, $p > .05$. The interaction between the scale and the time of day also was not significant, $F(3, 24) = 0.75$, $p > .05$. The Factor Vigorous scale, likewise showed no main effects, $F(2, 7) = 1.66$, $p > .05$. The interaction between Factor Vigorous and time of day was also not significant, $F(6, 21) = 1.00$, $p > .05$. And finally, there was no main effect for Factor Morning, F (1,

8) = 4.96, $p > .05$. However, there was a significant interaction between Factor Morning and the time of day, F (3, 24) = 3.23, $p < .05$. The ANOVA table for the whole curve means for Factor Rigidity is located in Appendix C. The ANOVA tables for the other factors were similar to this table.

Questionnaire Analysis - Experiment 2

Because sex differences were found with regard to the mean log attenuations for this experiment, it was decided to analyze the male and female questionnaires separately. Again, two factor ANOVA's (one between and one within) were performed for all three parts of the graphs 1) the first 11 minutes, 2) the last 19 minutes and 3) the whole curve means for all three of the factors from the questionnaire.

Males

Factor Rigidity

The independent variables were, again the time of day (the within factor) and the median splits on the Factor Rigidity scores. The dependent variable was the mean log attenuations for each particular part of the graph. The two factor ANOVA performed on the first 11 minutes of the Factor Rigidity scores revealed no significant main effects or interactions, F (1, 8) = 0.11, $p > .05$ and F (3, 23) = 2.61,

$p > .05$ respectively. There were also no significant main effects or interactions for the last 19 minutes of the curve, F (1, 23) = 0.03, $p > .05$ and F (3, 23) = 1.82, $p > .05$ respectively. And finally, again there was no significant main effect or interaction for Factor Rigidity and the time of day, F (1, 8) = 0.06, $p > .05$ and F (3, 23) = 1.31, $p > .05$ respectively. Again, the ANOVA table for the first 11 minutes of theis factor is located in Appendix C. The whole curve and last 19 minute ANOVA tables are similar to this table. The interactions are depicted graphically for both males and females in Figures 8, 9, and 10.

