THE SLEEP NEED: SLEEP DEPRIVATION IN THE RAT

By

ROBERT ALAN LEVITT

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INTRODUCTION

Using as our criterion the time spent in consummatory activity, sleep is by far the most important of man's activities (Cofer & Appley, 1964). And yet, although sleep is presently receiving a great deal of experimental attention, little is known of its biological function. Sir John Eccles, in opening an international symposium on sleep, put it this way. "When we consider the immense human significance of sleep, the absolute necessity for us to spend a considerable part of our lives in abject mental annihilation, it is remarkable how little we know about it, how little we can say to account for the necessity of sleep" (Eccles, 1960, p. 1).

The experiments to be reported in this paper are a beginning on an analysis of the necessity for sleep. The method utilized is to produce a heightened need through deprivation of sleep and to study the changes in behavior following this deprivation. The extirpation of an organ has long been a recognized method of determining function. Depriving the organism of a particular bodily activity (sleep) is the functional counterpart (Kleitman, 1963). The dependent variable in these experiments is the

characteristics of the sleep cycle as measured electrophysiologically or by means of an activity measuring unit. The basic assumption underlying this research is that the compensatory changes following sleep deprivation are part of the homeostatic bodily mechanisms which function during sleep. A number of investigators have suggested the existence of stages or levels of sleep. It may be that these various stages of sleep subserve different bodily functions.

Stages

The first major human electroencephalographic (EEG) study of sleep was made by Loomis, Harvey, and Hobart (1937). They classified the EEG into five stages, A to E, of increasing sleep depth. Other investigators have preferred somewhat different classifications (Kleitman, 1963; Oswald, 1962). The waking EEG is of a low amplitude and high frequency. As the depth of sleep increases (from stages A through E or 1 through 4), the amplitude increases and the frequency is reduced. Although most investigators have assumed that the function of sleep is accomplished mostly at increasing depths, at the present time no separate functions can be attributed to the different stages. The most that can be said for the function of sleep is that investigators tend to assume a restorative one, allowing the recovery from deficits produced by waking

activity (Kleitman, 1963; Oswald, 1962). However, new evidence has suggested a specific function for at least one stage of sleep.

Dreaming.--In recent years attention has been directed to a stage of sleep designated the rapid eye movement phase (REMP). This stage has also been called paradoxical, activated, rhinencephalic, or low voltage fast sleep (LVFS). These terms will be used interchangeably in this paper. REM sleep was first described in human subjects by Aserinsky and Kleitman in 1953. Since then, an analogous stage of sleep has been described in a number of other species, including the cat (Dement, 1958) and the rat (Hall, 1963; Swisher, 1962).

When Aserinsky and Kleitman (1953) awoke subjects from the different stages of sleep including the REMP, they made the exciting discovery that dreams were reported about 80 per cent of the time when the awakening interrupted REM sleep but only about 20 per cent when the awakening interrupted other sleep stages. This discovery led to a number of studies exploring this relationship (Dement, 1955; Dement & Kleitman, 1957a). Dement (1965), upon reviewing the evidence on dreaming, has concluded that although the probability of dream recall and by inference the probability of dreaming is much higher in REM than non-REM (NREM) sleep, dreams do occur during NREM sleep. However, Dement has

suggested that REM and NREM dreams differ in their essential nature with only REM dreams consisting of perceptual experiences (Class I experiencing). He has theorized that, "during the REM phase of sleep the brain is somehow generating the neurophysiological background for Class I experiencing" (Dement, 1965, p. 203). NREM dreams consist only of Class II experiencing (abstract thought, imagery) not dependent on sensory input. Thus, during REM dreams the brain is generating its own sensory input. Dement (1965) and Jouvet (1960) have suggested the existence of two qualitatively different phases of sleep.

Sleep phases.--Jouvet (1960; Jouvet & Jouvet , 1963) has demonstrated that the paradoxical phase of sleep is triggered by an area in the caudal brain stem. He has called this phase rhombencephalic sleep to differentiate it from slow wave sleep which he believes is telencephalic in origin. The paradoxical nature of rhombencephalic sleep is that the EEG is one of waking or light sleep, but the depth of sleep as determined by arousal threshold is deeper than during any other stage (Dement & Kleitman, 1957b; Dillon & Webb, in press).

Both Dement (1965) and Jouvet (1960) have concluded that there are two entirely distinct phases of sleep. One of these phases is the REM stage and the other is all the slow wave sleep that precedes it. Dement (1965) has

suggested that physiological variations within NREM sleep are essentially quantitative and do not warrant further subdivision. These two phases may be expected to subserve differing functions and may respond independently to experimental manipulations.

The measurement of sleep

Until now there have been two means of measuring the sleep cycle, behavioral observation and the electroencephalograph. Both of these methods have severe disadvantages. Behavioral observation requires the continuing presence of an observer to make a subjective judgment of whether the subject (S) is asleep or awake. The experimenter's very presence is likely to disturb the sleep cycle and also means a great expense in man hours. For this expense only a subjective judgment of sleep or waking is produced.

The EEG surmounts two of these difficulties; levels of sleep are discriminable and the judgment is objective. However, these advantages are obtained at considerable expense. An observer must constantly be present to monitor the equipment, and electrodes need to be attached to the subject. In the case of the rat, the animal used in these studies, the recording electrodes usually are surgically implanted under the skull. These electrodes are likely to alter somewhat the nature of the response being measured. This large expense in observer and equipment severely limits the length of EEG recording that it is practical to

obtain. As an alternative, the first experiment reported here presents an activity measuring device for recording sleep and waking. This method has the advantage that it is inexpensive, relative to the EEG, and does not require the continuing presence of an experimenter. This activity system may be operated continually for long periods of time. Since the activity system has the disadvantage of being unable to discriminate phases of sleep, both activity and EEG recording were used in these experiments.

The production of sleep deprivation

Another difficulty which had to be surmounted was that of producing sleep deprivation. Licklider and Bunch (1946) were unable to keep rats awake on beds of nails, but thought they could successfully maintain wakefulness by forcing the Ss to walk a treadmill partially submerged in water. The rats died after 3 to 14 days on the treadmill probably, at least partially, from fighting with each other (the rats were not in separate compartments). Evidence to be reported in this paper raises serious questions as to the effectiveness of the treadmill as a method of producing sleep deprivation.

Svorad and Novikova (1960) have reported successfully producing sleep deprivation lasting seven days in rats by periodically administering dextro-amphetamine. The authors do not state their criterion of sleep deprivation, nor do

they report data on the sleep cycle in their Ss. This procedure was part of a study on the effect of sleep deprivation on an experimentally induced neurosis in the rat. In a preliminary study the present author was successful in replicating the finding of Svorad and Novikova that sleep deprivation could be produced in rats with dextro-amphetamine.

According to Beckman (1961) dextro-amphetamine is used clinically, principally as a stimulant of the central nervous system and for its anorexigenic effect in the treatment of obesity. Bradley and Elkes (1957) have found a correlated EEG desynchronization and behavioral alertness to follow systemic dextro-amphetamine administration. This arousal was like that following electrical reticular formation stimulation and was dependent on an intact mesencephalon for its occurrence, implicating receptors in the ascending reticular activating system.

Williams, Lubin, and Goodnow (1959) have studied the effects of sleep loss in humans on performance at skilled tasks. They felt the impaired performance was due to the occurrence of brief sleep episodes in sleep deprived subjects during performance. This interpretation is supported by the experiments of Kornetsky, Mirsky, Kessler, and Dorff (1959), who found that dextro-amphetamine, which stimulates the reticular formation, greatly reduces performance impairment in sleep deprived Ss.

The results of sleep deprivation

The only report of a change in sleep behavior following sleep deprivation in the rat is that of Webb (1957), who found that rats sleep deprived on a water-immersed treadmill had shorter "sleep latencies" (time to fall asleep) than during control tests.

Kleitman (1963) and Oswald (1962) have reported an increase in sleep time following prolonged sleep deprivation in humans. This increase is not equal to the amount of sleep lost, but amounts to about 11 to 14 hours sleep the first night following up to 65 hours sleep deprivation. Berger and Oswald (1962) and Williams, Hammack, Daly, Dement, and Lubin (1963) have reported data on changes in the EEG stages of sleep following deprivation. Both studies reported an increase in stage 4 on the first recovery night, followed by an increase in REM sleep on the second recovery night. The authors of these articles infer that these rebound effects reflect an underlying "need" state of the organism. Webb and Agnew (1965), in an experiment on continued partial sleep deprivation in the human, found an increase in stage 4 sleep. This increase was at the expense, primarily, of stage 3 with essentially no change in REM or stage 2 sleep as a percentage of total sleep. This result is probably due to the Ss being allowed only three hours sleep per night. Since the stage 4 "need" apparently takes precedence

over the REM "need," the Ss did not have time to compensate for the REM loss.

Oswald (1962) has suggested that there may not be a fundamental requirement for adult humans to spend 8 out of every 24 hours asleep, since deprived subjects make up only a fraction of the deficit on the first night. It would seem this question cannot be answered at the present, since compensatory changes in sleep behavior may continue to occur for a number of days following deprivation. However, it may be that there is a difference in the "value" of the various stages or phases of sleep in alleviating a large sleep debt, and that following sleep deprivation the "most valuable" kind of sleep will occur in greater quantity than normal.

If, in fact, as Dement and Jouvet have suggested there are two distinct sleep phases differing in function, then this assumption of a change in sleep characteristics following total sleep deprivation is reasonable. There are a number of reports of deprivation of one or another stage of sleep. Dement (1960) has reported that REM (dream) deprivation by awakening human subjects every time they enter REM sleep for five consecutive nights results in the need for an increased number of awakenings on successive nights in order to prevent occurrences of REM sleep. He also found a compensatory increase in REM time following

deprivation. Dement also reported REM deprivation to produce "personality disturbances" in the subjects, to a degree that some subjects were forced to terminate this part of the experiment. These personality disturbances occurred in spite of a normal, or near normal, level of total sleep and did not occur when, in a control study, the same Ss were awakened only from NREM sleep.

These findings have recently been replicated by Siegel and Gordon (1965) in the cat and Khazan and Sawyer (1963) in the rabbit. Siegel and Gordon's Ss required an increased number of awakenings on successive days of deprivation in order to prevent paradoxical sleep, and compensated for the deprivation with an increased amount of paradoxical sleep after deprivation was discontinued. Siegel and Gordon recorded EEGs for 10 to 12 hours a day during a so-called "sleep period." Paradoxical sleep deprivation during this sleep period was produced by electrical stimulation of the reticular formation each time the S entered paradoxical sleep. During the other 12 to 14 hours per day, the authors report keeping the cats awake by placing them on a brick in the middle of a pan of water, which was the floor of the cage. Khazan and Sawyer (1963) discovered that a constant loud noise (80 decibels) would almost obliterate paradoxical sleep without influencing the amount of slow wave sleep. Following 20 hours of paradoxical sleep deprivation, a rebound increase occurred. After 20 hours, the paradoxical

sleep inhibition produced by the white noise tended to abate.

Agnew, Webb, and Williams (1964) have reported a compensatory increase in stage 4 sleep following deprivation of this sleep stage in humans. Current sleep research is centered on the importance and function of these two stages of sleep, REM and stage 4.

In order to answer the questions of (a) the length of time that compensatory changes continue following total sleep deprivation, (b) the amount of total compensation that occurs, and (c) the possibility of changes in the phase characteristics of sleep following total sleep deprivation, the following experiments were undertaken.

The first experiment details the design and development of an activity system to record the sleep cycle in small mammals. Experiment II studies the effect of varying lengths of dextro-amphetamine induced sleep deprivation on the sleep cycle measured by the ultrasonic activity units. Experiment III is concerned with the character of the EEG of rats walking on the water-immersed treadmill. Previous behavioral observation had suggested that rats may get some sleep on the treadmill, and this observation was confirmed in Experiment III. In spite of this finding, in Experiment IV sleep cycle was measured before and after treadmill deprivation using the activity units. It was

thought that although the Ss did obtain some sleep, the amount was less than normal, and this might be evident in post-deprivation compensation. In Experiment V sleep cycle was measured before and after dextro-amphetamine or treadmill deprivation, but the EEG was used instead of the activity units. Although it is not now considered reasonable to record EEG for the lengths of time we use the activity units, it was possible to get 24-hours' data before and after deprivation. Although these data are not as stable or reliable as the activity data, the EEG does permit the differentiation of paradoxical from slow wave sleep.

These experiments have required lengthy normative sleep cycle recording and enable the detailing of the normal parameters of sleep in our subject population.

EXPERIMENT I

THE DEVELOPMENT OF AN ULTRASONIC ACTIVITY DEVICE TO MEASURE SLEEP AND WAKING IN THE RAT

Dillon (1963) used an ultrasonic recording device developed by Peacock and Williams (1962) to record sleeping and waking in the rat. Higgins (1964) has built a transistorized version of the Peacock and Williams unit. This experiment presents the methodology for an ultrasonic activity measure of sleep. The system is a modification of Dillon's. A simple scoring system has been developed, a pendulum-type calibrator for adjusting sensitivity has been designed and tested, and permanent enclosures for the system have been built. Using these modifications a series of correlated EEG-activity recordings were collected.

Method

Subjects

Six male Long-Evans hooded rats were used. Three were 90-100 and three 180-200 days old. They were maintained on ad-lib. food and water throughout the experiment.

Apparatus

Sleep and waking were determined by a Grass-III-D

EEG unit. Behavioral observations were periodically recorded on the EEG record. Each minute was scored for waking, slow wave sleep, or paradoxical sleep. Whichever of these was most prevalent was the condition assigned to that minute.

Activity was measured with an ultrasonic device developed by Peacock and Williams (1962) or a transistorized version built by Alton Electronics (Higgins, 1964). These activity devices are intended for use in detecting the movement of small mammals. The devices are usable within a one-cubic yard area. The Peacock and Williams unit consists of a transmitting transducer, receiving transducer, power supply, and readout unit. The readout drives an ink writing recorder. In the Alton units the power supply drives the recorder directly.

The principle of operation is as follows. A 40-kilocycle sine wave signal is generated by the transmitter and radiated as ultrasonic sound (nonaudible) into the test volume by the transmitting transducer. These sound waves permeate the experimental volume, being reflected from its walls and from objects within the experimental volume.

At the receiver a transducer picks up the sound waves, converting them back into electrical signals which are then amplified. Any motion within the experimental volume into which the sound wave is directed produces disturbances in

the received portion of the wave, causing the receiver to produce electrical pulses. These electrical pulses may be recorded directly on a strip chart recorder (Alton unit) or (Peacock and Williams unit) used to operate a relay which in turn may operate a recording device (Higgins, 1964).

The ink writing recorder is an Esterline-Angus Operation Recorder, Model AW.

The magnitude of movement which causes a pulse can be adjusted through a broad range of behavior by manipulating a sensitivity control.

