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Response to Cold Challenge in Male Wistar Rats Maintained in Constant Illumination

Bryan G. Nadeau

A Thesis

in

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Abstract

Response to Cold Challenge in Male Wistar Rats Maintained in Constant Illumination

Bryan G. Nadeau

Fuller, Sulzman and Moore-Ede (1978; 1979b) reported that squirrel monkeys maintained in constant illumination and isolated from environmental time cues were unable to maintain core temperature in response to cold challenge. They attributed the impairment in ability to maintain core temperature in the squirrel monkeys to desynchronization of the circadian system. These findings have never been replicated by another laboratory or extended to another species. To this end, two experiments were conducted using male Wistar rats. In each experiment, 24 animals were housed for five weeks in either constant illumination (LL) or a light-dark cycle (LD). At the end of the five weeks, all animals were given a cold challenge. Groups of 12 animals were cold challenged either during the light or dark portion of the daily light cycle (for those subjects housed in LL correspondence to the LD 12/12 cycle was used). In the first experiment the cold challenge consisted of a half-hour immersion in a water bath at 27 ºC. Each rat was tested on four occasions. In the second experiment rats were housed in a refrigerator for 12 hours at 6 ºC. Each rat was tested on two occasions. Data acquisition was achieved via a telemetry system which monitored both core temperature and locomotor activity. In both experiments animals that were housed in LL maintained core temperature as efficiently as subjects who were housed in the LD cycle. The difference between these findings and those reported by

Fuller et. al. (1978; 1979b) are discussed within the context of the protocols used and differences between rats and squirrel monkeys in the response of the circadian system to LL.

Acknowledgments

Thank you to Dr. Amir, my thesis supervisor for your help, patience and support. Thank you to the members of my committee for your input and advice. Thank you Dena Davidson. And finally thank you to my wife Joanne for your effort on this project and for putting up with the gig.

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The body temperature of homeothermic animals is one of the most striking and easily observed examples of the homeostatic process. Body temperature regulation in homeotherms is achieved via the coordinated initiation of appropriate corrective responses in proportion to the magnitude of a detected deviation from a set point (the point around which body temperature is defended). Responses to both heat and cold include autonomic and behavioral mechanisms. The alteration in body temperature achieved by these responses is then fed back and the new body temperature compared to set point. Thermoregulatory response is then readjusted accordingly. Body temperature is thus maintained within narrow limits by a negative -feedback control system (Cabanac, 1975; Hammel, 1968; Satinoff, 1978).

Heat-loss or heat-gain mechanisms other than vasomotor tone are not initiated until a critical upper or lower bound of body temperature is reached. The difference between the upper and lower bounds allows for a small degree of body temperature variation. Added to this small potential for body temperature perturbation, is the circadian oscillation in average core body temperature (Aschoff, 1965, 1970). In general, higher average core temperatures are

associated with the active portion of the daily cycle (Aschoff, 1965, 1970).

The circadian rhythm in core body temperature, and in numerous other physiological phenomena, are the products of a self-sustained circadian pacemaker or pacemakers (Pittendrigh & Daan 1976). Normally the daily oscillation of the pacemaking system or systems is synchronized by 24-hour cues in nature, the most conspicuous of which is the 24-hour light-dark cycle (LD). When the temporal cues that synchronize this system or systems are removed, and when the subjects are maintained in constant darkness, the rhythms persist with a period of approximately 24-hours (Aschoff, Gerecke & Wever, 1967). When however an animal is left in constant light (LL) quite different effects may be observed. These include desynchronization (Aschoff, 1965; Aschoff, Gercke & Weaver, 1967; Czeisler, Weitzman, Moore-Ede, Zimmerman & Knauer, 1980; Czeisler, Zimmerman, Ronda, Moore-Ede & Weitzman, 1980; Wever, 1979), splitting (Pittendrigh & Daan, 1976; Rosenwasser & Adler, 1986; Schardt, Wilhelm & Erkert, 1989; Turek, Earnest & Swann, 1982) and arrhythmicity (Critchlow, 1963; Dempsey & Searles, 1943; Fioretti, Riccardi, Menconi & Martini, 1974; Klein & Weller,

1970).

The importance of the synchronization of the circadian time keeping system to 24-hour time cues for efficient physiological function has been only rarely examined (Fuller, Sulzman & Moore-Ede, 1979b). The loss of periodic time cues in LL has been shown to negatively effect the growth of tomato plants (Hillman, 1956) and the life span of fruit flies (Pittendrigh & Minis, 1972). Similarly it has been reported that in squirrel monkeys synchronization of the circadian system to a 24-hour light-dark cycle, or other potent time cue, may be essential to effective response to cold challenge (Fuller, Sulzman & Moore-Ede, 1979b). Two studies have reported that the desynchronization of components of the circadian system, induced in LL, results in a impaired response to a cold challenge in the squirrel monkey (Fuller, Sulzman & Moore-Ede, 1978, 1979b). The purpose of this thesis is to further explore this phenomenon in the laboratory rat.

In this introductory section, the circadian timing system and the neural substrates which are thought to mediate its operation will be discussed. The circadian rhythm in core body temperature will then be described. Work, which indicates that the squirrel

monkey's ability to maintain a constant core body temperature in response to cold challenge may be dependent on periodic inputs from the environment (in the circadian range) will then be reviewed. The hypothesis that squirrel monkeys housed in LL fail to maintain core temperature in response to cold challenge, because components of the circadian system desynchronize, will then be examined.

The Circadian System

The capacity to measure approximately 24-hour intervals of time has been demonstrated in organisms ranging from unicellular algae (Hastings & Sweeney, 1958) to humans (Aschoff, 1965). Rhythmic 24-hour changes that match the solar cycle are evident in a wide variety of biochemical, physiological and behavioural parameters (Aschoff, 1981; Brady, 1982; Moore-Ede, Sulzman & Fuller, 1982, Palmer, 1976; Saunders, 1977). In nature, these diurnal rhythms are synchronized with 24-hour environmental rhythms such as the light-dark cycle. Early explanations of the daily cycles focused on changes in the external environment as the causative or driving force underling 24-hour periodicity. This notion was challenged by experiments which demonstrated that rhythmic changes continued to be expressed by individuals housed in

environments which lacked 24-hour time cues (Aschoff, 1965). On the basis of these and other data, it is now generally accepted that behavioural and physiological rhythms are the products of an endogenous biological pacemaker or pacemakers (Rusak & Zucker, 1975).

Attempts to elucidate the physiological basis of circadian (i.e., about a day) rhythms have focused on the nervous system. Circadian oscillators, or pacemakers which are responsible for either the generation or driving of one or more circadian rhythms have been localized to specific neural structures in a number of species (Menaker, Takahashi, & Eskin, 1978; Page, 1981; Rusak & Zucker, 1979; Takahashi & Zatz, 1982). Although the circadian timing system in mammals may consist of one, or a number of coupled oscillators, at least one, perhaps the most important one, is thought to be located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Kafka, 1983).

The abolition of circadian rhythms in food intake, drinking, and wheel-running in rats, subsequent to antero-medial hypothalamic lesions was first reported by Richter (1965, 1967). Further work showed that the suprachiasmatic nuclei (SCN) of the anterior

hypothalamus were critical to these behavioral rhythms and to endocrine rhythms such as the corticosterone rhythm (Moore & Eichler, 1972; Stephan & Zucker, 1972a). In 1972 a direct retinal pathway terminating in the SCN was identified (Hendrikson, Wagner & Cowan, 1972; Moore & Lenn, 1972). The identification of this retino-hypothalamic pathway provided a potential anatomical substrate for the light entrainment of SCN-mediated circadian rhythms. Subsequent work has established that this pathway is probably sufficient for entrainment, as it has been demonstrated that circadian entrainment persists in the face of destruction of all other known components of the visual system (Dark & Asdourian, 1975; Moore & Klein, 1974; Stephan & Zucker, 1972b). The necessity of the retino-hypothalamic tract for circadian entrainment by a light dark cycle, however, has yet to be demonstrated due to inadequate means for its selective interruption (Rosenwasser & Adler, 1986).

The general hypothesis, that the circadian timing system of complex organisms may consist of multiple circadian oscillators which are coordinated by both hierarchical and non-hierarchical (mutual) coupling relationships, is the most influential heuristic

guiding research in the area (Rosenwasser & Adler, 1986). From this perspective, oscillators at or near the top of a hierarchical system have the major responsibility for the internal coordination of the system. Oscillators positioned lower in the hierarchy provide timing signals for the effector systems which directly drive the overt rhythmic functions. That overall circadian organization may be the product of interactions between oscillatory sub-systems which are normally coupled, is supported by two lines of research.

A number of experiments have demonstrated that occasionally, when animals are maintained in LL, rhythms that are normally synchronous with equal periods will separate and express different periods (Aschoff, 1965; Aschoff, Gercke & Weaver, 1967; Czeisler, Weitzman, Moore-Ede, Zimmerman & Knauer, 1980; Czeisler, Zimmerman, Ronda, Moore-Ede & Weitzman, 1980; Wever, 1979). The phenomenon, referred to as spontaneous internal desynchronization was first observed by Aschoff in 1965 with human subjects maintained in constant dim light (LL). In about 25% of these subjects, rhythms which normally displayed constant phase relationships and similar periods, would spontaneously desynchronize and exhibit different periods and, over the course of a

number of weeks, all 360º of possible phase relationships (Moore -Ede, 1983a). Not all rhythms separated from one another and ran with different periods. Instead, there appeared to be two groups of rhythms, each expressing an independent period. For example, Aschoff (1965) observed that the rhythms of activity and urinary calcium excretion exhibited a free-running period of about 33 hours while at the same time the rhythms of core body temperature, urine volume and urinary potassium excretion had a period of about 25 hours.

The fact that one group of rhythms may lap the other several times over the course of an experiment indicates that there may be at least two separate pacemakers in the human circadian system, each of which maintains phase control over a number of rhythmic variables. Under normal conditions, these two pacemakers or oscillators are mutually coupled to one another, however, in LL this coupling can break down and the different periodicity of these two pacemakers can be observed (Moore-Ede, 1983a; Sulzman, 1983).

Other studies have reported that maintenance in LL may result in a single rhythm splitting into two components which temporarily express different periods before stabilizing in an antiphase

relationship (Pittendrigh & Daan, 1976; Rosenwasser & Adler, 1986; Schardt, Wilhelm & Erkert, 1989; Turek, Earnest & Swann, 1982). With the establishment of the antiphase relationship, the overt appearance of the split rhythm could be described as a circa-12 hour rhythm (Rosenwasser & Adler, 1986). In spite of this appearance, it has been demonstrated that each component of a split rhythm is capable of independent response to dark pulses and has its own phase-response profile (Boulos & Morin, 1985; Boulos & Rusak, 1982). Independent response to dark pulses by each component of the split rhythm has been interpreted as being consistent with the output of two underlying circadian oscillators rather than a single circa-12 hour oscillator (Daan & Berde, 1978; Kawato & Suzuki, 1980; Pittendrigh & Daan, 1976).

Circadian Rhythm of Body Temperature

Hunter (1778) working with what was then the newly invented thermometer, was the first to recognize that body temperature exhibited a diurnal variation. Despite a strong relationship between wakefulness, food intake and rises in body temperature, research has demonstrated a self-sustaining rhythm in core body temperature exists which is independent of wakefulness and or food intake

(Aschoff, 1970).

In LL as well as in LD, circadian rhythms of core body temperature and activity are characterized by a phase-lag between the two rhythms (Aschoff, 1970). When entrained to a 24-hour LD cycle, the core body temperature of a human subject reaches its maximum towards the end of the active phase, and its minimum two to four hours before the beginning of the active phase (Aschoff, 1970; Moore-Ede, 1983b). In LL when both rhythms are expressing the same period, the phase relationship is altered. The maximum core body temperature occurs prior to subjective noon (as judged from the activity rhythm) and the minimum shortly after the subject goes to sleep (Aschoff, 1970). In support of the assertion that the temperature rhythm is independent of the activity rhythm is that the rhythm of core body temperature phase-lags the rhythm of activity and that the phase relationship is altered under free -running conditions. If the diurnal rhythm in core body temperature were passively driven by the daily activity cycle, one would expect that the daily peak and trough in core temperature would be concurrent with, or lag only slightly behind, the peak and trough in activity. Furthermore, if the core body temperature rhythm were a

passive response to the activity rhythm, then the phase relationship established in one set of conditions should be maintained under all conditions (Aschoff, 1970).

The expression of the circadian rhythm of core body temperature requires the timing of heat loss and heat production (Moore-Ede, 1983b). Both a low amplitude circadian rhythm in heat production as measured by basal metabolism and much larger diurnal adjustments in the rate of heat loss from the body as measured by skin temperature, have been established in a number of species ranging from birds to humans (Aschoff & Heise, 1972; Aschoff & Pohl, 1970; Fuller, Sulzman & Moore-Ede, 1979a; Kreider, Buskirk & Bass, 1958).

The Circadian Component of Effective Response to Cold Challenge

Until the late 1970's, spontaneous internal desynchronization had been observed only in humans and had been thought to reflect human volitional control over sleep and wakefulness rather than indicating the existence of multiple oscillators or pacemakers (Sulzman, 1983). This idea was refuted in the late 1970's by studies with squirrel monkeys maintained in LL in which internal desynchronization was observed (Sulzman, Fuller & Moore-Ede,

1977; Sulzman, Fuller & Moore-Ede, 1979).

During the course of an experiment with squirrel monkeys maintained in LL, the failure of a thermostat controlling ambient temperature led to the observation that the ability to maintain core body temperature in response to cold challenge may be compromised in the squirrel monkey in LL. Subsequent work showed that significant falls in core temperature in squirrel monkeys maintained in LL could be produced by drops in ambient temperature from 28 ºC to 20 ºC at all phases of the circadian cycle. The same drop in ambient temperature did not cause a significant fall in core body temperature if the animals were maintained in a 24-hour LD cycle no matter what phase of the cycle the cold challenge was initiated at (Fuller, Sulzman & Moore-Ede, 1978).

The authors advanced three potential explanations for their observation: First, the stress of the chair restraint the monkeys were kept in, or the isolation conditions (or both) could have resulted in impaired maintenance of core body temperature; second, constant bright light could have impaired the thermoregulatory system; third, the failure in the thermoregulatory system was a consequence of internal desynchronization induced in LL (Fuller,

Sulzman & Moore-Ede, 1978).

The stress option was discounted because monkeys maintained in the same restraint and chamber conditions who were kept in a LD cycle had no trouble maintaining core body temperature in response to the same cold challenge. Constant illumination was not considered to be the important variable in the observed impairment in the ability to maintain core body temperature as monkeys maintained in LL but entrained to 24-hour cycles of food availability were able to maintain core body temperature in response to cold challenge. Instead, the authors favored the third option and concluded that "Regulation against cold is more effective when animals are synchronized by periodic inputs from the environment" (Fuller, Sulzman and Moore-Ede 1978; p. 794).

The failure of squirrel monkeys in LL to defend core body temperature in response to cold challenge has been attributed to the desynchronization of the multi-oscillator circadian timing system of the squirrel monkey (Fuller, Sulzman & Moore-Ede, 1978; Fuller, Sulzman & Moore-Ede, 1979b; Moore-Ede, 1983b). Support for this idea has been provided by subsequent work showing that when internal desynchronization is forced in adrenalectomized squirrel

monkeys in LL by the infusion of cortisol for one hour per 24-hour period (the periodic infusion cortisol entrains a portion of the circadian system to a 24 hour period while the remainder expresses a period different from 24 hours), large falls in body temperature occurred whenever ambient temperature was reduced (Fuller, Sulzman & Moore-Ede, 1979b). In fact, in a group of squirrel monkeys in which internal desynchronization was forced in this way, maintenance of core body temperature in response to cold challenge was impaired to an even greater degree than in a group of monkeys just maintained in LL (Fuller, Sulzman & Moore-Ede, 1979b).

Objective

The finding that internal desynchronization is related to an impairment in the ability to maintain core body temperature in response to cold challenge in the squirrel monkey has never been replicated in another laboratory. Neither has the phenomenon been reported in another species. To this end two experiments were conducted using male Wistar rats. In each experiment, rats maintained in LL or a LD cycle, were subjected to a cold challenge. It was predicted, in light of the work previously reviewed, that

animals maintained in LL would, experience greater drops in core temperature in response to cold challenge than animals maintained in a LD cycle.

Experiment 1

Due to the thermal conductivity of water, small mammals are unable to maintain a constant core temperature when they are forced to swim unless the water temperature is very close to core temperature or the duration of the swim is very short (Dawson & Horvath, 1970). Thus, the half-hour immersion in water 10ºC below core temperature used in this experiment represented considerable thermal stress to the rat and provided sufficient opportunity to assess potential differences in ability to defend and recover core temperature. Rats were maintained in either constant illumination (LL) or on a normal light-dark schedule (LD).