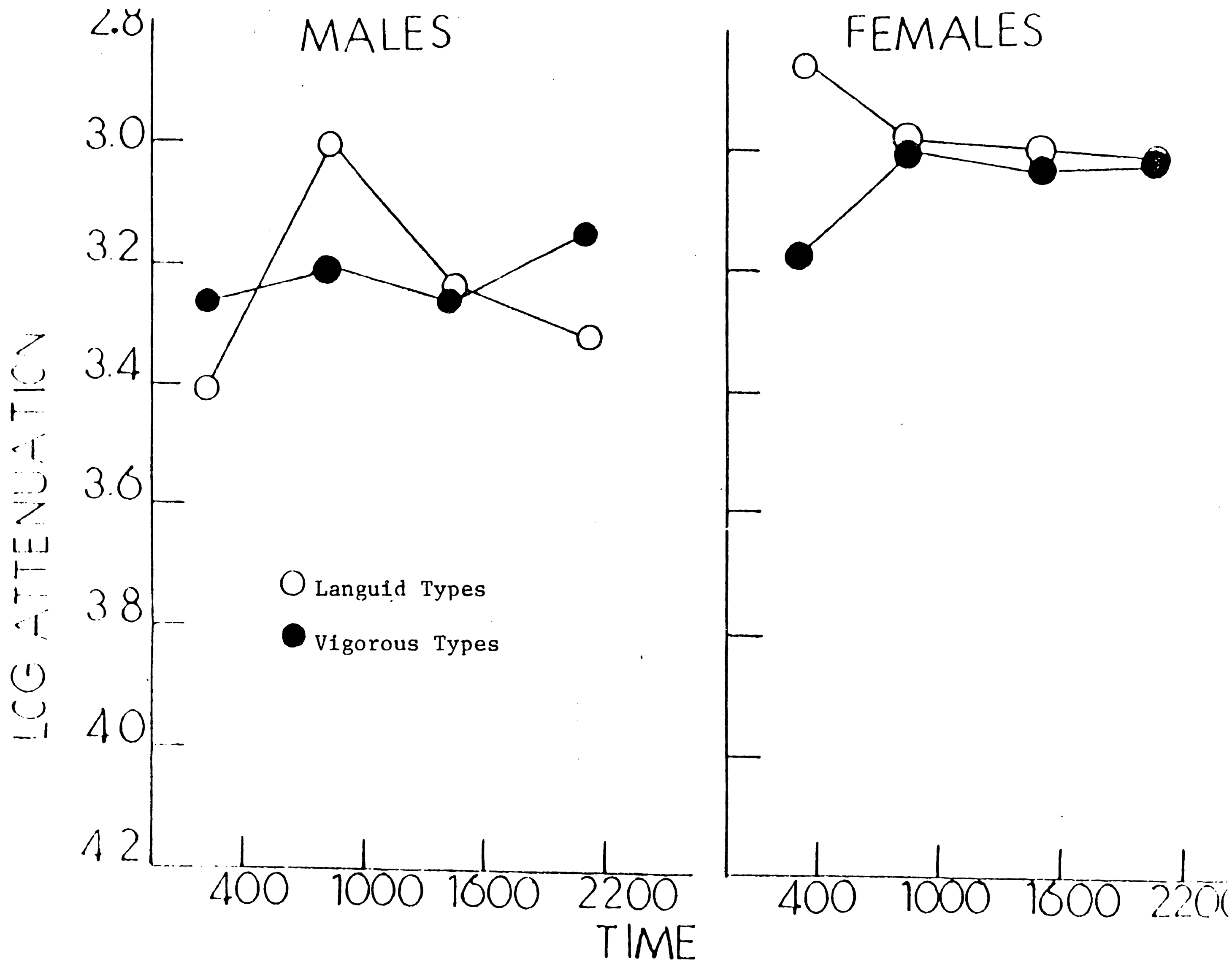
Insert Figure 8 about here

Insert Figure 9 about here

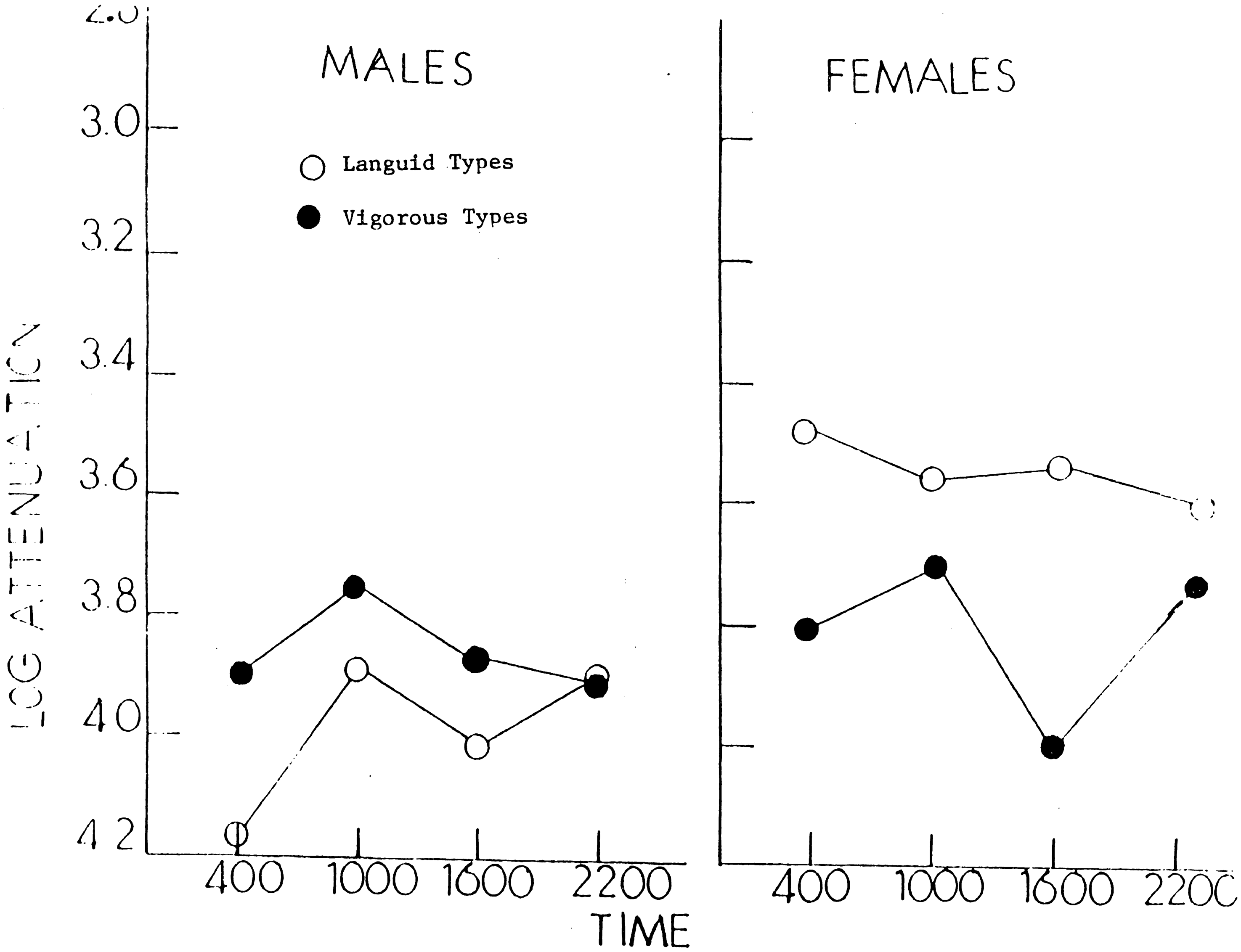
Insert Figure 10 about here

Factor Vigorous

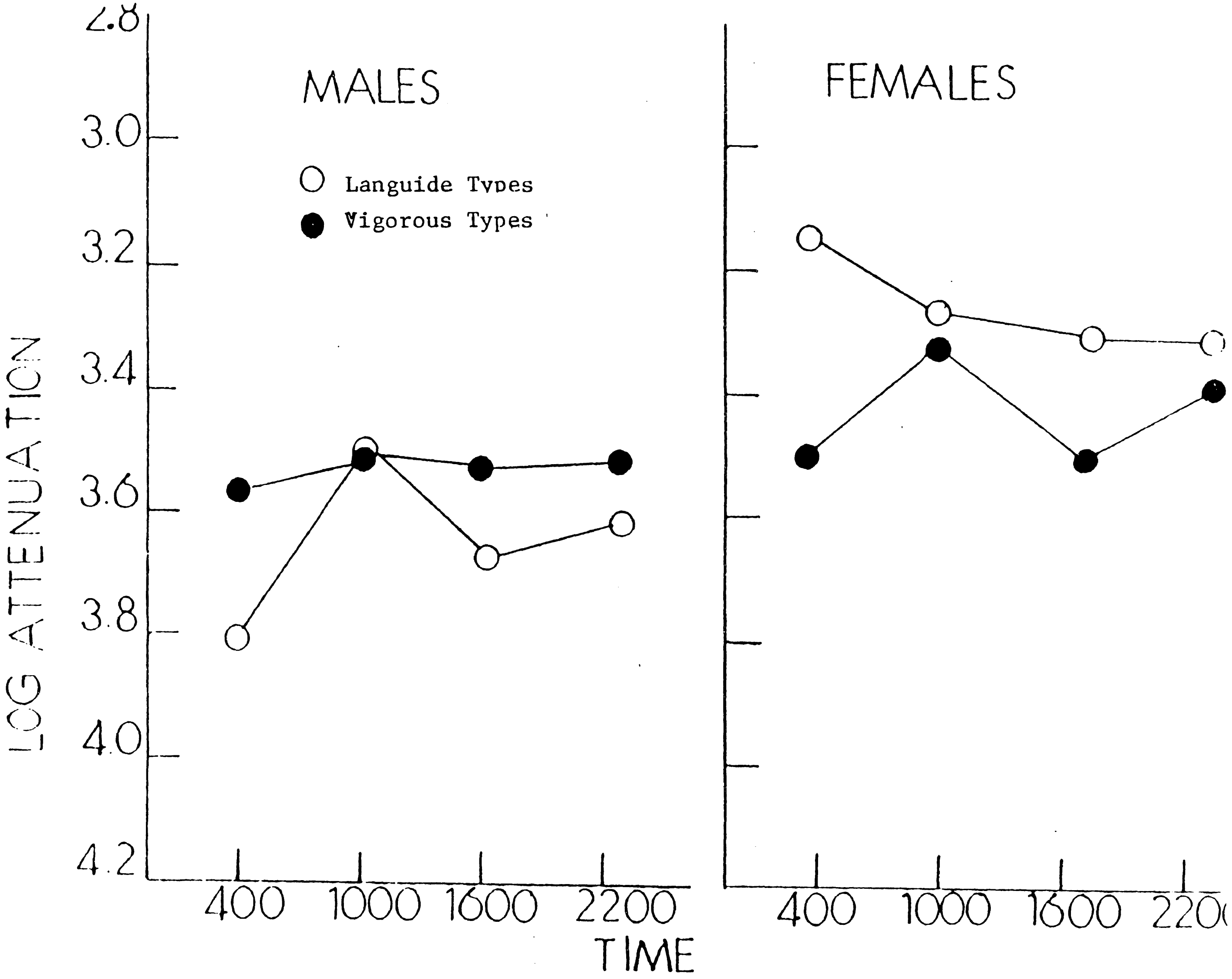
These two factor ANOVA's were performed on the time of day and Factor Vigorous median split. There was no significant main effect for the first 11 minutes for this factor, F (2, 7) = 1.48, $p > .05$. However, there was a



Factor Vigorous - First 11 Minutes
FIGURE 8



Factor Vigorous - Last 19 Minutes
FIGURE 9



Factor Vigorous - whole Curve Means
FIGURE 10

significant interaction, F (6, 20) = 4.33, p < .05. And again, for the last 19 minutes, there was no significant main effect, F (2, 7) = 1.32, p > .05. However, there was a significant interaction, F (6, 20) = 3.01, p < .05. The ANOVA for the whole curve means showed no significant main effects, F (2, 7) = 1.49, p > .05. However, again there was a significant interaction between time of day and Factor Vigorous, F (6, 20) = 4.80, p < .05. (Refer to the ANOVA table in Appendix C for Factor R. The ANOVA tables for Factor V are similar to this table.

Factor Morning

These ANOVA's looked at Factor Morning and the interaction between it and the time of day. The first 11 minutes of the curves revealed no significant main effects or interactions, F (1, 8) = 3.83, p > .05 and F (3, 23) = 0.74, p > .05, respectively. However, for the last 19 minutes of the curve, there was a significant main effect, F (1, 8) = 7.50, p < .05. Those people who were considered night people (i.e. scored low on Factor Morning) had numerically higher (i.e. could detect light better) mean log attenuations (m = 4.10) than those subjects who were considered morning people (m = 3.64). Figure 11 graphically depicts these results. There were, however, no significant interactions, F (3, 23) = 1.40, p > .05. And again, there were significant main effects for the whole curve means, F (1, 8) = 6.46, p < .05. As before, those subjects who were considered night people had significantly numerically higher means (m = 3.71) than did those subjects who were considered morning people (m = 3.34). This is graphically shown in Figure 12. And finally, there were no significant interactions, F (3, 23) = 1.38, p > .05. (Again refer to Appendix C for the Factor R ANOVA table).