Calibration

The activity units have a sensitivity adjustment for controlling the level of activity that will activate the system. A pendulum-type calibrator was used to determine the sensitivity. Figure 1 is a dimensional diagram of this calibrator. The pendulum was manually held at a given distance from the vertical and then released. The distance between the swing adjustment and pendulum screw in these experiments was 0.20 inch. The time from release until the activity unit stopped responding to the decreasing motion was the unit of calibration. A reading was made immediately before data collection began, and this reading was the calibration setting. A goal of this experiment was to determine the range of sensitivity settings at which an

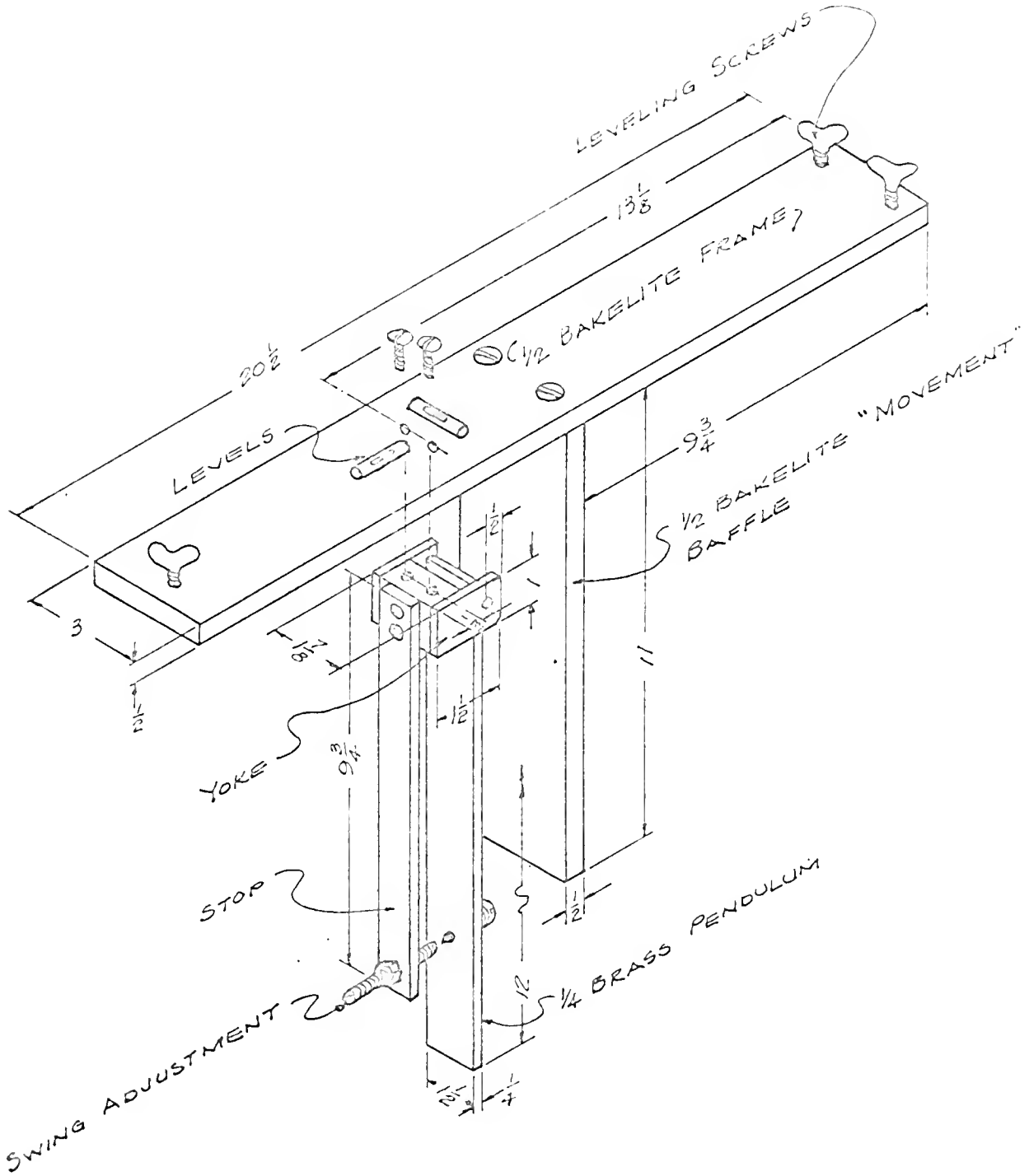


Figure 1. The Pendulum-type Calibrator.

acceptable correlation between EEG and activity is obtainable.

Recording procedure

Care must be taken to ensure that activity and EEG records correspond when both are being recorded. This means that sections of data from these two methods should be within 1 to 2 seconds of each other. Dillon (1963) has described a simple method to ensure this agreement; the system mainly involves using a stop watch to turn one system on an exactly known time after the other.

Environmental conditions

For EEG-activity recording the animal cage was placed inside an electrically shielded room. The cages were 9 in. high, 11 in. wide, and 16 in. long. The cage bottom was 1 in. above the floor allowing droppings to fall from the cage. All six sides were of wire mesh, permitting the ultrasonic waves to pass through. The cages were individually housed within a wall-board enclosure measuring 32-1/2 in. long, 14 in. wide, and 16 in. high. This enclosure was designed to bounce the waves throughout the cage and eliminate "dead" spots in the corners and also to prevent leakage of ultrasonic sound into neighboring units, causing interference and interaction. Figure 2 is a dimensional diagram showing the wall-board enclosure and a Peacock and Williams activity unit ready for operation. The Alton unit

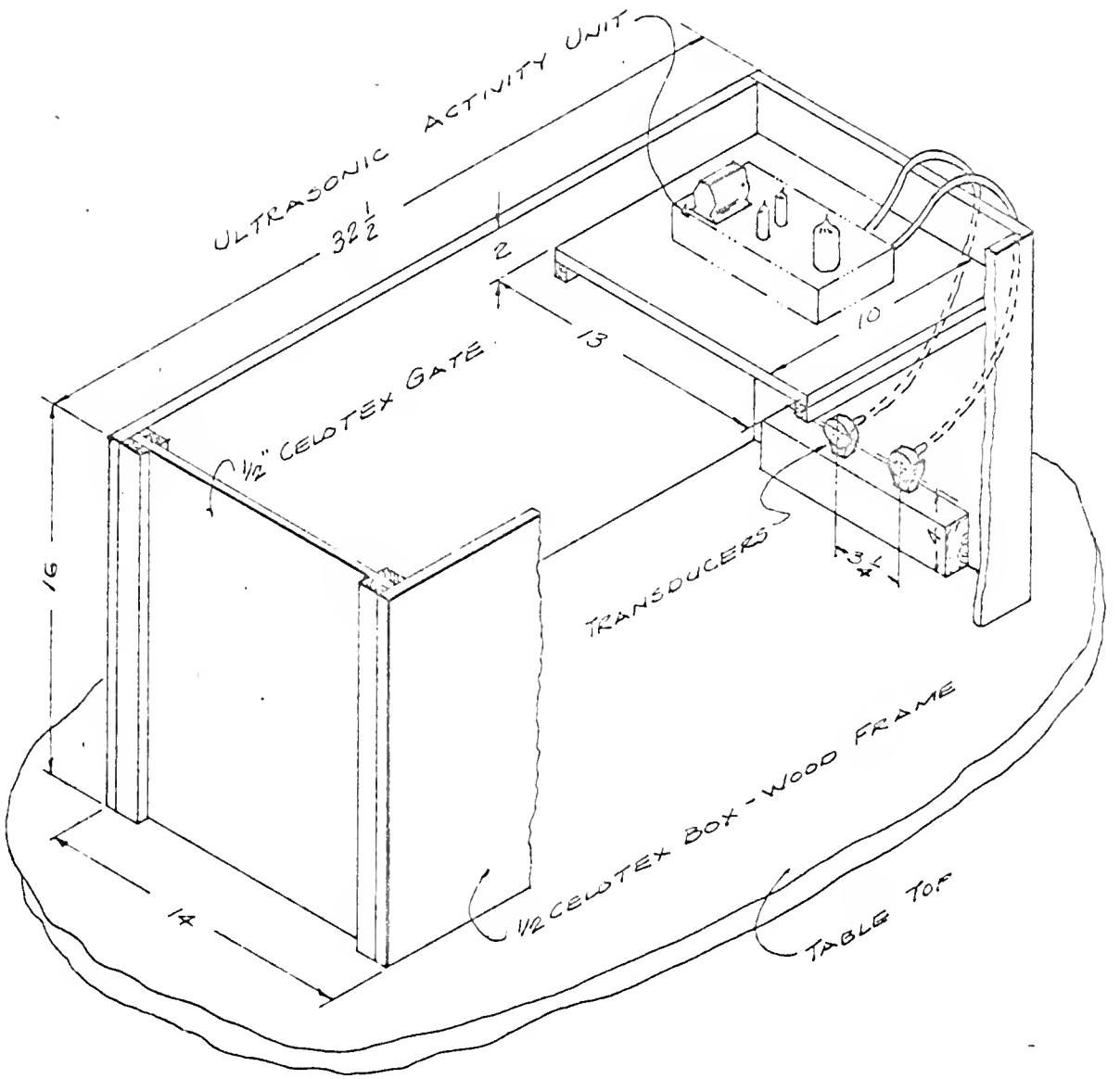


Figure 2. The Environmental Enclosure and Ultrasonic Activity Unit.

does not have the activity unit sitting on top of the enclosure but on the floor behind the transducers. Figure 3 shows the calibrator position in the enclosure during calibration. The "movement baffle" faces toward the activity transducers. The cage was removed from the enclosure for calibration.

The two activity transducers were 3-1/4 in. apart parallel to and 10 in. from the cage. The activity unit placement is shown in Figure 2. The activity transducers were at a level 3 in. above the cage floor. The recorder was housed inside an "ice chest" to reduce noise.

A light cycle was maintained in the room with lights on 9:00 A.M. to 9:00 P.M. and off 9:00 P.M. to 9:00 A.M. The room was air-conditioned with the temperature maintained between 66 and 69 degrees. The activity units are sensitive to temperature and function best at this level.

Implantations

Gold bipolar dural electrodes were fabricated and implanted under nembutal anesthesia at least four days prior to recording. The method is described in detail by Dillon (1963). Both electrodes were placed on the same side of the skull, one in the posterior and one in the frontal area. This electrode placement is important, since Swisher (1962) has found it difficult to differentiate paradoxical sleep from waking if symmetrical electrodes are

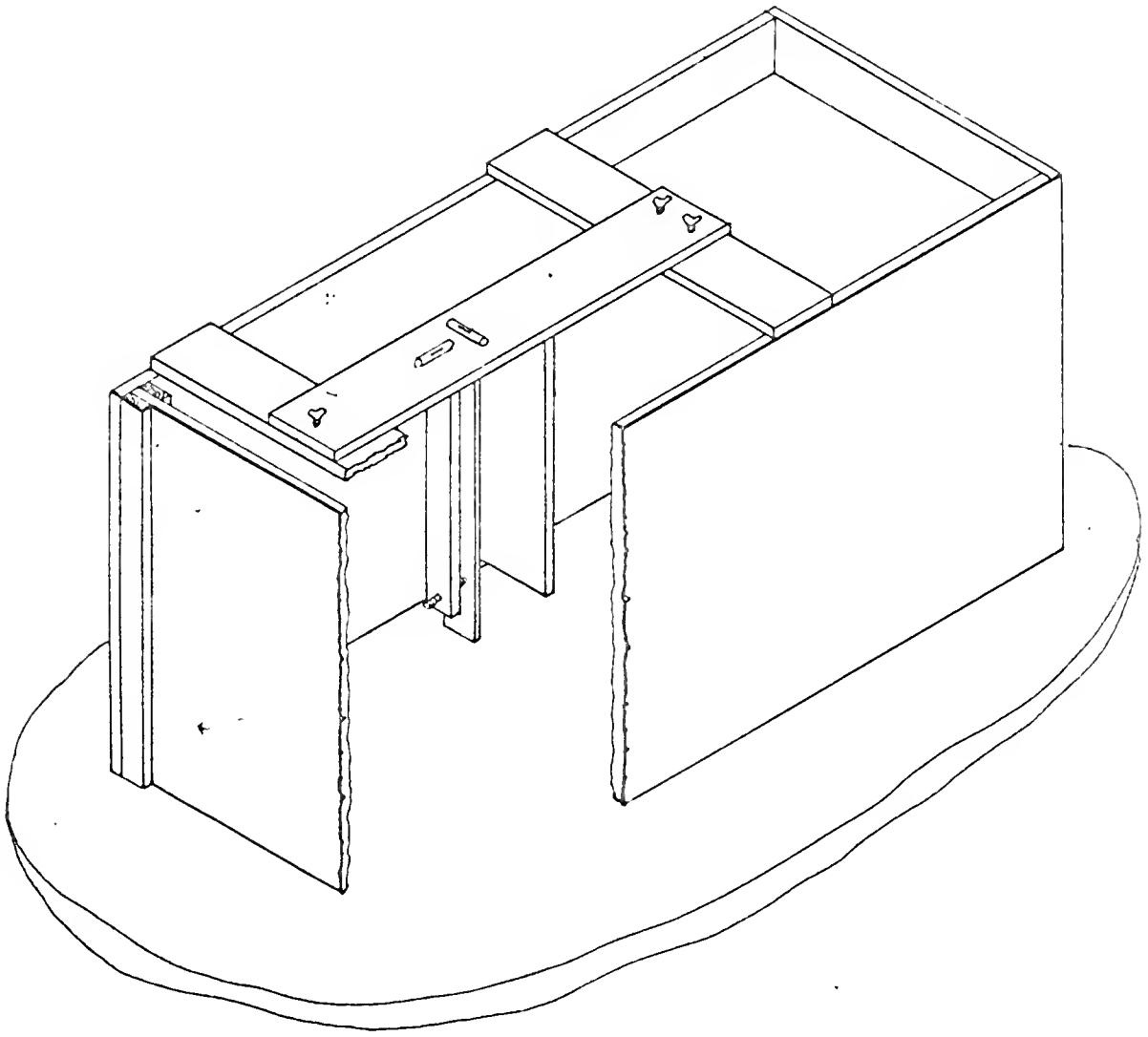


Figure 3. The Environmental Enclosure with Calibrator in Position.

used. For EEG recording, electrode leads were lowered through the wire mesh top of the cage and attached to S. The leads were suspended from a rubber band to maintain a constant tension and prevent the rat getting tangled in the wires.

Scoring procedure

The scoring rules were developed empirically by taking a correlated sample of 200 minutes of EEG-activity data and working out a set of rules that produced a high correlation. The scoring system assigns a number to each minute based on the amount of activity within the four 15-second periods making up the minute. Table 1 shows the scoring rules.

The point scores for the four 15-second periods making up a minute are added together, and the final number defines the activity level for that minute. Using this system a minute can have a score from 0 to 20. Any minute with a total point score from 0 to 3 is considered sleep, and any with a point score of 4 to 20 is waking except that one minute of 3 between two minutes of 4 or more is scored as waking, and one minute of 4 between two minutes of 3 or less is considered sleep. It should be remembered that paradoxical and slow wave sleep can be separated by the EEG but not with activity recording.

TABLE 1

An Activity Scoring System*

Points	Needle excursions (blips)
0	0
1	1-3
2	4-8
3	9-15
4	16-24
5	25 or more

* Per 15-second observation period

Reliability.--The experimenter and an independent observer both scored the same 150-minute sample of correlated EEG and activity data. The two scorers worked independently and scored the EEG and activity records separately. A 95 per cent agreement on EEG and 100 per cent agreement on activity was obtained when the records were scored on a minute by minute analysis for sleeping or waking only. LVFS was scored sleep, since it cannot be differentiated from sleep in the activity records. The scoring of EEG and ultrasonic activity records in this experiment was done separately without referring to the other record.

Results and Discussion

Table 2 presents data on the individual EEG-activity records. Each sample is between 100 and 110 minutes long.

From the data in Table 2 it is apparent that the EEG-activity correlation is satisfactory at all settings between 120 and 200 seconds (individual minute agreement ranges from 83 to 100 per cent and total correspondence from 93 to 100 per cent). The system would seem to function effectively for male hooded rats at least between 90 and 200 days old. The total correspondence measure may require explanation. This measure is defined as the percentage agreement between the EEG and activity units for a lengthy sample of data. For example, upon scoring a 100-minute sample, part of the errors are of the sleep-active type and part of the waking-nonactive type. On scoring for the entire sample, rather than minute by minute, some of these errors will offset each other, thereby increasing the percentage agreement. This total correspondence measure is important, since in succeeding experiments minutes of sleep and waking per 6-to 10-hour period is the dependent variable.

Table 3 summarizes the data from this experiment. Only data from the calibration settings between 120 and 200 seconds are included. The figures on minutes of waking, sleep, and LVFS are based on the EEG records.

TABLE 2

Agreement Between Individual Samples of Correlated EEG-Activity Records

Mean calibration reading	Minute by minute agreement	Total correspondence	Animal age
	Peacock and Williams Units		
75 seconds	80%	80%	90-100 days
98	46	46	180-200
99	89	89	90-100
108	78	80	180-200
119	94	97	90-100
139	90	100	180-200
161	92	98	90-100
176	98	98	180-200
192	91	93	90-100
196	83	95	180-200
215	87	87	90-100
	Alton Units		
102	49	49	180-200
125	86	100	90-100
127	99	99	180-200
130	100	100	90-100
140	88	94	180-200
155	94	96	90-100
157	92	94	180-200
198	97	99	90-100

TABLE 3

Averaged Data on Sleep Cycle and EEG-Activity Correlation from Experiment I

	Peacock and Williams Units	Alton Units
Total minutes recorded	610	708
Minutes waking	394	402
Per cent of time waking	65%	57%
Minutes waking errors	31	26
Per cent of waking minutes in error	7.9%	6.5%
Minutes sleeping	177	268
Per cent of time sleeping	29%	38%
Minutes sleeping errors	18	18
Per cent of sleeping minutes in error	10.2%	6.7%
Minutes LVFS	39	38
Per cent of time LVFS	6%	5%
Minutes LVFS errors	4	2
Per cent of LVFS minutes in error	10.3%	5.3%
Minute by minute agreement*	91%	94%
Total correspondence*	97%	97%

*Based on individual 100-minute samples.

There is an average 97 per cent correspondence for 100-minute samples with both the Peacock and Williams and Alton activity units. This correlation is sufficiently high to warrant using ultrasonically measured activity as a sleep cycle measure. Both units would seem to be equally accurate. The figures on the percentage of time spent in sleep, waking, or LVFS are not acceptable as normative data, since the animals were disturbed by having electrodes attached immediately before the 100-minute recording sessions, and, also, occasionally electrodes required manipulation during the session. These factors would be expected to increase the amount of waking. However, it is interesting to note that the percentage of sleep errors and LVFS errors is very similar.