Method

Subjects

Twenty-four male Wistar New Colony rats (Charles Rivers Breeding Laboratories, St. Constant, Quebec) were used. Their body weight upon arrival at the laboratory was between 275 and 300 grams. The animals were housed in the testing laboratory in

translucent plastic cages measuring 20 x 33 x 18cm, one animal per cage . The cage floor was covered with approximately one cm. of beta chips. The cage tops were stainless steel grids configured so as to hold a water bottle and food pellets. Standard lab chow pellets (AGWAY: ProLab) and water were available ad libitum. The room was maintained at 22 ºC. The experiment was run in two stages corresponding to animals kept in either LL or a LD 12/12 cycle. The lab was lit by overhead fluorescent lighting during the light phase (300 lux) (these lights were on continuously for the LL group) and by a 15 Watt red bulb (5 lux) during the dark phase of the cycle. During the LD cycle the lights came on at 0700 hours. Animals were kept housed as described for five weeks before the first cold water challenge test. Cleaning and maintenance was performed at irregular intervals and times of day.

Apparatus

The testing apparatus consisted of a set of cages of the type and dimensions described above. These were filled to within one centimeter of the rim with tap water (27 ºC). During the 30-minute test period the cage was covered with a transparent plastic cage measuring 39 x 50 x 20 to prevent the rat from escaping. One edge

of the cover was elevated above the rim of the test cage to allow for ventilation.

Measurement of Activity and Core Temperature. A telemetry system (Dataquest III) was used to collect activity and core body temperature data from each animal for the five weeks prior to the cold water challenge tests and during the cold water challenge tests. Samples of each variable (core temperature and activity) were taken at 10 minute intervals except during the cold water challenge tests when a sample of each variable were made every minute.

Procedure

Implantation of Mini-Mitter. Animals were allowed to adjust to the lab for 2 days before a telemetry transmitter (Mini-Mitter) was implanted in the intraperitoneal cavity. Prior to surgery, the area just lateral to either side of the midline of the abdomen was shaved. Animals were anesthetized with Methafane (Pitman-Moore Ltd./M. T. C., Pharmaceuticals, Mississaga, Ont.). An incision was made first through the skin then into the peritoneal cavity. The incision was of just sufficient size to allow passage of the Mini-Mitter into the peritoneal cavity. The muscle layer was then closed

with absorbable sutures and the skin with wound clips. Finally an antibiotic ointment (Panolog; Squibb, Canada Inc., Montreal, Quebec) was applied to the wound. Animals were then placed into a recovery cage for two hours before being returned to their home cages.

Cold Water Challenge Tests. Each group of rats (LL and LD) was further divided into two for the cold water challenge tests. One group was always tested during the light phase the other during the dark phase (for animals in LL correspondence to the LD cycle was used). Testing during the light phase commenced at 10 AM, and during the dark phase at 8 PM. Testing was conducted in the same room in which the rats were housed.

The testing procedure was as follows: A half-hour of baseline data were collected for core temperature and activity counts from each rat. At the end of the half-hour the rat was removed from the home cage and immersed in a previously prepared water bath (27 ºC), which was then covered as previously described. The water bath, like the home cage was positioned atop a telemetry receiver. The rat was left undisturbed in the water bath for 30 minutes. The rat was then removed from the water bath and returned to the home cage. A further one hour of data were collected from the rat as core

temperature returned towards normal. Each rat was cold water challenged on four occasions, with ten days between each session.

Two rats proceeded through the stages of the testing sequence simultaneously. Each pair of rats was entered into the testing sequence after the previous pair began the 30 minute session in the water bath. The testing procedure carried out in this way took 3 to 4 hours to complete. For each rat and each test, fresh water and clean cages were used.

Analysis of Data from the Cold Water Challenge Tests. The data from each test session were separated into three periods corresponding to baseline, test and recovery for each animal. The 30 temperature samples taken in the baseline period, were used to compute an average baseline core temperature for each animal. Similarly, averages for the final five minutes of the half-hour test period and the final five minutes of the one-hour recovery period were calculated separately for each animal. Due to equipment problems and the death of one of the animals, the total sample size was reduced from 24 to 20 animals or from six to five in each test condition.

Baseline core temperature data were analyzed in a 2X2X4

ANOVA. There were two between factors; lighting schedule (LD or LL) and time of day (AM or PM); and one within factor, testing session (4 levels). Core temperature data for the test and recovery period of the cold water challenge test were analyzed in a single 2X2X4X2 ANOVA. There were two between factors lighting schedule (LD or LL) and time of day (AM or PM); and two within factors, testing session (4 levels), and period (test or recovery).

Baseline activity data were analyzed in an ANOVA identical in structure to the one described for baseline core temperature data. Activity counts recorded per minute during the test period were summed and then averaged for the entire test period for each animal, data from the recovery period was similarly treated. Activity data for the test and recovery periods of the cold water challenge test were then analyzed in a single ANOVA of identical structure to the one described for the core temperature data.

Results and Discussion

Figures 1 through 4 represent examples of the daily rhythms in core temperature and activity of animals kept in either a LD cycle or LL for four weeks. Animals housed in the LD cycle exhibit a regular rhythm in core temperature and level of activity displaying one

clear peak and trough in each variable every 24-hours. In contrast, the animals housed in LL display multiple daily peaks and troughs in core temperature and level of activity per 24-hours (Figures 3 and 4). This visual interpretation is backed by the results of Fourier analysis which shows that there is one cycle in average core temperature and locomotor activity each 24-hours for the animals housed in the LD cycle and as many as six for those housed in LL (Figures 5 through 8).

The rhythm data for both groups are consistent with previous reports. A single oscillation in average core temperature and level of activity each 24-hours is normally associated with maintenance in a LD cycle (Aschoff, 1970). Similarly the breakdown of clear 24-hour rhythmicity in rats housed in LL has been observed (Eastman & Rechtschaffen, 1983; Honma & Hiroshige, 1978a, 1978b).

Analysis of the baseline core temperature data for the cold water challenge tests did not yield any significant results (Appendix A). This was unexpected. In nocturnal rodents such as the rat higher average core temperatures are associated with the dark portion of the daily light cycle (Abrams & Hammel, 1965). No significant difference in average baseline core temperature was observed as a

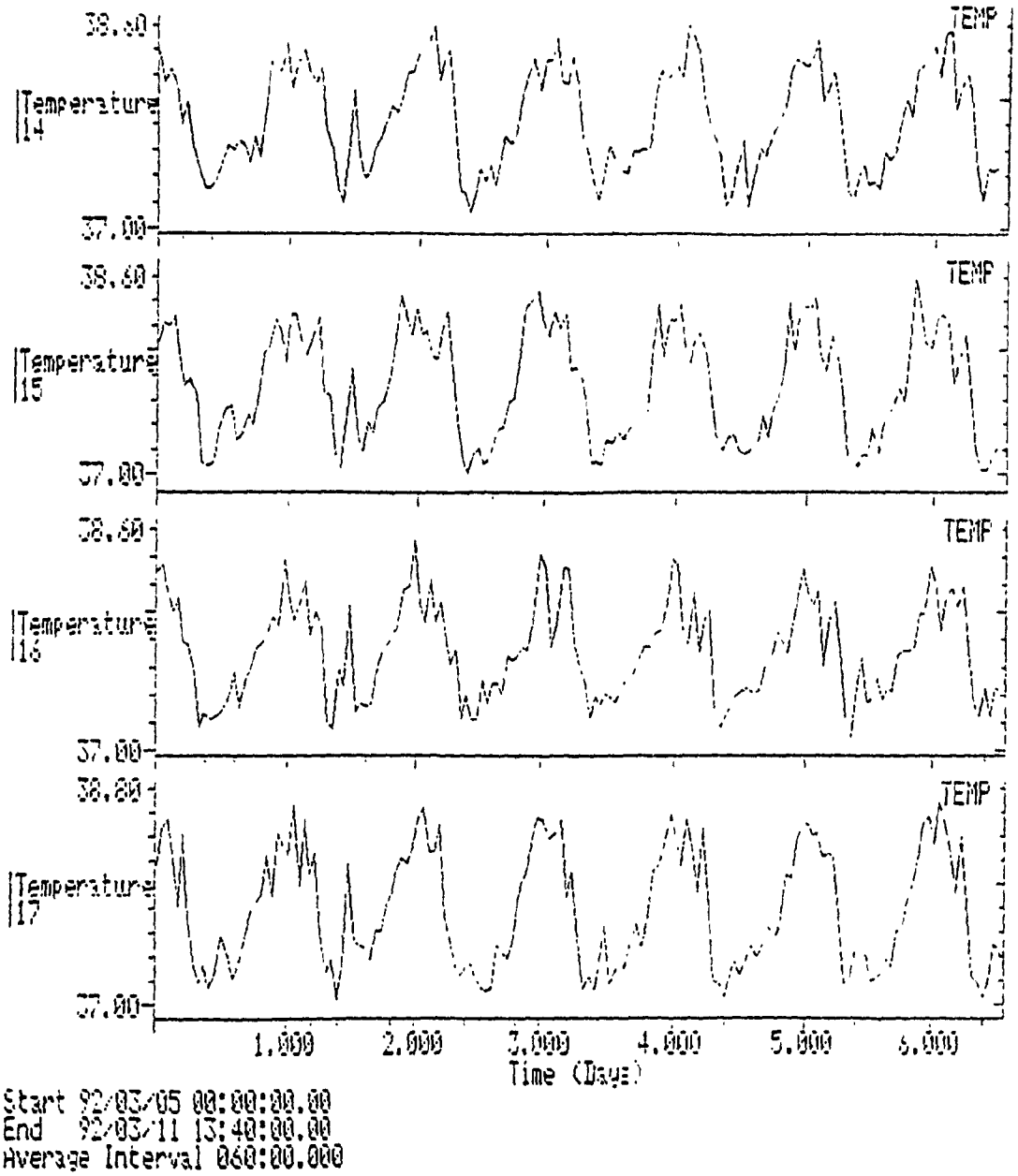


Figure 1 Temperature rhythm of four rats in their fourth week of exposure to a light-dark cycle (LD 12/12). Traces were clipped at 37.0 ºC and 38.8 ºC.

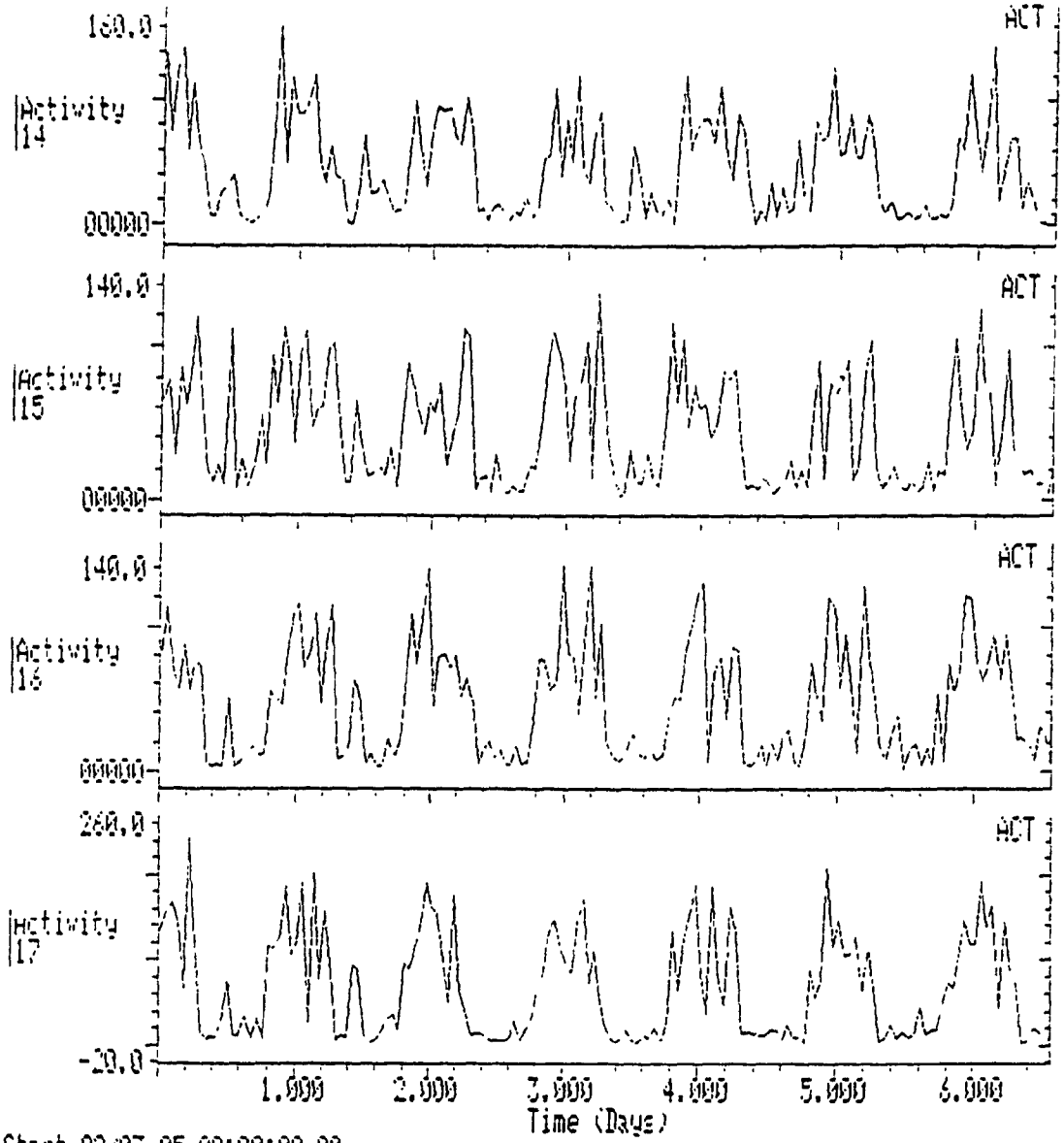


Figure 2. Activity rhythm of four rats in their fourth week of exposure to a light-dark cycle (LD 12/12).

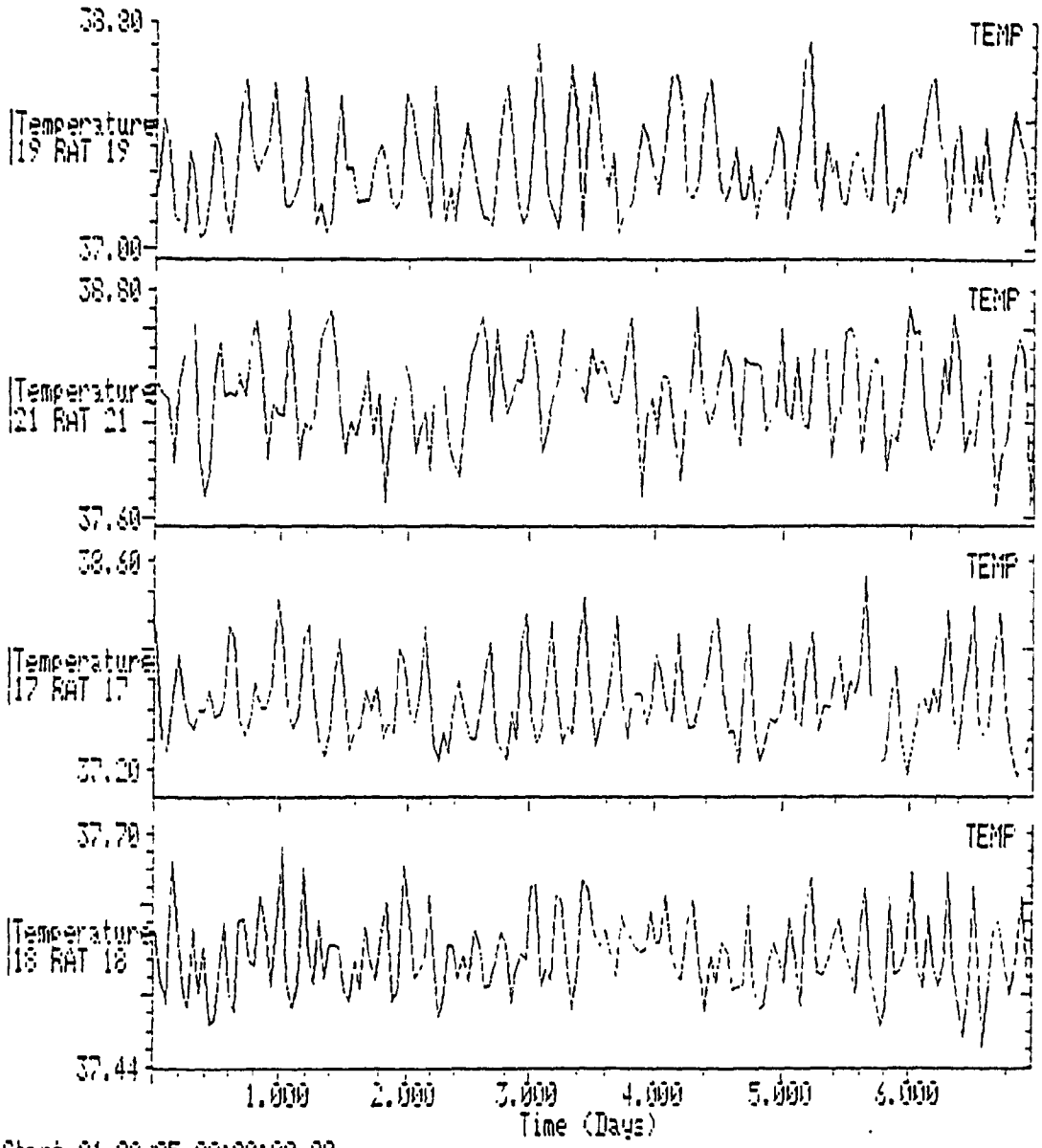


Figure 3 Temperature rhythm of four rats in their fourth week of exposure to constant illumination. Traces were clipped at 37.0 ºC and 38.8 ºC.