Insert Figure 11 about here

Insert Figure 12 about here

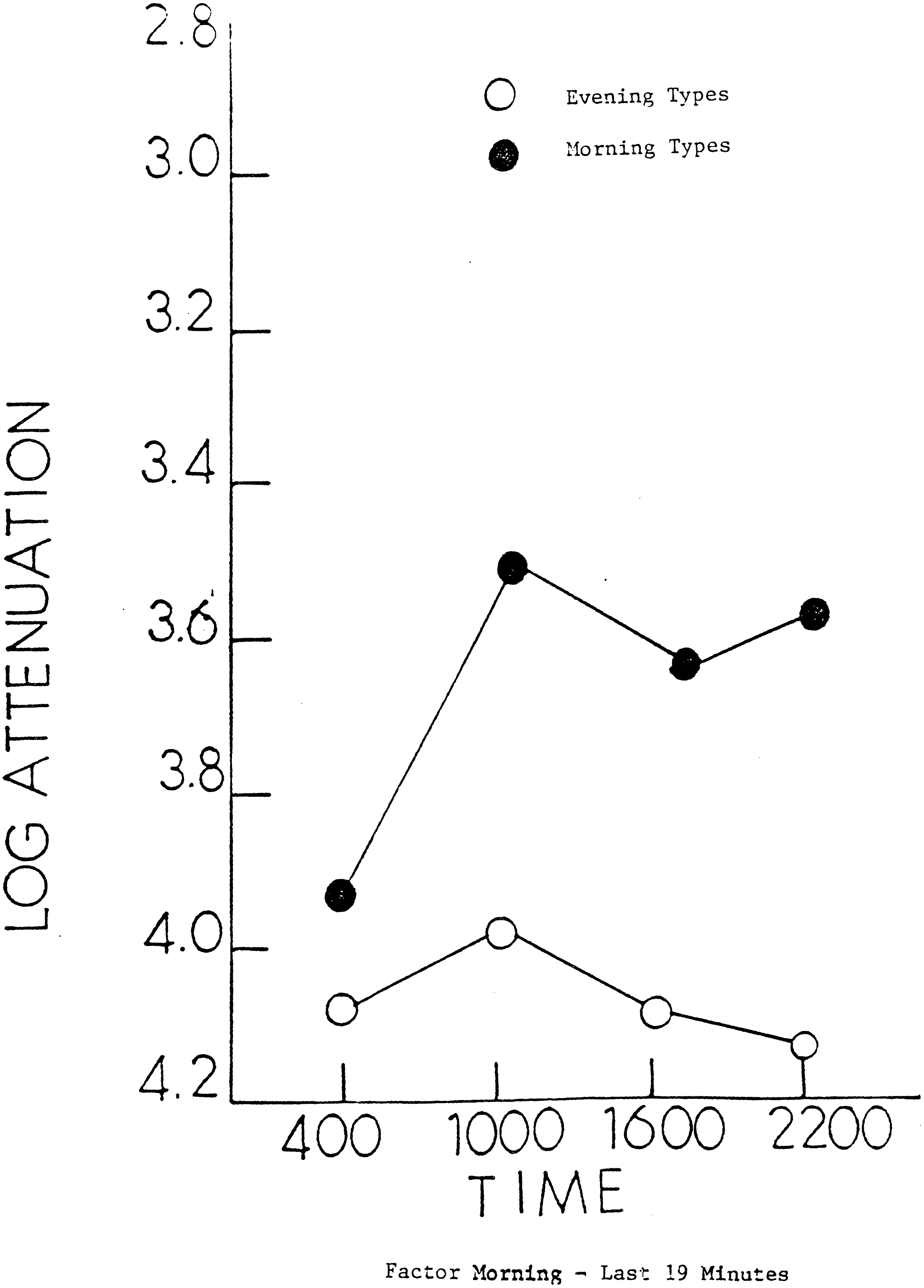
Females

Factor Rigidity

These ANOVA's dealt with the female subjects' Factor Rigidity scores and the time of day. There were no significant main effects or interactions for the first 11 minutes, F (2, 7) = 0.37, p > .05 and F (6, 21) = 0.41, p > .05. And also, there were no significant main effects or interactions for the last 19 minutes, F (2, 7) = 1.20, p > .05 and F (6, 21) = 1.42, p > .05. And finally, there were no significant main effects or interactions for the whole curve means for Factor Rigidity, F (2, 7) = 0.83, p > .05 and F (6, 21) = 0.58, p > .05. The ANOVA table for this factor and the first 11 minutes is located in Appendix C.

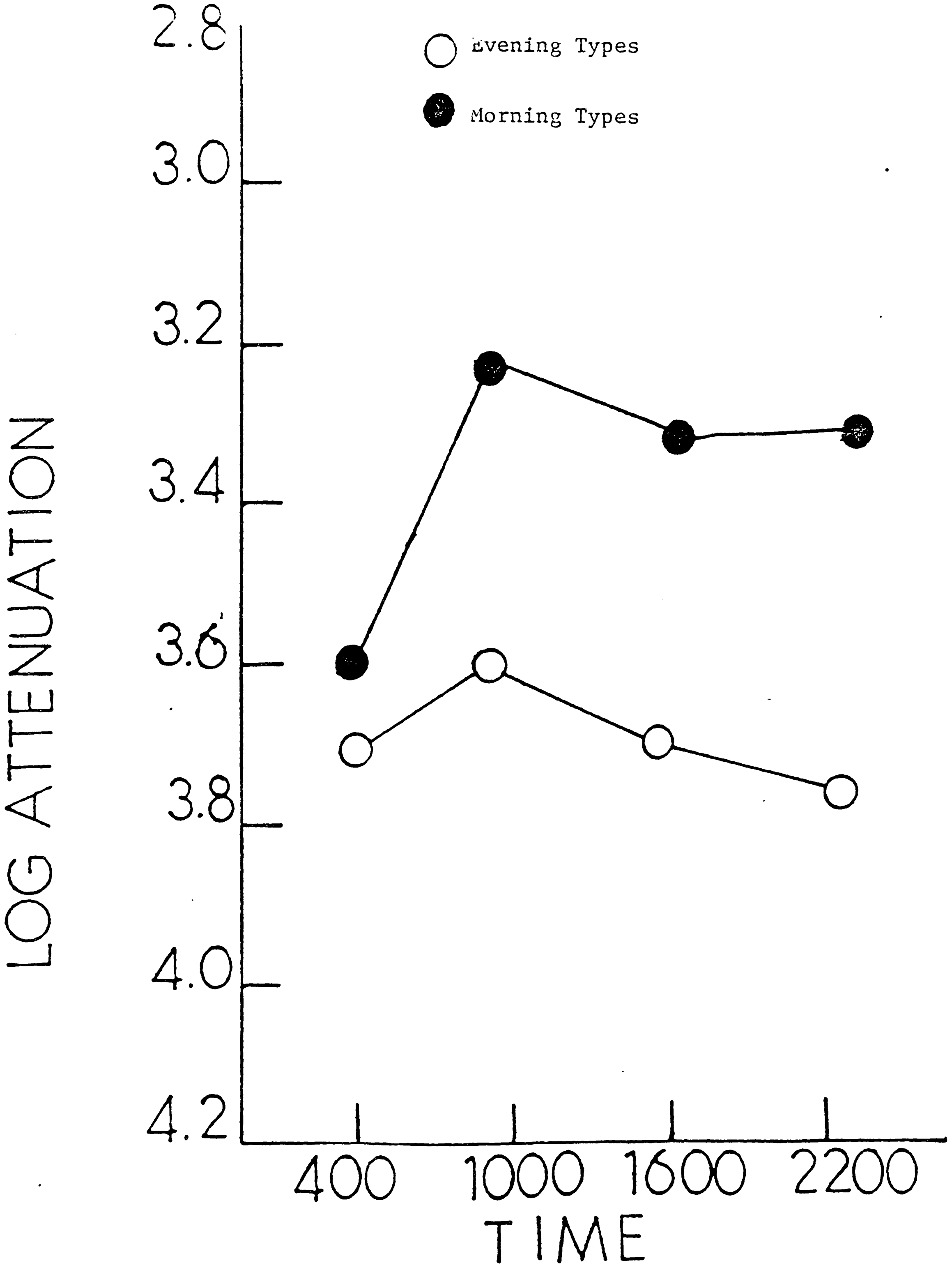
Factor Vigorous

The ANOVA's for the first 11 minutes on the Factor Vigorous scores showed no main effect for the factor, $F(2, 7) = 1.35$, $p > .05$. However, there was a significant interaction, $F(6, 21) = 6.73$, $p < .05$, for the first 11



Factor Morning - Last 19 Minutes

Males
Figure 11



Factor Morning - Whole Curve Means
Males
FIGURE 12

minutes. There was no significant main effect or interaction for the last 19 minutes of the curve, F (2, 7) = 1.93, p > .05 and F (6, 21) = 1.88, p > .05, respectively. For the whole curve means, there was no significant main effect, F (2, 7) = 2.31, p > .05. However, there was a significant interaction for the whole curve means, F (6, 21) = 5.87, p < .05. Again, refer to Figures 8, 9, and 10 and also to Appendix C for the ANOVA table for Factor R.

Factor Morning

The ANOVA pertaining to the first 11 minutes of the curve and testing Factor Morning found no significant main effect or interaction, F (2, 7) = 0.30, p > .05 and F (6, 21) = 0.22, p > .05, respectively. There were also no main effects or interactions for the last 19 minutes, F (2, 7) = 0.81, p > .05 and F (6, 21) = 0.57, p > .05. And finally, there were no significant main effects or interactions for the whole curve means, F (2, 7) = 0.32, p > .05 and F (6, 21) = 0.28, p > .05. (Please refer to Appendix C and the Factor R ANOVA table).

