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ACTIVITY IN A RANDOM LIGHT-DARK ENVIRONMENT

by

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A large compilation of literature has been accumulated in the last twelve years demonstrating the survival value of circadian (circa: about, dies: day; hence, about one day) and other cycles. The problem of biorhythmicity has been cited as "one of the five most important questions in biological science" (Pinc, 1966). A large percentage of an organism's behavior is traceable to biological rhythms. The fact that all humans sleep and waken implies vast physiological and psychological changes in behavior.

Circadian and certain other cycles, e. g. reproductive cycles, can be differentiated from such physiological rhythms as heartbeat and breathing by two criteria: Their natural period is an evolved approximation to an environmental cycle and their period remains stable over a wide range of temperatures (Pittendrigh & Bruce, 1959).

Perhaps the major survival value of circadian and related cycles has been summarized by Cloudsley-Thompson (1961). He described the primary value of cyclic behavior as a form of pre-adaption. An organism which feeds only at a specific time of day, as at dawn and dusk or at high tide, has an obvious advantage over its competition for the available food supply if it is able to anticipate feeding time. The advantage of internal cycles is also apparent when environmental cues may be temporarily disrupted, as during long storms.

A second survival value lies in the directional sense of certain insects and birds. This sense of direction is often based on knowledge of sun position. Obviously the organism must somehow correct for the passage of the sun overhead as well as the sun's north-south movements throughout the year. Certain birds are able to migrate either north or south, depending on the season, on the basis of increment or decrement of daylight (Wolfson, 1959, 1965).

A third survival value of circadian cycles pertains to mating. If the female members of a species are only receptive a certain times, e. g. during two weeks in spring, then it is to the advantage of the males to develop means of anticipating this receptive period. Such anticipation not only directly benefits the male but increases the probability of the survival of the species.

The general susceptibility of the whole organism to environmental agents, as toxicants, bacterial and virus invasions, physical damage, and other factors, has been verified for plants, insects, rats, humans; indeed the whole range of living organisms from single cells to whole populations of organisms. Cole and Adkisson (1964, 1965) demonstrated that the boll weevil exhibited an infradian and circadian rhythm of susceptibility to an insecticide, methyl parathion. When subjected to the insecticide at dawn only 10 per cent of the insects died; the same dosage three hours later proved fatal to 90 per cent.

Pizzarello, Witcofski, & Lyons (1963) subjected white rats to X-irradiation at 0900 and 2100 hours of the day. The twenty rats radiated at 2100 died within 14 days; the twenty rats radiated at 0900 were still in good health 52 days later.

Eight of 17 male rats forcibly immobilized for six hours during their normally active phase developed gastric erosions; none of 13 rats immobilized during their normally resting phase developed gastric erosions (Ader, 1964).

The circadian rhythmicity of an organism is complicated by the fact that different organs and different tissues within the same organism show different circadian cycles, i. e. different in phase synchronization and adaptibility (Halberg, 1964). Different points on the skin of a human show different circadian temperature cycles. Some areas, as the mouth and neck, maintain relatively constant temperatures while areas like the ankles and wrists show wide variations over a day (Halberg *et al.*, 1959).

More obvious evidence of the survival value of circadian cycles in human behavior has been cited by Halberg (1960) and Reinberg (1965). The hour of birth is related to the rate of infant mortality. Post-surgical mortality is least from 1200-1500 hours (about two per cent) and greatest from 2100-2400 hours (about six per cent). Skin reaction to histamine show a definite circadian cycle, as does urine chemistry. The rate and seriousness of industrial accidents is greater for night shifts than for day shifts (Aschoff, 1965).

Dissociation in human rhythmic patterns occurs when the *S*s live in abnormal time routines, e. g. a 27 hour day. The amount of water, sodium, and chloride in urine and body temperature showed a 27 hour period whereas potassium in urine showed a 24 hour period (Lobban, 1965). Aschoff (1965) found one *S* who showed a free-running period of 32.6 hours for activity and calcium excretion in continuous light. Body temperature, water excretion, and potassium excretion for the same S showed a period of 24.7 hours. It appears that potassium excretion is more resistant to periodicity change than activity, body temperature, and calcium excretion.

In humans the effect of circadian cycles is complicated by social cyclic behavior as well as physiological cycles. Thus, normal working hours are from nine to five. It is likely that the selection of these particular hours of work, and their 24 hour period, are ultimely traceable to physiological and environmental cycles.

Aschoff (1965) isolated individual human Ss for several days from time cues. Among the results obtained it was noted that individual cycles within a single S tended to drift from a 24 hour cycle, i. e. cycles developed which differed slightly from 24 hours in period. Crawford & Nicora (1964) and Thor & Crawford (1964) isolated groups of men, women, and children from time cues for several days. Activity of the group showed a definite 24 hour pattern with little drifting. Presumably, drifting of individual cycles by one person was corrected by the group.

Other aspects which have been demonstrated to occur in circadian patterns include time estimation and time perspective (Thor & Baldwin, 1965; Thor, 1962a, 1962b). Kleitman (1963) tested human Ss on several motor tasks throughout the waking day. A well marked rhythm of performance was observed in card dealing (speed), card sorting (speed), mirror drawing (speed and accuracy), code transcription (speed and accuracy), multiplication (speed and accuracy), and hand steadiness. Peak performance fell between 1000 and 1200 hours. The shape of the performance curves showed a marked similarity to the rhythm of oral temperature taken concurrently with the tests. Reaction time showed an inverse relationship to body temperature, and hence an inverse rhythm with respect to the performance tasks. It is likely that related tasks, as performance on a verbal test, show similar circadian rhythms.

Two general theories have been formulated to account for cyclic behavior. The first and most generally accepted among biologists states that cyclic behavior is intrinsic or endogenous but is synchronized by environmental cues. The second theory holds that cyclic behavior is extrinsic or exogenous, that the organism is responding to environmental cues, some of which may be very subtle and pervasive.

There are many different hypotheses about the nature of the endogenous "clock." It is generally believed that cyclic behavior results from at least two coupled oscillators ; for very general cycles, as activity cycles, there are undoubtably many oscillators. In addition, the coupling (dependence) between oscillators may also be cyclic, i. e. the coupling between oscillators is a function of their phase relationship and strength, resulting in a non-linear, cyclic interaction.

Pittendrigh & Bruce (1959) suggest two oscillators function to regulate the endogenous clock. The primary oscillator is light sensitive and the secondary oscillator is sensitive to temperature. Normally the light oscillator regulates the organism, but in certain circumstances, as in extreme temperatures, abnormal light schedules, disease, the temperature oscillator assumes regulation of the organism. A natural extension of this model is that additional oscillators, differentially coupled to the primary oscillator, are sensitive to other environmental timing cues. In humans some of these oscillators may be sensitive to conditioning.

The cyclic behavior of a single cell has been studied extensively. The most important, perhaps the cell's "master" oscillator, involves RNA synthesis by the cell nucleus. Essentially, the cell nucleus synthesizes RNA from specific enzymes and the RNA then moves into the cell cytoplasm. Eventually, however, the amount of substrates needed for new RNA synthesis falls below the required level and RNA synthesis decreases. If a certain level of decrease signaled the cell to acquire additional enzymes from another source, as the bloodstream, then RNA synthesis would again rise to a high level (Hasting & Sweeney, 1959; Hasting & Keynan, 1965). Recent experiments have indicated that the regulation of the cellular clock does depend on the exchange of material between the nucleus and the cytoplasm (Schweiger, Wallraff, & Schweiger, 1964; Schweiger & Schweiger, 1965). The RNA synthesis model is characterized as a prey-predator, all or none, or relaxation type of oscillator. This type of oscillator has a stable amplitude and an unstable period; it is thus easily synchronized to environmental cues (Bünning, 1964).

The second important oscillator is of the harmonic (pendelum, sine) type. An example is the cell's accumulation of waste byproducts of metabolism; eventually the cell discharges the byproducts and again begins to accumulate waste. This type of oscillation is characterized by a stable periodicity but unstable amplitude (Cloudsley-Thompson, 1961).