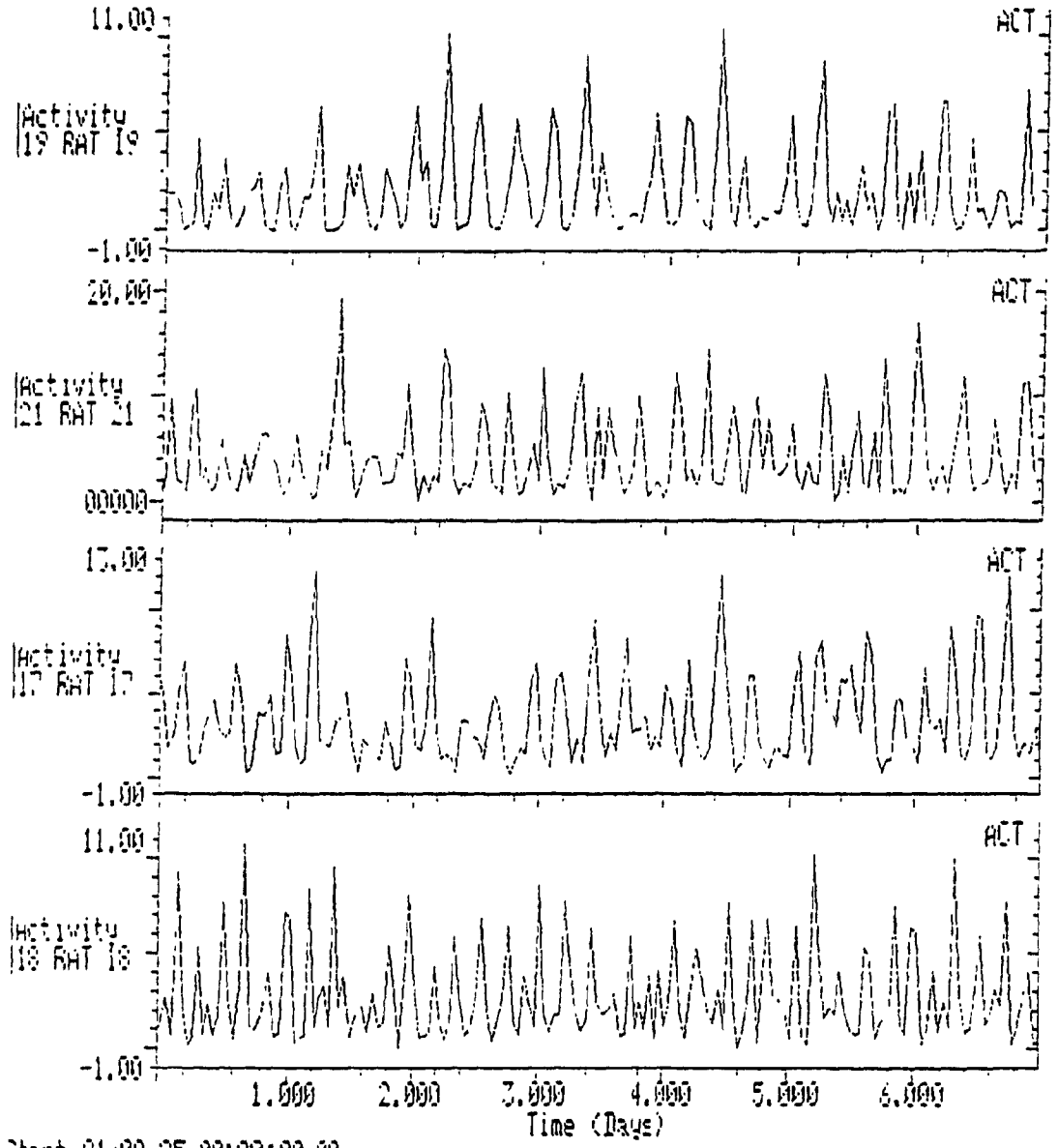


Figure 4 Activity rhythm of four rats in their fourth week of exposure to constant illumination.

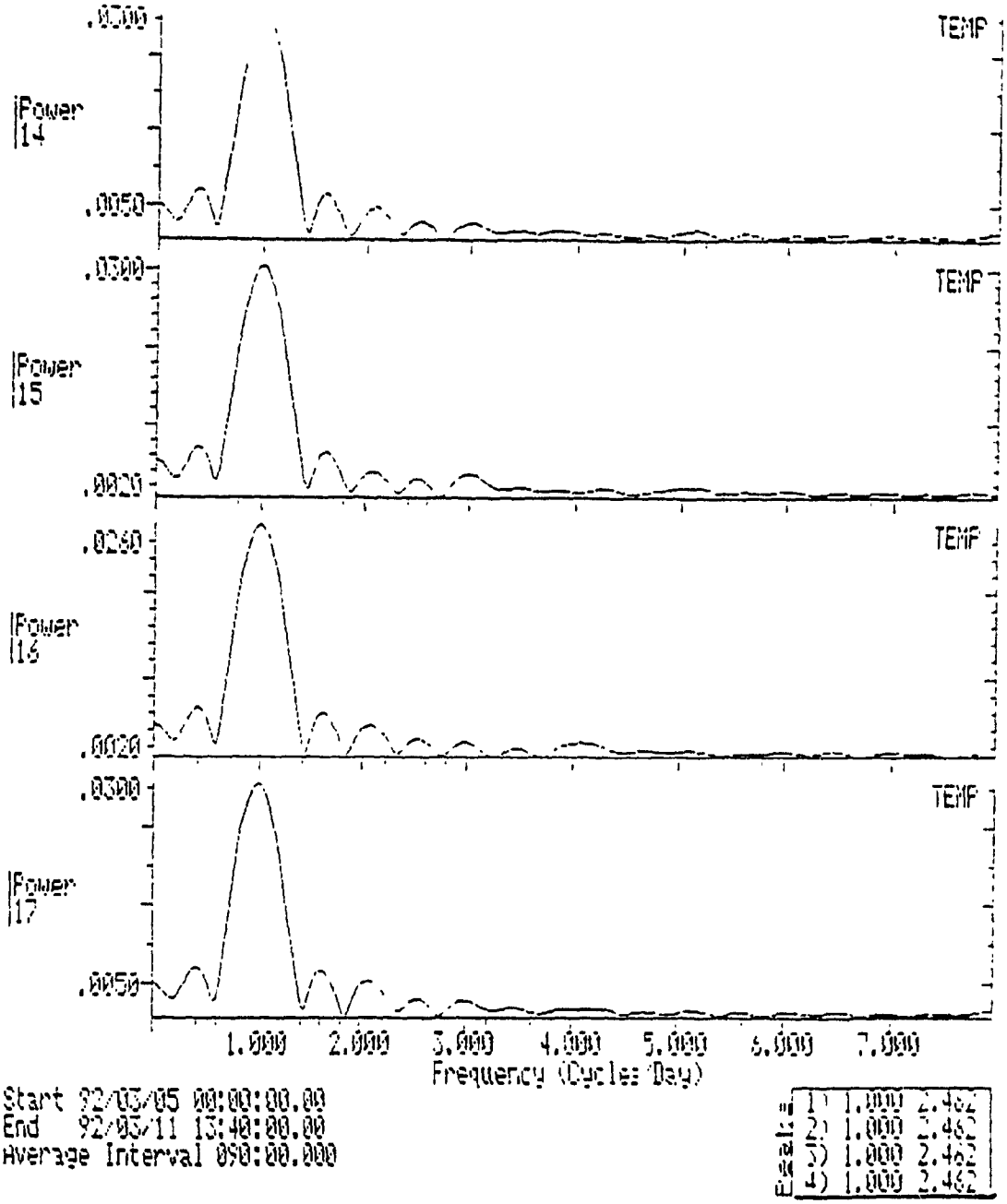


Figure 5 Results of Fourier analysis of core temperature data from four rats in their fourth week of exposure to a light-dark cycle (LD 12/12).

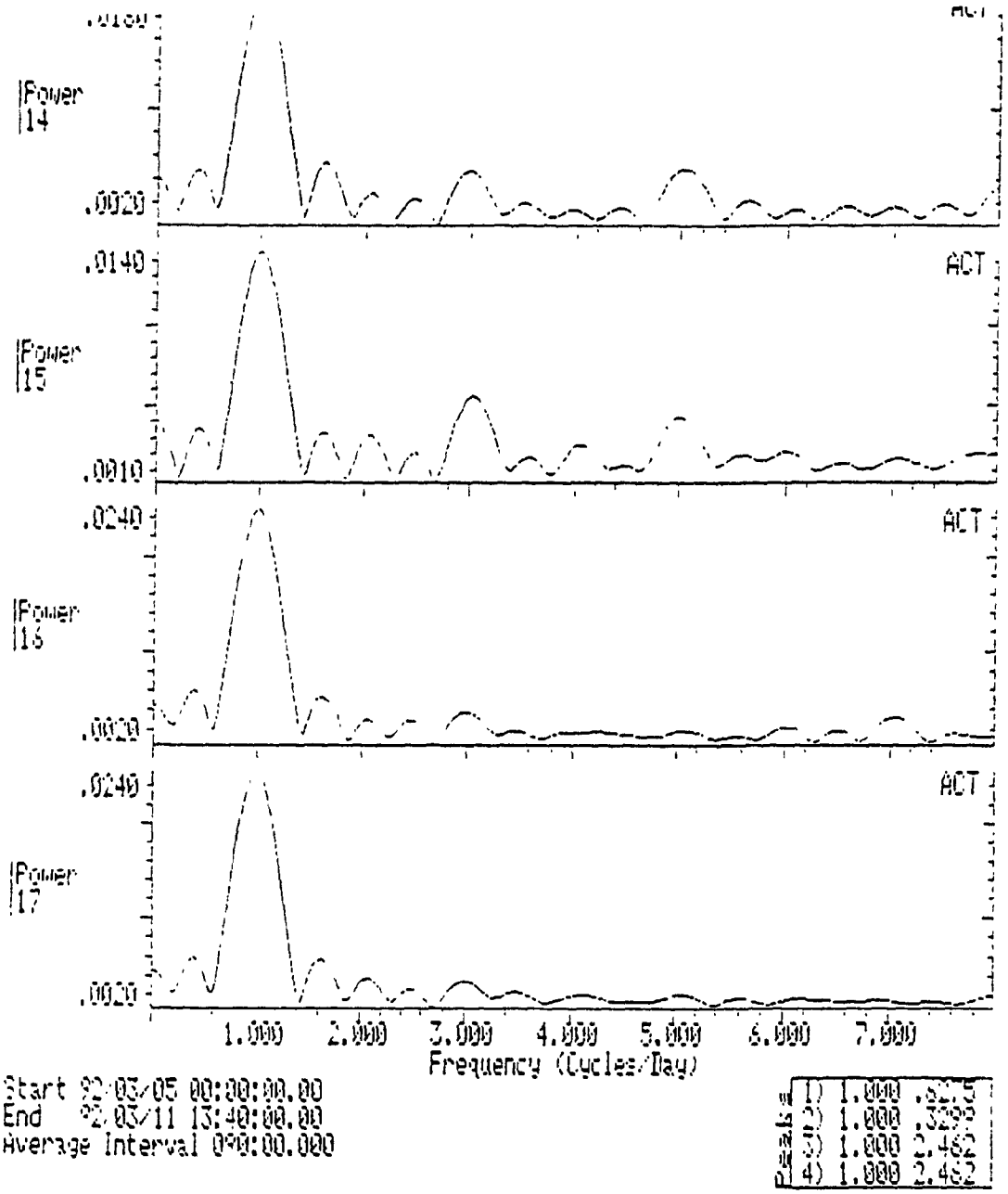


Figure 6 Results of Fourier analysis of activity count data from four rats in their fourth week of a light-dark cycle (LD 12/12).

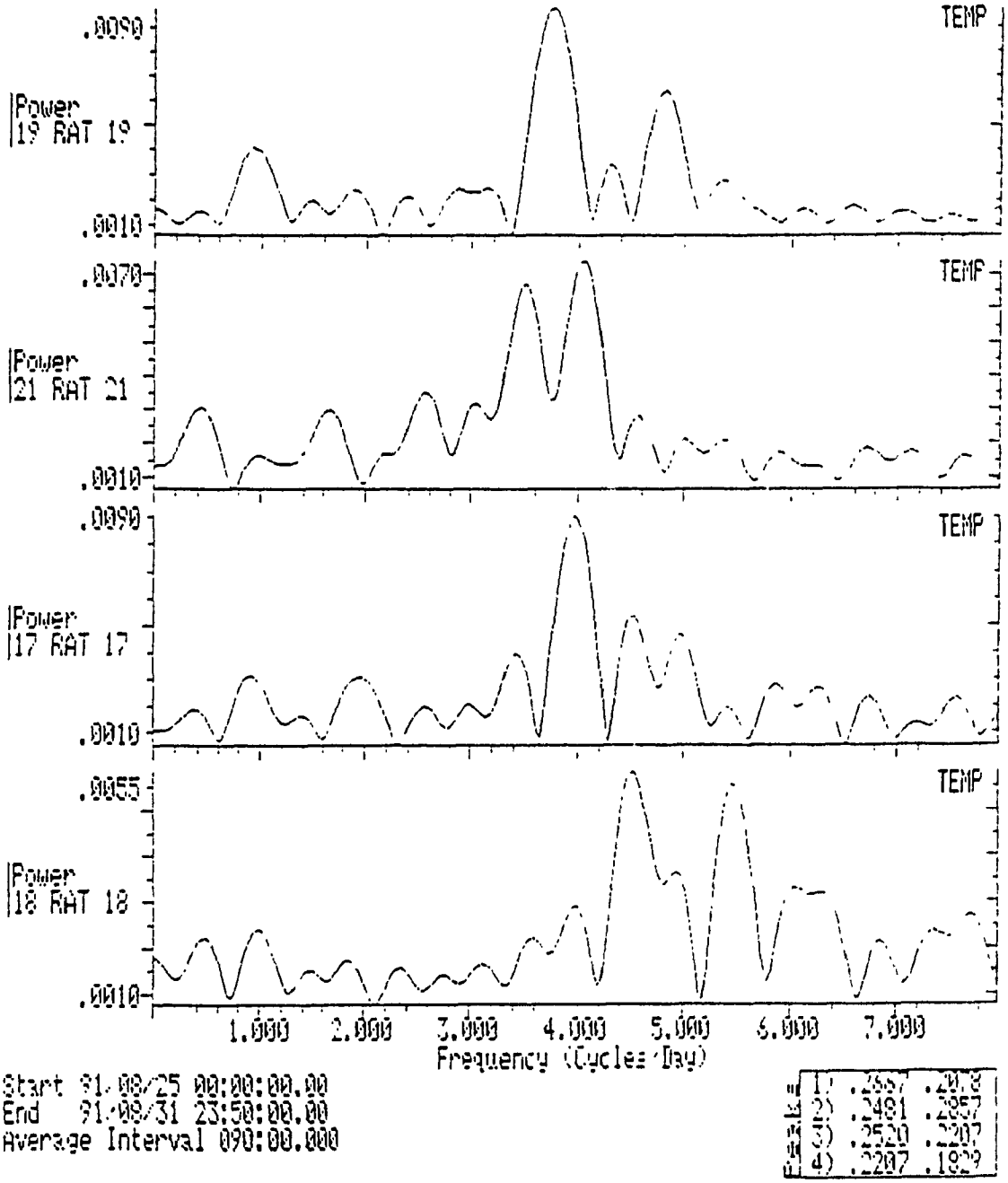


Figure 7 Results of Fourier analysis of core temperature data from four rats in their fourth week of exposure to constant illumination.