A number of investigators have found muscle tone to be at a minimum during REM sleep in humans (Dement & Kleitman, 1957b), in cats (Jouvet, 1960), and in rats (Hall, 1963). The incidence of body movement seems to peak just prior to REM sleep and be at a minimum during the REMP. In contrast to gross body movements both human and animal subjects show a maximum of small twitches (fingers, tail, vibrissae) during the REM. Also, it should be remembered that depth of sleep as measured by threshold for arousal is higher during the REMP than slow wave sleep (Dement & Kleitman, 1957b; Dillon & Webb, in press; Jouvet, 1960).

These factors might suggest an imbalance in activity errors between slow wave and paradoxical sleep. The lack of this imbalance can do no more than suggest that a number of factors are present and perhaps neutralize each other.

EXPERIMENT II

THE EFFECT OF DEXTRO-AMPHETAMINE INDUCED SLEEP DEPRIVATION ON THE SLEEP CYCLE (ACTIVITY MEASURE)

In a pilot study the present author confirmed Svorad and Novikova's (1960) report of obtaining sleep deprivation with periodic dextro-amphetamine injections. Rats were kept awake up to six days, obtaining only small amounts of sleep between the time an injection wore off and the time this change in behavior was noticed by an observer. Samples of EEG were taken and these showed desynchronization during drug action and highly synchronized activity when the drug was allowed to wear off. Figure 4 is a comparison of EEG recordings for two rats during these various states of the sleep cycle. During the drugged state, the Ss appeared quite active, shaking their heads from side to side incessantly, but not moving around the cage much. When startled, the S would characteristically react violently with a jump, followed by rapidly running around the cage for a few seconds then resuming the head shake. Following drug withdrawal the animals went into what seemed to be a deep sleep, from which they were difficult to awaken.

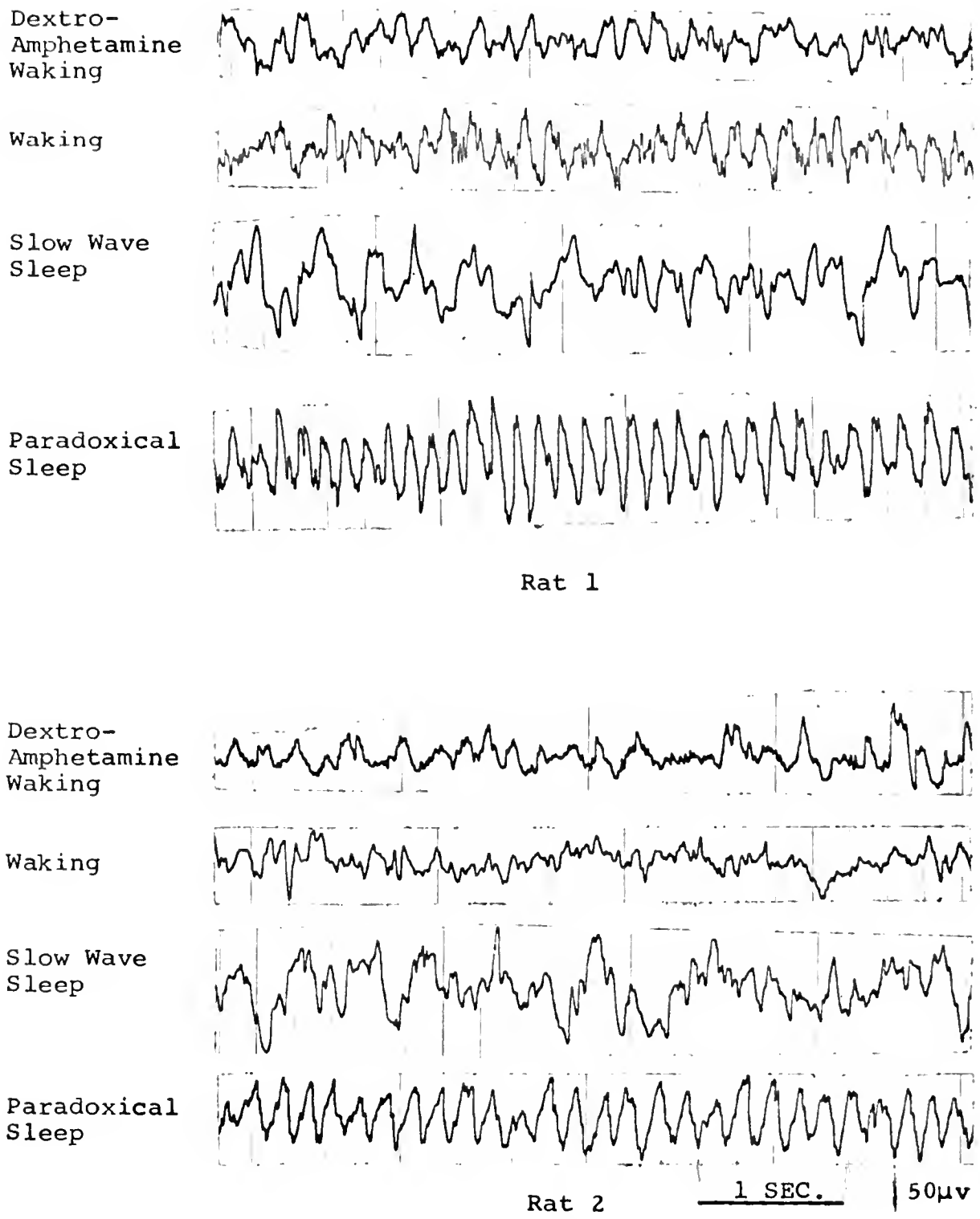


Figure 4. EEG Recordings from Two Rats.*

* Right frontal to right visual bipolar electrodes

It was expected that sleep deprivation would increase the rats' sleep need and that, as a result, sleep time during recovery would increase above normative values. Also, it was thought that this experiment would answer the question of whether Ss completely make up for missed sleep or compensate by making up only a part of the loss. This experiment was also expected to provide data on the relationship between length of sleep deprivation and amount of compensatory sleep obtained.

Measures of food and water intake and body weight were also made, since the anorexigenic action of dextro-amphetamine was expected to influence them. These measures were made both in the morning and evening, since the circadian rhythm has been found to strongly influence these behaviors. A circadian rhythm in behavior is an association between the day-night cycle and the level of the behavior under observation. Since rats are known to be relatively active at night and relatively inactive during the day, highest levels of waking, eating, and drinking were expected during the night, and body weight should be higher following an evening's activity than a day's sleep (Munn, 1950).

Method

Subjects

Twelve male Long-Evans hooded rats 110-120 days old

at the beginning of the experiment were used.

Apparatus

The apparatus was the same as in Experiment I. Both Alton and Peacock and Williams activity units were used in this and the succeeding studies. Occasionally a malfunction developed in an activity unit. This usually consisted of a unit becoming insensitive to movement, or less frequently, picking up "random noise" and responding although there was no movement within the enclosure. Spare units were available so that a rat could be transferred to an operable unit. The maximum amount of lost data for an individual animal was ten hours. Usually at least the first part of the recording period (2 to 8 hours' data) was usable. If fewer than ten hours' data were usable, the data were prorated to ten hours to produce a number which would fit into the data analysis.

Calibration

The same pendulum-type calibrator as that shown in Figure 2 was used in all succeeding activity recording experiments. A sensitivity setting between 120 and 200 seconds was used.

Design and procedures

There were four subjects at each of the deprivation levels, 24, 72, or 120 hours. The environmental conditions were the same as in Experiment I except that an electrically

shielded room was not required. The Ss were fed powdered Purina rat chow and received water from metered glass bottles. The chow consistency and water bottles were different from what the rats had experienced in the main colony. A ten-day adaptation period was utilized during which Ss were housed in experimental cages in the experimental room and adapted to the new food and water procedures.

The lights were on approximately 9:00 A.M. to 9:00 P.M. and off 9:00 P.M. to 9:00 A.M. The room was completely blacked out during the lights off period. Activity recordings were made from 10:00 A.M. to 8:00 P.M. and 10:00 P.M. to 8:00 A.M. During the morning and night two-hour non-recording periods (8:00 to 10:00 A.M. and 8:00 to 10:00 P.M.), the activity units were calibrated, the food and rats weighed, and water intake measured. The food, water, and body weight measures were made in only 2 of the 4 animals at each deprivation level. The calibration procedures took about 1 to 2 hours to complete. This sometimes required that the lights be turned on a few minutes before 9:00 A.M. and turned off a few minutes after 9:00 P.M. Except for the injection phase of the experiment, the experimenters (Es) were not in the experimental room except to calibrate.

Data were not scored until at least 15 minutes had passed since calibration and measurement procedures had been completed and E had left the room. Following the

deprivation period, data were collected for an additional eight days.

Injections

Preliminary studies had utilized both the subcutaneous (SQ) and intraperitoneal (IP) routes. The SQ route produced a longer response and was used in the present study. The dose was 10 mg./kgm. of dextro-amphetamine sulfate SQ, under the skin on the dorsal surface (upper back between shoulders). A stock solution containing 10 mg./cc. was prepared.

In the preliminary studies higher doses had produced circling and backing-up behavior, followed by coma and death at still higher doses.

The inter-injection time was determined by Ss response. When a S first showed signs of sleep, it received another injection. The observers were directed to constantly watch the recorder. When a minute of inactivity was seen, they would observe the rat, and, if asleep, give an injection (occasionally the S would be awake but in such a position that the units did not record the wakefulness). If S was awake, the observer would note this on the recording paper. Figure 8 shows the average injection intervals.

Scoring

The data were scored for sleeping and waking according to the rules illustrated in Table 1. The daily 20 hours

of recording were divided into ten daylight hours and ten dark hours. The total minutes of sleep per ten-hour period (600 minutes) was the measure used for statistical analysis. Occasionally the full 600 minutes of data was not obtained. In this case the data were interpolated to 600 minutes. For example, if calibration procedures had taken an inordinately long time, perhaps 100 minutes' data would be lost. If the animal was found to have been asleep 300 out of 500 minutes, this number (300) would be interpolated to a 600-minute total and 360 would be assigned for this S. Table 4 illustrates the schedule in this experiment by deprivation groups. A finding of this study was a reduction in circadian rhythm following five days' deprivation. In other words, the difference between the amount of sleep obtained during the day and the amount of sleep obtained during the night was reduced. For this reason three five-day deprived animals were recorded on post-drug days 19 and 20 to determine if the circadian rhythm reduction was still present.

TABLE 4

Recording Schedule for the Deprivation Groups in Experiment II*

Deprivation group	Pre-drug	Drug	Post-drug
24-hours	4 days	1 day	8 days
72-hours	4 days	3 days	8 days
120-hours	4 days	5 days	8 days

* Four Ss per group

Replications

The Ss in this study were run in two separate groups; the replications were approximately six months apart. Each experiment consisted of six rats, two at each level of deprivation. Food, water, and body weight data were recorded only in the original experiment. All other aspects of the studies were identical. Since the results of the two replications were essentially identical, supporting all of the conclusions reached from the grouped data, the data will be presented in grouped form only.

Results and Discussion

Normative sleep cycle data

Figure 5 is a graph of the daily average sleep time during this experiment for the three deprivation groups. Figure 6 shows the circadian rhythm with the deprivation groups combined. Table 13 in Appendix A contains the significance levels for the statistically significant effects in five separate analyses run on these data.

If we look at the four pre-deprivation days in Figures 5 and 6, it can be seen that there is a strong circadian rhythm in sleep cycle (Figure 6) and also a significant variation in sleep time over days. The analysis of variance of sleep time for days 1-4 pre-deprivation also shows significant subject differences in sleep time.