The two types of oscillators discussed here occur in a single cell; however, it is not believed that single cells working in phase determine the circadian cycles of complex animals.

Many physiological systems in the human body display rhythmic behavior: the cardiovascular system, the respiratory system, the alimentary cardiorenal, and others. It is generally believed that the nervous system functions as a "master clock," exherting control over the whole organism (Cloudsley-Thompson, 1961; Bruce, 1965). It is very possible that a center in the brain, or a number of centers, functions as a master clock by controlling endocrine activity, mediating sensory signals, and receiving feedback information from various parts of the body. In humans and other high animals these brain centers could very well interact with memory and learning centers, thus enabling exogenous cycles to be conditioned.

While the cyclic behavior of an organism may be endogenous the organism is not unaffected by environmental conditions. However, the endogenity of the cycle places limits on the organism's ability to adapt to changing environmental conditions. Furthermore, it is also evident that individual differences are great, both between individuals of the same species and between species. Different cycles within the same species, even within the same organ in an organism, display differential ability to change phase relationships, periods, and amplitudes. Thus, placing the organism in any kind of experimental environment will likely disrupt some cycles more than others. It then becomes important to identify precisely the cycle being investigated and the method used to investigate it. The different temperature cycles for different areas of the skin of a human, mentioned previously, is an example.

Researchers have often cited their results as support for the endogenous theory, even through their results do not either prove the correctness of the endogenous theory or disprove the exogenous theory. There is, however, certain evidence which supports the view of endogenous oscillators.

Bolles & Stojkiewicz (1965) placed white rats on a strict, one hour a day feeding schedule. Within a few weeks the rats began to anticipate their feeding hour by becoming very active during the hour preceeding feeding. This anticipation (pre-adaption) was most likely learned, although the Ss could have been cued by some unknown environmental factor.

Aschoff (1955a, b) isolated several generations of mice in constant dim illumination. The second and third generations of mice continued to show a circadian pattern of activity. Furthermore, each generation showed individual circadian cycles, i. e. each generation drifted slightly from an exact 24 hour period and from each other. The drifting of activity cycles strongly indicates that environmental cues, including pervase geophysical factors, played no role in determining the activity cycles of the mice. Browman (1952) raised 25 generations of mice in constant illumination. In the 25th generation 11 of 16 mice showed a definite circadian cycle of voluntary activity. However, several uncontrolled aspects of this study render it inconclusive.

A cockroach has two sets of photoreceptors, the eyes and the ocelli. Removal of the ocelli did not affect activity cycles; blinding of the eyes resulted in a free-running circadian cycle with a period of about 23.5 hours. After eyesight was restored the cockroach rapidly resynchronized to the 24 hour lighting schedule (Roberts, 1965a).

Hoffman (1965) gives a summary of the relationship between intensity of light in constant light conditions and the length of period for diurnal and nocturnal animals. Essentially, period decreases with greater light intensity for diurnal animals and increases for nocturnal animals. It is widely recognized that light is the most important environmental cue, followed by temperature, for most organisms.

White rats are able to synchronize with an environmental lighting schedule and alter their activity period length up to a point. Generally speaking, rats can adjust to a 16 hour day

(8 hours light, 8 hours dark) and to a 28 to 30 hour day. However, for extreme deviations from 24 hours large individual differences are to be expected (Bünning, 1964; Tribukait, 1954).

The endogenous theory is flexible enough to recognize and attempt to account for these and other sources of variance of cyclic behavior. Halberg (1960) gives a summary, stating that the organism provides the basic patterns of behavior and then synchronizes these patterns to each other and to enviornmental cues. For some organisms, as certain insect orders, birth may be the major event which synchronizes the organism and initiates the endogenous rhythm. After that first synchronizing event all other potential synchronizers are ignored (Cloudsley-Thompson, 1961; Rensing, 1965). Other organisms, e. g. humans, may never develop a stable circadian periodicity for some behavior, as general activity.

The exogenous theory has been largely expounded by Brown (1965) and his associates. Very briefly, Brown holds that cyclic behavior results for the most part from the organism responding to environmental cues.

For Swiss mice the solar day (24 hours) is the major synchronizer. However, when the mice are place in constant, dim illumination a lunar day (24.8 hours) component becomes evident, although it does not equal the component strength of the solar day (Terracini & Brown, 1962). It is implied in a later summary (Brown, 1965) that the organism is capable of synchronizing to a number of environmental cues; if light is held constant the organism will respond to temperature fluctuations, if temperature is then controlled the organism will respond to another environmental cue, as lunar gravitation. A re-examination of some of Brown's data on which he bases his conclusions by a proponent of the endogenous theory (Enright, 1965) fails to confirm the existence of a lunar day component.

Exactly how an organism such as a mouse is able to respond to lunar gravitation is unknown, but Brown (1965) believes that the organism acts as a variable frequency transformer. Other possible subtle, yet pervase, environmental cues may be the atmospheric tides and associated changes in barometric pressure, magnetic fields, electrostatic fields, and gamma fields.

Evidence favoring the exogenous theory is less abundant than evidence for the endogenous theory. Fiddler crabs display a solar day rhythm of color change. When both eyestalks are removed the rhythmic color change is no longer apparent (Abramowitz, 1937). This would seem to be a case of purely exogenous timing; similar eyestalk operations did not affect a rhythmic consumption of oxygen, indicating independence of these two cycles (Brown et al., 1954).

New Haven oysters open at high tide. When transported to Evanston, Illinois, they continue to open at regular intervals, but gradually shift until, about two weeks after transport, they are synchronized to open at high tide in Evanston (Brown, 1958). The oysters must, somehow, be receptive either to lunar gravitation or to changes in their environment (sea water) produced by lunar gravitation. It remains possible that the opening cycle is endogneous but is synchronized by lunar gravitation. The fact that the oysters required two weeks to resynchronize to high tide at a different longitude indicates at least some endogenity of the rhythm of opening.

Hamner et al. (1962) transported several types of organisms (higher and lower plants, insects, and mammals) to the south pole for observation of their circadian rhythms. Diurnal variation of geophysical factors, as gamma fields, lunar gravitation, and electromagnetic fields, was controlled by placing some of the orgnaisms on a table which rotated once every 24 hours against the earth, thus making the position of these animals constant with respect to rotation of the earth. The results indicated that the organisms did not respond to any of the geophysical factors mentioned but continued their circadian cycles of behavior.

A very broad generalization with respect to the two theories is that the exogenous theory seems to be more valid for plants and lower animals than it does for vertebrates. Most of Brown's work has been with snails, oysters, crabs, worms, and other invertebrates.

Bolles & Stojkiewicz (1965) indicated that rats could learn to anticipate availibility of food and water. It seems reasonable to assume that rats could also learn to anticipate light onset and offset. However, a random pattern of light onset and offset would not enable the rat to learn anticipation and would tend to extinguish whatever anticipation had been acquired. If the anticipation were the result of subtle environmental cues then these cues would no longer be reliable for predicting light onset and offset and the rat would extinguish on them.

Removal of environmental cues, as in constant light or dark, causes a gradual drifting of the individual's activity (Aschoff, 1965; Roberts, 1965b; Rawson, 1959). Presenting environmental cues in a random sequence may cause disruption of the established circadian cycle if S synchronized with them. On the other hand S may very well ignore the false cues and remain on a 24 hour activity cycle.

The present study investigated the effect of a random light-dark cycle on the gross motor activity of a white rat. Since each light and dark period was of randomized length and hence unpredictable the S could either ignore the lighting schedule, thus lending support to the endogenous theory, or S could respond to the light, becoming active when the light went off and inactive when it came on, and thus supporting the exogenous theory.

An additional hypothesis was that the method of randomizing timing cues is a valid method for investigation of cyclic behavior in both animals and humans.

METHOD

Subjects: The subject was one, experimentally naive, albino, male rat, about 120 days old, from the animal colony maintained by the Psychology Department at Western Michigan University. The colony is kept on a natural lighting schedule.