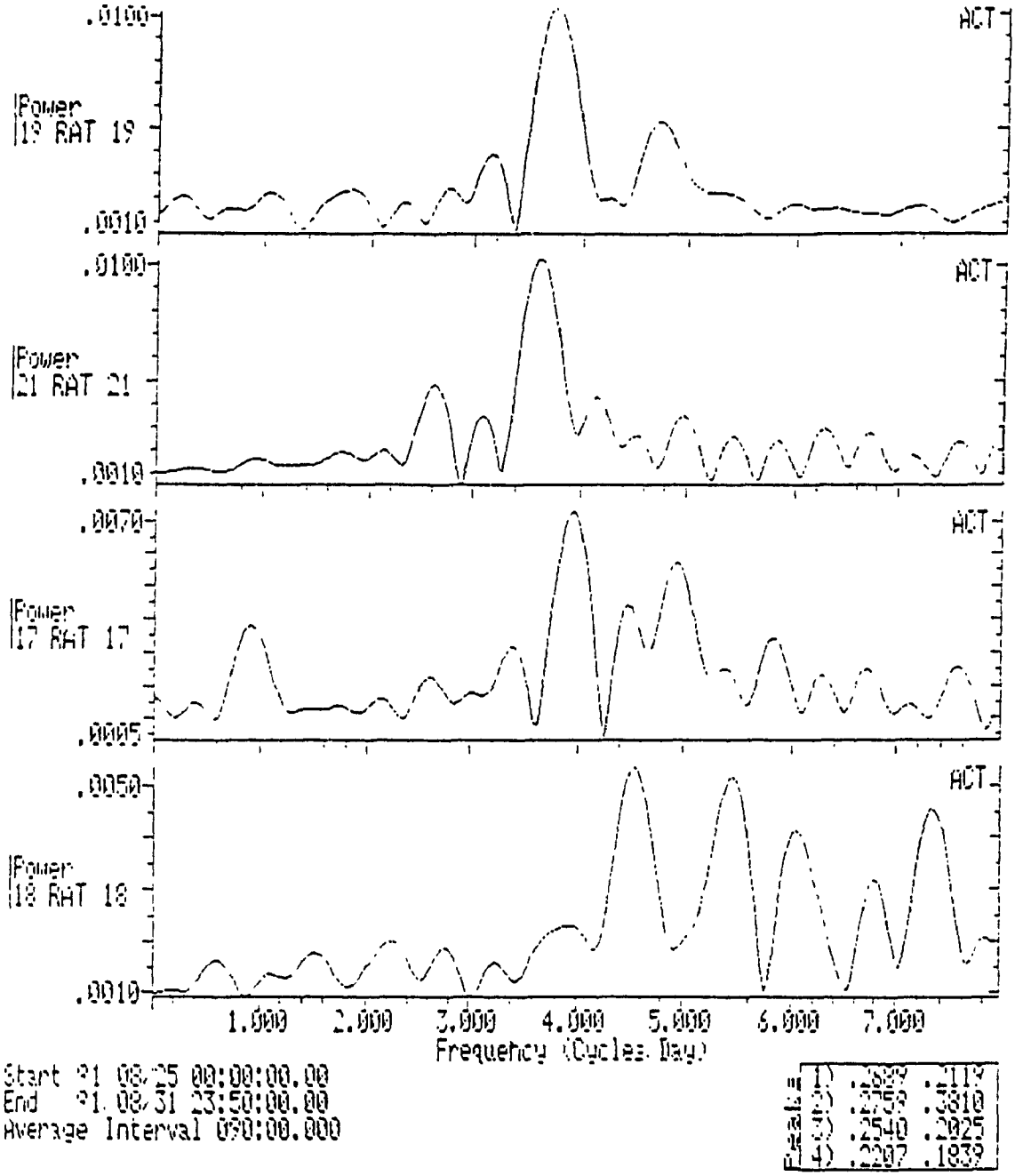


Figure 8 Results of Fourier analysis of activity count data from four rats in their fourth week of exposure to constant illumination.

function of the phase of the light cycle an animal was cold water challenged in. This may have occurred because a light-dark difference in average core temperature in the LD groups was obscured by the absence of differences in baseline core temperature by time of day tested in the LL groups. This possibility does not receive strong support from the data as the lighting group by time of day interaction was not significant (Appendix A). However there is a greater mean difference in core body temperature between AM and PM tests in the LD group (Table 1).

Analysis of the baseline activity data for the cold water challenge tests produced two significant effects (Appendix B). These were a main effect for lighting group (LL versus LD), $F(1, 16) = 3.336$, $p < .05$, and an interaction between lighting group (LL or LD) and time of day tested (AM or PM), $F(1, 16) = 24.221$, $p < .05$. The LD group was significantly more active than the LL group (LL, $M = 8.51$, $SD = 7.46$; LD, $M = 10.64$, $SD = 7.93$). This is consistent with previous work which has shown that activity is reduced in nocturnal species maintained in LL (Pittendrigh, 1967a). The significant interaction between lighting group and time of day tested can be attributed to a significant AM, to PM increase in activity exhibited

Table 1

Mean Core Temperatures at Baseline for LL and LD Rats for the AM and PM Tests, Experiment 1.

Group	Mean	Standard Deviation
LL AM	38.39	.50
LL PM	38.30	.49
LD AM	38.08	.56
LD PM	38.47	.44

Table 2

Mean Activity Counts at Baseline for LL and LD Rats for the AM and PM Tests, Experiment 1.

Group	Mean	Standard Deviation
LL AM	10.59	9.11
LL PM	6.42	4.70
LD AM	6.99	3.47
LD PM	14.28	9.44

by the LD group but not the LL group (Table 2). These results were expected as the LD group should demonstrate a clear daily oscillation in average level of activity which is tied to the light cycle with higher levels of activity being observed in the dark phase of the light cycle (Bolles & Duncan, 1969). The lack of an AM, PM difference in activity in the LL group was also expected as daily rhythms of activity have been reported to disappear in rats in LL (Honma & Hiroshige, 1978a).

Figures 9A through D illustrate the results of the cold water challenge tests for core body temperature data. In each cold water challenge test core temperature drops below baseline while the animal is in the water bath and returns close to baseline by the end of the hour-long recovery period. Significant differences between groups (LL and LD) in maintenance and recovery of core temperature in the cold water challenge test were not observed. Rats housed in LL were as efficient as those housed in LD in maintaining and recovering core temperature in the cold water challenge test $\underline{F}(1, 16) = 3.17$, $p > .05$ (Figures 9A-D, 10; Appendix C). The failure to find significant differences between groups is reflected in the variable rank order of the groups in maintenance and recovery of

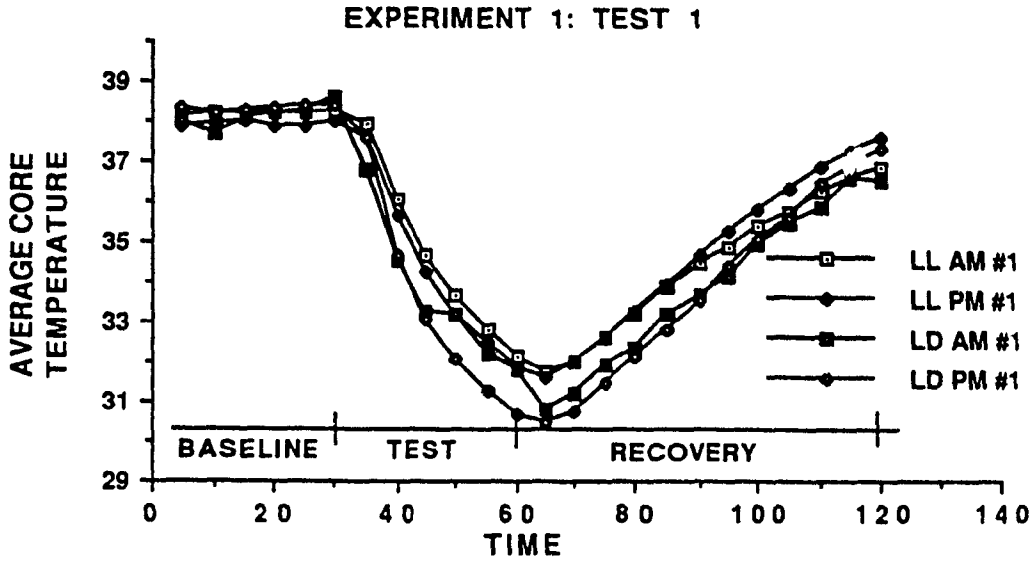


Figure 9A. Average core temperature for the three phases of Experiment 1, session 1. Time scale is in minutes.

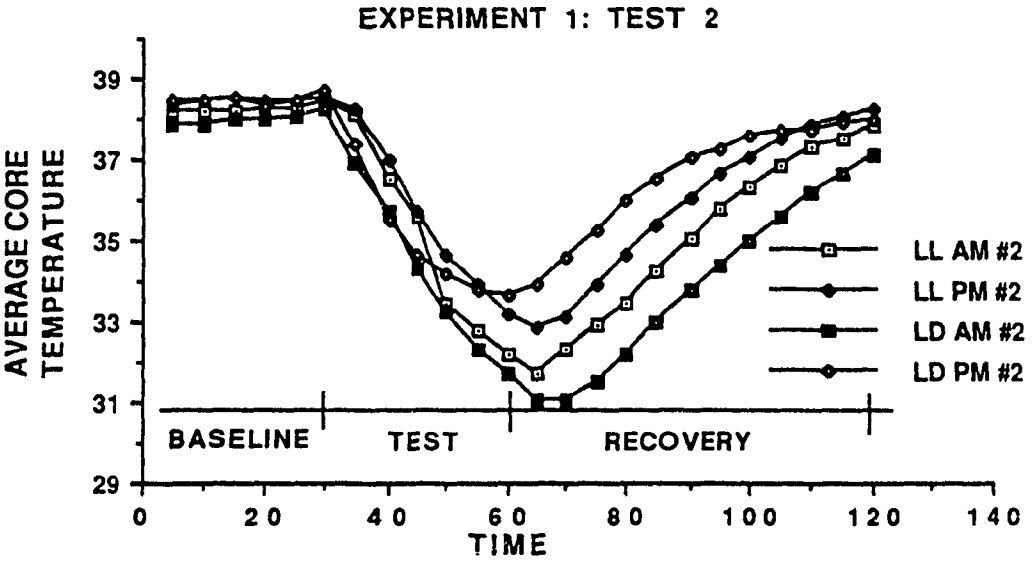


Figure 9B. Average core temperature for the three phases of Experiment 1, session 2. Time scale is in minutes.

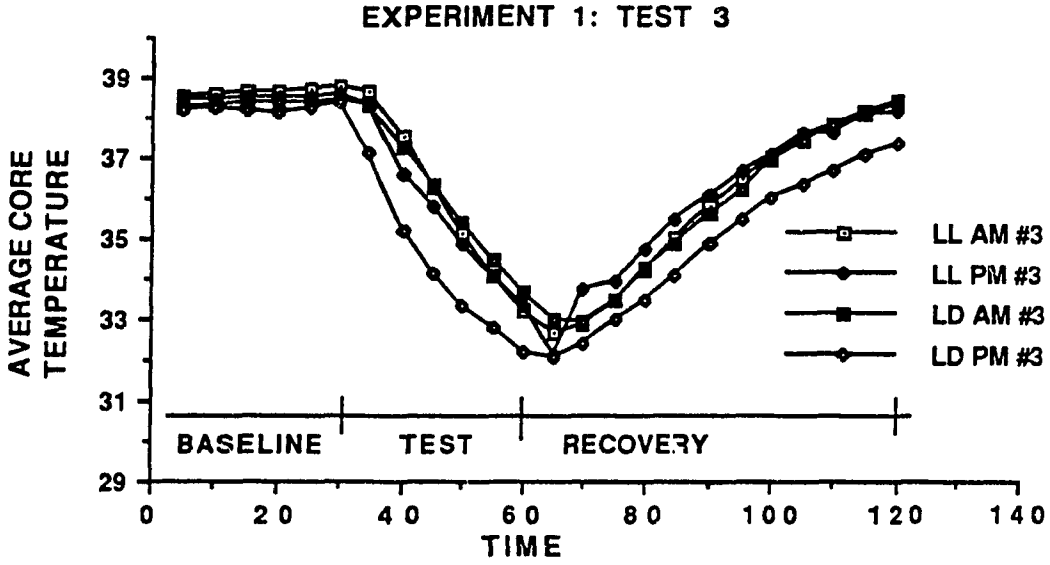


Figure 9C. Average core temperature for the three phases of Experiment 1, session 3. Time scale is in minutes.

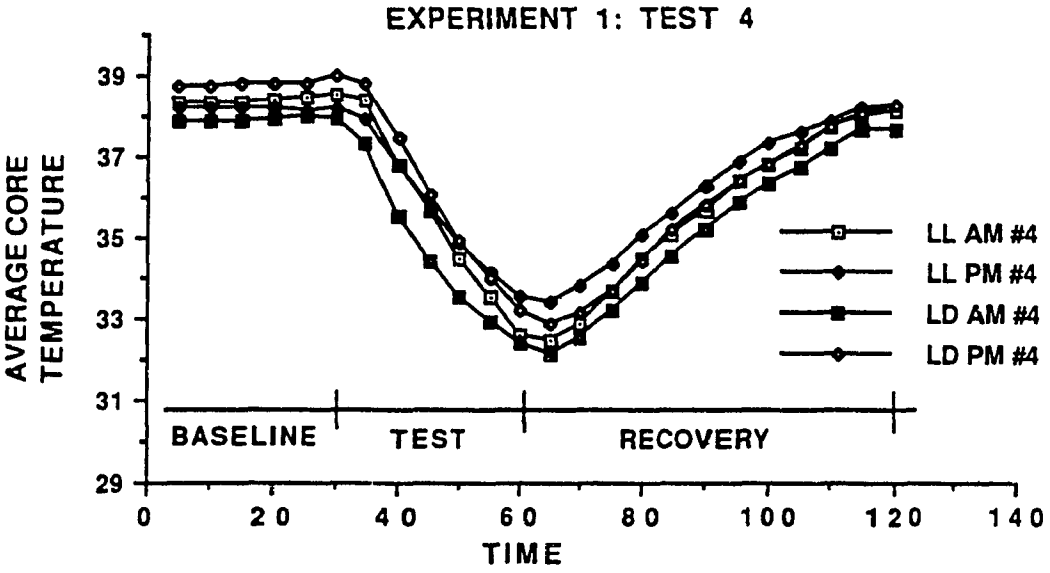


Figure 9D. Average core temperature for the three phases of Experiment 1, session 4. Time scale is in minutes.

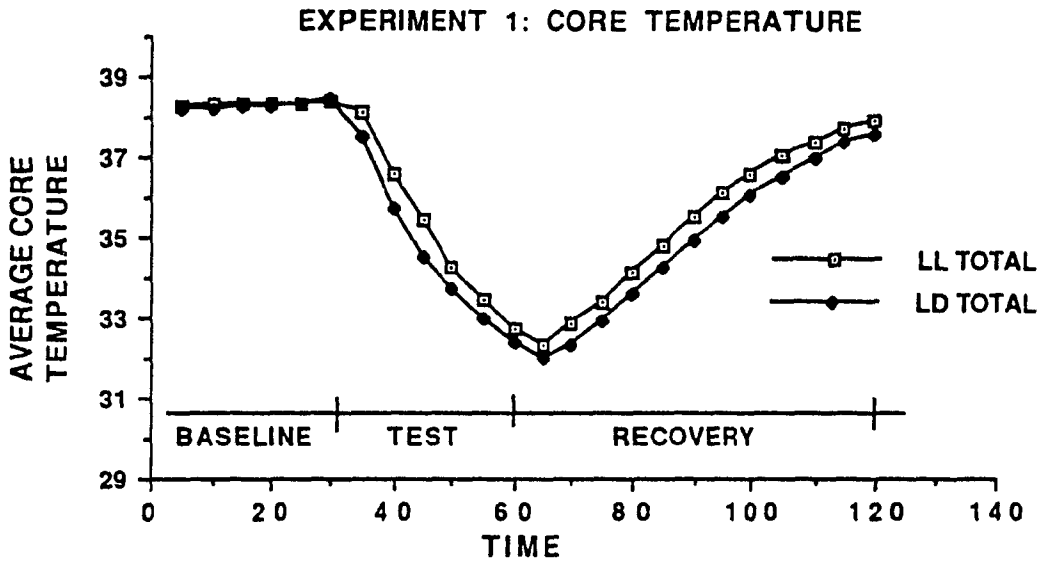


Figure 10. Core temperature data averaged over the AM and PM testing groups and the four testing sessions of Experiment 1. Time scale is in minutes.

core temperature across the four cold water challenge tests (Figures 9A-D).