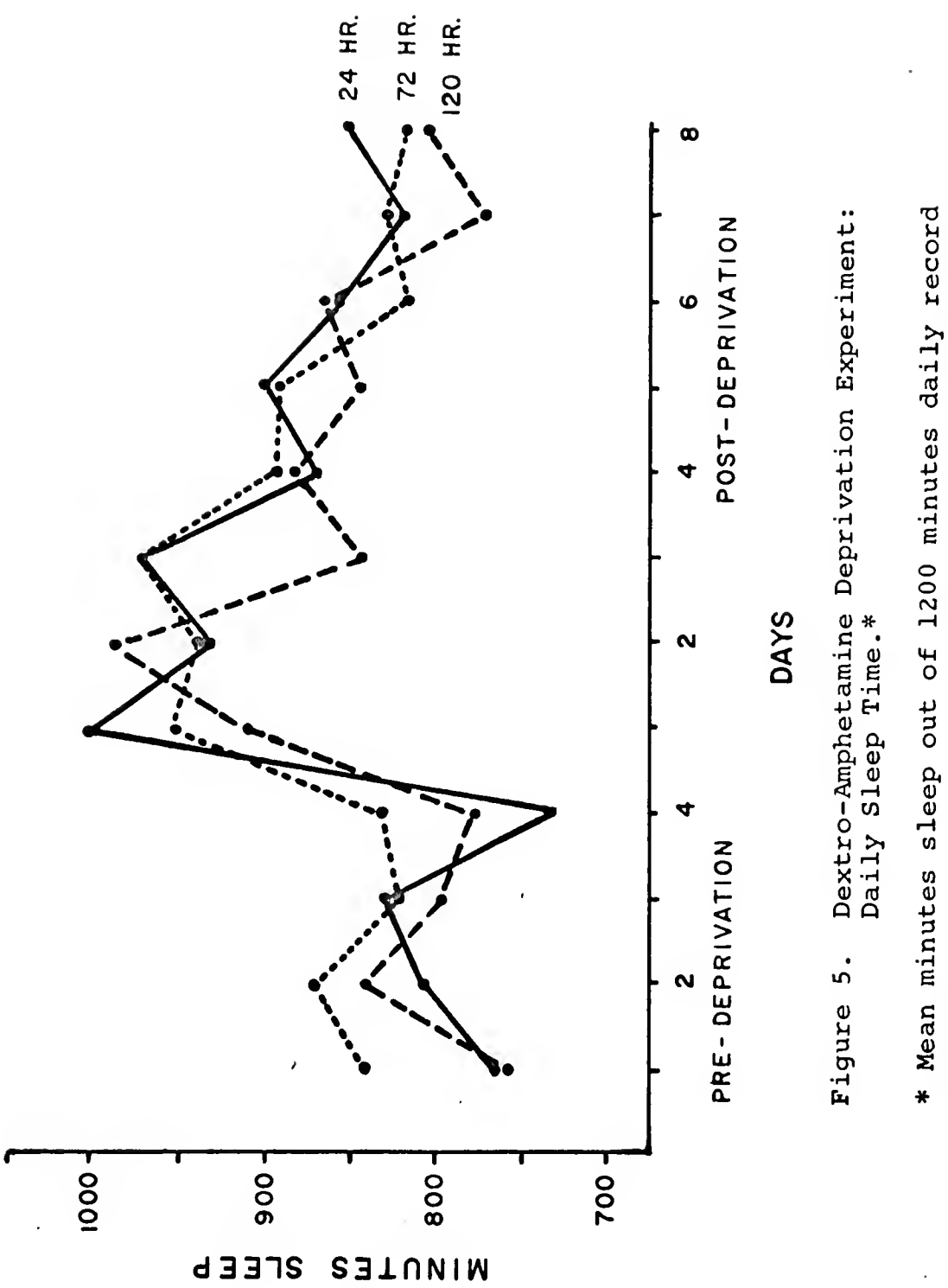


Figure 5. Dextro-Amphetamine Deprivation Experiment: Daily Sleep Time.*

* Mean minutes sleep out of 1200 minutes daily record

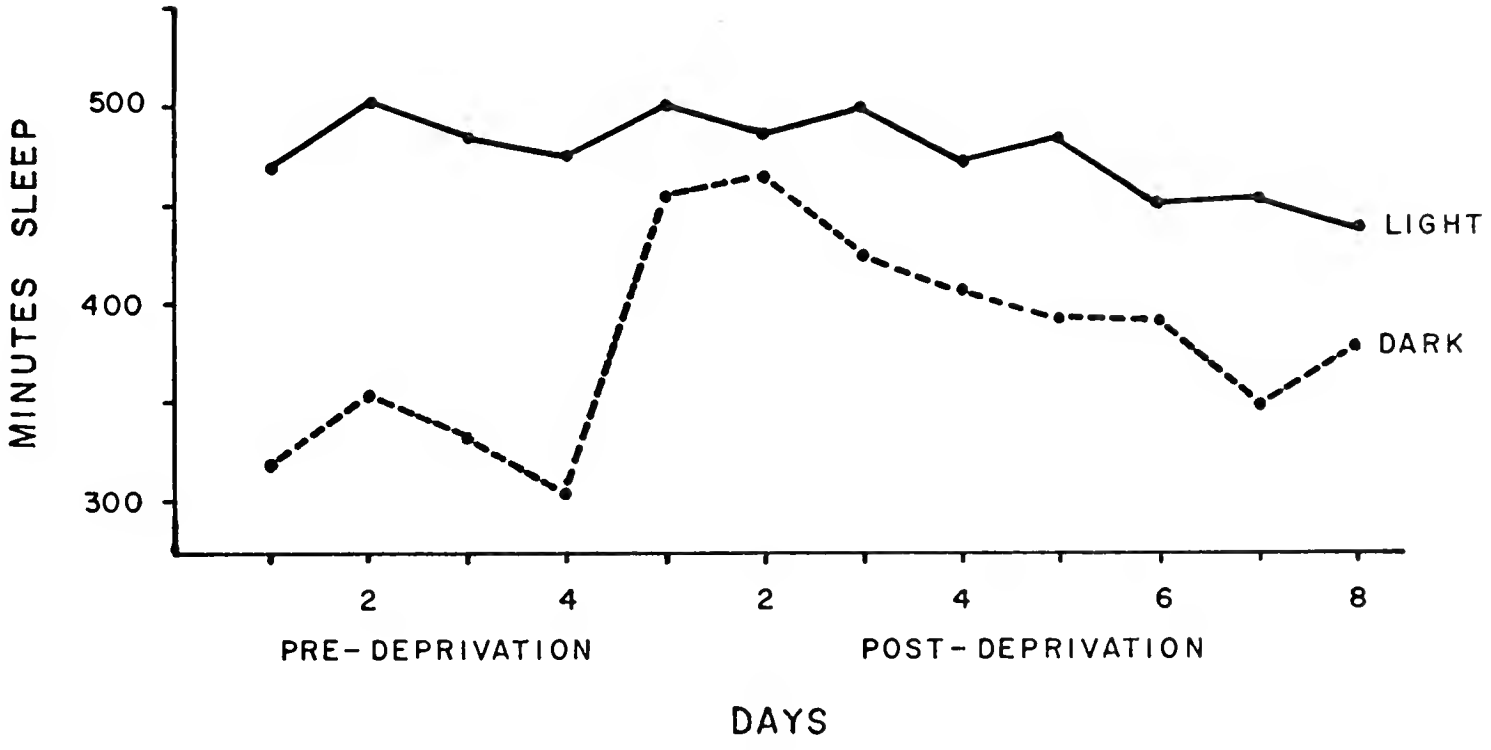


Figure 6. Dextro-Amphetamine Deprivation Experiment: Circadian Rhythm.*

* Mean minutes sleep out of each 600 minute recording period

These animals slept an average of 68 per cent of the time (811 minutes out of 1,200 minutes recorded). They slept 80 per cent of the day (483 minutes out of 600 minutes recorded) and 55 per cent of the night (328 minutes out of 600 minutes recorded). The Ss obtained a mean of 60 per cent of their total sleep during the day and 40 per cent during the night.

Sleep deprivation effects

The significant circadian rhythm continued throughout the experiment. However, this rhythm was considerably reduced following deprivation. Figure 6 shows these effects. Sleep deprivation did increase sleep time above the pre-deprivation level; however, this effect is statistically significant only during the first four post-deprivation days.

There is no difference in total compensatory sleep due to deprivation level (24, 72, or 120 hours). This can be seen in Figure 5 where there is no divergence in sleep time between the deprivation level groups. The days' effect is significant on days 5-8 post-deprivation, but not on days 1-4 post-deprivation. However, it can be seen in Figure 5 that sleep time is considerably elevated on day one post-deprivation, and gradually returns to the pre-deprivation level. The 24- and 120-hour deprivation groups return to a level of daily total sleep within their

pre-deprivation range on day 7 post-deprivation, and the 72-hour group does this on day 6 post-deprivation. Table 5 is an attempt to estimate the average amount of compensatory sleep obtained by each deprivation group. Compensation is arbitrarily considered to have ended on the first day that the mean sleep time for a deprivation group overlaps its pre-deprivation range. The most important point to be made is that total compensation is not significantly different between the deprivation groups. Deprivation level failed to have an effect in spite of the sleep deprivation periods being 3 and 5 times as long, respectively, in the 72- and 120-hour groups as in the 24-hour deprivation group. Again, there is no increase in amount of compensatory sleep obtained with increasing sleep deprivation.

There are two possible confounding influences which may account for this finding. The first is shown in Figure 7. Although the amount of sleep obtained during deprivation was small throughout the drug-injection period, the amount of sleep obtained does increase after the first day. This may be due to the increasing sleep need of the Ss as the deprivation period progressed. It seems possible that during this high need state one minute of sleep may be "more valuable" in reducing the post-deprivation compensation than would normally be expected. However, this

TABLE 5

Estimated Post-Dextro-Amphetamine Deprivation Compensation

	24-hour group	72-hour group	120-hour group
Mean pre-deprivation sleep time (minutes)	784	839	794
Days of deprivation	1	3	5
Minutes sleep loss	784	2,517	3,970
Post-deprivation compensation*			
Day 1	218	113	117
2	150	99	194
3	186	133	49
4	85	54	88
5	117	51	50
6	73	-	70
Total compensation	829	450	568
Per cent compensation	(100%)	18%	14%

* Minutes sleep above the pre-deprivation mean.

sleep during deprivation amounts to only about 20 minutes per day, which is a very small amount when compared to the 800 minutes sleep these animals normally get each day. It seems unlikely that this small amount of sleep would be sufficient to eliminate increasing compensation with increasing amounts of deprivation.

Another consideration is a possible interaction between the hunger and sleep drives following dextro-amphetamine. In Figures 9 and 11 it can be seen that the drug reduced food intake and body weight considerably, suggesting that after deprivation the Ss may awaken to eat because of a strong hunger drive. This could have changed the relationship between hours of drug administration and compensatory sleep. However, it can be seen in Figure 9 that food intake did not increase above normal after deprivation. Actually, food intake appears to be slightly depressed. This may be due to stomach shrinkage during the drug treatment. If stomach shrinkage did occur, it is possible that the Ss ate more frequently, but consumed smaller amounts at each "meal."

A curious effect is illustrated in Figure 6. There is no increase in sleep time during the post-deprivation light period. As a matter of fact, there is a slight decrease in sleeping time during the day. The entire compensation is accounted for by an increased sleep time

during the dark or night phase of the light cycle. An explanation of this phenomenon may be that the rat, being a nocturnal creature, sleeps maximally during the day, awakening only to satisfy other need states which require attention. The rat, according to this hypothesis, cannot constantly remain asleep for 8-12 hours because of other need requirements. Thus, only at night is there time available to compensate for the heightened sleep need. Only at night does the rat indulge in activities which can be curtailed to satisfy an increased need.

Table 6 illustrates the circadian rhythm reduction following dextro-amphetamine administration. This effect is particularly noticeable at the longer periods of deprivation. The Ss in the 120-hour group obtained 61 per cent of their daily sleep during the daylight period on the control days and only 54 per cent on days 5-8 post-deprivation. Two of these Ss had their sleep cycles recorded on days 19 and 20 following 120-hours deprivation. These are not the same Ss who received a drug injection on day 11 post-deprivation (see below). A 57 per cent figure for these two days suggests that this is only a temporary effect. This circadian rhythm reduction was not observed in the treadmill deprivation study (Experiment IV), suggesting that it is specifically related to the drug treatment.

Since the reduction continues after post-deprivation compensation has ended, the reduction cannot simply be accounted for by an increased amount of night sleep with no change in day sleep. On days 7 and 8 post-deprivation (Figure 6) after compensation has ended, the Ss are obtaining normal amounts of total sleep, but this amount is achieved by sleeping less than normal during the day and more than normal during the night.

TABLE 6

Mean Percentage of Daily Sleep Obtained During Lighted Hours

Days		Deprivation level 24-hours	72-hours	120-hours
Pre-deprivation	(1-4)	59%	59%	61%
Post-deprivation	(1-4)	53	53	52
	(5-8)	57	53	54
	(19-20)	-	-	57

Sleep during drug administration

Figure 7 shows the mean minutes sleep over days during deprivation. The diurnal, days and subjects variations were not statistically significant, although there does appear to be an increase in sleep time over days, especially

between day 1 and days 2 to 5. On the average the Ss received about 3 per cent of their normal daily sleep time during deprivation.

Injection parameters

Figure 8 is a graph of the mean length of sleep deprivation per injection over the five deprivation days. An analysis of the original waking times showed the decrease in length of drug action over days to be significant ($p < .01$). This effect could be due either to true drug tolerance or to an increased sleep need produced by the increasing deprivation. As a possible test of these alternatives, a tolerance test was conducted on six of the Ss (two at each deprivation level) 11 days following drug withdrawal. A single injection of dextro-amphetamine was administered and the length of its action determined. The mean length of wakefulness during the tolerance test was 357 minutes compared to a mean of 354 minutes for the drug on deprivation day one. It appears that the Ss drug responsiveness had returned to its initial level; thus, it is not possible to choose between the alternatives. This effect may be due to the dissipation of either tolerance or sleep need. If the length of drug effect had still been shortened, tolerance would be suggested since indications are that the sleep need is normal on day 11 post-deprivation (the 120-hour Ss used in this test

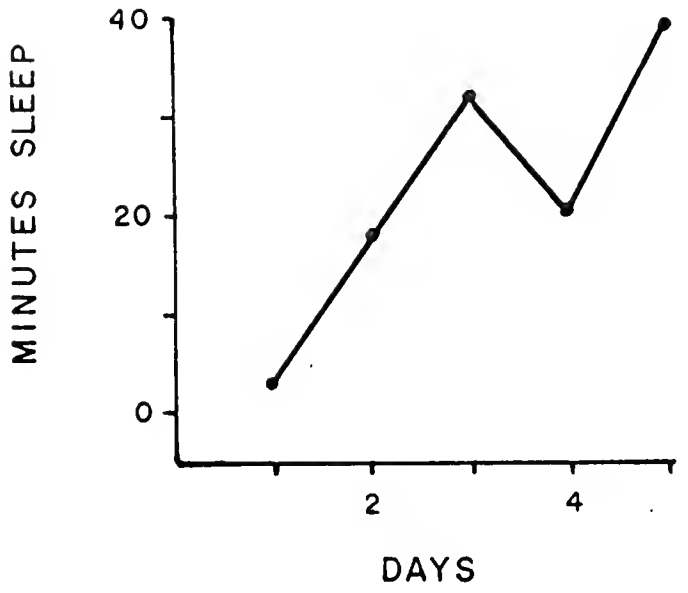


Figure 7. Mean Minutes Sleep Obtained per Subject During Dextro-Amphetamine Deprivation.

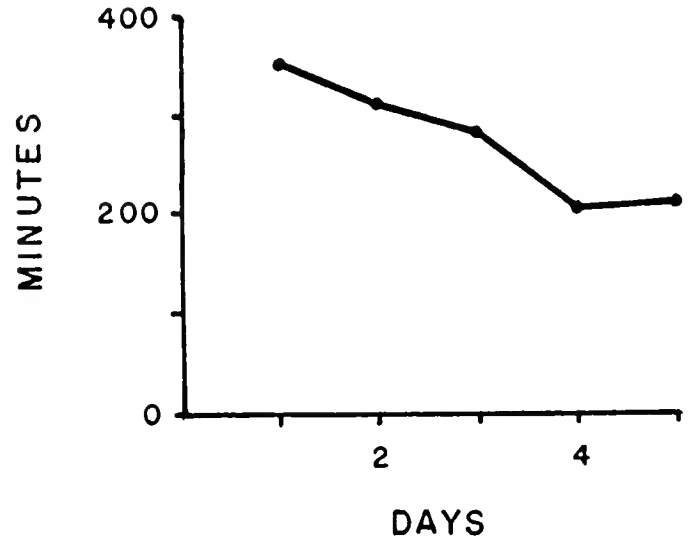


Figure 8. Minutes Sleep Deprivation per Dextro-Amphetamine Injection.

were not the same rats as those whose sleep cycle was recorded on days 19 and 20 post-deprivation).

Drug toxicity

Some of the Ss in the 72- and 120-hour deprivation groups lost some toes during deprivation. The paws became white, and the Ss constantly worried and nibbled at them during deprivation. The most likely explanation for this seems to be that the dextro-amphetamine produced peripheral vasoconstriction, reducing circulation and causing numbness. Tetracycline (10 mg./kgm. twice a day) was administered on days 1-3 post-deprivation to the injured Ss. There was no evidence that this side effect had any confounding influence on the data. All Ss recuperated and appeared normal following the experiment.

Food and water intake and body weight data

Figures 9, 10, and 11 illustrate the mean food, water, and body weight measures for each deprivation group over the entire experiment. These data are for only 6 of the 12 Ss, two at each level of deprivation. Tables 14, 15, and 16 in Appendix A are summaries of the variance analyses of these data. The effects of the drug treatment on these measures are determinable from an inspection of the figures and tables. The most important conclusions to be reached are: (a) the drug treatment almost completely

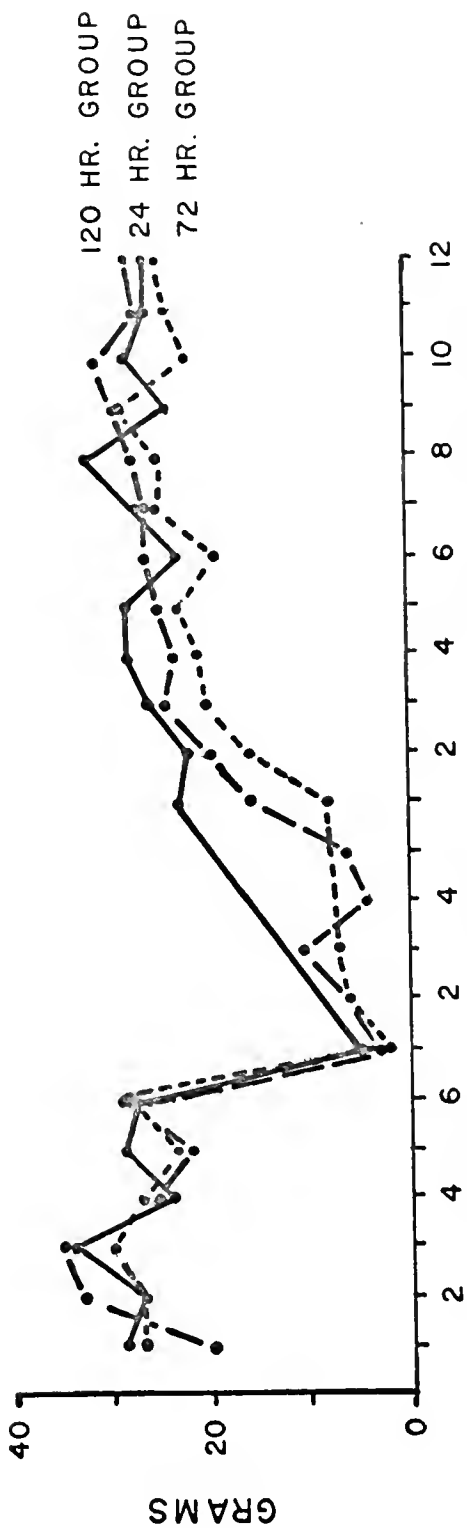


Figure 9. Food Intake During Dextro-Amphetamine Experiment.