Apparatus: The apparatus consisted of several components. The *S* was confined to a 29 by 23 by 20 cm. activity cage throughout the experiment. The cage floor consisted of four triangular, wire mesh partitions which moved vertically about .5 cm. as *S* moved about the cage. A simple lever extended beyond the cage walls and opened a leaf switch whenever a floor partition was depressed. The activity cage thus measured gross locomotor activity, *S* being required to move from one part of the cage to another part.

The activity cage was placed inside an attenuated chamber, an 8.2 cubic foot refrigerator. The chamber was lightproof and dampened noise, vibration, and temperature variations in the room. The experimental room itself was kept locked and darkened and was relatively isolated from foot traffic. A blower provided continuous venilation inside the chamber and also served as a masking noise.

The leaf switches on the activity cage connected to a four-channel Rustrak event recorder, one channel for each floor partition. The recorder and its power supply were regulated by a Hunter timer which operated the recorder for 30 sec. Since the timer has a maximum time period of 99.99 sec. it was necessary to wire it to a second timer which turned the Hunter timer on and off every five minutes. The second timer consisted of a synchronuous, one revolution per hour motor which opened and closed a microswitch through a gear arrangement every five min. The complete apparatus was thus programmed to record a 30 sec. sample of activity once every five min. throughout the day. A diagram of the components is presented in Fig. 1.

A Paragon 24 hour timer (Model 4001-0) turned the light inside the isolation chamber on or off according to a predetermined schedule. The light inside the chamber was provided by a 15 watt incandescent bulb approximately 40 cm. above the cage. The _S_ was shaded from the direct rays of the bulb. Illumination level of the cage was approximately 2.2 footcandles (.2 lux). Investigations by Lockard (1963) indicate that this level of illumination is not aversive to white rats.

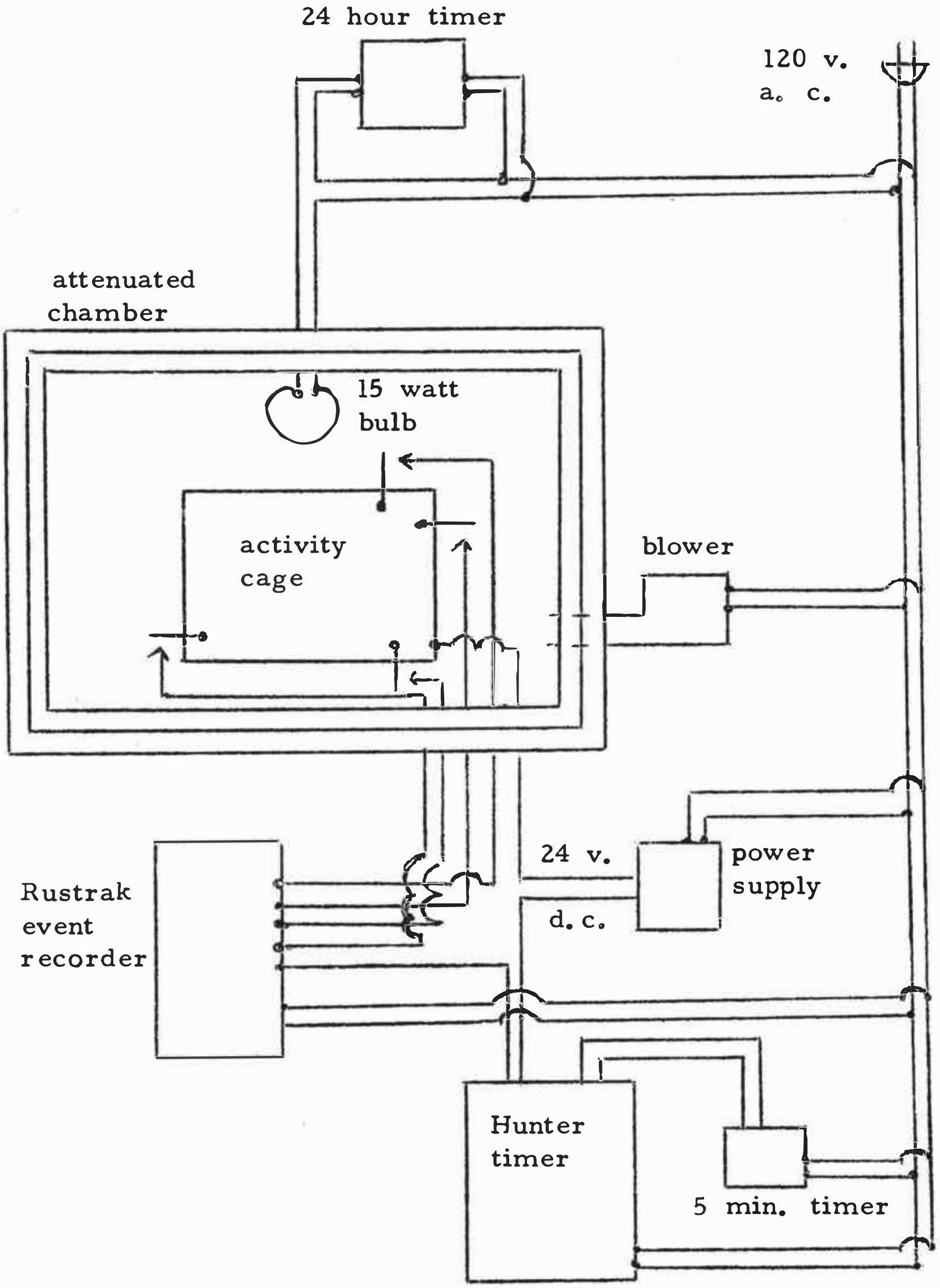

Fig. 1. Apparatus and electrical schematic.

The temperature difference resulting from the light being on was 1.3 degrees C., obtained by spot check. The maximum temperature was 23.3 C.; the minimum temperature of 19.9 C. coincided with a cold spell and a lowering of heat level throughout the building during weekends. The normal temperatures ranged between 21.4 C. (light off) and 22.7 C. (light on).

Procedure: A rat was placed in the activity cage from Nov. 24 through Dec. 12, 1965. During this time apparatus adjustments were made. On Dec. 13 the S was placed in the activity cage and put on a 12 hour on-12 hour off light-dark schedule.

On Jan. 4, 0900 hours, recording of activity began and proceeded for five days. On the sixth day (Jan. 9) the timer was programmed to switch the light according to a predetermined, randomized schedule. The apparatus was checked daily at different times. Routine care of S took place only when the light was on and the recorder off. No strict schedule of care was maintained; food and water were available ad lib. Inspection of the charts revealed no unusual rise or fall in activity during periods of care.

After 16 days of randomized lighting the 12:12 light-dark schedule was reinstated. An additional eight days of 12:12

lighting was imposed. On the 30th day (Feb. 3) S was removed from the activity cage.

Two equipment breakdowns occurred. On the 19th day the food cup inside the activity cage fell onto the floor and depressed all four floor partitions, thereby preventing recording of activity. On the 23rd day the incandescent bulb blew out and was replaced after 45 hours. The bulb most likely ceased functioning either at 24:0600 (24th day, 0600 hours) or at 25:0600.

The light and dark durations during the randomized condition was determined from a rectangular distribution of numbers with a mean of 12. The range of durations was 1.5 to 22.5 hours. The normal and randomized lighting schedules are shown in Figs. 2 and 3.

In certain instances the activity cage measured very minor activity which was not considered as part of S's general gross activity. These measurements were eliminated by counting only the first depression of any floor partition during any 30 sec. recording period. Thus, for every hour activity was rated on a 49 point scale (0-48).

RESULTS

The activity level of S throughout the 30 experimental days is shown in Figs. 2 and 3. Analysis of this type of data can take several forms; the following analyses and tests were made. Data obtained during the two equipment breakdowns were not included in the analyses.

Table 1 shows the mean activity and standard deviations for the normal and randomized conditions and for lights on versus lights off. An F test for difference between variances of the conditions was significant (F(362, 249) = 1.357, $p < .01$). A matched t test between means of conditions was not significant ($t < 1.00$).