Despite the failure to find group differences in the maintenance and recovery of core temperature in the cold water challenge test, four other effects were significant. These were testing session $F(3, 48) = 9.557$, $p < .05$; period $F(1, 16) = 2827.73$, $p < .05$; and interactions between testing session and time of day tested $F(3, 48) = 3.29$, $p <. 05$; and testing session, period and time of day tested $F(3, 48) = 8.99$, $p < .05$ (Appendix C).

Maintenance and recovery of core temperature improved significantly across testing sessions for rats housed in LL and LD (Figures 11, and 9A-D). The bulk of this improvement is made between test 1 and test 2 with little improvement being made thereafter (Table 3). Why this effect may have been observed will be discussed in conjunction with the activity data from the cold water challenge tests.

The significant effect for period simply reflects that the animals were warmer at the end of the recovery period than at the end of the test period. The other significant results, interactions between testing session and time of day tested and testing session,

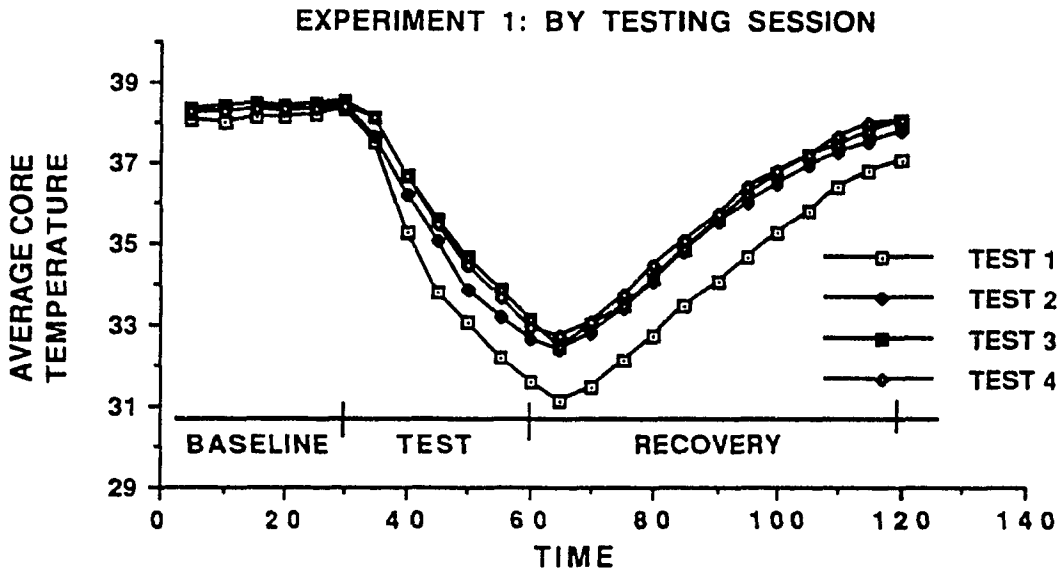


Figure 11. Core temperature for each testing session averaged across illumination groups (LL and LD), and the time of day tested (AM or PM). Time scale is in minutes.

Table 3

Mean Core Temperature Maintained and Recovered for Each Testing Session Across Groups (LL and LD), and Time of Day Tested (AM and PM), Experiment 1

Test	Mean	Standard Deviation
Test 1	34.36	3.05
Test 2	35.32	2.75
Test 3	35.53	2.70
Test 4	35.59	2.76

period and time of day tested were not pursued as neither accounted for as much as 5% of the total variance (SS Test Session X Time of Day/ SS Total = .01; SS Test Session X Period X Time of Day / SS Total = .006).

Figures 12A through D illustrate the results of the cold water challenge tests for activity data. In each cold water challenge test, activity initially increases over baseline level when the animal is placed in the water bath then returns to a level close to that observed during the baseline period. Activity again transiently increases above baseline levels immediately after removal from the water bath before returning to level close to baseline for the remainder of the recovery period. The results of the analysis of activity data for Experiment 1 are contained in Appendix D.

Subjects maintained in LL were significantly less active in the cold water challenge test than subjects maintained in LD $F(1, 16) = 8.786$, $p < .05$ (Figure 13). This is consistent with what was observed in the baseline period. This difference between groups may be attributed to the general reduction of activity in nocturnal species in LL (Aschoff, 1960; Pittendrigh, 1967a).

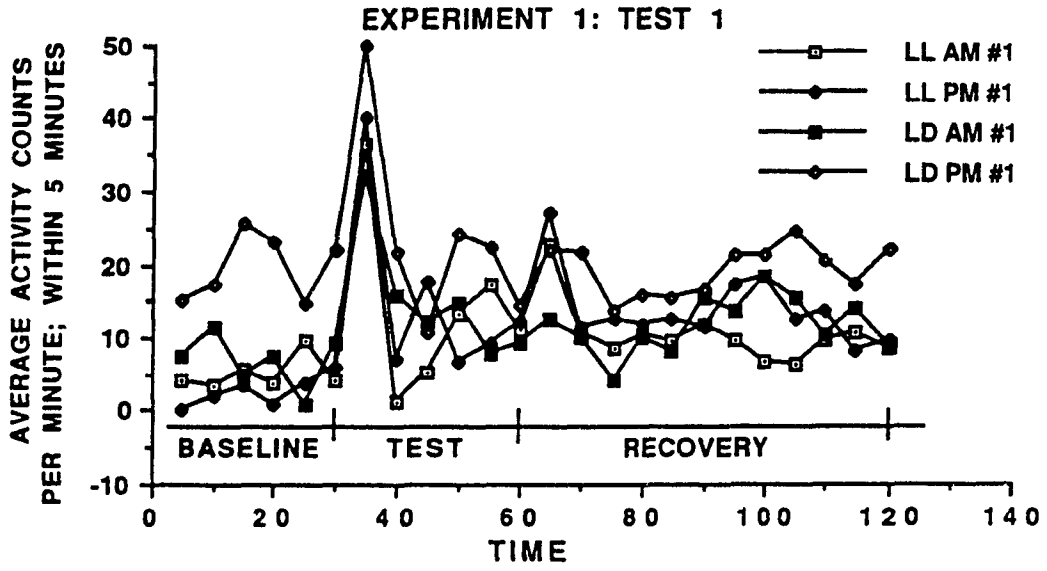


Figure 12A. Average activity counts per minute within a five minute interval for the three phases of Experiment 1, testing session 1. Time scale is in minutes.

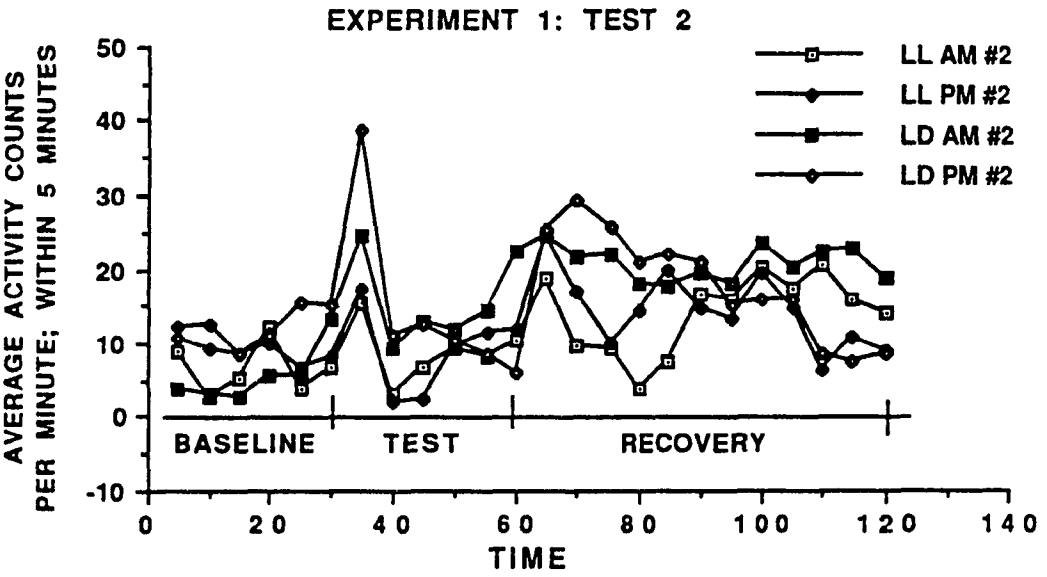


Figure 12B. Average activity counts per minute within a five minute interval for the three phases of Experiment 1, testing session 2. Time scale is in minutes.

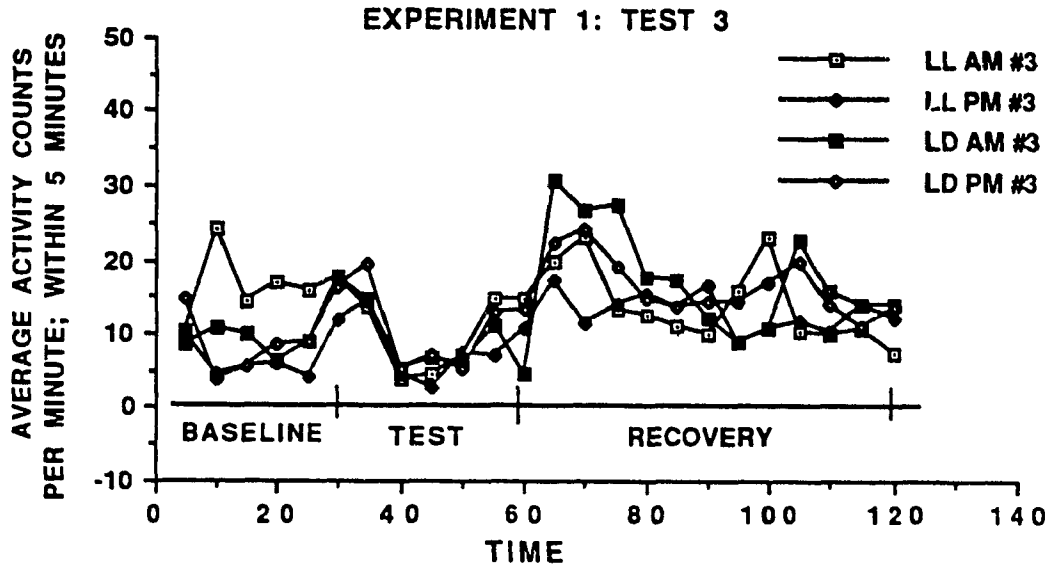


Figure 12C. Average activity counts per minute within a five minute interval for the three phases of Experiment 1 testing session 3. Time scale is in minutes.

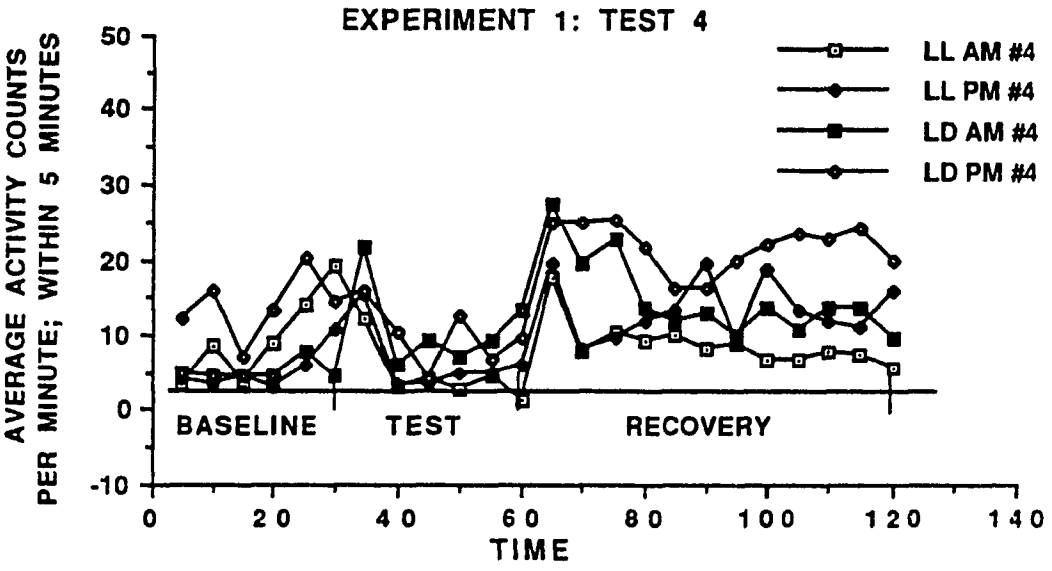


Figure 12D. Average activity counts per minute within a five minute interval for the three phases of Experiment 1, testing session 4. Time scale is in minutes

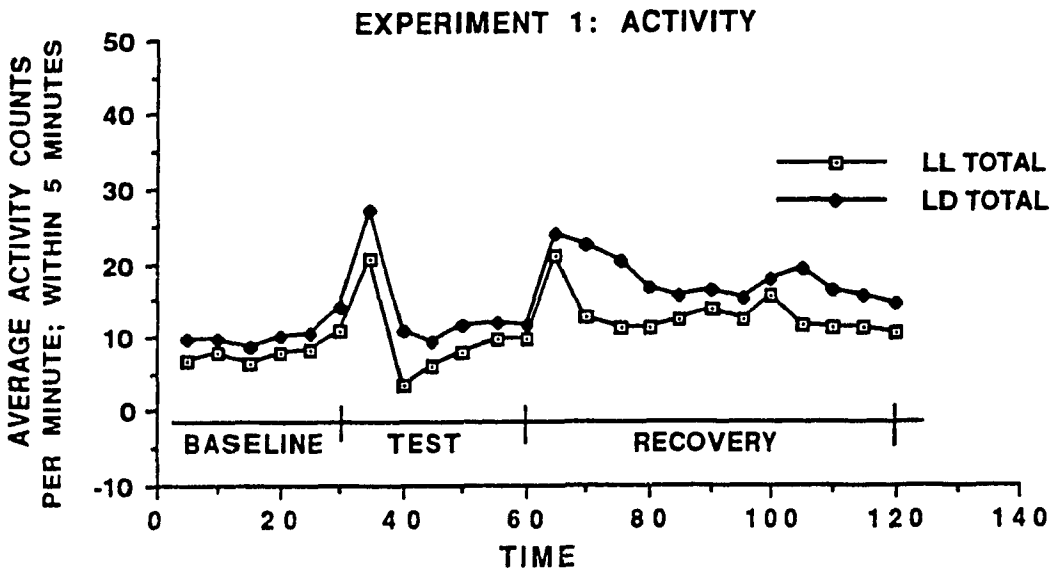


Figure 13. Activity data averaged over AM and PM testing groups and the four testing sessions of Experiment 1. Time scale is in minutes.

The average level of activity in the cold water challenge test declined significantly across testing sessions for animals housed in LL and LD (Figures 12A- D, 14; Appendix D). The decline in activity across the four testing sessions of the cold water challenge test was not consistent over the test and recovery periods. This is reflected in the significant test by period interaction (Appendix D). Average level of activity declines across the four test periods of the cold water challenge test but remains relatively stable across the four recovery periods (Table 4).