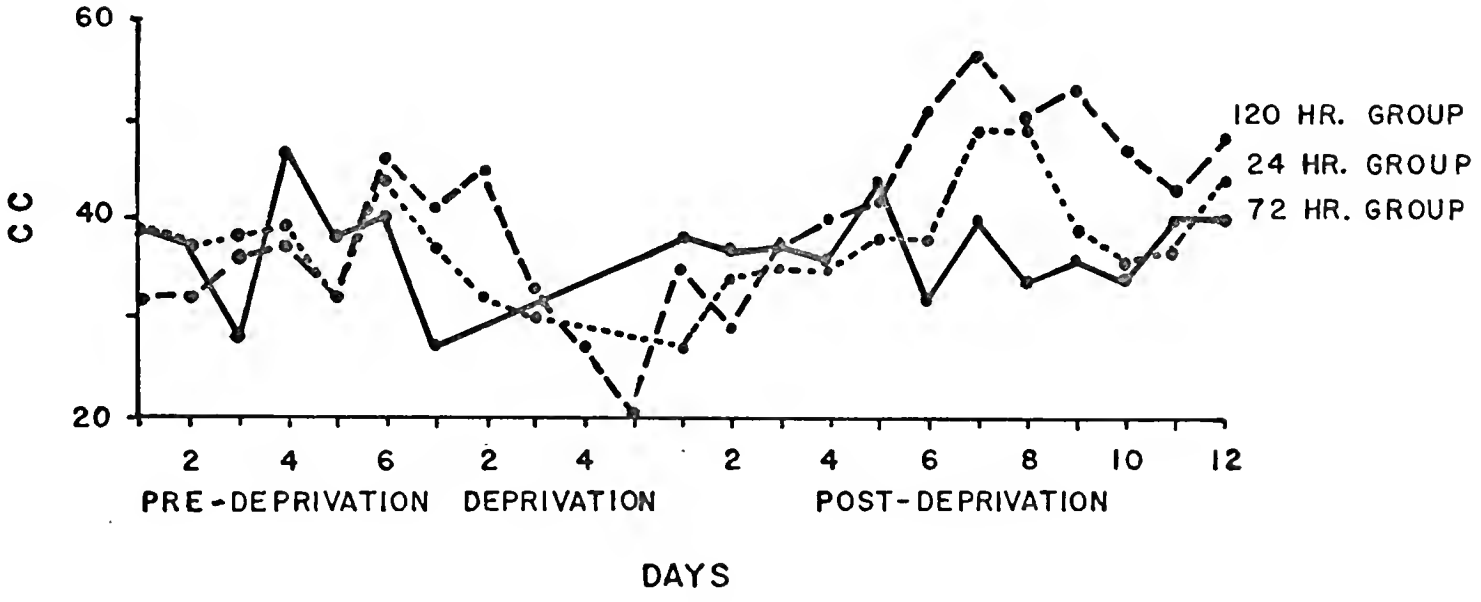


Figure 10. Water Intake During Dextro-Amphetamine Experiment.

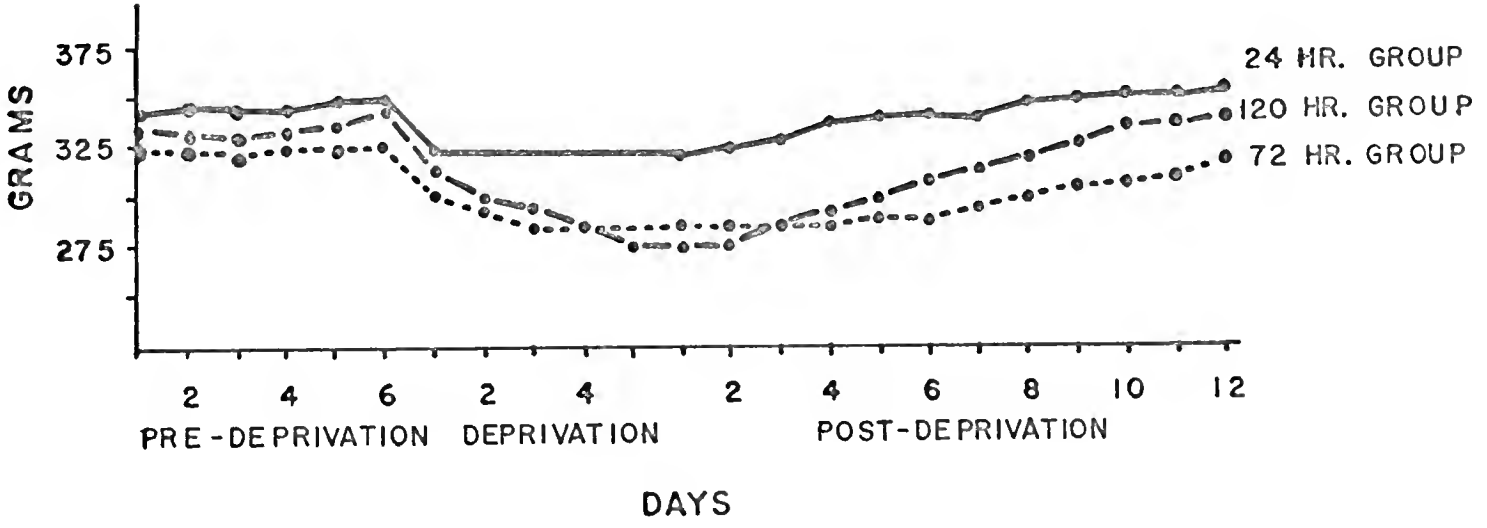


Figure 11. Body Weight During Dextro-Amphetamine Experiment.

eliminates eating, and (b) water intake and body weight are also decreased secondary to the drug effect on hunger. These measures all return to normal by the end of the 12 post-deprivation days (readings of these measures were started two days before and continued four days after the sleep cycle recording). A reduction in circadian variation, similar to that seen in the sleep cycle following drug treatment, is present in the food and water intake data (Tables 7 and 8).

Tolerance does not seem to have developed to the anorexigenic effect of dextro-amphetamine during the five days of treatment.

TABLE 7

Mean Percentage of Daily Food Intake Obtained During Dark Hours

Days		Deprivation level 24-hours	72-hours	120-hours
Pre-deprivation	(1-6)	63%	69%	70%
Post-deprivation	(1-6)	66	56	56
	(7-12)	65	61	58

TABLE 8

Mean Percentage of Daily Water Intake Obtained During Dark Hours

Days		Deprivation level 24-hours	72-hours	120-hours
Pre-deprivation	(1-6)	65%	68%	68%
Post-deprivation	(1-6)	65	57	58
	(7-12)	73	64	56

Body weight was lost during the drug treatment. The Ss were at approximately 92 per cent of pre-deprivation body weight after 24 hours, 87 per cent after 72 hours, and 82 per cent after 120 hours. A diurnal rhythm in body weight is present throughout the experiment, and body weight has returned to the pre-deprivation level by the end of the experiment. It is interesting that for the 72- and 120-hour groups body weight continues to fall for a day or two following drug withdrawal. Food intake is also relatively low during this period. It is possible that although there is not enough drug remaining in the body to maintain wakefulness there is enough to reduce food intake. The continued low food intake on post-deprivation days 3-10 possibly results from shrinkage of the stomach during deprivation.

The inversion of the diurnal effect on body weight during deprivation is an artifact resulting from the procedure having been started in the morning. Each consecutive weighing was lower than the preceding, and the daytime weighing followed the night weighing on each day.

The most to be said about the analyses in Tables 14, 15, and 16 in Appendix A is that these measures seem to be quite variable, and most sources of variances did produce significant effects. An interesting finding is that there are significant individual differences in body weight in spite of no subject difference in food or water intake prior to deprivation. This would suggest that differences in body weight among same aged animals are due to other than intake factors.

Another curious finding is that, although sleep time increased above pre-deprivation levels following dextroamphetamine deprivation, food intake did not. The drug has deprived the Ss of both of these goal objects, but apparently the Ss compensate, in this particular situation, only for the lost sleep, not for the lost food. This may be due to the aforementioned stomach shrinkage.

EXPERIMENT III

EEG AND BEHAVIORAL OBSERVATION ON RATS WALKING THE WATER-IMMERSED TREADMILL

In some preliminary observations periodic sleep waves were found in the EEG of animals on the treadmill. The rats were seen moving to the front of the wheel to remain stationary as long as 3 or 4 seconds while riding to the rear, followed by walking to the front (taking 2 to 4 seconds) to repeat the process. It appeared that there was good agreement between the EEG synchronization and behavioral motionlessness. This experiment was designed to further study this phenomenon.

Method

Subjects

Three 110-120-day-old male Long-Evans hooded rats with bipolar EEG electrodes implanted were used (right frontal to right visual). Food and water were available throughout the experiment.

Apparatus

This apparatus is the same as the water-immersed treadmill used by Levitt and Webb (1964). The rats were placed in individual 5.5 by 9.5 inch cubicles, on wheels

two-thirds submerged in water, which rotated at a constant speed of 2 r.p.m. Food trays were available in each cubicle. The animals remained on these wheels continuously. The total distance covered by an animal was approximately 0.7 mile per 24-hour period. The upper sides of the apparatus were of plexiglass to facilitate behavioral observations. The animals remained on the wheels for 32 hours.

Results and Discussion

Seven hours of EEG data were collected on each of the three Ss during their 32 hours on the wheel. Figure 12 shows the increase in "sleep" prevalence during the course of the experiment. The increase in sleep during the time on the wheel was statistically significant ($p < .05$) as was the difference in sleep prevalence between subjects ($p < .01$). The sleep on the treadmill differed from normal slow wave sleep mainly in the length of each burst. This "micro-sleep" occurred in bursts of only 1 to 4 seconds separated by 2 to 5 seconds of waking activity, while normally, bursts of sleep last for a number of minutes. There was no micro-sleep exhibited on the stopped wheel before the experiment or during the first hour on the moving wheel. It was possible to inspect the EEG records and estimate the amount of micro-sleep obtained by the Ss. By hour 32 Ss

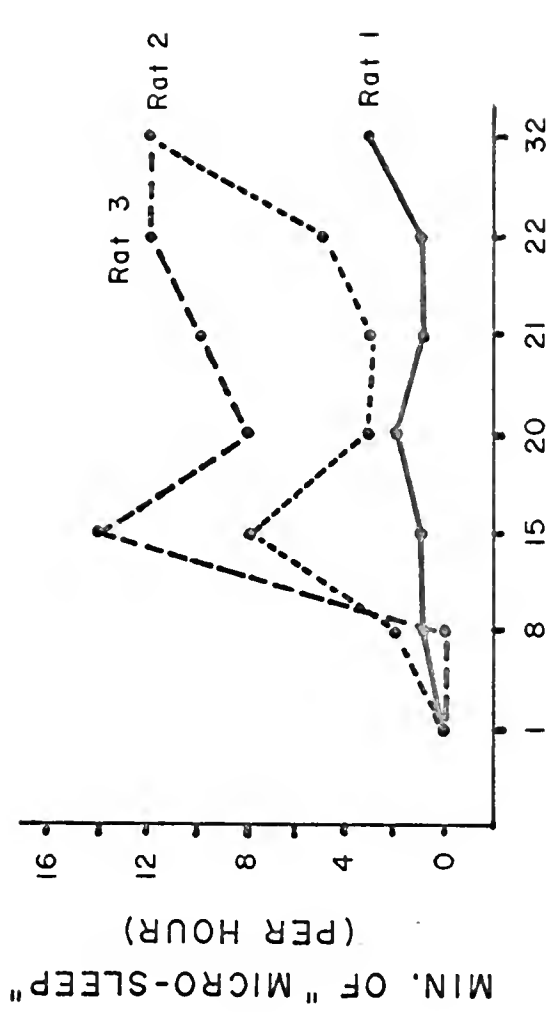


Figure 12. Micro-Sleep Prevalence During Treadmill Deprivation.

2 and 3 were micro-sleeping approximately 20 per cent of the time. This is compared to a home cage value of about 65 to 70 per cent of total time asleep for the rats used in Experiment II. As suggested before, this amount of sleep may have more value in reducing compensation than would be expected by the objective amount.

Also, it must be recognized that the relationship between normal sleep and micro-sleep has not been established. However, there is evidence that micro-sleep is not equivalent to paradoxical sleep. First, rats have not been observed to enter paradoxical sleep directly, but only via slow wave sleep (Hall, 1963; Swisher, 1962). Since micro-sleep occurs in very short bursts separated by a waking record, it is unlikely that it is similar to paradoxical sleep; the Ss simply do not have time to pass through slow wave sleep and into paradoxical sleep. Second, Hall (1963) for the rat and Dement (1958) for the cat have found muscle tension to be at its lowest during paradoxical sleep. Both investigators reported that whenever the S entered paradoxical sleep it collapsed due to the reduced muscle tension. This phenomenon would be inconsistent with the erect position maintained by the rats on the treadmill during micro-sleep.

The data discussed in the preceding paragraph would tend to support a skeptical attitude toward the normative data on paradoxical sleep reported by Siegel and Gordon

(1965). These authors reported control levels of paradoxical sleep, for the cat, ranging from 27 to 42 per cent of total sleep. These figures are compared to about 20 per cent in the human (Dement, 1960) and approximately 10 per cent reported later in this paper for the rat (Experiment V). The peculiarity of the Siegel and Gordon study is that during 12 to 14 hours out of each day (at night) the cats were kept awake by being placed on a brick in the middle of a pan of water. The authors report that the cats could not lie down; however, they do not mention having recorded the EEG of these cats on the brick. The evidence on the occurrence of micro-sleep reported in this dissertation suggests that Siegel and Gordon's cats did obtain short bursts of sleep on the brick. However, it is unlikely that any of this sleep was paradoxical sleep, since the reduced muscle tension would cause the cat to fall in the water. Therefore, the normative data on paradoxical sleep as a percentage of total sleep reported by Siegel and Gordon are probably artifactually high as a result of the cats being differentially deprived of paradoxical sleep for 12 to 14 hours a day while on the brick.

Figure 13 shows examples of micro-sleep and a behavioral correlation with motionlessness. This behavioral reading was obtained by an observer watching the rat and activating a channel on the EEG record whenever the S

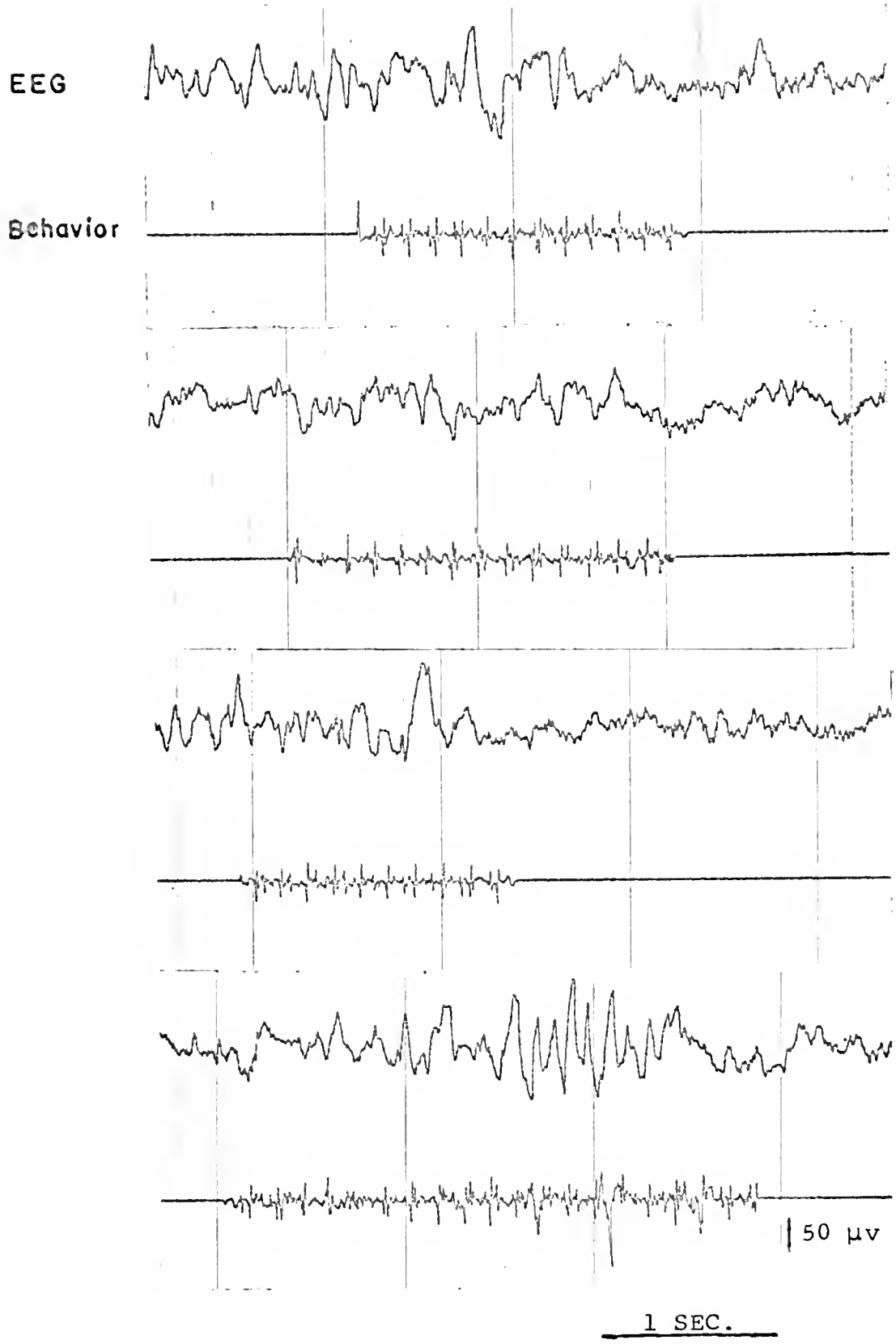


Figure 13. Micro-Sleep and Behavioral Motionlessness on the Sleep Deprivation Treadmill.*