Inspection of Figs. 2 and 3 indicate that S followed a regular, circadian pattern of activity throughout the experiment but that a phase shift occurred at the onset of the randomized condition and a second phase shift occurred at the reinstatement of the normal condition.

An autocorrelation analysis (Kendall, 1948) revealed that this is exactly what happened. The autocorrelation function for both conditions is plotted in Fig. 4. It is readily evident that

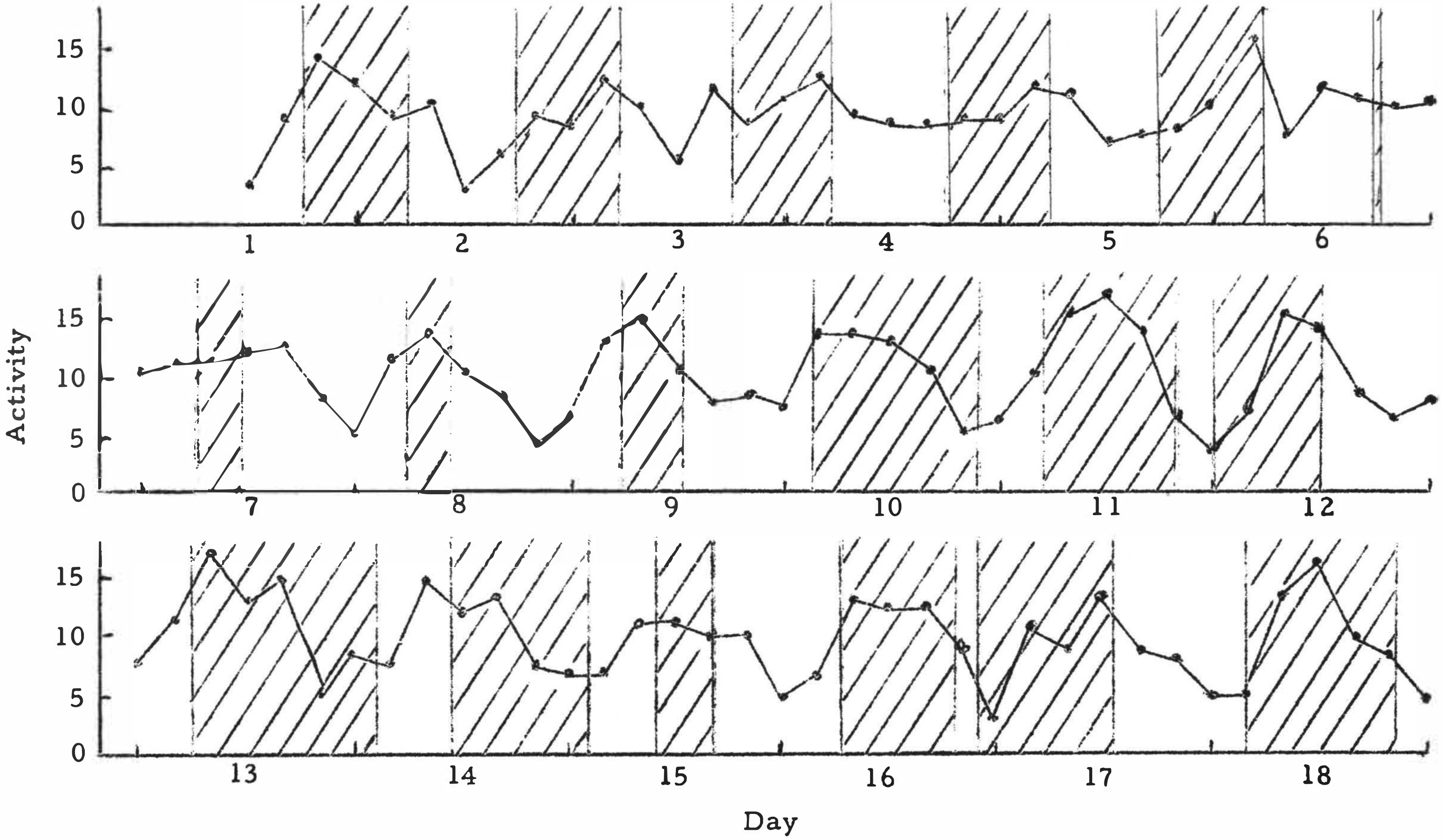

Fig. 2. Mean activity of S, four unit moving average. Shaded portions indicate light was off.

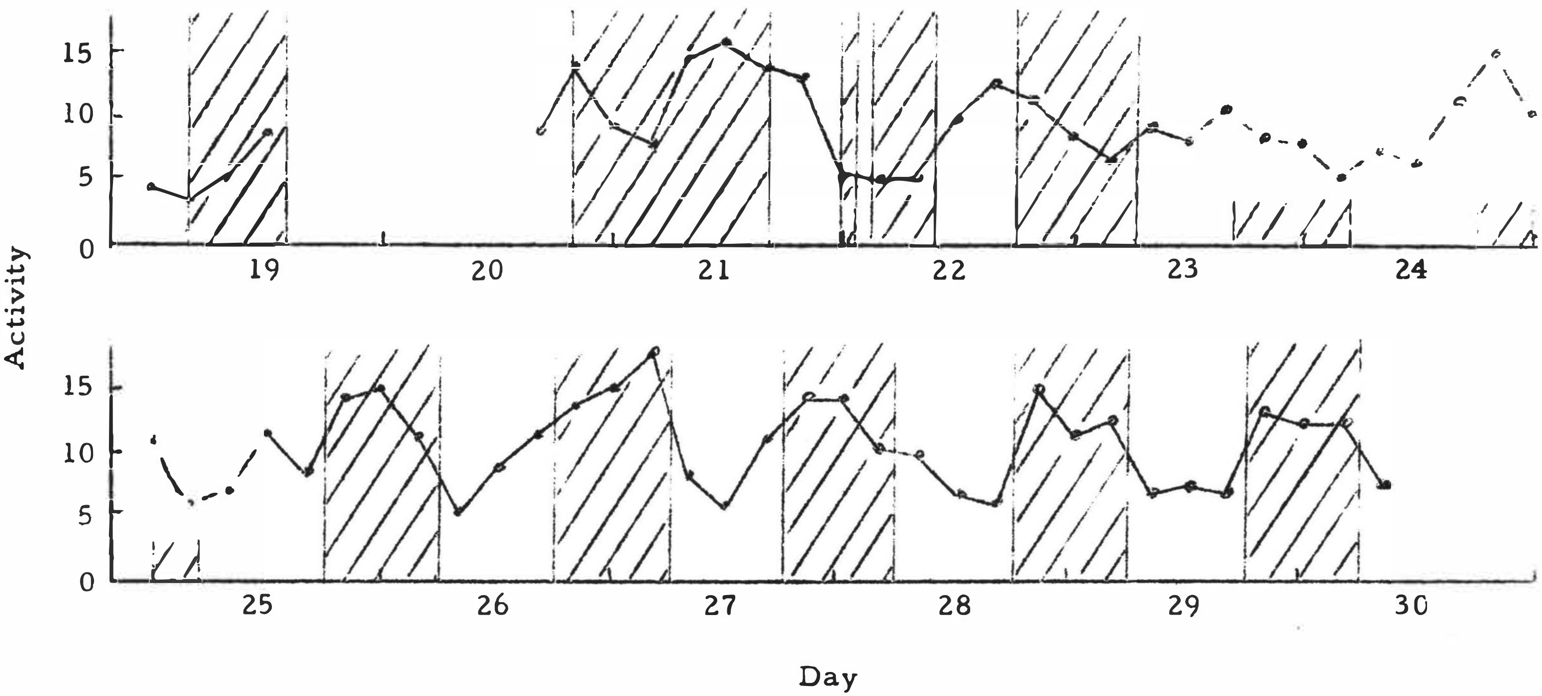

Fig. 3. Mean activity, continued from Fig. 2.