The reduction of activity during the test portion of the cold water challenge test in sessions subsequent to the first is consistent with data reported by other authors (Prosolt , Bertin, Blavet, Deniel & Jalfre, 1979). These authors have reported that rats and mice will become relatively immobile when forced to swim in a restricted space. The onset of relative immobility is more rapid and complete in test sessions subsequent to the first (Prosolt, 1979). It has been argued that the immobility seen in the second and subsequent sessions represents a state of behavioral despair (Prosolt, Le Pichon, Jalfre, 1977) analogous to learned helplessness (Seligman, 1976). Other authors have argued that immobility

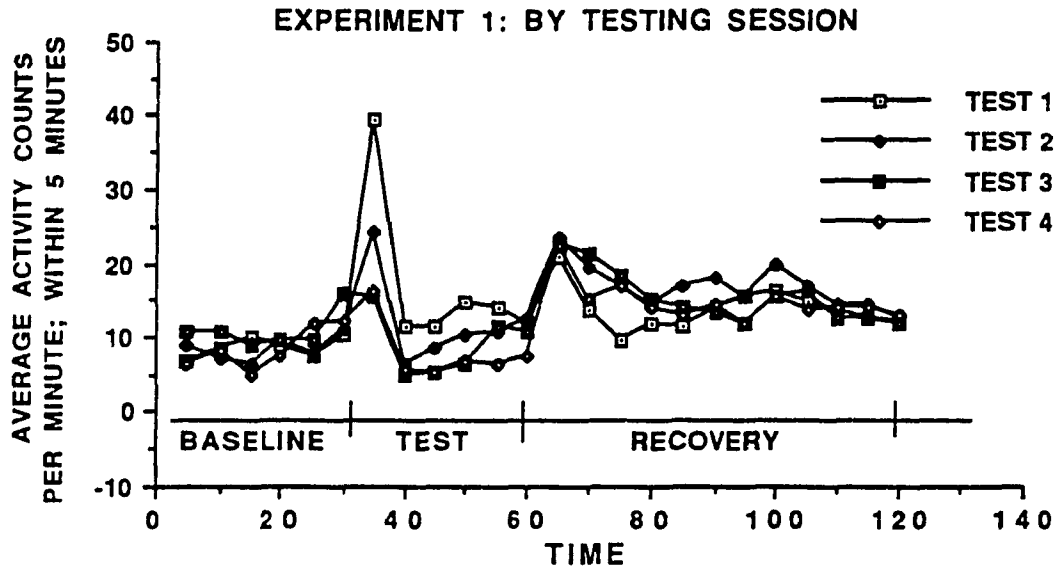


Figure 14. Activity data for each testing session averaged across illumination groups (LL and LD), and the time of day tested (AM and PM). Time scale is in minutes.

Table 4

Mean Activity Counts for Test Session and Recovery in Each Testing Session Across Groups (LL and LD), and Time of Day Tested (AM and PM)

Test	Test		Recovery	
	Mean	Std. Dev.	Mean	Std. Dev.
Test 1	16.54	9.09	14.68	7.04
Test 2	11.60	6.62	17.44	6.54
Test 3	9.54	6.39	15.14	4.19
Test 4	8.32	4.85	14.82	8 54

is an adaptive response (Nishimura, Tsuda, Oguchi, Ida & Tanaka, 1988). The results of this study concur with this view. While the level of activity in the test portion of the cold water challenge test is declining, the mean core temperature maintained while the animal is in the water is increasing (r = -.49). This suggests that immobility while the animal is in the water represents an adaptive response which aids in the maintenance of core body temperature.

The average level of activity for subjects housed in both LL and LD is significantly lower in the test period than in the recovery period over the four occasions subjects were tested. The mean level of activity during the test period was 11.496 (S = 7.471) and during the recovery period 15.521 (S = 6.727). The effect is not however consistent across testing sessions, hence the significant period by testing session interaction which has already been reported and discussed.

The interaction between testing session and time of day was not pursued further as this effect accounted for less than 5% of the total variance (SS test session X time of day/ SS total = .044).

The results of this experiment did not support the hypothesis that rats housed in LL would be less efficient in maintaining and

recovering core temperature in response to cold challenge than rats housed in a LD cycle. No significant differences in maintenance and recovery of core temperature in the cold water challenge test were seen between subjects housed in LL and LD (Figure 10). This finding appears to contradict previous studies (Fuller, Sulzman & Moore-Ede, 1978, 1979b) which reported that maintenance of core temperature in squirrel monkeys maintained in LL was impaired relative to animals maintained in LD.

The variance between the current and previously published data might be attributable to a number of factors. These factors can in turn be grouped into one of two general categories: 1) squirrel monkeys and rats differ in important ways with respect to the input of the circadian system to the thermoregulatory system and/or thermoregulatory capacity; 2) the cold challenge used in this experiment either provided opportunity to employ thermal effectors not available in the other protocol or that the nature of the test provoked a different kind or class of response. The latter possibility was examined in Experiment 2.

Experiment 2

The cold water challenge test used in Experiment 1 may have represented a very different kind or degree of cold stress than that used in the studies with squirrel monkeys (Fuller, Sulzman & Moore-Ede, 1978, 1979b). As described by Fuller, Sulzman and Moore-Ede (1978, p. 794), "The monkeys were subjected to mild 6-hour cold exposures". The drop in ambient temperature in these studies from 28 ºC to 20 ºC resulted in at most a 2 ºC drop in core temperature in the squirrel monkeys (Fuller, Sulzman & Moore-Ede, 1978, 1979b). In contrast, the cold water challenge test used in Experiment 1 produced marked hypothermia with drops in core temperature as large as 7 ºC being observed (Figure 9A). In addition, the cold water challenge test may have provoked emotional stress not present in the studies with squirrel monkeys, in that declines in ambient temperature are not accompanied by an innate fear response to drowning (Dawson & Horvath, 1970). For these reasons a second experiment, the refrigerator cold challenge test was conducted. It was predicted that rats housed in LL for five weeks prior to the first refrigerator cold challenge test would maintain a lower average core temperature than rats housed in a LD cycle for five

weeks prior to the first test.

Methods

Subjects A total of 24 male New Colony Wistar rats (Charles Rivers Breeding Laboratories, St. Constant, Quebec) were used. As before subjects were housed in either constant light (LL) or a LD 12/12 (LD) cycle (N=12/group). All aspects of housing and protocol prior to the commencement of testing were identical to those described for Experiment 1.

Apparatus The testing apparatus consisted of large display case type refrigerator (Foster; model GH-45-G-T). Ventilation was provided by two open ports, one on each lateral aspect of the refrigerator with a diameter of 4cm.

Procedure

Refrigerator Cold Challenge Test. Each group of rats (LL and LD) was divided in two and each subgroup run either in the light or dark phase of the cycle (for animals in LL correspondence to the LD cycle was used).

The cold challenge involved the placement of each subgroup of animals in the refrigerator for the 12 hour duration of the phase of the light cycle in which they were tested. Cages in the

refrigerator, as in Experiment 1, were positioned atop a telemetry receiver. Records of core body temperature and locomotor activity were logged by Dataquest III. A ten minute sampling period was used for all phases of data collection in Experiment 2.

The refrigerator was set to maintain a temperature of 6 ºC. Each group of animals was tested on two occasions which were separated from each other by a ten day period. A neon tube in the refrigerator was used for the illumination of the refrigerator (300 lux) interior for tests in the light phase and for the LL groups. Equipment failures resulted in a reduction in sample size from six animals per lighting schedule (LL or LD) and time of day tested (AM or PM) to four.

Data Analysis. Averages of core temperature and activity were computed for each hour of the testing session and one hour prior to the beginning of the session. Data from the hour before the test session was used as a baseline comparison value. Activity and core body temperature data were analyzed in separate ANOVAs.

Data for core temperature and locomotor activity were each analyzed in a 2X2X2X13 ANOVA. The effects examined in the analysis corresponded to the following factors; lighting schedule (LL

or LD), time of day tested (AM or PM), testing session (one or two), and time elapsed (from the hour of baseline prior to placement in the refrigerator, through the twelve hour duration of the test).

Results and Discussion

Rhythm data for Experiment 2 were consistent with Experiment 1. Animals housed in the LD cycle displayed regular 24-hour oscillations in mean level of activity and core body temperature whereas animals housed in LL displayed multiple cycles in both variables over a 24-hour period.

Figures 15 A and B illustrate the results for core body temperature data for the refrigerator cold challenge test. No significant differences in maintenance of core temperature between animals kept in LL and LD were observed (Figure 16) $F(1, 12) = .44$, $p > .05$ (Appendix E). As was the case in Experiment 1, rank order of the groups was not maintained across testing sessions (Figure 15, A and B).

Three interaction terms for core temperature data from the refrigerator cold challenge test did achieve significance, $p < .05$. These were time elapsed by lighting schedule, time elapsed by time of day and time elapsed by lighting schedule by time of day

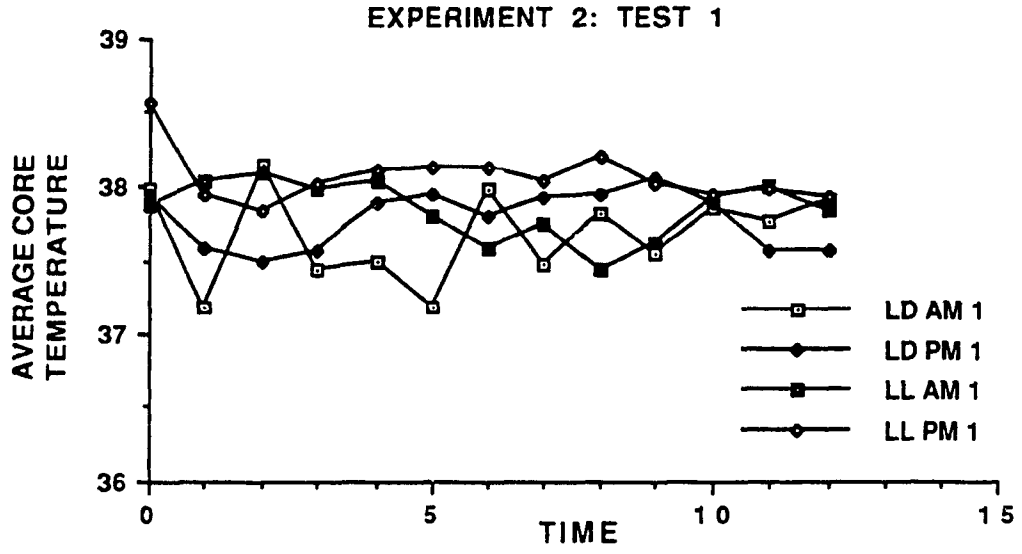


Figure 15A. Average core temperature per hour for testing session1, of Experiment 2. Time scale is in hours.

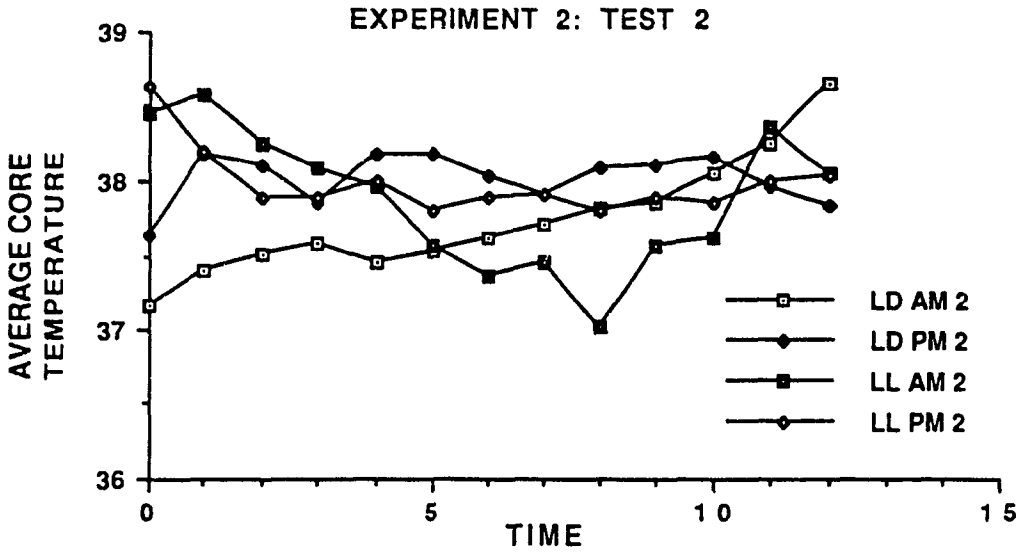


Figure 15B. Average core temperature per hour for testing session 2, of Experiment 2. Time scale is in hours.

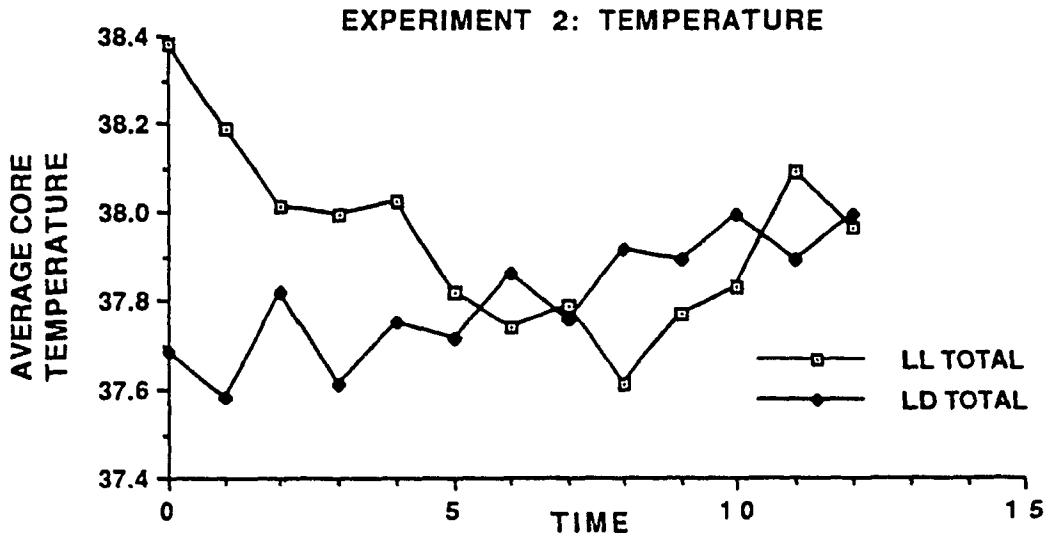


Figure 16. Average core temperature for Experiment 2 averaged over the time of day tested and testing session. Time scale is in hours.

(Appendix E). None of these interactions accounted for as much as 5% of total variance and were not pursued further.

Figures 17A and B illustrate the activity data for the refrigerator cold challenge test. Analysis of these data produced three significant results, an interaction between time of day tested and lighting schedule, $F(1, 12) = 5.87$, $p < .05$, an interaction between test (one or two) and lighting schedule, $F(1, 12) = 4.73$, $p < .05$ and a main effect for time elapsed, $F(12, 144) = 1900.59$, $p <. 05$. Group differences (LL versus LD) were not observed, $F(1, 12) = .84$, $p>.05$ (Appendix F).

Neither of the interactions (time of day tested by lighting schedule and test by lighting schedule) accounted for as much as 5% of total variance and so were not pursued further. The main effect for time elapsed is illustrated in Figure 18. The general downward trend in level of activity over the course of the testing session was exhibited by both groups (LL and LD) in each of the testing sessions. The reduction in activity over the course of the test session may represent the adoption of a cold defense posture. When exposed to cold environments, rats assume a ball-like posture which conceals appendages from direct exposure to the cold and thereby reduces

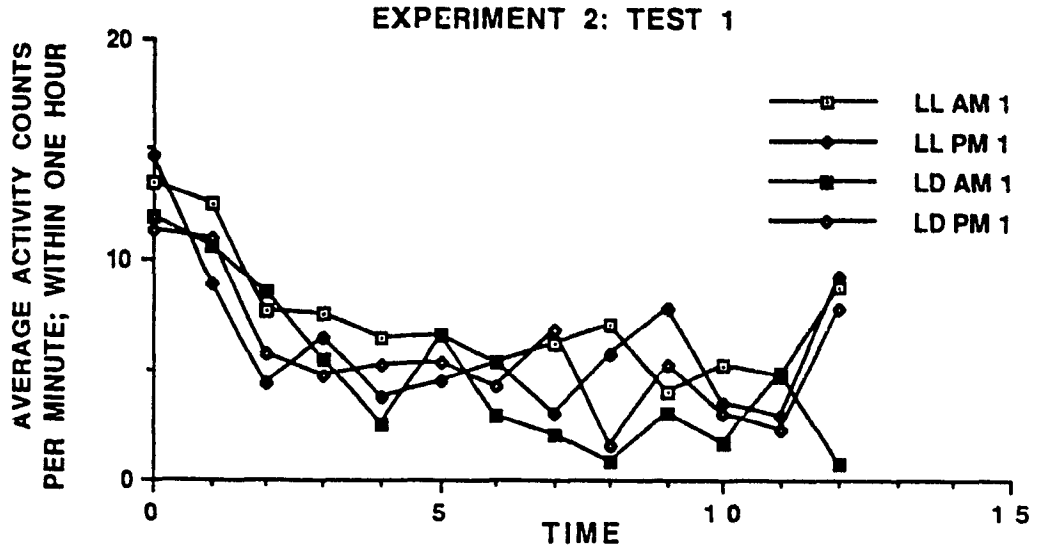


Figure 17A. Average activity per minute within an hour for testing session 1 of Experiment 2. Time scale is in hours.