DISCUSSION

The experiments conducted in this thesis were aimed at determining if a circadian rhythm existed for visual thresholds. From this main objective, three hypotheses were generated and tested. First, it was thought that rods would have a peak sensitivity level at 1000h and a trough at 0400h. It was also thought that cones would demonstrate a circadian rhythm, but would have peaks and troughs at different times from the rods. Second, it was hypothesized that there would be sex differences for the circadian rhythms with one sex exhibiting a periodic change in the rhythms which would be different from the other sex. This was based on the notion that the female menstrual cycle might somehow influence the circadian rhythms of vision. And third, it was thought that a questionnaire, developed specifically for circadian rhythm research, would relate to the visual threshold circadian rhythms. This was based on the idea of group differences such as the morning lark and night owl type individuals.

Hypothesis 1 - Rod Thresholds - Males

Sex differences were found for scotopic thresholds. However, they will not be discussed until the next section which will include the discussion of the female data. This section will only discuss rod thresholds for males. It was hypothesized that rods would be most sensitive at 1000h and least sensitive at 0400h. This hypothesis was formulated by both past research in circadian rhythms, which dictates that low points usually occur at 0400h and peaks occur sometime in the morning, the results of a pilot study (performed on two male subjects) and the results of the Powers *et al.* (1982) study. The results, nevertheless, did not support this hypothesis. Thresholds were found to exhibit large individual differences as was shown in Figure 5. However, a circadian rhythm for the rods was clearly established with differences on the order of .26 log units. However, instead of the rods being most sensitive at 1000h they were found to be least sensitive at this time. And instead of the rods being least sensitive at 0400h, they were most sensitive at this time. This turn of events came as a complete surprise to the experimenters. However, the results can be explained and in fact have been independently supported by another lab.

In a poster session at the seventh annual European Conference on Visual Perception (ECVP), Powers & Bassi (1984) presented a study examining the circadian rhythmicity of scotopic thresholds. Upon a more complete examination of their previous study (Powers *et al.*, 1982, in which subjects were found to be least sensitive to light at 0400h) they found that the results were unreliable and probably due to some artifact of the study. However, the 1984 study was reliable and the results follow the results of this thesis quite nicely.

The Powers and Bassi (1984) experiment again tested human visual sensitivity in a signal detection paradigm. Four subjects (two male and two female) were kept in total darkness for 24 hours and were to detect a test series of 502 nm peripheral flashes of light every hour. They found that from hour to hour the sensitivity was highly variable but nonetheless there was a significant difference between sensitivity from night to day which was on the order of 0.1 log units of attenuation on the average and 0.2 log units from peak to trough. They found that the rods were most sensitive at 0400h and least sensitive around 1100h. Essentially, they found exactly the same results as is reported for rod thresholds in this study. Powers and Bassi also recorded body temperature and found significant correlations between body temperature and visual sensitivity. They found that higher sensitivity was always correlated with lower body temperatures. Typically, body temperature is lower at night than during the day, thus, visual sensitivity was lower during the night. The fact that scotopic vision follows the body temperature rhythm so closely lends support to the notion that vision does, indeed, follow a circadian rhythm.

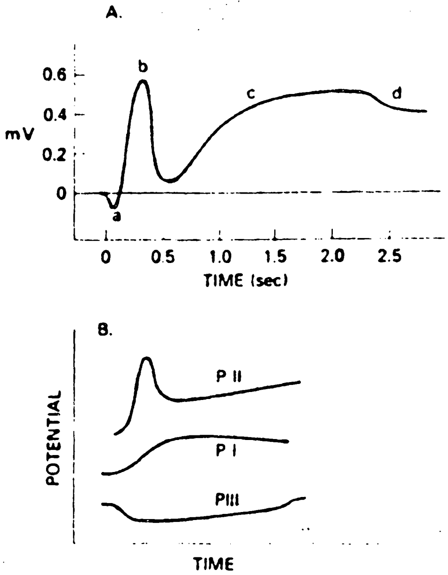
There are several ways in which these results can be accounted for. The most plausible and supported explanation is that of rod outer segment shedding. Rod outer segments are important in that they are the place where light is absorbed in the rods. They are composed of disks which are encased in plasma membrane. Rhodopsin, the photoreceptor molecule of the rods, is contained in the disks of the outer segments. It is well known that these disks are continually renewed with the new ones created at the bottom of the stack and old ones breaking off from the top (Ludel, 1978). It was only recently, however, discovered that these outer segments probably shed in a circadian rhythmic pattern. As was stated in the introduction of this thesis, Besharse *et al.* (1977) and others have found evidence for disk shedding that follows a circadian rhythm in many different animals. La Vail (1976), in a study of rat rod disk shedding, found that the number of phagosomes (organelles which contain shedded receptor disks) also vary in a diurnal rhythm. More phagosomes were present immediately at the onset of light than at any other time.

Birch, Berson & Sandberg (1984) found evidence that human rod electroretinograms (ERG) also show a circadian rhythm. An ERG is produced by placing an electrode on the cornea of a human dark adapted observer and then placing a reference electrode on the scalp. The subject is then exposed to a brief flash of light and the resultant electrical response constitutes the ERG. A graph of an ERG consists of four components (see Figure 13).

Insert Figure 13 about here

Birch *et al.* examined the a and b wave components which probably correspond to rod and cone receptors and bipolar cells respectively (Shichi, 1983).

Birch *et al.* (1984) entrained one eye for each of six subjects to a 14 hour light and 10 hour dark schedule by placing a patch over the eye for 10 hours. The other eye, the unentrained eye, was kept in light 14 hours per day for the entire seven days of the experiment. The full field rod ERGs were elicited by 10 microsecond flashes of 440nm light waves that varied in retinal illuminance by .3 log unit steps. Subjects were tested at 0730h, 0930h, 1600h and 2100h. Birch *et al.* found that all six subjects entrained eyes showed significantly higher thresholds (13% higher) for the b wave at 0930h than at any other time including 0730h. Three of the six subjects showed higher thresholds (17% higher) for the a wave at 0930h than at any other time. There were no significant differences in the unentrained eyes, however. Birch *et al.* estimated that if 10% of the rods are shed between 0800h and 0930h, the maximum increase in threshold due to a decrease in rhodopsin should be .05 log units. However, they found an average increase of .13



The electroretinogram. (A) The electrical response shown begins with a decrease (hyperpolarization) in the membrane potential (a wave), followed by three depolarizing waves (b, c, and d). (B) The electroretinogram represents a sum of three components, PI, PII, and PIII. PIII is responsible for the a wave, PII corresponds to the b wave, and PI is related to the c wave.

FIGURE 13

(from Shichi, 1983)

log units. They postulated that this difference can be accounted for by a possible impairment of shedding rods or a nonuniformity of photoresponse along the rod outer segment length.

These studies all support the theory that rods have a circadian rhythm with high thresholds in the morning, about an hour or two after the onset of light, and low thresholds at most other times. The results of this thesis, then, can be explained in terms of those studies examining rod outer segment shedding.

Hypothesis 2 - Sex Differences

It was hypothesized that the two sexes would exhibit different thresholds. This did indeed happen for rod thresholds. Females demonstrated higher thresholds (on the order of .14 log units at the least to .39 log units at the most) than males did. Not only that, but males exhibited significant time of day effects while females did not. However, upon examination of individual graphs, it appeared that females did exhibit time of day effects. It was thought that females might have different circadian visual cycles during particular points of their menstrual cycle. Therefore, when the data were averaged, a flattened curve appeared.

To test the hypothesis regarding the menstrual cycle influence, visual thresholds for the first fourteen days of the cycle were compared to the last fourteen days. No significant differences were found. This could be due to several reasons. First, the data for females were combined from the two experiments for this analysis. This was performed to increase the cell sizes. But in doing this it is possible that the real rhythm was muddled by the two experiments.

A second reason is that the point of ovulation might not have been a critical period for visual thresholds. Redgrove (1971) indicates that studies linking performance to the menstrual cycle have come up with very mixed results. Some studies have found the lowest performance to occur at menstruation, others at premenstruation and still others found the lowest performance to occur at various periods throughout the cycle. However, Redgrove cited several studies which did test sensory thresholds. One study examined sensitivity for the taste of quinine. Maximum sensitivity was found to be 1 to 5 days after the onset of menstruation. However, considerable individual differences were found. In another study, two point thresholds for pain and tactile sensitivity were determined for females. It was found that maximum sensitivity occurred at the premenstrual period.