* Right frontal to right visual bipolar electrodes

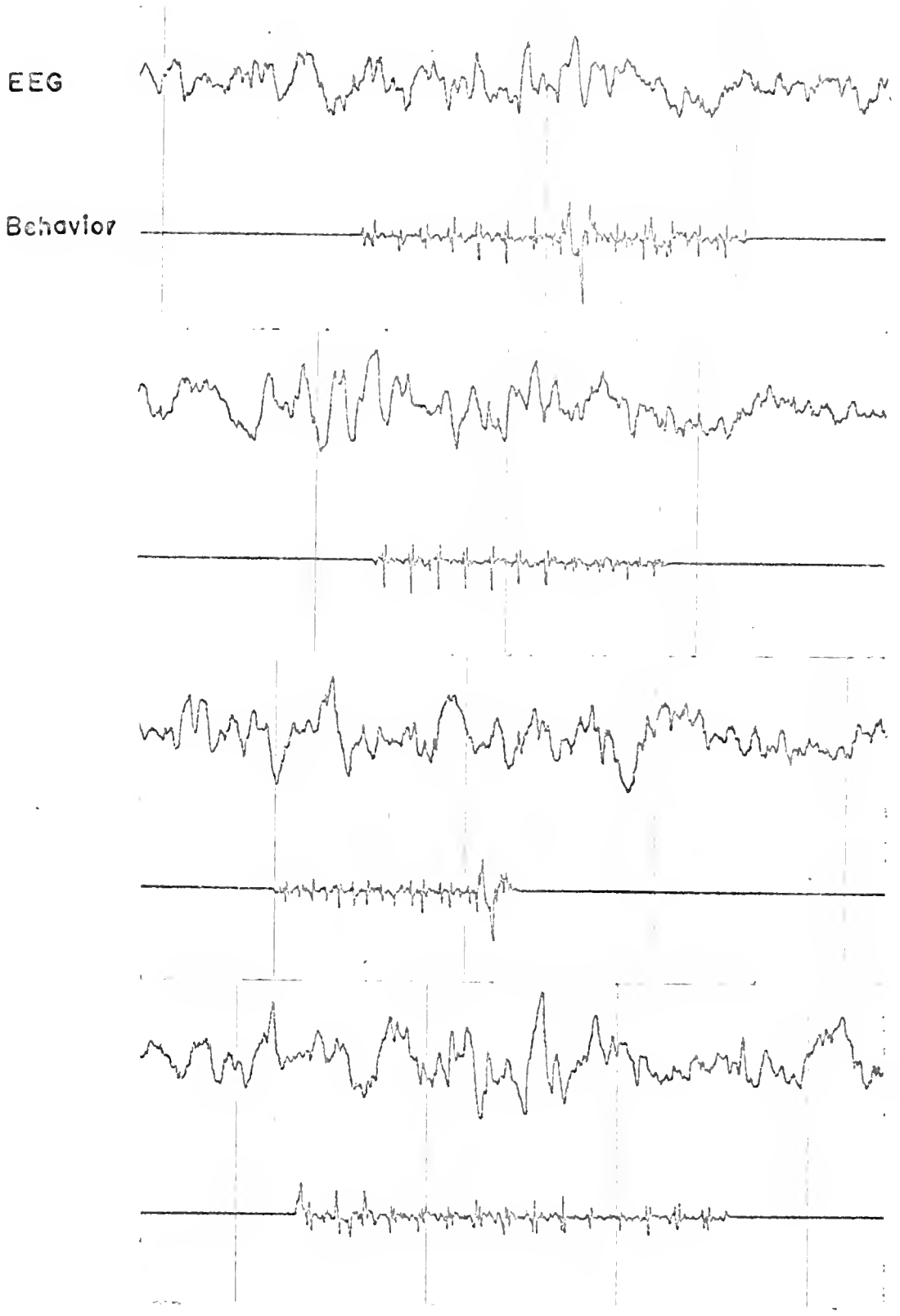


Figure 13. Continued

appeared motionless. The E pressed a switch to activate the behavior channel without observing the EEG record. This correlation appeared to be essentially 100 per cent. There were certain sources of error such as latency of observer's response and the necessity of the S being motionless for a short period before E could make a decision and activate the switch. These error sources seemed to account for any deviation between the EEG and behavioral records.

It is most interesting to find the rats on the treadmill able to sleep 1 to 4 seconds, wake up for 2 to 5 seconds to perform a goal directed act, and instantaneously return to sleep. This finding would seem to be of some theoretical interest. First, these results raise a serious question as to the sleep deprivation produced on the water-immersed treadmill (Levitt & Webb, 1964; Licklider & Bunch, 1946; Webb & Agnew, 1962) and on the brick surrounded by water (Siegel & Gordon, 1965). Second, broadly conceived, this micro-sleep phenomenon may be interpreted as an instrumental response (walking to the front of the wheel) at least partially motivated by the drive for sleep (micro-sleep). The author recognizes that escape from water is also a motive for treadmill walking and initially the only motive. However, he prefers the interpretation that during the

course of the experiment a second motive for micro-sleep also comes into force. The possibility of instrumentally conditioning the sleep response is certainly deserving of further study. Clemente, Sterman, and Wyrwicka (1963), and also the present author (Levitt, 1964) have previously been successful in classically conditioning sleep.

EXPERIMENT IV

THE EFFECT OF TREADMILL INDUCED SLEEP DEPRIVATION ON THE SLEEP CYCLE (ACTIVITY MEASURE)

Although the recording of EEGs on the treadmill showed a sleep-like pattern, it still seemed advisable to study the effect of this deprivation technique on the sleep cycle using the ultrasonic activity method, especially since the Ss may only experience a sleep state analogous to slow wave sleep on the treadmill. If this were the case, rats on the treadmill would be deprived of paradoxical or "dream" sleep at the same time they were receiving some slow wave sleep. This situation would be similar to the previously discussed "dream" or paradoxical sleep deprivation studies (Dement, 1960; Khazan & Sawyer, 1963; Siegel & Gordon, 1965).

Method

Subjects

Ten male Long-Evans hooded rats 110-120 days old at the beginning of the experiment were used.

Apparatus

Sleep cycle recording was by the same ultrasonic activity units used in Experiments I and II. The sleep

deprivation treadmill was described in Experiment III. The calibration procedure was the same as that used in Experiment II.

Design

There were five Ss at each of two deprivation levels (24 or 72 hours). Environmental conditions and scoring methods were described in Experiment II. Table 9 illustrates the design of this experiment.

TABLE 9

Recording Schedule for the Deprivation Groups in Experiment IV

Deprivation level	Pre-deprivation	Deprivation	Post-deprivation
24-hours	3 days	1 day	5 days
72-hours	3 days	3 days	5 days

Results and Discussion

Figures 14 and 15 illustrate the sleep cycle data from this experiment. Table 17 in Appendix A summarizes the analyses of variance, and Table 10 in the text is an estimate of sleep compensation following treadmill deprivation.

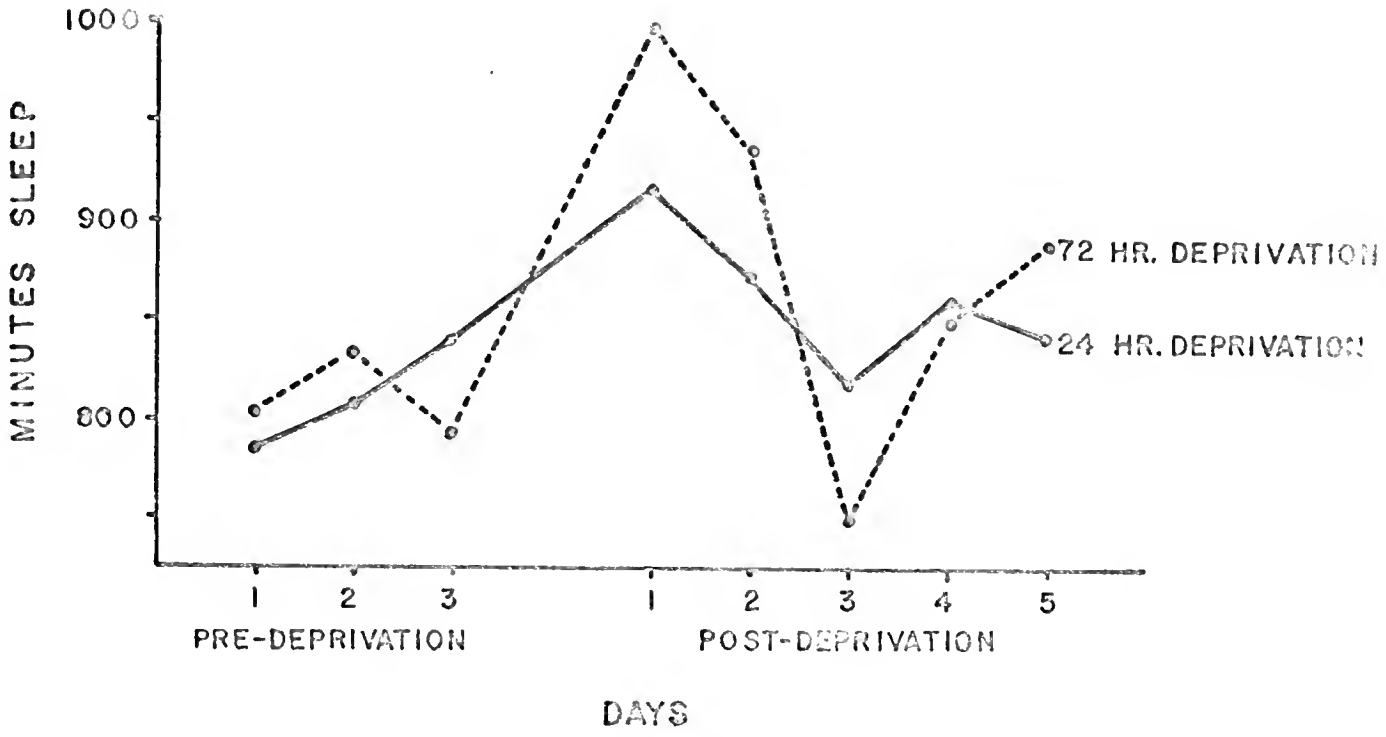


Figure 14. Treadmill Deprivation Experiment: Daily Sleep Time.*

* Mean minutes sleep out of 1200 minutes daily record

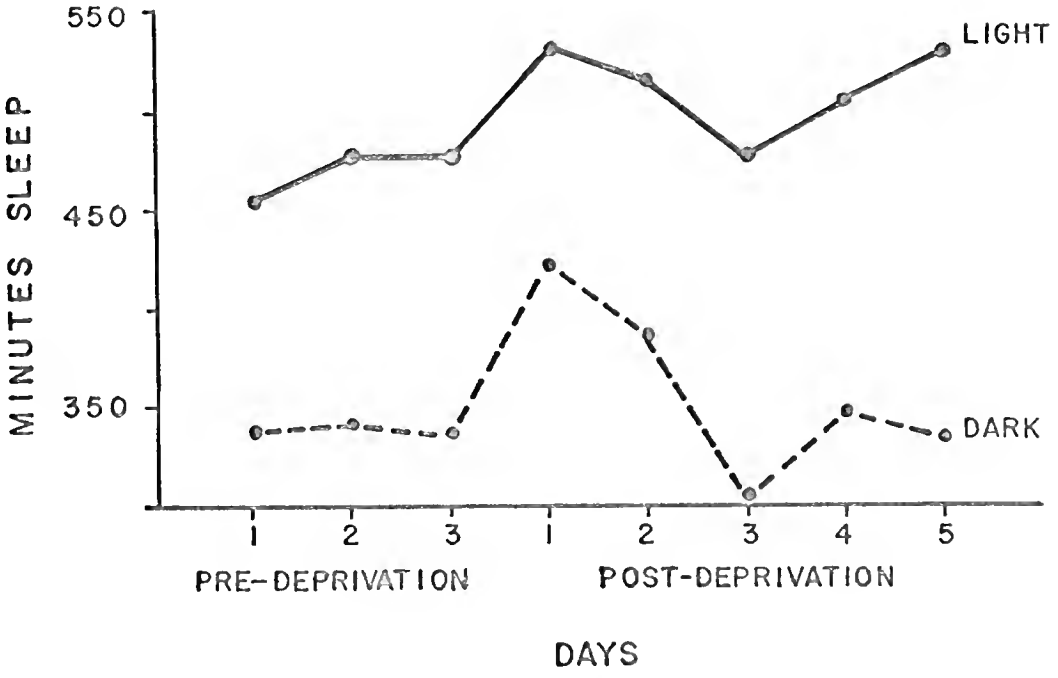


Figure 15. Treadmill Deprivation Experiment: Circadian Rhythm.*

* Mean minutes sleep out of each 600 minute recording period

TABLE 10

Estimated Post-Treadmill Deprivation Compensation

	24-hour group	72-hour group
Mean pre-deprivation sleep time	810	810
Days of deprivation	1	1
Minutes sleep loss	810	2,430
Post-deprivation compensation*		
Day 1	105	187
2	63	126
Total compensation	168	313
Per cent compensation	21%	13%

* Minutes sleep above the pre-deprivation mean.

There was a strong circadian rhythm throughout the experiment, but unlike Experiment II there was not a circadian rhythm reduction following treadmill deprivation (Figure 15). See Figure 5 for a comparison. As a matter of fact, the circadian rhythm on post-deprivation days 3 to 5 was greater than it was pre-deprivation ($p < .05$). It was not possible to maintain treadmill deprivation for as long as dextro-amphetamine deprivation, since many Ss

at this age would be expected to reach exhaustion between 72 and 120 hours on the wheel (Levitt & Webb, 1964; Webb & Agnew, 1962).

The major conclusions suggested by the results of this experiment are: (a) that sleep time temporarily increased following treadmill deprivation and had returned to normal by the end of the study, and (b) that there was no significant effect of deprivation level on sleep deprivation compensation. Both these findings confirm the dextro-amphetamine experiment.

Table 10 shows the estimated compensation. It appears to be somewhat less than in Experiment II. The Ss returned to their normal sleep time range on day 3 post-deprivation, while the Ss in Experiment II did not do this until day 6 or 7 post-deprivation. Also, the total minutes compensation and percentage compensation are smaller in Table 10 than in Table 5. This is especially noticeable for the 24-hour deprivation groups.

EXPERIMENT V

DEXTRO-AMPHETAMINE OR TREADMILL INDUCED SLEEP DEPRIVATION AND EEG MEASURED SLEEP CYCLE

The experiments detailed thus far have produced some interesting and suggestive data on the response of the sleep cycle to sleep deprivation. A question remaining to be answered is whether there is a difference in the response of the two sleep phases (slow wave and paradoxical) to the two deprivation techniques utilized in this paper. In particular, dextro-amphetamine induced sleep deprivation deprives the S equally of both slow wave and paradoxical sleep. The present experiment will enable us to answer the question of whether the post-deprivation compensation consists of the two sleep phases in their normative proportions or consists predominantly of one or the other sleep phase. Unlike dextro-amphetamine induced sleep deprivation, treadmill induced deprivation seems to differentially deprive Ss of paradoxical sleep. Although the Ss also receive less slow wave sleep than normal, the treadmill seems to deprive them of relatively more paradoxical than slow wave sleep. This experiment will enable the description of the response of the two sleep phases to treadmill deprivation. The information

reported in this experiment should provide a beginning at answering the question of which phase of sleep is most "needed," and hopefully also provide part of a foundation for a functional analysis of the sleep phases.

This experiment will also serve as a partial replication using the EEG of Experiments II and IV which measured the sleep cycle with the ultrasonic activity units. The data on compensation during the first 24 hours in this experiment can be considered a replication of the compensation findings on day one post-deprivation in Experiments II and IV.

Method

Subjects

Six male Long-Evans hooded rats 110-120 days old at the beginning of the experiment were used. All Ss had bipolar cortical electrodes implanted. This procedure is the same as that in Experiments I and III.

Apparatus

The EEG, treadmill, and dextro-amphetamine procedures have been described earlier in this paper.

Procedures

All six Ss were placed in the experimental cages 48 hours prior to EEG recording. EEG recording was begun

at 8:00 A.M. on day one. Data were not scored until 2:00 P.M. This six-hour period was used as an adaptation procedure. The EEG attachment would occasionally come off the rat and require reapplication. An observer was always in the experimental room monitoring the Ss and EEG equipment. The behavioral appearance of S (sleeping or waking) was periodically noted and marked on the EEG record.

These procedures would be expected to disturb the Ss somewhat and cause them to be awake more than normal. Although this did occur, the change would not seem large enough to effect generalization from these findings. During the control days in the first dextro-amphetamine study, the Ss averaged 68 per cent total sleep. During the 24 control hours in this study, the Ss averaged 52 per cent total sleep.