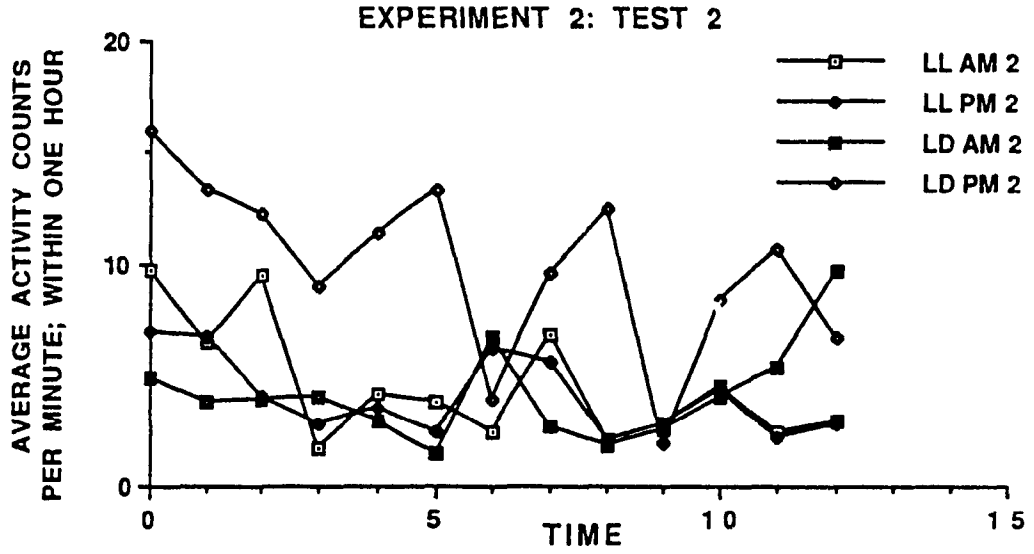


Figure 17B. Average activity per minute within an hour for testing session 2 of Experiment 2. Time scale is in hours.

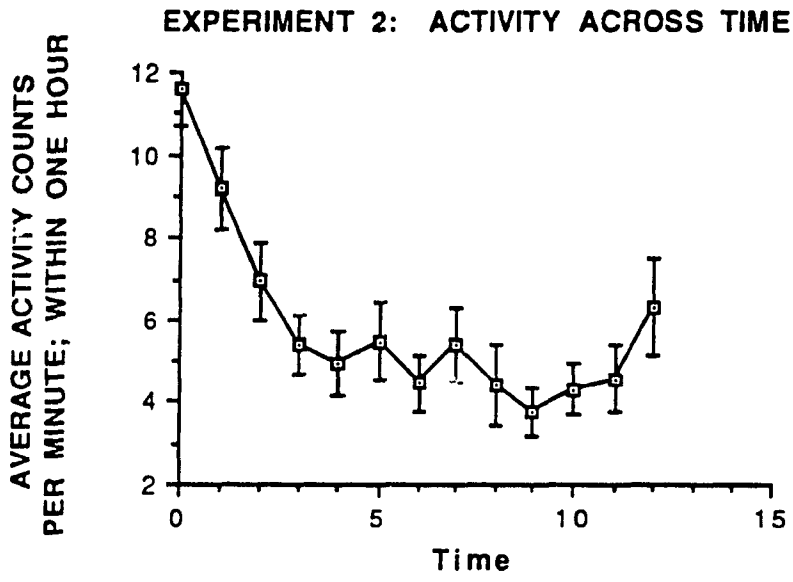


Figure 18. Average activity counts for Experiment 2 averaged over the time of day tested and illumination group. Time scale is in hours.

heat loss (Gordon, 1989). The adoption of a ball like posture would necessarily lead to a reduction in activity. Though subjects were often observed immobile in their cages by the experimenter this kind of behaviour is difficult to quantify and as such no data can be offered to support this explanation.

The results of the second experiment reaffirm those of the first. Rats kept in LL maintain core body temperature as efficiently against cold challenge as those kept in LD. These results are again at odds with previously published data (Fuller, Sulzman & Moore -Ede, 1978, 1979b). The hypothesis that the results of the first experiment may be at least in part attributable to the severity or stress of the cold challenge was not supported. The less severe and stressful cold challenge used in Experiment 2 produced results consistent with Experiment 1.

General Discussion

The data reported from the two experiments which comprise this study did not support previous findings. Animals maintained in LL defended core temperature as efficiently as animals maintained in a LD cycle. The variance between the results of the current study and previous work may be ascribed to a number of factors. These

factors include differences in protocol and species used.

Differences in Protocol

Each of the previous studies in which maintenance of animals in LL was found to be associated with impaired maintenance of core body temperature in response to cold challenge has utilized chair-acclimatized squirrel monkeys (Fuller, Sulzman & Moore-Ede, 1978, 1979b). The major aspects of the technique can be summarized as follows. During the course of the experiment, the squirrel monkeys sit restrained in a metabolism chair. The animal is housed within an isolation chamber in which ambient lighting and temperature are tightly controlled. The isolation chamber minimizes contact with the external environment and the time cues this contact may provide (Moore-Ede & Herd,1977; Moore-Ede, Kass & Herd,1977).

The animals used in the present study, male Wistar rats, were not similarly restrained but were free to move about the extent of their cages during all phases of each experiment including the test sequences. Differences between the results of the current study and previous ones (Fuller, Sulzman & Moore-Ede, 1978, 1979b) may in part rise from the relative behavioral freedom inherent in the current design.

Fuller et. al. (1978; 1979b) attributed impaired ability to maintain core temperature in squirrel monkeys kept in LL to the desynchronization of circadian timing system in these animals. The rhythmic processes pointed to by Fuller et. al. (1978) as possibly exhibiting altered temporal relationships, were the rhythms of heat production and heat loss.

In homeotherms there is both a rhythm of heat production (metabolism) and of heat loss (skin conductance) each of which is timed by the circadian system (Aschoff & Pohl, 1970; Aschoff & Heise, 1972; Aschoff, Biebach, Heise & Schmidt, 1974). In a natural light-dark cycle higher levels of heat production are associated with the animal's active period; the rhythm of heat loss from the body's extremities is a virtual mirror image of that of heat production (Aschoff, 1970; Aschoff & Heise, 1972). Though the rhythm of heat loss from the extremites is a mirror image of heat production this daily pattern of heat loss is not consistent over the entire body surface. In humans, for example, it has been shown that although heat loss from the hands and feet is in antiphase to the rhythm of core temperature, heat loss from the trunk is in phase with core temperature (Aschoff & Heise, 1972). Aschoff and Pohl

(1970) using data provided by a number of authors from a number of species, have demonstrated that, although conductance from the extremities is lower during an animal's active phase, the minimum overall thermal conductance is higher in the active phase of the cycle because of the increase in heat loss from the trunk.

The rhythms of heat production and conductance can desynchronize in constant illumination (Fuller, Sulzman & Moore -Ede, 1978; 1979a). Response to cold challenge in an animal in which desynchrony has occurred might be impaired because the cold challenge commences with or overlaps a period when the animal is at the trough of the rhythm of heat production and at the active phase minimum of thermal conductance. In such circumstances, the animal would be impaired in its ability to generate heat metabolically and in its ability to retain heat once it was generated.

Making up for an impairment, behaviorally, like that described above is within the bounds of possibility. It has, for example, been demonstrated that rats with preoptic anterior hypothalamic lesions which severely disrupt the autonomic thermoregulatory system can maintain a normal core temperature in both hot and cold environments behaviorally, if provided with the opportunity to do so

(Carlisle, 1969; Lipton, 1968; Satinoff & Rutstein, 1970).

Although the rats used in the current study were not provided with a direct behavioral means such as the turning on of a heat lamp via a bar press response, they did perhaps enjoy a greater opportunity than the chair-restrained squirrel monkeys to compensate behaviorally for an inefficiency in the autonomic thermoregulatory system if one did in fact exist.

Muscular activity is associated with a rise in core temperature at all phases of the circadian cycle (Aschoff, 1970). LL does not disrupt this relationship (Honma & Hiroshige, 1978b). That the subjects maintained in LL may have been using muscular activity as it is reflected in activity to compensate for a autonomic thermoregulatory deficit is not supported by the results of either Experiment 1 or 2. In Experiment 2, no significant differences in activity were observed between rats housed in LL and those housed in a LD cycle. The results of Experiment 1 do show that there is a significant difference in the level of activity between rats maintained in LL and a LD cycle. The rats housed in LL exhibit a lower average level of activity at all phases of the experiment (Figure 13). This is opposite to what would be expected if heat

generated by muscular activity was being used to compensate for an autonomic inefficiency.

The failure to find differences in the level of activity to support the idea that muscular effort might be being used to maintain core temperature in animals housed in LL, does not preclude the potential importance of adjustments in behaviour to the outcomes of the two experiments. Dataquest III does not record changes in muscular tension or posture, only locomotor activity. As such, differences in these or other behavioral variables between subjects housed in LL and LD may not have been reflected in the data. Visual observation of subjects did not indicate this to be the case, but neither did it provide a strong case against this possibility.

The contribution that adjustments in behavioral parameters can make to maintenance of core temperature is illustrated in Experiment 1 by animals housed in both lighting conditions. The results of Experiment 1 show that there is a steady increase in the maintenance of core body temperature in the water and the degree of recovery of core temperature in an hour across the four testing sessions. A proportion of this change can be attributed to a steady decrease in activity while the animal is in the water (R^2 = .24)

(Figures 12A-D, 14, 19). The LL group is less active in the cold water challenge test than the LD group (Figure 13, Appendix D). This effect does not necessarily reflect heightened behavioral adjustment made by LL group, as LL has been shown to produce a reduction in the level of activity in nocturnal species (Aschoff, 1960; Pittendrigh, 1967a) and the same group difference was observed at baseline (Appendix A).

Species Differences

It has been asserted, as was reviewed in the introduction, that the primate circadian system is driven not by the output of a single self-sustained oscillator or pacemaker, but by at least two mutually coupled self-sustained oscillators each of which drives its own subpopulation of rhythms (Moore-Ede, 1983a). This perspective on the circadian timing system in primates is based on the observation of spontaneous internal desynchronization between two groups of rhythms in humans (Aschoff, 1965) and squirrel monkeys in LL (Sulzman, Fuller & Moore-Ede, 1977).

Similarly, the rodent circadian timing system is hypothesized to be composed of multiple circadian oscillators. The basis for this in the rodent is not spontaneous internal desynchronization. Rather,

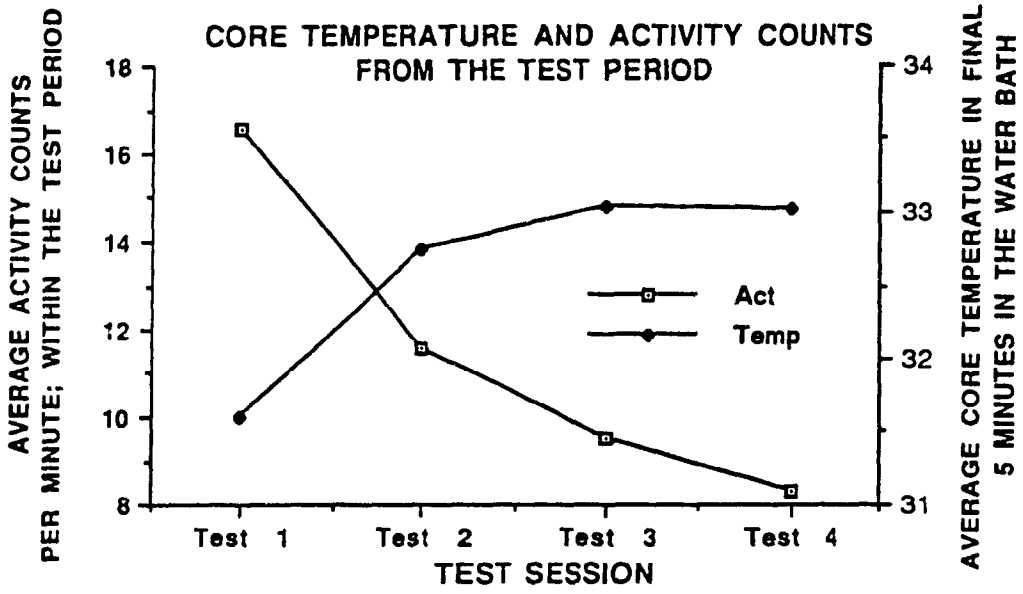


Figure 19. Average core temperature and activity counts from the four testing sessions of Experiment 1. Core temperature represents an average of the final five minutes of the test period. Activity counts represent an average per minute for the 30 minute test period.

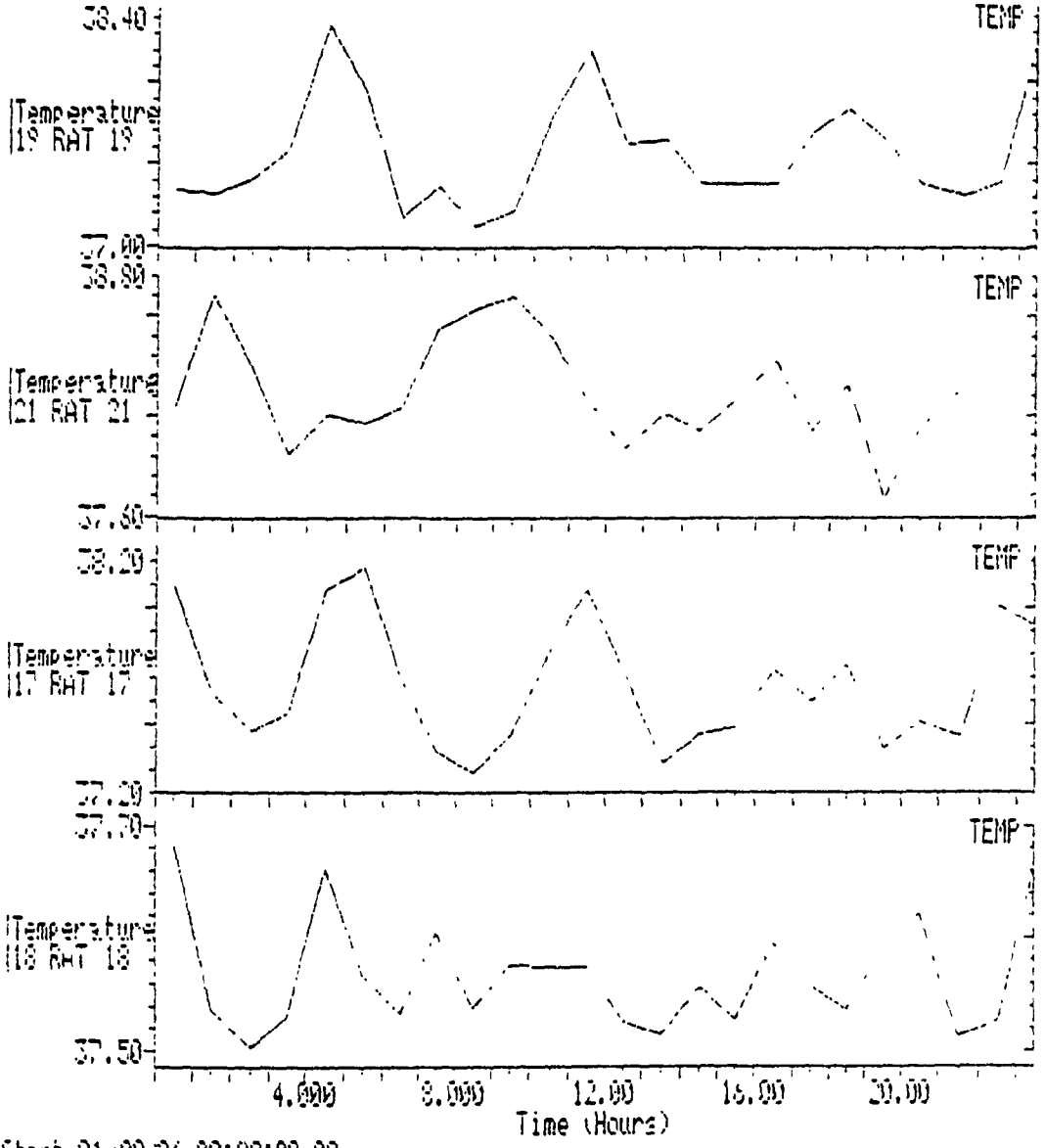
in rodents a different phenomena is sometimes observed after prolonged exposure to LL, namely splitting. In this case, in contrast to internal desynchronization in which two oscillators each driving their own set of rhythms appear tc become uncoupled and express a different period, a single rhythmic output dissociates or splits into two distinct components (Turek, 1983). The two components of the previously unimodal rhythm often express different periods for a time which is characterized by changing phase relationships between the two components (Earnest & Turek, 1982; Pittendrigh & Dann, 1976). The two components usually become recoupled about 12 hours out of phase with each other and thereafter assume an identical period.