These basic findings suggest that female sensitivity and performance probably does change in a rhythmic pattern over the menstrual cycle. These changes might not be consistent across women and could possibly vary even within the subjects over several months. Powers *et al.* (1984) did use two female subjects in their study. They did find a time of day effect for women, but it was far more variable and less defined than for the males. They also found that the female rhythm correlated less with body temperature than did the males and that the body temperature rhythm was also less defined than the males. Powers also felt that this difference for males and females was related to a menstrual cycle influence.

It is interesting that there were no sex differences for cone thresholds in experiment 1. Exactly why this occurred is unknown. However, it indicates that both male and female cones can detect light just as well at the different times of the day. Yet, males have significantly lower rod thresholds than females at all times of the day. And females seem to have extremely variable rod thresholds over the course of the menstrual period.

Hypothesis 1 - Cone Thresholds

Time of day effects for cone thresholds were tested using two different methods. First, one entire experiment was conducted using a long wavelength light as the test stimulus. Rods are very insensitive to this wavelength, and thus the thresholds for the test stimulus should be high. In fact they should be as high as the first 11 minutes of the second study which was the second technique used to measure cone thresholds. By comparing the mean log attenuations for the three parts of the curve (the first 11 minutes, the last 19 minutes, and the whole curve means) of the first experiment to the first 11 minutes of the second experiment (including both males and females) it can be seen that the mean log attenuations are similar.

There were no sex differences in the first experiment, thus data for males and females were combined. In that experiment, time of day effects were also found for the first 11 minutes and for the whole curve. These differences indicate that cones are significantly *less* sensitive at 1000h than at any other time of the day. The differences range from, at the least .14 log units to, at the most, .37 log units of attenuation.

The independent groups ANOVA revealed significant time of day differences for the last 19 minutes. These differences again demonstrated that cone thresholds were highest at 1000h with 1600h and 0400h falling in the middle range of sensitivity and 2200h was shown to be the most sensitive time. The differences here, however, were much smaller than for the previous analysis with differences of between .04 and .14 log units of attenuation. As was stated in the results section of this thesis, these results must be taken with caution because of the appropriateness of the analysis.

A different story emerges when the first 11 minutes of the second experiment are examined. This part of the curve is typically associated with photopic vision and should produce results similar to the first experiment. However, there were no significant time of day differences for either males or females for the first 11 minutes. This becomes difficult to explain in light of the results from the first experiment. However, the differences in the test stimuli for the two experiments could be the key to the differences in results.

It appears that the mean log attenuations for both males and females in the first 11 minutes of the second experiment are similar to the last 19 minutes of the second experiment. The last 19 minutes of the first experiment produced significant differences only in the independent groups ANOVA and these differences were small. Thus, it is possible that the second experiment was not sensitive enough to detect the time of day differences in the cone thresholds. The first experiment used a long wavelength test stimulus which red and green cones can detect easier than blue cones (given the trichromatic theory of color vision). A heterochromatic light source was used as the test stimulus in the second experiment. This light source could be detected by all three types of cones and rods. Thus, it is possible that the different cones could have different circadian rhythms. The blue cones may have some how inhibited the circadian rhythm effect of the red and green cones in the second experiment. This theory is supported by the fact that the mean log attenuations for the first 11 minutes of the first study are higher than the mean log attenuations for the first 11 minutes of the second study. Thus, the test stimulus in the first study was quite likely producing a wavelength that only some of the cones could detect. If all of the cones could detect it, the log attenuations should have been the same for the first 11 minutes of both studies.

The results do support a circadian rhythm in cone activity with a low point at 1000h and a peak probably at 2200h. It also appears that these time of day differences in the cone thresholds diminish over a thirty minute period. Thus, cones appear to exhibit a greatest time of day effect within the first 11 minutes of dark adaptation. And finally, there do not appear to be any sex differences for cone thresholds over the times of the day.

These results can, as with the rods, be discussed as a function of cone outer segment shedding. Cone outer segments, like rod outer segments, are made up of disks containing photopigments which react to light stimulation. Unlike the rod disks, cone disks are not independent of each other but rather are continuous with the plasma membrane. It has long been thought that cone outer segments do not shed but rather go through a process of replacement of proteins in the already existing disks. This, however, has recently been shown to be a fallacy stemming from the process that is normally used for detecting disk replacement (Young, 1978).

Typically, radioactive amino acids are injected into the animal and and are incorporated into proteins in the inner segments of rod receptors. These labeled proteins are used by the inner segments in the creation of new disks for the outer segments. Over time, the newly labeled disks can be seen to move up the outer segment and finally shed off to be dissolved by phagosomes. In cone outer segments, radioactive amino acids are not incorporated into a single disk, but rather, because of the continuity of the disks, the radioactive amino acids are diffused throughout the whole disk (Shichi, 1983). This process has led many researchers to believe that cone outer segments simply renew proteins, but do not shed.

Young (1978) was one of the researchers that originally believed that cones do not shed. However, he noted that there were several discrepancies with the theory. First, in the salamander and chick, an autoradiographic band failed to appear in developing outer segments. For developing segments, this band should have developed. The conclusion was that cones manufactured outer segments without localizing the new protein in them. This is because the disks are continuous segments.

In 1974 it was discovered that human cones may shed, but in other animals the shedding appeared to be rare (Young, 1978). After the discovery of rhythmic rod outer segment shedding, Young (1978) hypothesized that cones may also shed in a rhythmic way. This would account for the varying results of the cone studies. O'Day and Young (1978) found evidence for cone membrane shedding in the goldfish. Apparently, the tip of the cone membrane sheds and the discarded debris is ingested by phagosomes in much the same way as rods. The only difference that O'Day and Young found between the rods and cones was that this process occurs after the lights are turned off. This research has been confirmed in lizards and chickens (Young, 1978).

Given these results, one would expect highest cone visual thresholds around nightfall with lower thresholds during the day. The results of this thesis do not support that notion. The tendency was for highest thresholds at 1000h and lowest thresholds between 2200h and 0400h. It therefore appears that if cone outer segment shedding is occurring, it is not affecting visual thresholds at the tested times; or at least in an inhibitory fashion. It is possible that the testing times were such that cone shedding was not a problem, and something else caused the decrement in thresholds. The decrement could also have been due to a rhythmic fluctuation by certain biochemicals that are required for cone functioning.

It is also possible that the effect could be due to a change in the waveguide properties of the cones. This refers to a phenomenon known as the Stiles-Crawford effect (Riggs, 1965). Basically, the effect states that a light wave entering the center of the pupil will be more luminous efficient than a light wave reaching the same point on the retina but passing through a different point on the pupil. The reason the effect occurs is that light waves entering certain regions of the cones are better trappers of light (thus leading to better light detection) than other areas. The properties of light trapping and conduction are referred to as waveguide properties. And cones in the inner fovea are longer and narrower (because of crowding) than other cones. This shape of the inner foveal cones dictates that light waves that enter the center of the pupil will be more efficient than light waves entering other parts of the pupil (Riggs, 1965). If these waveguide properties exhibit a circadian rhythm then visual thresholds would also change. This hypothesis could be tested by measuring the size of the Stiles-Crawford effect over a twenty-four hour period.

Hypothesis 3 - Questionnaire Data

It was hypothesized that if there was a circadian rhythm for visual thresholds, then the Folkard *et al.* (1979) questionnaire would relate to these rhythms. Essentially, this thesis would act as a type of validation study for the questionnaire. Two factor ANOVA's were used in the analyses and the results should be taken with caution for several reasons. First, the cell sizes were extremely small. A total of only ten subjects was used in any of the analyses. This violates the usual 10 subjects per cell design. Second, a median split was used for the categorization of subjects on the factors. This method was chosen because of lack of normative data for the questionnaire and the need for using all of the subjects in the analyses. And finally, for a correct validation of the questionnaire, a correlational type procedure, with an n of one hundred or more, should have been used. Thus, the major fault with analyses on the present data is one of small sample size. For practical reasons (time, money, and subject recruitment), a larger sample size was not used. However, the results of these analyses can be taken as an indication that more research should be performed.

A significant main effect for any of the factors indicates that, regardless of the time of day, the mean log attenuation is significantly higher for one level of the factor than the other. A significant interaction between a factor and the time of day indicates that the effect of time of day on the mean log attenuation depends on the level of the factor in question. For ease in interpretation, the factors will be discussed one at a time and include the results of both experiments.