Control EEG recordings were made from 2:00 P.M. on day 1 to 2:00 P.M. on day 2 at which time 3 Ss received their initial dextro-amphetamine injection and the other 3 Ss were put on the treadmill. Deprivation continued for 24 hours until 2:00 P.M. on day 3. Post-deprivation EEG recording began at this time and continued for 24 hours. Each minute of the EEG record was scored waking, paradoxical sleep, or slow wave (normal) sleep.

Results and Discussion

Figures 16 through 23, Tables 11 and 12 in the text, and Tables 18, 19, and 20 in Appendix A contain summaries and analyses of these data. For purposes of analysis the day was divided into four six-hour periods beginning at 6:00 A.M. Table 18 is a summary of nine analyses of variance performed on these data. Figure 16 illustrates these effects. Dextro-amphetamine deprivation significantly increased the amount of both paradoxical and slow wave sleep and decreased waking as compared with their control values. Treadmill deprivation increased paradoxical sleep without significantly altering the amount of either waking or slow wave sleep. Both these findings (dextro-amphetamine and treadmill) are consistent with those of Experiments II and IV. The bottom half of Table 18 is a summary of three analyses comparing dextro-amphetamine to treadmill deprivation. The lack of a significant method effect indicates that the two groups did not differ significantly before treatment. The treatment effect on waking and normal sleep is known from the analyses at the top of Table 18 to be completely accounted for by the dextro-amphetamine group. The significant M x T interaction on waking and normal sleep is an expression of the drug, but not the treadmill altering these measures. The lack of a M x T interaction on paradoxical sleep confirms that both deprivation methods increased paradoxical sleep equally.

TABLE 11

Sleep Cycle Totals Before and After Deprivation

	Dextro-amphetamine deprivation		Treadmill deprivation	
	Pre	Post	Pre	Post
Minutes				
Waking	728 min.	387 min.	653 min.	587 min.
Paradoxical sleep	71	172	86	176
Normal sleep	641	881	701	677
	1,440	1,440	1,440	1,440
Per cent of total record				
Waking	51%	27%	45%	41%
Paradoxical sleep	5	12	6	12
Normal sleep	44	61	49	47
Paradoxical sleep as a per cent of total sleep	10	16	11	21

TABLE 12

Estimated Post-Deprivation Compensation

	Dextro-amphetamine group	Treadmill group
Pre-deprivation 24-hour sleep time		
Normal sleep	641 min.	701 min.
Paradoxical sleep	71	86
Total minutes sleep loss	712	787
Post-deprivation compensation*		
Normal sleep	240	-24
Paradoxical sleep	101	90
Total minutes compensation	341	66

* Minutes of normal sleep and paradoxical sleep above the pre-deprivation level.

Figure 17 shows the deprivation effects as a percentage of control readings for the two deprivation methods, and Table 11 presents pre- and post-deprivation waking, paradoxical, and normal sleep averages for each deprivation method. Table 12 is an attempt to estimate the amount of post-deprivation compensation. Again we see that dextro-amphetamine deprivation increased both normal and paradoxical sleep and decreased waking; however, the major

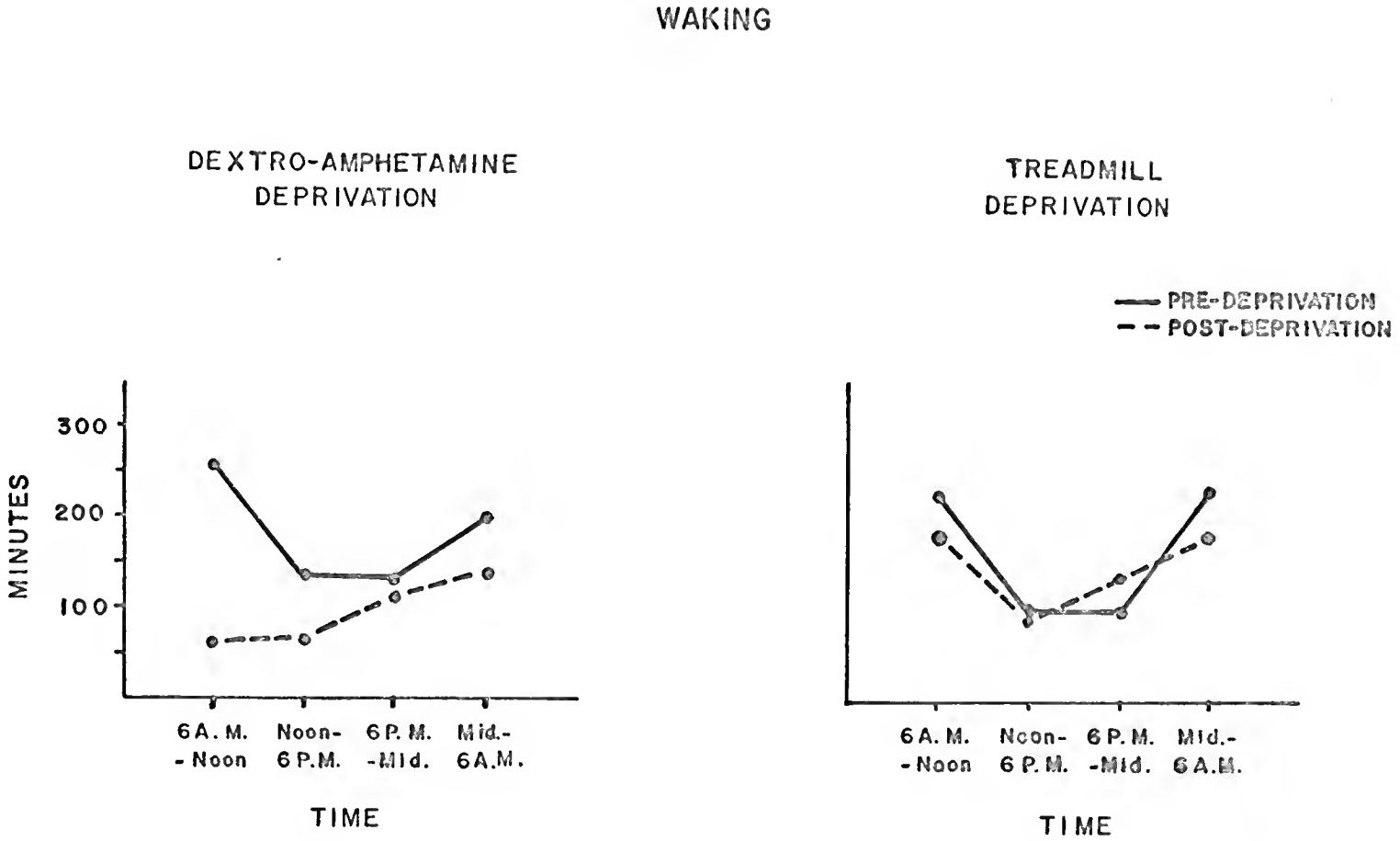


Figure 16. Dextro-Amphetamine or Treadmill Deprivation and Sleep Cycle: Waking, Paradoxical Sleep, and Normal Sleep.

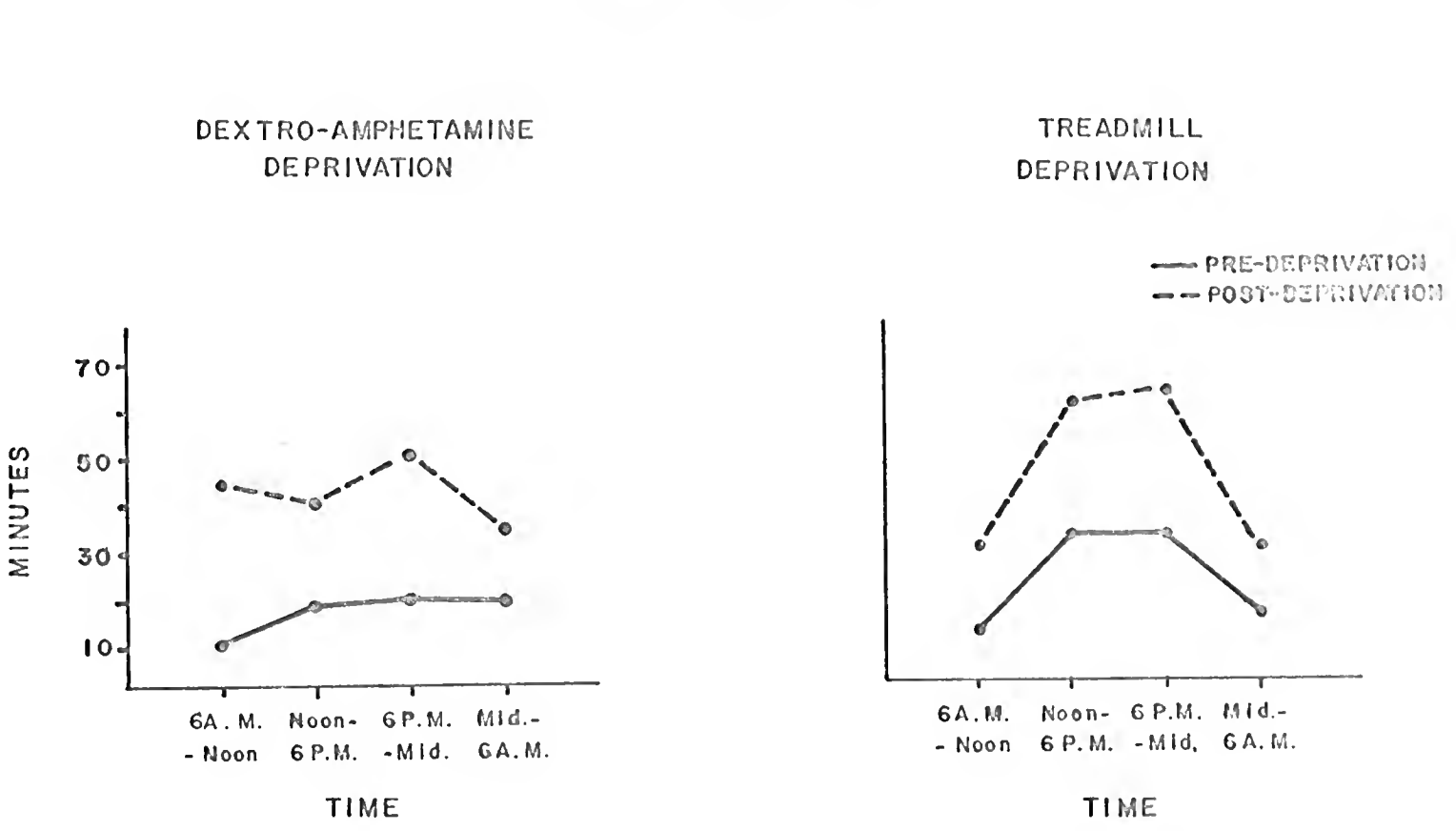
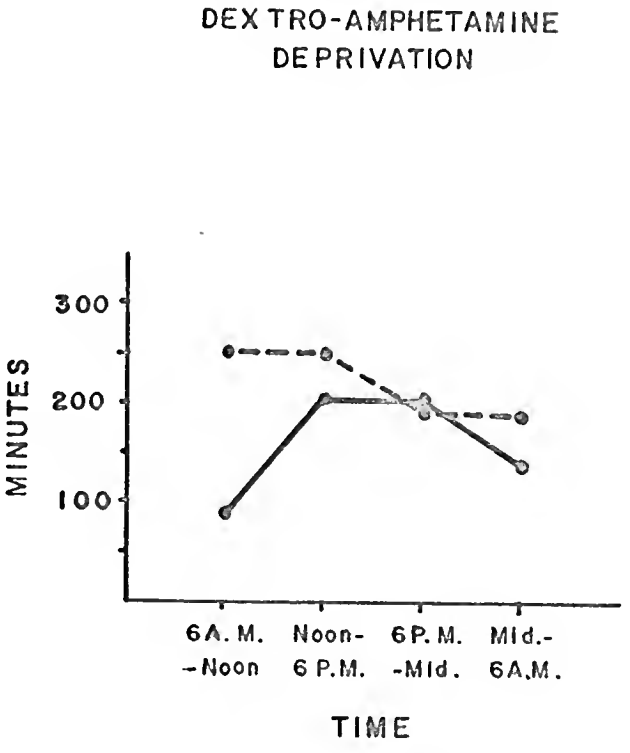


Figure 16. Continued.

NORMAL SLEEP



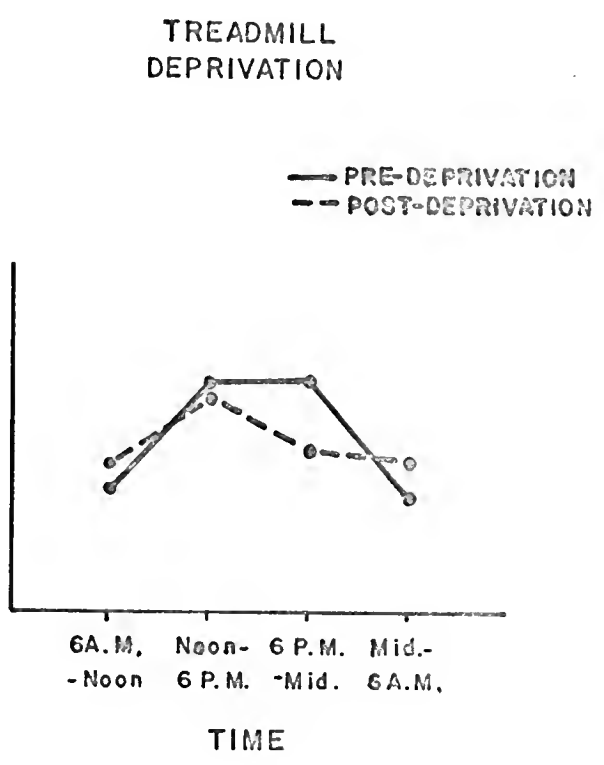


Figure 16. Continued.

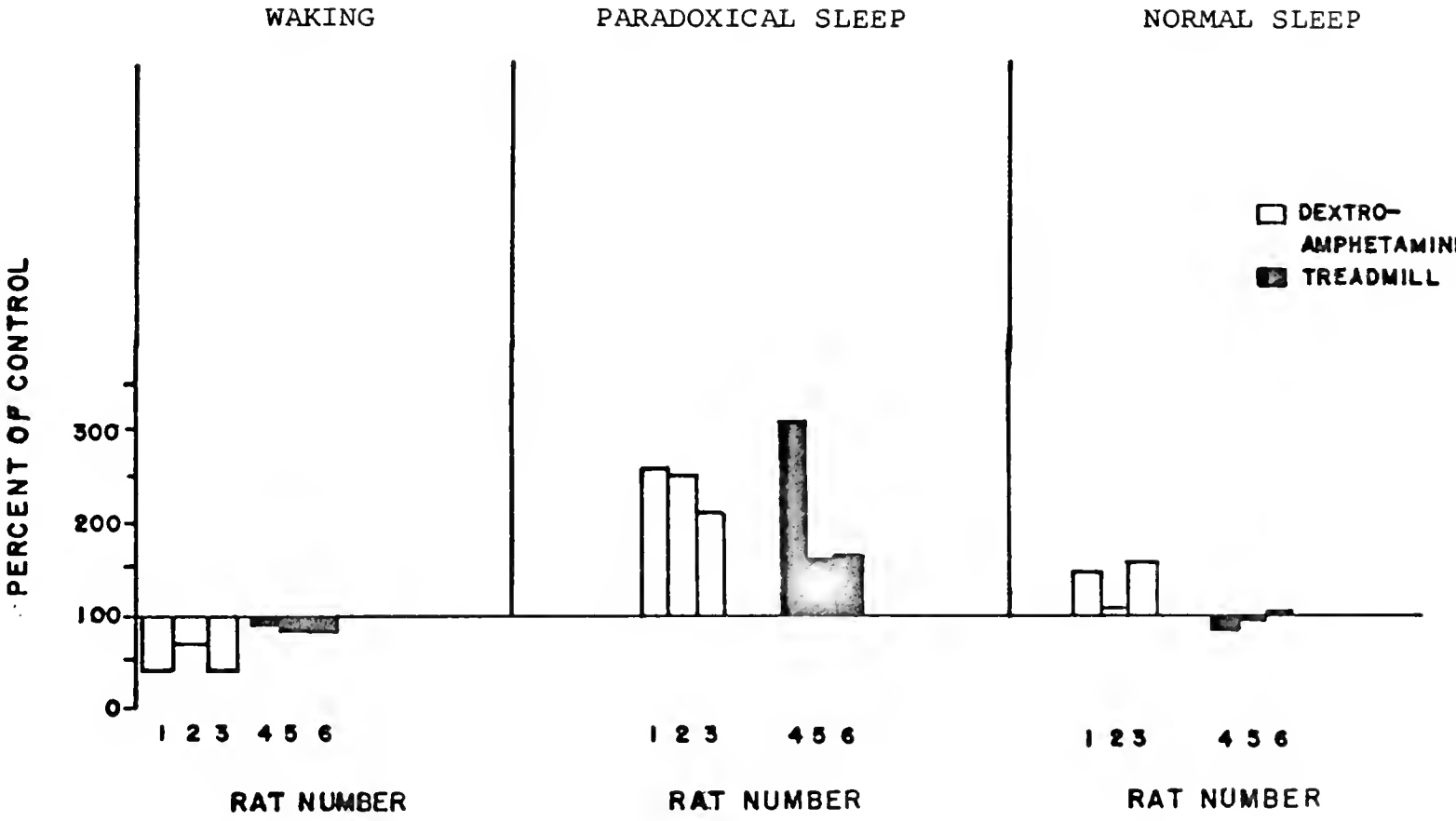


Figure 17. Post-Deprivation Sleep-Waking Cycle as a Percentage Change from Control Values.

effect is on paradoxical sleep which increases from 10 to 16 per cent of total sleep. Treadmill deprivation strongly increased paradoxical sleep, and this increase was allowed for by small but nonsignificant decreases in both waking and normal sleep. Paradoxical sleep increased from 11 to 21 per cent of total sleep after treadmill deprivation. The increases in paradoxical sleep produced by dextro-amphetamine or treadmill deprivation were not statistically different.