TABLE 1

Activity during light on and light off for both conditions.

	Light on		Light off		Total	
	M	SD	M	SD	M	SD
Normal	7.98	4.26	12.12	4.36	9.97	4.28
Randomized	8.57	5.72	11.19	5.67	10.01	5.81
Total	8.30	5.09	11.56	5.20	9.98	5.15

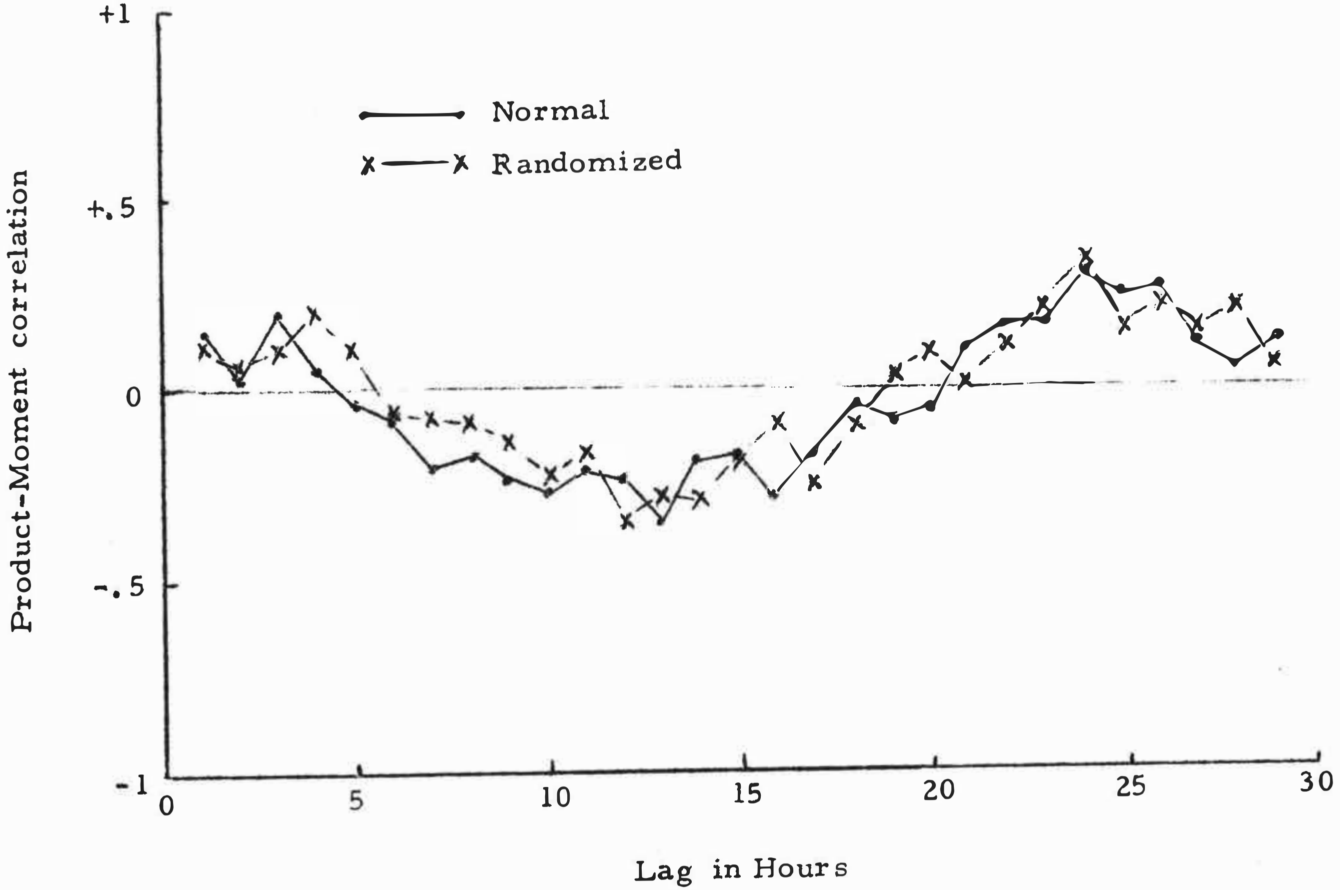

Fig. 4. Autocorrelation functions.

the two functions are very similar. Both show maximum lag correlations at 24 hours, indicating the period of the activity cycle is 24 hours for both conditions. The minimum correlations at 12 and 13 lag hours support this conclusion.

The mean daily activity for each hour and condition is shown in Fig. 5. The normal condition shows a cycle very similar to that obtained by Hodge, Peacock, & Thomas (1966), who obtained daily activity cycles from 10 rats for 38 days using an ultrasonic device.

The randomized means show a pattern which is phase shifted with respect to the normal means. They also show much greater variance between adjacent means as well as greater overall variance.

The evidence for a phase shift was further tested by an F test. The variance of the randomized means about the normal means is 382.72; the variance of the randomized means about the phase shifted normal means is 86.71. A test for equality of variances shows F to be highly significant ($F(23, 22) = 4.41$, $p < .001$).

This F ratio and the autocorrelation functions strongly indicate that S maintained a circadian cycle of activity with an 11 hour positive phase shift occurring upon imposition of the

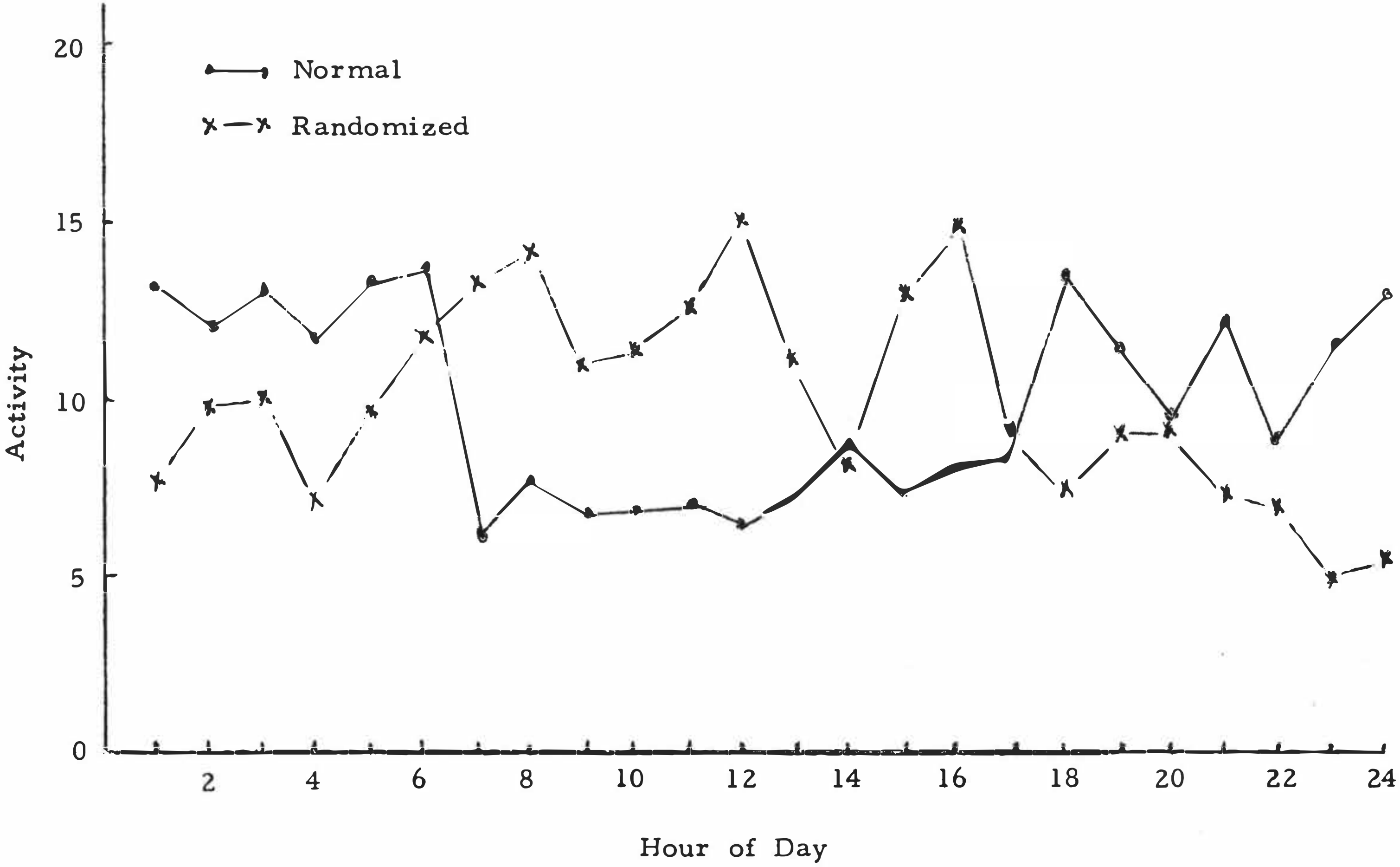

Fig. 5. Mean hourly activity, in arbitrary units.

randomized schedule and a second, 13 hour positive phase shift occurring upon reinstatement of the normal schedule.