The phenomenon of impaired maintenance of core body temperature in squirrel monkeys maintained in LL was attributed by Fuller et. al. (1978, 1979b) to internal desynchronization. Although splitting has been observed in primates (Schardt, Wilhelm & Erkert, 1989), spontaneous internal desynchronization has never, even after prolonged exposure to LL, been observed in rats (Eastman & Rechtschaffen, 1983; Honma & Hiroshige, 1978a, 1978b).

Figures 20 and 21 contain examples of one day's data for

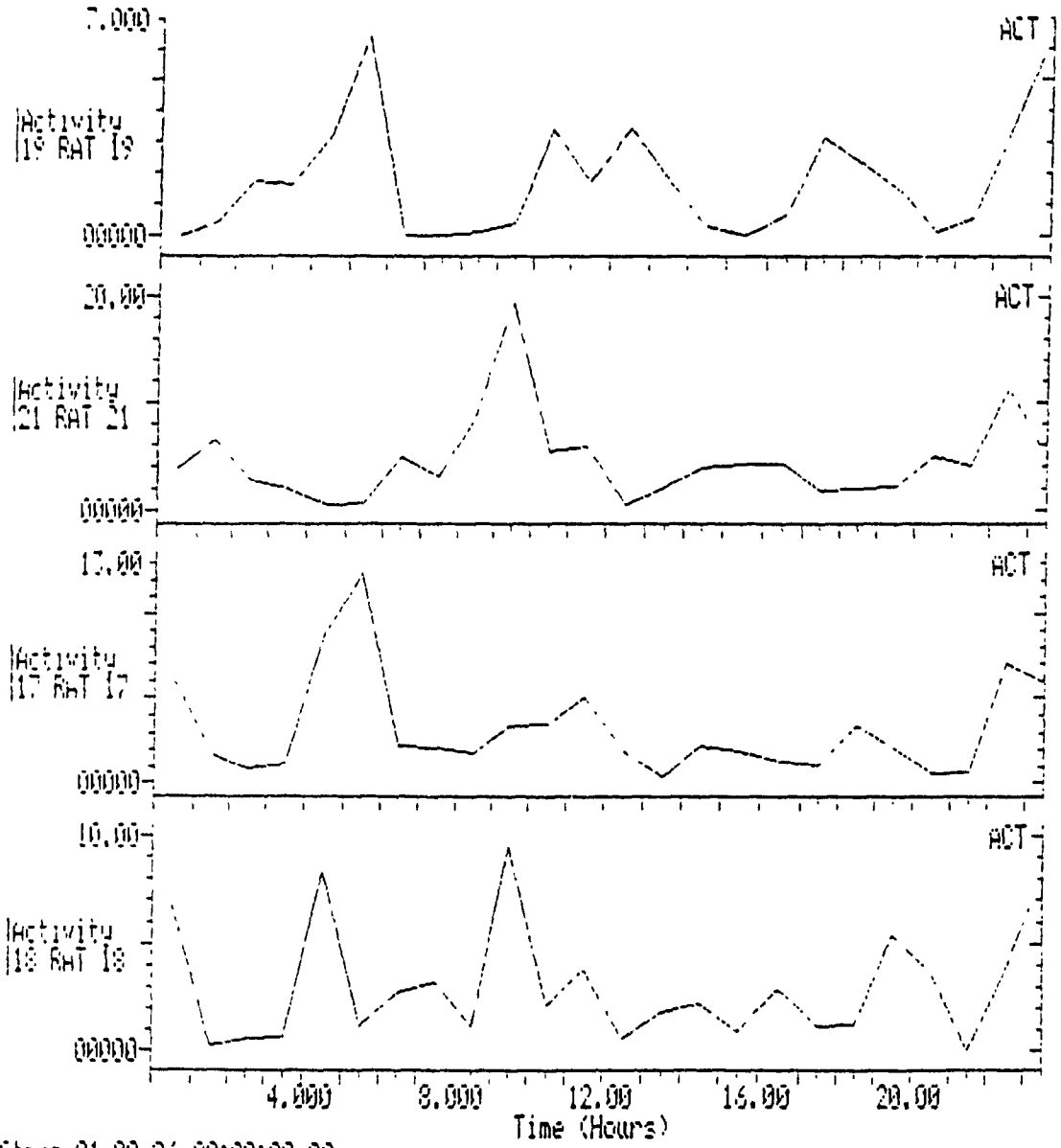
temperature and activity of subjects maintained in LL. These graphs make it apparent that within a 24-hour time span, the subjects maintained in LL exhibited several oscillations in the average level of both variables. Further, as determined using cosinor analysis (Halberg, Tong & Johnson, 1967), no periodicity which accounted for more than 15% of the variance in raw data points collected over the course of week four in LL could be found for the core temperature or activity data of the group maintained in LL.

The sum of the data reported above is that, although LL is having a dramatic effect on the rhythms of core body temperature and activity in the animals used in these experiments, the analysis does not support desynchrony. The defining characteristic of internal desynchrony, that two rhythms or groups of rhythms express different periods (Sulzman, 1983; Turek, 1983), was not observed. Rather, with respect to the two measured variables, the analysis makes it plausible that the animals maintained in LL became arrhythmic (no cyclicity in the average level of core body temperature or activity which has a period of approximately 24



Start 91/08/26 00:00:00.00
End 91/08/26 23:50:00.00
Average Interval 000:00.000

Figure 20 Record of one days core temperature data from four rats in their fourth week of exposure to continuous illumination. Traces were clipped at 37 °C and 38.8 °C.



Start 91/03/26 00:00:00.00
End 91 03 28 23:50:00.00
Average Interval 060:00.000

Figure 21 Record of one days activity counts (same day and rats as in Figure 20) from four rats in their fourth week of exposure to continuous illumination.

hours). Arrhythmicity does not preclude an altered phase relationship between core temperature and activity, but this eventuality is not supported by visual inspection of the data (Figures 20 and 21).

The results of this study provide at least indirect support for the conclusions of Fuller et. al. (1978; 1979b). Exposure to LL when unaccompanied by internal desynchronization, is not associated with impaired maintenance of core body temperature in response to cold challenge in the rat. This assertion is made on the basis that rats maintained in LL which may have become arrhythmic, as opposed to desynchronous, demonstrated no impairment in the ability to maintain core body temperature in response to two different cold challenges.

Suggestions for For Further Research

Desynchronization between groups of circadian rhythms is characterized by the maintenance of different periods by at least two groups of rhythms and ever-changing phase relationships between the member rhythms of the groups (Moore-Ede, 1983a). Impaired maintenance of core temperature in response to cold challenge in LL, was attributed to the alteration of the phase

relationship between the rhythms of heat production and vasomotor heat loss (Fuller, Sulzman & Moore-Ede, 1978).

Disruption of the normal phase relationships between various rhythms in LL as a consequence of internal desynchrony, has not been observed in rats (Eastman & Rechtschaffen, 1983; Honma & Hiroshige, 1978a, 1978b). This does not preclude the use of this species to test the hypothesis that it is desynchrony which accounts for the impairment in maintenance of core temperature, in response to cold challenge observed in squirrel monkeys maintained LL (Fuller, Sulzman & Moore-Ede, 1978; 1979b).

The resetting of the pacemaker or pacemakers following a phase shift is almost instantaneous (Pittendrigh,1967b). The various physiological rhythms do not shift immediately but require several transient cycles before adopting the new local time. Further, the number of transient cycles required to resynchronize to the new light schedule is not consistent among the various physiological rhythms (Moore-Ede, Kass & Herd, 1977). As a consequence of the unevenness with which different rhythms re-establish their original phase-angle relationship with the new light-dark cycle, a state of transient internal desynchronization is

induced by phase shifts (Moore-Ede, Kass & Herd, 1977) Unlike spontaneous or forced internal desynchronization (Fuller, Sulzman & Moore-Ede, 1978; 1979b) transient desynchronization has been observed in rodents (Finkelstein, Baum & Campbell, 1978; Zucker & Stephan, 1973).

Spontaneous internal desynchronization and transient internal desynchronization are not isomorphic. Transient internal desynchronization probably reflects the temporary uncoupling of subordinate oscillators from their pacemaker rather than the uncoupling of two or more master pacemakers (Moore-Ede, Sulzman & Fuller, 1982). In spite of this difference, the other feature of spontaneous internal desynchronization, altered phase relationships among rhythmic variables, is maintained. If effective response to cold challenge requires the precise internal synchronization of the circadian time keeping system. Then, cold challenge following a shift in lighting schedule while the rhythmic variables are still in transition to their original phase-angle relationships could produce larger drops in core temperature than when they are in a stable phase relationship. This is, of course, provided that the rhythms of heat production and heat loss transiently desynchronize following a

phase shift and that this phenomena occurs in the rat.

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Appendices

Appendix A

Source Table for the ANOVA for Baseline Core Temperature, Experiment 1

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	.119	.119	.375	.5491
Time of Day	1	.456	.456	1.431	.2490
Light X Time of Day	1	1.172	1.172	3.677	.0732
Error	16	5.101	.319		
Within					
Testing Session	3	.846	.282	1.241	.3052
Testing Session X Lighting Schedule	3	.571	.190	.838	.4797
Testing Session X Time of Day	3	1.006	.335	1.477	.2326
Testing Session X Lighting Schedule X Time of Day	3	.472	.157	.693	.5609
Testing Session X Lighting Schedule X Time of Day X Subjects	48	10.902	.227		
Total	79	20.645			

Appendix B

Source Table for the ANOVA for Baseline Activity, Experiment 1

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	90.525	90.526	3.336	.0865
Time of Day	1	48.516	48.516	1.788	.1999
Light X Time of Day	1	657.231	657.231	24.221	.0002
Error	16	434.149	27.134		
Within					
Testing Session	3	55.439	18.480	.324	.8077
Testing Session X Lighting Schedule	3	398.060	132.687	2.329	.0862
Testing Session X Time of Day	3	229.135	76.378	1.341	.2722
Testing Session X Lighting Schedule X Time of Day	3	65.956	21985	.386	.7179
Testing Session X Lighting Schedule X Time of Day X Subjects	48	2734.371		56.966	
Total	79	4713.382			

Appendix C

Source Table for the ANOVA for Core Temperature, Experiment 1

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	5.798	5.798	3.175	.0937
Time of Day	1	3.909	2.141	2.141	.1628
Light X Time of Day	1	.822	.822	.450	.5118
Error	16	29.212	1.1826		
Within					
Testing Session	3	39.539	13.180	9.547	.0001
Testing Session X Lighting Schedule	3	.909	.303	.219	.8825
Testing Session X Time of Day	3	13.629	4.543	3.291	.0284
Testing Session X Lighting Schedule X Time of Day	3	2.805	.935	.677	.5702
Testing Session X Lighting Schedule X Time of Day X Subjects	48	66.262	1.380		
Period	1	1083.02	1083.02	2827.7	.0001
Period X Lighting Schedule	1	.005	.005	.014	.9081
Period X Time of Day	1	.097	.097	.254	.6215
Period X Lighting Schedule X Time of Day	1	.253	.253	.660	.4284

Source Table for the ANOVA for Core Temperature, Experiment 1

Appendix C Continued...

Period X Lighting Schedule X Time of Day X Subjects	16	6.126	.383		
Testing Session X Period	3	1.418	.473	1.644	.1916
Testing Session X Period X Lighting Schedule	3	1.160	.387	1.344	.2712
Testing Session X Period X Time of Day	3	7.756	2.585	8.990	.0001
Testing Session X Period X Lighting Schedule X Time of Day	3	.772	.257	.894	.4510
Testing Session X Period X Lighting Schedule X Time of Day X Subjects	48	13.804	.288		
Total	159	1277.298			

Appendix D

Source Table for the ANOVA for Activity, Experiment 1

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	787.656	787.656	8.786	.0091
Time of Day	1	203.401	203.401	2.269	.1515
Light X Time of Day	1	33.856	33.856	.378	.5475
Error	12	1434.432		89.652	
Within					
Testing Session	3	422.318	140.773	5.405	.0028
Testing Session X Lighting Schedule	3	93.854	31.285	1.201	.3194
Testing Session X Time of Day	3	381.348	127.116	4.881	.0048
Testing Session X Lighting Schedule X Time of Day	3	150.417	50.139	1.925	.1381
Testing Session X Lighting Schedule X Time of Day X Subjects	48	1250.114		26.044	
Period	1	648.025	648.025	10.18	.0057
Period X Lighting Schedule	1	42.025	42.025	.660	.4284
Period X Time of Day	1	1.122	1.122	.018	.8960
Period X Lighting Schedule X Time of Day	1	29.412	29.412	.462	.5064

Source Table for the ANOVA for Activity, Experiment 1

Appendix D continued...

Period X Lighting Schedule X Time of Day X Subjects	16	1010.518		63.657	
Testing Session X Period	3	465.271	155.090	5.006	.0042
Testing Session X Period X Lighting Schedule	3	3.359	1.120	.036	.9907
Testing Session X Period X Time of Day	3	139.080	46.360	1.497	.2274
Testing Session X Period X Lighting Schedule X Time of Day	3	41.942	13.981	.451	.7176
Testing Session X Period X Lighting Schedule X Time of Day X Subjects	48	1486.976		30.979	
Total	159	8633.162			

Appendix E

Source Table for the ANOVA for Core Temperature, Experiment 2

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	2.164	2.164	.444	.5178
Time of Day	1	3.071	3.071	.630	.4427
Light X Time of Day	1	.051	.051	.010	.9202
Error	12	58.486	4.874		
Within					
Testing Session	1	.561	.561	.572	.4642
Testing Session X Lighting Schedule	1	.634	.634	.646	.4373
Testing Session X Time of Day	1	.004	.004	.004	.9529
Testing Session X Lighting Schedule X Time of Day	1	.694	.694	.707	.4170
Testing Session X Lighting Schedule X Time of Day X Subjects	12	11.777	.981		
Time Elapsed	12	2.819	.235	1.342	.2111
Time Elapsed X Lighting Schedule	12	7.832	.653	3.678	.0001
Time Elapsed X Time of Day	12	6.797	.566	3.192	.0005
Time Elapsed X Lighting Schedule X Time of Day	12	4.220	.352	1.982	.0299

Source Table for the ANOVA for Core Temperature, Experiment 2

Appendix E continued...

Time Elapsed X Lighting Schedule X Time of Day X Subjects	144	25.556	.177		
Testing Session X Time Elapsed	12	3.223	.269	1.326	.2101
Testing Session X Time Elapsed X Lighting Schedule	12	2.759	.230	1.135	.3368
Testing Session X Time Elapsed X Time of Day	12	1.235	.103	.508	.9070
Testing Session X Time Elapsed X Lighting Schedule X Time of Day	12	1.701	.142	.699	.7501
Testing Session X Time Elapsed X Lighting Schedule X Time of Day X Subjects	144	29.177	.203		
Total	415	162.761			

Appendix F

Source Table for the ANOVA for Activity, Experiment 2

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between					
Lighting Schedule	1	58.651	58.651	.838	.3780
Time of Day	1	127.729	127.729	1.825	2.016
Light X Time of Day	1	410	410.629	5.868	.0322
Error	12	839.760	69.980		
Within					
Testing Session	1	2.895	2.895	.025	.8772
Testing Session X Lighting Schedule	1	549.452	549.452	4.729	.0504
Testing Session X Time of Day	1	149.279	149.279	1.285	.2792
Testing Session X Lighting Schedule X Time of Day	1	79.509	79.509	.684	.4243
Testing Session X Lighting Schedule X Time of Day X Subjects	12	1394.375		116.198	
Time Elapsed	12	1900.594		158.383	10.369
.0001					
Time Elapsed X Lighting Schedule	12	126.170	10.514	.688	.7607
Time Elapsed X Time of Day	12	67.145	5.595	.366	.9732
Time Elapsed X Lighting Schedule X Time of Day	12	289.645	24.137	1.580	.1035

Source Table for the ANOVA for Activity, Experiment 2

Appendix F continued...

Time Elapsed X Lighting Schedule X Time of Day X Subjects	144	2199.469	15.274	
Testing Session X Time Elapsed	12	281.622	23.468	1.541	.1160
Testing Session X Time Elapsed X Lighting Schedule	12	332.429	27.702	1.819	.0502
Testing Session X Time Elapsed X Time of Day	12	323.003	26.917	1.767	.0589
Testing Session X Time Elapsed X Lighting Schedule X Time of Day	12	311.263	25.939	1.703	.0717
Testing Session X Time Elapsed X Lighting Schedule X Time of Day X Subjects	144	2193.067	15.230	
Total	415	11636.686		