Factor Rigidity

This factor related to the rigidity or flexibility of sleeping habits. Rigid types needed regular meal and sleep hours, while flexible types could eat and sleep at any time. No significant main effects or interactions were found for either experiment on any of the three parts of the dark adaptation curve (the first 11 minutes, the last 19 minutes or the whole curve means). Thus, it appears that this factor does not relate to visual thresholds, whether it is scotopic or photopic. This factor, however, did account for the most variance (28%) in the Folkard *et al.* (1979) study.

Factor Vigorous

This factor related to one's ability to overcome drowsiness; low scorers were considered languid types while high scorers were vigorous types. Vigorous types could overcome drowsiness and were little affected by sleep loss, while languid types felt drowsy after reduced sleep and had trouble overcoming this. There were no main effects or significant interactions of this factor with the first experiment. There were no significant main effects for both males and females on the second experiment. There were, nonetheless, significant interactions for all parts of the curve for the males and for the first 11 minutes and whole curves for the females. Thus, this factor does relate to visual thresholds for both scotopic and photopic (as defined by experiment 2) vision and for both males and females.

Figures 8, 9 and 10 graphically depict the interactions for the first 11 minutes, the last 19 minutes and the whole curve means for both males and females. As can be seen, males and females have almost totally opposite graphs. For males, the languid types typically have lower log attenuations for 0400h. Females languid types, however, typically have their highest log attenuations at 0400h. Also, the vigorous type males exhibit far less circadian rhythmic effect than the languid types. In females, just the opposite is true. Here, the vigorous types show a larger circadian rhythmic effect than the languid types.

Folkard *et al.* (1979) felt that vigorous types would have an easier time adjusting to shift work because they showed less variable circadian rhythms. The present results, at least for males, tends to uphold this notion. Vigorous types may not exhibit the visual sensitivity that languid types exhibit, but they tend to be very consistent in their thresholds. Apparently, it doesn't make much difference what time of day they must detect light; they will typically be just as good at one time of the day as another. For females, just the opposite is true. Languid types will probably be better able to adjust to shift work requiring consistent visual thresholds.

An interesting finding with respect to this factor was that the languid subjects in the Folkard *et al.* (1979) study had lower circadian rhythm body temperatures than did the vigorous types. Powers *et al.* (1984), as was said above, found that better visual sensitivity always followed lower body temperatures. Thus, at least for males, this may hold true. Powers *et al.* also found that females body temperature was less well defined than the males. This may be an indication as to why the female data are not as "clean" as the male data.

Factor Morning

This factor related to the morningness or eveningness of subjects. Morning individuals find it easier to work at normal times of the day and to wake up early in the morning. Evening types could work at unusual times and could get a second wind if staying up late.

Again, there were no significant main effects or interactions with visual thresholds for the first experiment. There were also no significant main effects or interactions for females on this factor. And there were no significant interactions for the males on this factor. However, there were significant main effects for the last 19 minutes of the curve and the whole curve means for males. This is graphically depicted in Figures 11 and 12. It appears that evening types have significantly lower thresholds than the morning types. This, of course, would be what one would hope to find. This factor, curiously enough, accounted for the least amount of variance (17%) in the Folkard *et al.* study and, in fact, only related significantly to one of the variables used by them for validation. This variable was the subjective ratings of alertness for the part time shift work nurses.

As was said at the beginning of the questionnaire discussion, these results must be taken with caution. However, the results do indicate that a questionnaire of this nature does relate to the circadian rhythms of visual thresholds. Factor Morning and Factor Vigorous did relate to visual thresholds. Considering that the questionnaire was not designed to detect differences in all of the circadian rhythms that have been studied on humans, it is a credit that the questionnaire could relate to the visual rhythms. This relationship gives credence to the notion of underlying rhythms that drive any one circadian rhythm and offers support for the notion of morning 'larks' and night 'owls'. And it is in some small way a check on the validity of the questionnaire to determine adjustment to shiftwork. It is quite possible that with a larger subject size, the effects could have been greater.

It does appear that the questionnaire does not relate to the first experiment. This may have occurred because the two sexes were combined and, as was seen in the second study, there were major sex differences on the questionnaire. Also, unlike the Folkard *et al.* (1979) study, Factor R was not significantly related to any of the visual threshold parameters. The female data were not as correlated with the questionnaire as the male data. This may have been due to the fact that the females, as a group, did not show any time of day effect. The same problem that was affecting time of day might have also affected their factor scores.

What It all Means

Circadian rhythms have been around as long as life itself. However, only recently have we begun to take notice of them. We sleep at night because, according to our evolution, we survive best by not wandering around during the night. We eat three times a day because our bodies need a constant intake of nutrients. And our various senses are most efficient at times when they would be needed the most. All of these things make intuitive sense. In the past we have ignored the influence of these rhythms in the hope that the effects were not large. In today's society, with the increased emphasis on quality control, and the exact limits that man must work within, the effects of circadian rhythms can no longer be ignored. Many factories are producing 24 hours a day and employ several continuous shifts of workers. A growing concern is felt for the accident and sickness rate of workers as well as the absenteeism rate.

According to the National Safety Council, approximately 2.2 million people are hurt each year. The total cost is $23 billion a year in production losses, insurance claims, and other damages. Two hundred and forty-five million person days per year are lost due to accidents. Figure 14 shows the dollar value of goods and services which are required to offset a $500 work accident loss. Figure 15 indicates how much a good industry must produce to offset a $500 work accident loss (Alliance of American Insurers, 1980).

Insert Figure 14 about here

Insert Figure 15 about here

Smith, Colligan & Tasto (1982) examined the relationship between shiftwork and health in the food processing industry. They compared day, afternoon, night and rotating shiftworkers on several health variables. Over 1000 workers were questioned and their health records examined. They found that night workers received the least amount of sleep - 6.4 hours, while the afternoon shift received the most sleep - 7.4 hours. A higher percentage of day and afternoon workers reported interrupted sleep than did night or rotating workers. More rotating and night shiftworkers rated sleep as poor than the other shifts. And 44% of those on rotating shifts said that it took at least a week to adjust their sleeping pattern when rotated. They indicated that the night shift was the toughest to adjust to.

Smith *et al.* (1982) also found that male night shiftworkers more frequently reported gas pains than male workers on other shifts. Female rotating shiftworkers reported more often a tight feeling in their stomachs than did females on other shifts. Day workers had the best appetites while afternoon and rotating shiftworkers had the

How much does a work accident really cost in terms of production? Manufacturers' and suppliers' costs in the following examples have been estimated on the basis of current retail prices and may vary somewhat from the figures used, depending on outside factors affecting a particular operation's actual production cost. In this representative sample of manufacturers and suppliers of typical goods and services, here is the production needed to offset a $500 work accident loss:

DOLLAR VALUE

Dollar value of goods and services required to produce $500 profit by industrial group.

Aerospace	$10.638
Air transport	29.412
Amusements	4.132
Autos, trucks	19.231
Automotive parts	11.111
Baking	13.889
Brewing	11.905
Building, heating, plumbing equipment	8.065
Cement	4.854
Chemical products	8.065
Clothing, apparel	10.000
Common carrier trucking	14.286
Construction	16.129
Dairy products	14.706
Department, specialty stores	14.706
Distilling	8.197
Drugs, medicines	4.717
Electrical equipment, electronics	9.615
Electric power, gas	5.618
Farm, construction, material handling equipment	8.621
Food chains	38.462
Food products	13.158
Furniture, fixtures	15.625
Glass products	7.[illegible]
Hardware, tools	9.434
Household appliances	12.195
Instruments, photo goods	5.814
Iron, steel	14.706
Lumber, wood products	6.410
Machinery	8.475
Meatpacking	45.455
Metal mining	2.538
Metal products	11.905
Nonferrous metals	6.944
Office equipment, computers	4.505
Paint, allied products	18.519
Paper, allied products	7.576
Petroleum products, refining	8.065
Printing, publishing	7.143
Quarrying, mining	7.463
Railroads	14.286
Restaurants, hotels	8.197
Rubber, allied products	26.316
Shoes, leather goods	14.706
Soap, cosmetics	8.197
Soft drinks	7.937
Stone, clay products	9.259
Sugar	10.417
Telephone and communications	4.310
Textile products	15.625
Tobacco products	8.333
Variety store chains	20.833
Wholesale houses	20.833

FIGURE 14

(From Alliance of American Insurers, 1980)

TO OFFSET WITH PROFITS EVEN A $500 WORK ACCIDENT LOSS BUSINESS MUST:

A builder must construct 538 square feet of housing space.