Lockard & Lockard (1965) suggested that white rats respond to specific environmental conditions, as darkness, rather than to environmental changes, as light onset. Mean activity for each hour following a light onset or offset is shown in Table 2. Since the mean and standard deviation shown in the total column includes the hours of onset and offset a simple $\underline{z}$ score will give an indication of the extent of differences: $\underline{z} = .379$, $p = .648$. Thus, it appears that the hour immediately following a light onset or offset is not characterized by higher activity.

TABLE 2

Mean hourly activity following light onset and offset

	Light onset	Light offset	Total
Mean	10. 13	11. 93	9. 98
SD	5. 15	5. 63	5. 15

DISCUSSION

It appears that the results of the present study support the endogenous theory more than the exogenous theory. The initial circadian cycle of activity may have been due either to an endogenous cycle of behavior synchronized with environmental cues or to an exogenous cycle of behavior regulated by the lighting schedule.

On the sixth day at 1800 the light went off; at 1930 the same day it came back on. At this point S began to make a phase shift; it would have been most interesting to have been able to monitor body temperature and other indicants of the physiological experience undergone by S, as an EEG. The mean activity during this phase shift was 9.93, almost the exact mean of activity throughout the entire experiment. The first phase shift was thus characterized by continuous, moderate activity. S may have been experiencing certain conflicts between cycles. Some physiological cycles may have preadapted to the light offset; when the light came back on at 1930 other, more easily modified cycles may have begun to adapt to the unanticipated light onset. One result of these conflicting cycles could have been a moderate level of activity. In effect, some systems are telling S to become active (he has just rested for several hours) and other systems (perhaps mediated by the visual sense, as a past history of conditioning to rest during daylight) are telling S to rest.

A second possible explanation is that S was engaging in aviodance behavior; he was seeking some means by which to avoid the light. Similar explanations have been offered for cockroaches and other organisms (Hoffman, 1965).

After the phase shift is completed peaks of activity during the randomized condition occur during late morning on a regular basis (see Figs. 2 and 3). Correspondingly, troughs of activity occur around midnight. The last trough of activity during the randomized condition occurs at 21:2200, the last peak at 22:1600; the normal schedule is reinstated at 22:1800.

During the first dark periods of the second normal condition (22:1800 to 23:0600) a peak of activity occurs at 22:2200 and a trough occurs at 23:0200; S is still on his phase shifted cycle. Unfortunately at this critical point the light bulb blew out; from personal experience it is felt the bulb most likely ceased functioning either at 23:0600 or at 24:0600. In any event recording of activity continued uninterrupted, shown in Fig. 3 as the dashed line. A distinct trough occurs at 24:0700, a five hour shift from a trough at 23:0200. During day 26 a one hour shift occurred, during day 27 a three hour shift occurred. Days 28, 29, and 30 show a cycle of activity similar to days 1 through 4, more pronounced but in phase with the environmental lighting schedule.

Unlike the first phase shift the second phase shift occurred more gradually, an hour on this day, three hours on the next day, etc. S may have extinguished whatever anticipation had been learned during the first normal condition and so did not maintain a moderate level of activity over a long period of time. The fact the the second phase shift occurred at all indicates that S did not entirely ignore the environmental lights as he seemed to be doing during the randomized condition.

Table 1 points out the inattention given to the environmental lights during the randomized condition. The normal means shown are the means for the combined normal conditions. Although activity during darkness was not statistically greater than activity during light for either condition, the difference between light and dark activity for the randomized condition (2.62) is less than the same difference for the normal condition (4.14). Additionally, the normal condition had less variance than the randomized condition for both light and dark periods.

Brown's (1965) hypothesis that organisms respond to a hierarchy of timing cues could have been the case here. S may have responded to the lighting cues during the normal conditions and to some other environmental cues during the randomized condition. Thus, it would have been the phase relationship of the two strongest environmental cues which determined the phase shift and not a conflict or avoidance condition within the organism.

Brown & Terracini (1959) and Terracini & Brown (1962) indicated that rats and mice could respond to lunar forces as well as to cues related to the solar day. Rats and mice were more active at lunar nadir and less active during lunar zenith during their experiments.

Times of moonrise and moonset were obtained from January and February 1966 issues of the *Kalamazoo Gazette*, the local daily newspaper, and plotted against the activity of S. It was readily evident that S was not responding to lunar gravitational forces during either condition in a manner corresponding to Brown & Terracini's animals. During the first normal condition activity was generally greater when the moon was above the horizon than when it was below, a result contradictory to the results of Brown & Terracini (1959). However, S may have been responding to the light since light would probably be the primary cue. During the randomized schedule it is also evident that S was not responding to lunar forces. At 10:1800 a trough of activity occurred; at approximately 10:1900 lunar nadir occurred. Examination of other points strongly indicates that the first phase shift was not related to lunar gravitation in a manner discussed by Brown & Terracini (1959).

Although there is no evidence for a lunar day component comprising part of *S*'s cyclic behavior this does not preclude the possibility that *S* was responding to other geophysical forces which have the same period as the solar day. Additionally, it does not preclude the possibility that *S* was or is able to respond to lunar and synodic monthly factors and other multiday cycles. However, the autocorrelation functions (Fig. 4) indicate that, if *S* was responding to a geophysical cue, this cue had a solar day period.

The autocorrelation function is a standard technique for determining the period or frequency of a repeating phenomenon. Each point is the Pearson product-moment correlation between successive lags. Thus, lag 1 is the correlation between all possible adjacent hours, lag 2 the coreelation between all possible hours with a difference of two, e. g. between hours 0300 and 0500, 0400 and 0600, 0500 and 0700, etc. The lag at zero hours is +1 since the correlation of a score with itself in necessarily +1.

The autocorrelation function obtained here, plotted in a periodogram in Fig. 4, appears to be a sinusoidal curve. The maximum point indicates the period of the data, in this case the maximum falls at 24 hours. The autocorrelation in Fig. 4 indicates that one could predict S's activity for the next hour better by knowing what he did 24 hours ago than by knowing what he did during the immediately previous hour. The reliability between the same hour on successive days is greater than the reliability between successive hours on the same day.

It is evident from inspection that the normal and randomized condition have very similar autocorrelations; the phase shifts did not affect the autocorrelation since they fell at the times of the randomized condition imposition and normal condition reinstatement.

There was some question of a lunar day component contributing to the phase shift; such a component would be shown in the autocorrelation function as an increased correlation coefficient at 25 lag hours since a lunar day is 24 hours 50 minutes long. For the randomized condition, lag 24 correlation is .330 and lag 25 correlation is .179. A Fisher's Z test between correlation coefficients (Guilford, 1956) yields 1.877, just below the five per cent level of significance for a two-tailed test. A t test between correlated correlation coefficients (Walker & Lev, 1953) yields 2.346 ($p <$ .01). The correlation between the two coefficients was taken as .157, the correlation between adjacent hours (lag 1).

Activity was collapsed across days to give mean activity for each hour over one day, shown in Fig. 5 for both conditions. The phase shift of the randomized condition has been discussed. It is evident that the randomized condition showed much greater variance between successive hours that did the normal condition. Hodge, Peacock, & Thomas (1966) obtained activity cycles from 10 rets over 38 days using an ultrasonic device, which measures activity very precisely. Their obtained means and the normal means of the present study are presented in Fig. 6 for comparison. Inspection shows the two cycles are similar in periodicity and order of magnitude (2). Since the units of activity are not equilivant a statistical comparison of the two studies is not applicable. A certain amount of the variance in the present study is undoubtably due to the method of measuring activity, the sampling technique used (against continuous monitoring of activity), and the recording device (an event recorder versus automatic card punch) as well as the smaller number of Ss and fewer experimental days. Dispite these drawbacks the obtained results remain valid.

A replication of this study is currently being undertaken by D. H. Thor, R. O. Baldwin, and other researchers. Certain aspects of the procedure will undoubtably be changed, as the distribution from which the length of the light and dark periods are

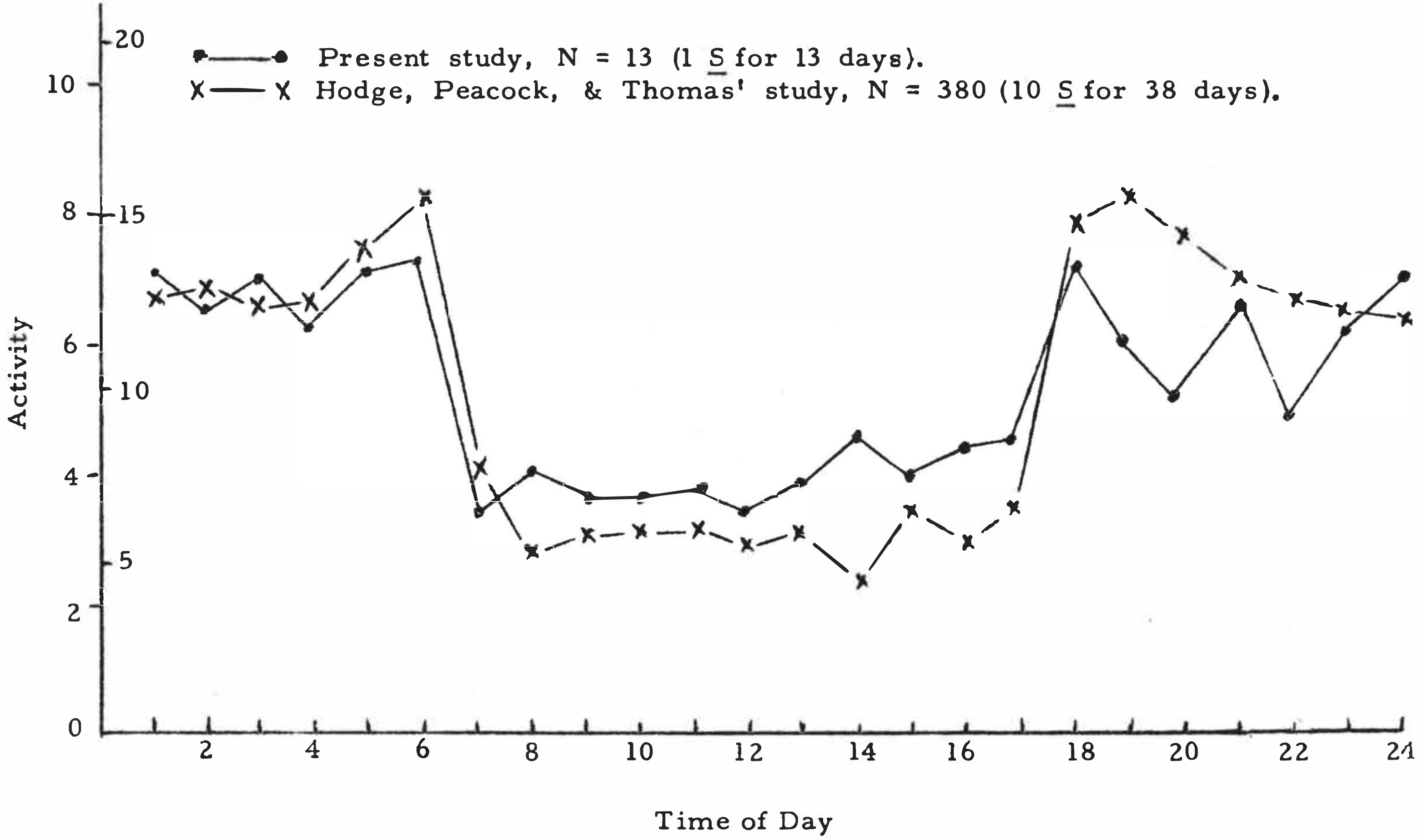

Fig. 6. Comparison of present study with a previous study (Hodge, Peacock, & Thomas, 1966).

drawn. However, results grossly different from the results of the present study are not to be expected; the only result which may be different is the phase shift. If the phase shift is partially a function of the first few light-dark periods of the randomized condition (and partially a function of the organism's physiology and history) then the degree of phase shifting will vary for different initial randomized light-dark period lengths. In any event it seems reasonable to expect S to maintain a circadian cycle of activity throughout the experiment.

The application of randomization of timing cues as a method of investigation is valid. Because of the limited response repertoire of a rat and other animals the method may be limited for these Ss. However, it may be possible to get an estimate of a rat's preference strength for a normal lighting schedule through prior training, as on a bar press.

The method appears to have special significance for use with humans since almost all human activiy takes place in some kind of light, natural or artificial. A randomized lighting schedule would not enable S to plan future behavior.

SUMMARY

Cyclic behaviors, especially circadian cycles, persist in the absence of overt environmental cues. The *endogenous* theory states that cyclic behaviors originate within the organism. These cycles are independent of environmental cues and, once started, need only occasional resynchronization. The *exogenous* theory holds that cyclic behaviors result from the organism responding to environmental cues, e. g. lunar gravitation.

By presenting timing cues in a random pattern, as contrasted to the normal cyclic pattern, it was hypothesized that one theory or the other would be supported.

One white male rat was placed in an activity cage inside an attenuated chamber. As the animal moved about portions of the cage floor activated an event recorder which sampled 30 sec. of activity every five min. for 30 days. A normal light schedule (12 hour on, 12 hours off), controlled by a timer, was maintained for 30 days, of which the last five days were recorded. A randomized light schedule was then imposed with light and dark durations ranging from 1.5 to 22.5 hours. After 16 days the normal light schedule was reinstated.

Mean activity during the randomized condition showed a phase displacement and greater variance but a circadian component was still very much evident. Autocorrelations for both conditions showed a period of 24 hours with no evidence for any periods different from 24 hours contributing to the cyclic behavior.

The consistency of the phase shift during the randomized condition supports the endogenous theory. The second phase shift occurring upon reinstatement of the normal condition may also be considered as support for the endogenous theory. The method of randomization of time cues may be considered a useful method of investigation of cyclic behavior.

REFERENCES

Abramowitz, A. A. The chromatophorotropic hormone of the crustacea: standardization, properties, and physiology of the eyestalk glands. Biol. Bull., 1937, 72, 344-365.

Ader, R. Gastric erosions in the rat: effects of immobilization at different points in the activity cycle. Science, 1964, 145, 406-407.

Aschoff, J. Exogene und endogene Komponente der 24-Stunden-Periodik bei Tier und Mensch. Naturwiss., 1955a, 42, 569-575.

Aschoff, J. Tagesperiodik von Mäusenstammen unter konstanten Umgebungsbedingungen. Pflug. Arch. ges. Physiol., 1955b, 262, 51-59. Cited by Cloudsley-Thompson, J. L. Rhythmic activity in animal physiology and behaviour. New York: Academic Press, 1961. Pp. 145-146.