A publisher must sell 35,715 newspapers.

A bakery must bake 46,297 loaves of bread.

A furniture manufacturer must make 312 $50 chairs.

A restaurant must serve 2,732 three-dollar lunches.

A paint manufacturer must produce 2,645 gallons of paint.

A department store must sell 11,765 pairs of boys' socks.

A garment manufacturer must sell 667 men's $15 shirts.

An electronics factory must build 32 black and white TV sets.

A supermart must ring up 1,538 sales of $25 each.

A telephone company must handle 21,552 local pay-phone calls.

An auto manufacturer must build 3 ³/₄ popular low-priced sedans.

A soap manufacturer must produce 40,985 bars of soap.

An appliance factory must make 1,220 electric irons.

A meat packer must process 32,468 pounds of hamburger.

A tool maker must manufacture 943 hammers at $10 each

FIGURE 15

(From Alliance of American Insurers, 1980) poorest. Fifty-three percent of the rotating workers said that it took one day to adjust to a new time, while 35% said it took between two days to one week to adjust.

The number of injuries, according to Smith *et al.* (1982), was significantly higher for the rotating shiftworkers than for any other shift and this was not due to accident repeaters. Thirty-eight percent of the males and sixty percent of the females on rotating shiftwork had one or more injuries in a six month period! The range for injuries in other shifts was from 15 to 26%. These findings have been supported by Koller, Kundi & Cervinka (1978); Meers, Maasen & Verhaegen (1978); and Colquhoun (1976) among others. However, it is possible to reduce many of the more unpleasant side effects of shiftwork and actually increase productivity by using principles developed from circadian rhythm research as Czeisler *et al.* (1982) have shown. Thus, the practical implications of circadian rhythms for industry in terms of reducing absenteeism, health care costs, and accident rates as well as increasing productivity are enormous.

The increase in today's technology requires very exact detection and responses for humans. Quality control personnel must detect flawed materials at a rapid pace, radiologists must detect the presence of tumors in X rays, systems monitors must monitor complex systems for infrequent malfunctions and radar operators must detect infrequent contacts of aircraft. It is critically important that a radar operator on the Distance Early Warning (DEW) System be sure whether a blip is an enemy plane, a cloud or a friendly plane. And just as critical is the radiologist who must detect whether or not a tumor exists in a cluttered X ray. Pilots must detect critical pieces of information from various displays both in the cockpit and through Head Up Displays (HUD). Without careful detection, the lives of many people could be endanger.

Each of these jobs is performed at all hours of the day, not just between the hours of nine to five. And each requires a high degree of sensitivity. Thus, any circadian rhythmic effect upon the senses has important implications for effective performance on these jobs. Individuals, who work in jobs requiring critical sensitivity, should obviously work at times when their senses are most acute. However, many jobs must, for practical reasons, be performed at times other than those that are optimal for the sense required. When faced with this problem, there are at least two methods which can reduce the effects of circadian rhythms on performance. The first method involves entraining the optimal time for the sense to a new time. The second method involves the selection of individuals who are able to change shifts with little circadian rhythm effect.

Entrainment is the resetting of the biological clock so as to be in phase with some Zeitgeber. This occurs everyday as we entrain our 25 hour bodies to the 24 hour light-dark cycle. In the area of the senses it might be possible to entrain our bodies so that the maximum sensitivity of vision, audition, or any other sense coincides with the time of work. Entrainment has been successful in changing peak body temperature as well as performance (Colquhoun, 1971) and has shown its practical significance with the Czeisler *et al.* (1982) study. Basically, the method involves using a phase delay slowly rotating shift system along with sleeping at times compatible with the shift system and an attempt to socialize at times that are again compatible with the system. In other words, it becomes important to keep a normal life with the exception that it is shifted with the normal 24 hour day. By implementing this method ones biological circadian rhythms change (Colquhoun, 1971). And further research may show that one's sensory circadian rhythms also change.

The second method involves selecting individuals for shift work who will not exhibit the detrimental side effects of shiftwork. The Folkard *et al.* (1979) questionnaire is an attempt at determining exactly what the variables are that determine ability to adjust to shiftwork. There are large individual differences in the ability to adjust to shift work. Many individuals do not exhibit any health problems while others do. As was said earlier, the worker who shows diminished circadian rhythmic effects would probably adjust better to shift work than the individual who demonstrates a large circadian rhythmic effect. Thus, questionnaires that can differentiate among these individuals on the relevant rhythms for the jobs would be invaluable as a selection tool. A great deal of research is still needed to develop reliable and valid questionnaires which can be used for this purpose, however.

CONCLUSIONS

A circadian rhythm exists for visual thresholds. The nature of this rhythm is one with high thresholds at 1000h and low thresholds at most other times. Both rods and cones appear to exhibit this same rhythm. It is possible to account, at least in part, for the rod threshold rhythm in terms of rod outer segment shedding. However, this same explanation can not account for the action of the cones. It is possible that the cone waveguide properties change over a twenty-four hour period. This can be tested with the Stiles-Crawford effect. It is just as likely that a biochemical, important in cone functioning, may also vary in a circadian rhythmic fashion. This might cause cone thresholds to vary in a rhythmic fashion. Further study into the rhythmic effects of some of the visual biochemicals is needed to substantiate this claim.

When the data for females were grouped, they did not exhibit circadian rhythms. However, individual data suggest that they do exhibit diurnal rhythms which are lost when grouped. Another study should be performed which examines the effect of the menstrual cycle on visual circadian thresholds to determine if this is the cause of the discrepant results.

A questionnaire, developed by Folkard *et al.* (1979), did relate to visual thresholds. This relationship indicates that it may be possible to develop questionnaires which can discriminate between individuals who can adjust rapidly to shift work from those who can not. Further research is needed in this area to develop such a reliable and valid test. It might also be possible to entrain individuals such that the point of lowest thresholds coincides with their work schedule.

And finally, further research is needed to determine if other senses also exhibit circadian rhythms and what the times for highest and lowest thresholds are. If these other senses do vary in a diurnal pattern this will have enormous implications for jobs which require detection of critical stimuli.

Circadian rhythm research has moved from the theoretical perspective to the practical application towards increasing human effectiveness. Industry will save money and increase productivity as well as accuracy of job performance. And the individual will benefit in terms of bettered health and increased job proficiency. Thus, the study of sensory circadian rhythms will be of great benefit to both industry and the individual.

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APPENDICES

APPENDIX A. CIRCADIAN RHYTHM QUESTIONNAIRE

Developed by

Folkard, Monk and Lobban (1979)

Factor Rigidity

1. How easy do you find it to take short 'cat naps' at odd times of the day?
Very easy   Very Difficult
1 2 3 4 5 6 7

2. If you have been out very late at a party, how easy do you find it to 'sleep in' the following morning if there is nothing to prevent you doing so?
Very easy   Very Difficult
1 2 3 4 5 6 7

3. After you've had several late nights in a row, how easy do you find it to get to sleep if you go to bed early to try to 'catch-up'?
Very easy   Very Difficult
1 2 3 4 5 6 7

4. Do you have phases, *i.e.* several nights in a row, when you find it difficult to get to sleep?
Seldom   Frequently
1 2 3 4 5 6 7

5. How easy do you find it to sleep during the day if you have to?
Very easy   Very Difficult
1 2 3 4 5 6 7

6. Do you go to bed at a regular time and get up at a regular time even if you don't have to?
Never   Always
1 2 3 4 5 6 7

7. To what extent do you prefer to have your meals at regular times?
No Preference   Strong Preference
1 2 3 4 5 6 7

8. When you are away on holiday, to what extent do you stick to your normal times of getting up and going to bed?
Very Different   Exactly the Same
1 2 3 4 5 6 7

Factor Vigorous

9. If you have very little sleep one night, do you feel

drowsy the following day?
Very Much So Hardly At All
1 2 3 4 5 6 7

10. To what extent are you better at working at certain times of the day or night than at others?
Very Much So No Difference
1 2 3 4 5 6 7

11. Are you the sort of person who can easily miss out a night's sleep?
Definitely Not Definitely
1 2 3 4 5 6 7

12. If you are woken up at an unusual time can you 'wake up' properly and do what ever it is you have to do?
Only With Great Difficulty Very Easily
1 2 3 4 5 6 7

13. If you have something important to do but feel very drowsy can you overcome your drowsiness?
Only With Great Difficulty Very Easily
1 2 3 4 5 6 7

Factor Morning

14. Do you get a 'second wind' if you stay up very late?
Always Never
1 2 3 4 5 6 7

15. How do you react to working at odd times of the day or night?
Enjoy it a Lot Dislike it a Lot
1 2 3 4 5 6 7

16. Are you the sort of person who feels far livelier during the day than early in the morning or late at night?
Definitely Not Definitely
1 2 3 4 5 6 7

17. If you don't have an alarm clock, can you successfully 'tell yourself' to wake up at a certain time?
Never Always
1 2 3 4 5 6 7

18. Do you find it easy to get up early in the morning if, for example, you are setting off on holiday?
Very Difficult Very Easy
1 2 3 4 5 6 7

19. When you have had to get up at a regular time for several days in a row do you start waking up just before your alarm goes off?