Aschoff, J. Circadian rhythms in man. Science, 1965, 148, 1427-1432.

Bolles, R. C., & Stojkiewicz, L. W. The rat's anticipation of diurnal and a-diurnal feeding. J. Comp. Physiol. Psychol., in press.

Browman, L. G. Artificial sixteen-hour day activity rhythms in the white rat. Amer. J. Physiol., 1952, 168, 694-697.

Brown, F. A., Jr. The rhythmic nature of animals and plants. Tri-Quarterly (Northwestern University), 1958, 1, 1, 35-47.

Brown, F. A., Jr. A unified theory for biological rhythms: rhythmic duplicity and the gensis of "circa" periodisms. In J. Aschoff (Ed.). Circadian clocks. Amsterdam: North-Holland, 1965. Pp. 231-261.

Brown, F. A., Jr., Bennett, M. F., & Webb, H. Marguerite. Persistent daily and tidal rhythms of O_2 consumption in fiddler crabs. J. Cell. Comp. Physiol., 1954, 44, 477-505.

Brown, F. A., Jr., & Terracini, Emma D. Exogenous timing of rat spontaneous activity periods. Proc. Soc. exp. Biol. Med., 1959, 101, 457-460.

Bruce, V. G. Cell division rhythms and the circadian clock. In J. Aschoff (Ed.). Circadian clocks. Amsterdam: North-Holland, 1965. Pp. 125-138.

Bünning, E. The physiological clock. New York: Academic Press, 1964.

Cloudsley-Thompson, J. L. Rhythmic activity in animal physiology and behaviour. New York: Academic Press, 1961.

Cole, C. L., & Adkisson, P. L. Daily rhythm in the susceptibility of an insect to a toxic agent. Science, 1964, 144, 1148-1149.

Cole, C. L., & Adkisson, P. L. A circadian rhythm in the susceptibility of an insect to an insecticide. In J. Aschoff (Ed.). Circadian clocks. Amsterdam: North-Holland, 1965. Pp. 309-313.

Crawford, M. L. J., & Nicora, B. D. Measurement of human group activity. Psychol. Rep., 1964, 15, 227-231.

Crawford, M. L. J., & Thor, D. H. Circadian activity and noise comparisons of two confined groups with and without reference to clock time. Percpt. mot. Skills, 1964, 19, 211-216.

Enright, J. J. Accurate geophysical rhythms and frequency analysis. In J. Aschoff (Ed.). Circadian clocks. Amsterdam: North-Holland, 1965. Pp. 31-42.

Guilford, J. P. Fundamental statistics in psychology and education. New York: McGraw-Hill, 1956. P. 194.

Halberg, F. Temporal coordination of physiologic function. *Cold spring harbor symposia on quantitative biology.* Vol. 25. Cold Spring Harbor, L. I., N. Y.: Cold Spring Harbor Laboratory of Quantitative Biology, 1960. Pp. 289-310.

Halberg, F. Physiologic rhythms. In J. D. Hardy (Ed.). *Physiological problems in space exploration.* Springfield, Ill.: Charles C. Thomas, 1964. Pp. 298-322.

Halberg, F., Halberg, E., Barnum, C. P., & Bittner, J. J. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen, and daily routine. In R. B. Withrow (Ed.). *Photoperiodism and related phenomena in plants and animals.* Washington, D. C.: Amer. Assoc. Adv. Sci., 1959. Pp. 803-878.

Hamner, K. C., Finn, J. C., Siroki, G. S., Hoshizaki, T., & Carpenter, B. H. The biological clock at the south pole. *Nature* (Lond.), 1962, 195, 476-480.

Hastings, J. W., & Keynan, A. Molecular aspects of circadian systems. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 167-182.

Hastings, J. W., & Sweeney, Beatrice M. The gonyaulax clock. In R. B. Withrow (Ed.). *Photoperiodism and related phenomena in plants and animals.* Washington, D. C.: Amer. Assoc. Adv. Sci., 1959. Pp. 567-584.

Hodge, M. H., Peacock, L. J., & Thomas, R. R. Ultrasonic measurement and automatic analysis of general activity in the rat. in press.

Hoffman, K. Overt circadian frequencies and the circadian rule. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 87-94.

Kendall, M. G. *The advanced theory of statistics.* Vol. 2 (2nd ed.). New York: Hafner, 1948. Pp. 396-439.

Kleitman, N. Sleep and wakefulness (Rev. ed.). Chicago: Univ. of Chicago, 1963.

Lobban, Mary C. Dissociation in human rhythmic functions. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 219-227.

Lockard, R. B. Some effect of light upon the behavior of rodents. *Psychol. Bull.,* 1963, 60, 6, 509-529.

Lockard, R. B., & Lockard, Joan S. Stimulus-change versus preference for light. *Psychol. Rep.,* 1964, 15, 191-198.

Pinc, B. quoted in *Kalamazoo Gazette* , Jan. 27, 1966.

Pittendrigh, C. S., & Bruce, V. G. Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism. In R. B. Withrow (Ed.). *Photoperiodism and related phenomena in plants and animals.* Washington, D. C.: Amer. Assoc. Adv. Sci., 1959. Pp. 475-506.

Pizzarello, D. J., Witcofski, R. L., & Lyons, E. Ann. Variations in survival time after whole-body radiation at two times of day. *Science,* 1963, 139, 349.

Rawson, K. S. Experimental modification of mammalian endogenous activity rhythms. In R. B. Withrow (Ed.). *Photoperiodism and related phenomena in plants and animals.* Washington, D. C.: Amer. Assoc. Adv. Sci., 1959. Pp. 791-800.

Reinberg, A. Hours of changing responsiveness in relation to allergy and the circadian adrenal cycle. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 214-218.

Rensing, L. Ontogenetic timing and circadian rhythms in insects. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 406-412.

Roberts, S. K. Photoreception and entrainment of cockroach activity rhythms. *Science,* 1965a, 148, 958.

Roberts, S. K. Significance of endocrines and central nervous system in circadian rhythms. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965b. Pp. 198-213.

Schweiger, H. G., & Schweiger, E. The role of the nucleus in a cytoplasmic diurnal rhythm. In J. Aschoff (Ed.). *Circadian clocks*. Amsterdam: North-Holland, 1965. Pp. 195-197.

Schweiger, E., Wallraff, H. G., & Schweiger, H. G. Endogenou circadian rhythm in cytoplasm of acetabularia: influence of nucleus. *Science,* 1964, 658-659.

Terracini, Emma D., & Brown, F. A., Jr. Periodisms in mouse "spontaneous" activity synchronized with major geophysical cycles. *Physiol. Zool.*, 1962, 35, 1, 27-37.

Thor, D. H. Diurnal vaiability in time estimation. *Percpt. mot. Skills,* 1962a, 15, 451-454.

Thor, D. H. Time perspective and time of day. *Psychol. Rec.,* 1962b, 12, 417-422.

Thor, D. H., & Baldwin, R. O. Time of day estimates at six times of day under normal conditions. *Percept. mot. Skills,* 1965, 21, 904-906.

Thor, D. H., & Crawford, M. L. J. Time perception during a two-week confinement: influence of age, sex, IQ, and time of day. *Acta Psychologica,* 1964, 22, 78-84.

Tribukait, B. Aktivitätsperiodik der Maus im künstlich verkürzten Tag. *Naturwiss.,* 1954, 41, 92-93.

Walker, Helen M., & Lev, J. *Statistical inference.* New York: Henry Holt, 1953. Pp. 256-257.

Wolfson, A. The role of light and darkness in the regulation of spring migration and reproductive cycles in birds. In R. B. Withrow (Ed.). Photoperiodism and related phenomena in plants and animals. Washington, D. C.: Amer. Assoc. Adv. Sci., 1959. Pp. 679-716.

Wolfson, A. Circadian rhythm and the photoperiodic regulation of the annual reproductive cycle in birds. In J. Aschoff (Ed.). *Circadian clocks.* Amsterdam: North-Holland, 1965. Pp. 370-378.