Never Frequently
1 2 3 4 5 6 7

Name ______________________ Date ________________

Please complete the following questions.

1. What time do you usually wake up? ________________
2. What time would you prefer to wake up? ____________
3. What time do you normally go to bed? ______________
4. What time would you like to go to bed? ____________
5. How many hours of sleep is optimal for you? ________
6. Have you ever been on shift work before? __________
7. If yes, what shift did you prefer? (Please state times) ____________

   And why? ____
8. At what times do you prefer to work on school work (put beginning

   time)? ________________

Please answer the following questions regarding your menstrual cycle. This information is necessary for the experiment. All information will remain confidential.

1. When did your last period begin (please be specific)? ____________
2. How long does your period usually last? ____________________
3. How many days does your cycle vary (e.g. 28 + or - 1) ? __________
4. Are you currently taking any medication to regulate your menstrual cycle (e.g. the Pill)? ________________
5. Are you pregnant? ______________________
6. Do you suffer from pre-menstrual syndrome (PMS)? ________________

APPENDIX B. CONSENT FORM

INFORMED CONSENT

This study involves research on the influence of circadian (twenty-four hour) rhythms on visual thresholds (ability to detect a light stimulus in a dark room). Your participation is required for 1 fifteen minute orientation session and four experimental sessions.

Each experimental session will last approximately 45 minutes and will take place over a two consecutive day period. You will be asked to come in at 10 a.m., 4 p.m., and 10 p.m. on the first day and 4 a.m. on the second day. You will be asked not to take any drugs, including caffeine, for twenty-four hours prior to the beginning of the experiment. You will also be asked not to eat for at least two hours before each testing session.

The study involves sitting in a small, light tight room and adjusting the intensity of a light stimulus for thirty minutes. A bright light will also be shone in the right eye for three minutes. You will also be required to fill out a questionnaire regarding usual wake up, bed, meal and work times. Females will also be requested to fill out a short questionnaire regarding their menstrual cycles.

Your identity and any records of sessions will remain confidential. None of the preceding procedures is expected to produce any adverse reactions. *Your participation is voluntary and you may discontinue participation at any time.* However, we would like to emphasize the importance to us of your completion of this task and ask you to do everything in your power to bring your participation to a successful conclusion.

For more information, or if you have any questions, please call
Timothy J. O'Keefe at 961-7310 or 552-0355,
Dr. A. M. Prestrude at 961-5673, or
Human Subjects committee (Dr. Bill Schicht) at 961-5346.

Under the conditions set forth above I ____________________ voluntarily agree to participate in the above study.

Signed ____________________ Date ______________

I.D. # ____________________ Phone ______________

APPENDIX C. ANALYSIS OF VARIANCE TABLES

Two Factor ANOVA Determining Sex Differences for Experiment 1 First 11 Minutes

Source	DF	SS	MS	F	P
Sex	1	0.000	0.000	0.00	0.99
Subject(Sex)	8	2.119	0.264		
Time of Day	3	0.718	0.239	8.32	0.00
Sex*Time of Day	3	0.151	0.050	1.75	0.18
Subject*Time of Day(Sex)	24	0.691	0.028		
Total	39	3.680			

Single Factor ANOVA for Determining Time of Day Differences Experiment 1 - First 11 Minutes

Source	DF	SS	MS	F	P
Subject	9	2.119	0.235		
Time of Day	3	0.718	0.239	7.68	0.00
Subject*Time of Day	27	0.842	0.031		
Total	39	3.680			

Four Factor Independent Groups ANOVA - Experiment 1 First 11 Minutes

Source	DF	SS	MS	F	P
Sex	1	0.000	0.000	0.060	0.81
Subject(Sex)	8	50.54	6.317	179.0	0.00
Time of Day	3	1.790	0.599	16.92	0.00
Time Within Session	18	17.90	0.994	28.19	0.00
Subject*Time Within Session	27	13.68	0.506	14.36	0.00
Error	690	24.34		0.035	
Total	747	108.26			

Two Factor ANOVA Determining Sex Differences for Experiment 2 First 11 Minutes

Source	DF	SS	MS	F	P
Sex	1	0.562	0.562	3.41	0.08
Subject(Sex)	18	2.969	0.165		
Time of Day	3	0.381	0.127	2.20	0.09
Sex*Time of Day	3	0.147	0.049	0.85	0.47
Subject*Time of Day(Sex)	53	3.059	0.057		
Total	78	7.118			

Single Factor ANOVA for Determining Time of Day Differences Experiment 2 - First 11 Minutes Males

Source	DF	SS	MS	F	P
Subject	9	2.329	0.258		
Time of Day	3	0.295	0.098	2.49	0.08
Subject*Time of Day	26	1.028	0.039		
Total	38	3.653			

Three Factor Independent Groups ANOVA - Experiment 2 Males First 11 Minutes

Source	DF	SS	MS	F	P
Subject	9	76.56	8.506	243.5	0.00
Time of Day	3	4.995	1.665	47.66	0.00
Time Within Session	18	30.22	1.678	48.06	0.00
Subject*Time Within Session	26	33.34	1.282	36.71	0.00
Error	673	23.51	0.035		
Total	729	168.63			

Single Factor ANOVA for Determining Time of Day Differences
Experiment 2 - First 11 Minutes
Females

Source	DF	SS	MS	F	P
Subject	9	0.639	0.071		
Time of Day	3	0.233	0.077	1.03	0.39
Subject*Time of Day	27	2.031	0.075		
Total	38	3.653			

Three Factor Independent Groups ANOVA - Experiment 2
Females
First 11 Minutes

Source	DF	SS	MS	F	P
Subject	9	45.45	5.050	86.58	0.00
Time of Day	3	0.159	2.719	0.910	0.44
Time Within Session	18	14.79	0.821	14.09	0.00
Subject*Time Within Session	26	19.02	0.732	12.54	0.00
Error	672	39.19	0.058		
Total	728	118.63			

Two Factor ANOVA Examining Time of Day and Beginning of Period Experiments Combined First 11 Minutes

Source	DF	SS	MS	F	P
Period	1	0.223	0.223	0.69	0.42
Subject(Period)	12	3.873	0.322		
Time of Day	3	0.354	0.118	1.65	0.19
Period*Time of Day	3	0.238	0.079	1.11	0.35
Subject*Time of Day(Period)	36	2.577	0.072		
Total	55	7.267			

Two Factor ANOVA Examining Time of Day and Factor Rigidity - Experiment 1 Whole Curve Means

Source	DF	SS	MS	F	P
Factor R	1	0.000	0.000	0.00	0.99
Subject(Factor R)	8	2.244	0.281		
Time of Day	3	0.327	0.109	6.26	0.00
Factor R*Time of Day	3	0.039	0.013	0.75	0.53
Subject*Time of Day(Factor R)	24	0.418	0.017		
Total	39	3.029			

Two Factor ANOVA Examining Time of Day and Factor Rigidity - Experiment 2 Males - First 11 Minutes

Source	DF	SS	MS	F	P
Factor R	1	0.033	0.033	0.11	0.74
Subject(Factor R)	8	2.296	0.287		
Time of Day	3	0.295	0.098	2.95	0.05
Factor R*Time of Day	3	0.261	0.087	2.61	0.07
Subject*Time of Day(Factor R)	23	0.767	0.033		
Total	38	3.653			

Two Factor ANOVA Examining Time of Day and Factor Rigidity - Experiment 2 Females - First 11 Minutes

Source	DF	SS	MS	F	P
Factor R	2	0.061	0.031	0.37	0.70
Subject(Factor R)	7	0.579	0.082		
Time of Day	3	0.233	0.077	0.90	0.46
Factor R*Time of Day	6	0.212	0.035	0.41	0.87
Subject*Time of Day(Factor R)	21	1.819	0.086		
Total	39	2.904			

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