An attempt was made to further analyze the change in paradoxical sleep following deprivation. Figure 18 shows the mean sleep epoch length before and after deprivation. Table 19 is an analysis of variance which confirmed that the sleep epochs were longer after deprivation than on the control day. A sleep epoch is defined as four or more consecutive minutes of sleep (either slow wave or paradoxical). In order for a sleep epoch to be terminated, four consecutive minutes of waking must interrupt the epoch. It is known from numerous studies of sleep in humans that REM sleep tends to occur in the second half of a night's sleep (Aserinsky & Kleitman, 1953; Dement & Kleitman, 1957a). This relationship has not heretofore been demonstrated in rats, but, if it existed, an increase in length of sleep epoch might be expected to produce increased paradoxical sleep as a nonspecific effect.

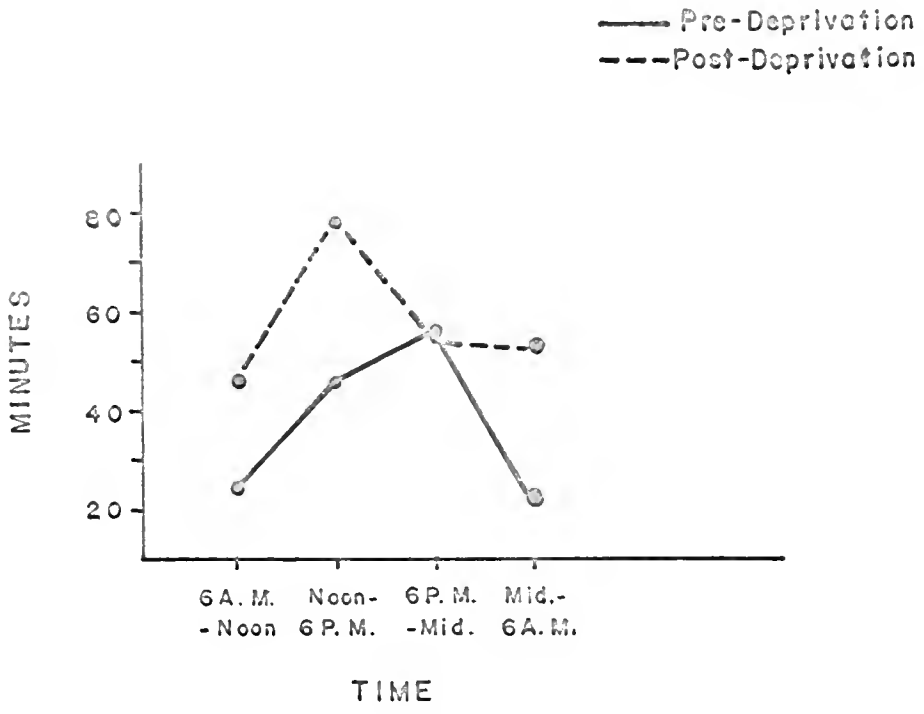


Figure 18. Length of Sleep Epochs.

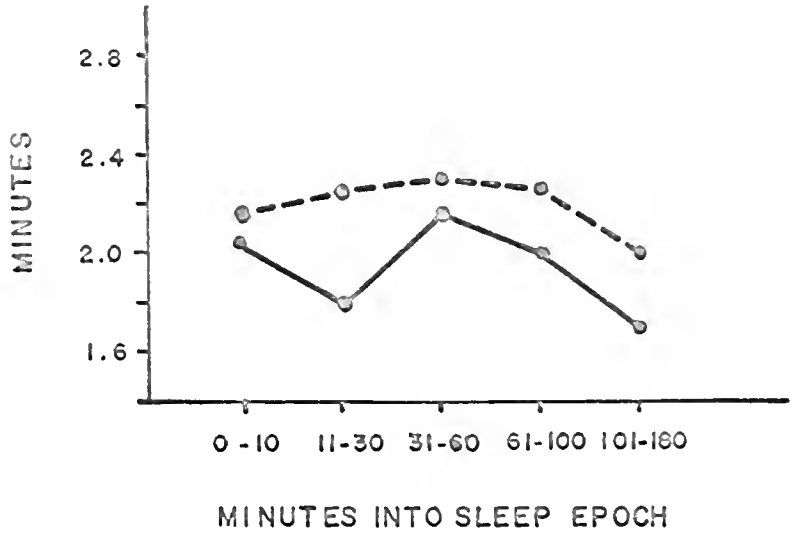


Figure 19. Length of Paradoxical Sleep Bursts.

An attempt was made to further analyze the paradoxical sleep data so as to test this hypothesis. The pre- and post-deprivation data were matched according to length of sleep epoch. Figures 19 through 22 summarize these data. Table 20 in Appendix A summarizes the variance analyses for these parameters. Following deprivation paradoxical sleep bursts occurred significantly more frequently during a sleep epoch and occupied a larger percentage of total sleep. Although the length of each paradoxical sleep burst was not significantly longer, it was in that direction (Figure 19). Also, the first minute of paradoxical sleep did not occur significantly earlier in the sleep epoch following deprivation, although again the data are in that direction (Figure 22). These results suggest that sleep deprivation specifically acts to increase paradoxical sleep. In Figures 19 to 21 it can be seen that the amount of paradoxical sleep does not increase with increasing length of sleep epoch. This suggests that the increase in paradoxical sleep produced by deprivation is not simply the result of an increase in sleep epoch length. Also, it can be seen in these figures that at the same point in a sleep epoch paradoxical sleep is more likely to occur following deprivation. This also suggests a specific activation of the paradoxical sleep state.

Many investigators have reported increased movement and twitching during paradoxical sleep in the rat (Hall,

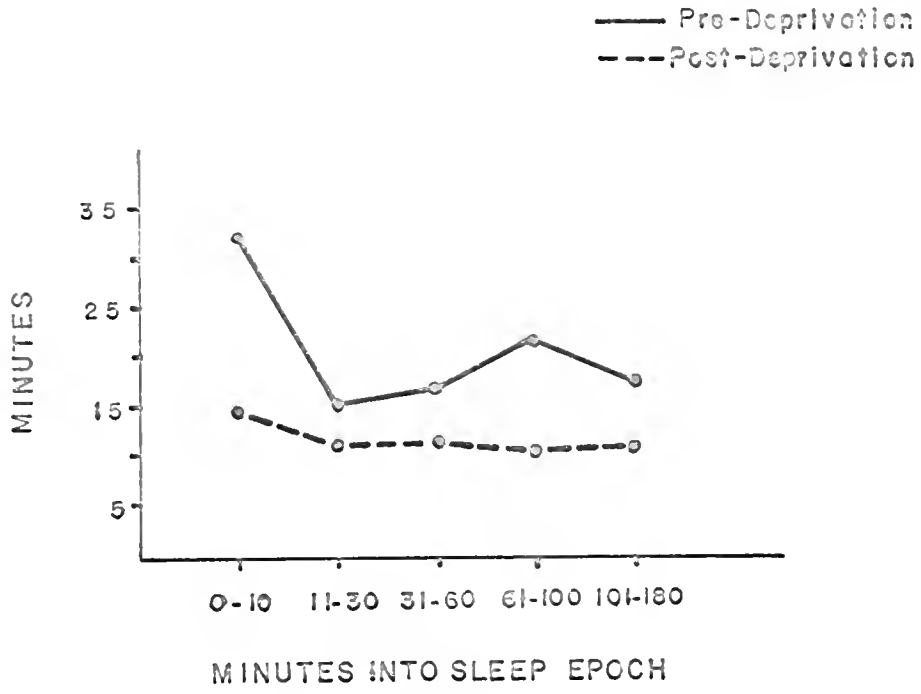


Figure 20. Minutes of Slow Wave Sleep Separating Paradoxical Sleep Bursts.*

* Frequency

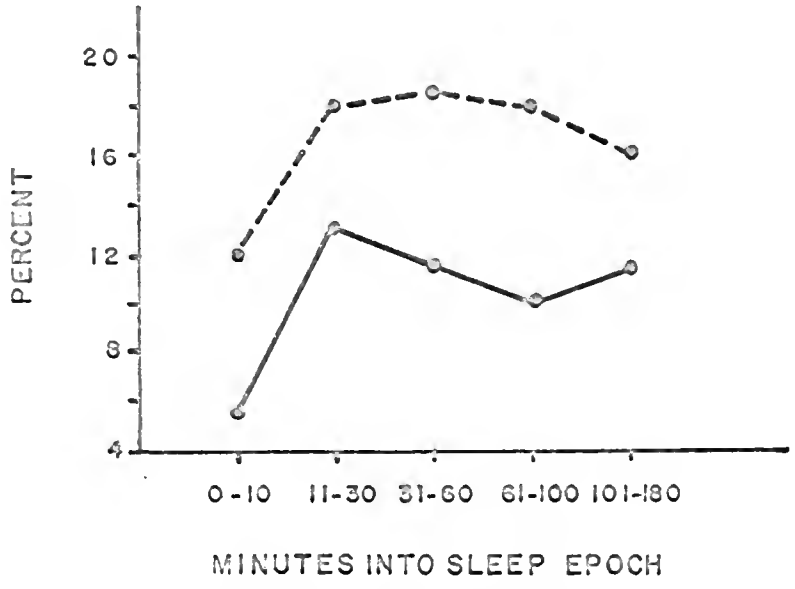


Figure 21. Percentage of Total Sleep Time Spent in Paradoxical Sleep.

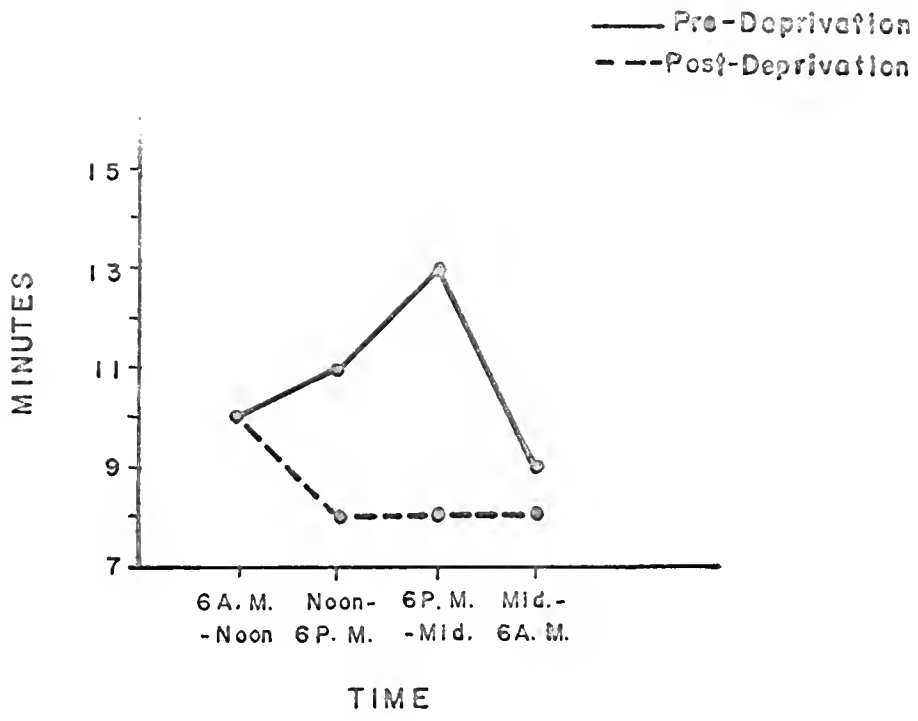


Figure 22. Minutes into Sleep Epoch When First Minute of Paradoxical Sleep Occurs.

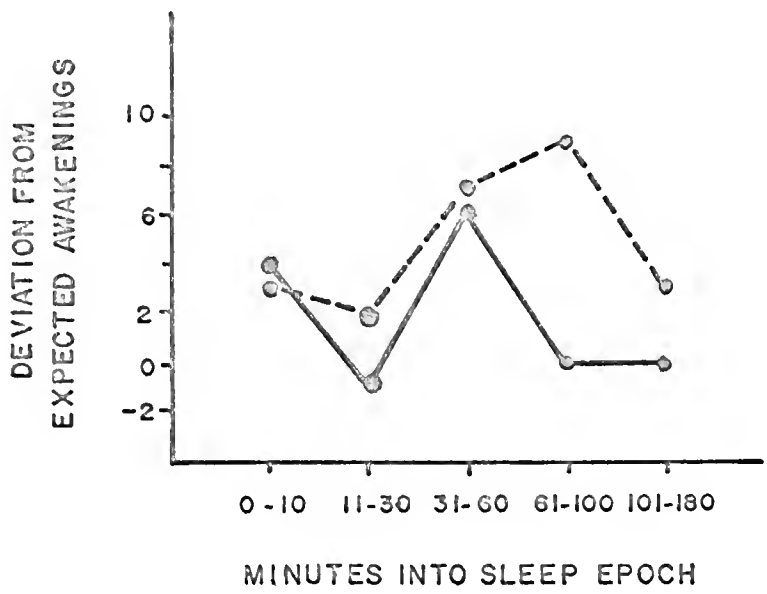


Figure 23. Difference Between Observed and Expected Awakenings from Paradoxical Sleep.

1963; Swisher, 1962). This was not confirmed in Experiment I, since activity during paradoxical and slow wave sleep as measured by ultrasonic activity errors was about equal. However, the hypothesis that Ss awoke more frequently from paradoxical than slow wave sleep was tested and confirmed ($x^2 = 60.2$, $df = 5$, $p < .001$). If the rats awoke from sleep epochs ten times during 100 minutes of sleep and paradoxical sleep amounted to 10 per cent of sleeping time, then we would predict one awakening from paradoxical sleep. Figure 23 presents these data. The Ss awoke significantly more frequently from paradoxical sleep than would be expected. This finding perhaps suggests a disturbing influence (dreams?) during this stage of sleep.

SUMMARY AND CONCLUSIONS

Experiment I

The Development of an Ultrasonic Activity Device to Measure Sleep and Waking in the Rat

This experiment established the ultrasonic activity system as a useful and reliable means of measuring the sleep cycle. These units are especially valuable for long term recordings for which the EEG is impractical. It is realized that it is still necessary to refer to the EEG periodically to recheck the EEG-activity correlation and also in order to differentiate sleep stages.

The ultrasonic activity unit can introduce new experimental possibilities into sleep research. With these units it should be possible to continuously monitor the sleep-waking cycle of small mammals for months or longer. The disadvantages of the EEG, which are eliminated by ultrasonic activity recording, include: (a) large expense in time and money, (b) the necessity of surgically implanting electrodes, (c) the expense and difficulty of reading and interpreting EEG records, and (d) the necessity for the animal to trail electrode wires, which probably disrupt the naturalness of the response under study. The major disadvantage of ultrasonic activity recording--not

being able to differentiate sleep phases--has already been mentioned.

The interesting finding of no difference between the percentage of high voltage slow sleep minutes and LVFS minutes, which produce errors, would lead to the conclusion that the Ss were equally active during these two sleep stages.

Experiment II
The Effect of Dextro-Amphetamine Induced Sleep Deprivation on the Sleep Cycle (Activity Measure)

The stability of the sleep cycle in this experiment and also in Experiments IV and V is quite rewarding. It must be realized that these are the first explorations utilizing the techniques of ultrasonic activity and prolonged EEG recording. It is expected that future experiments will use longer recording periods and also that, as the Es become more familiar with the equipment, an even more stable baseline will be found. This consideration is important, since the magnitude of experimental effect required to produce statistical dependability is partially determined by the stability of the baseline measure. The fact that results of both statistical and psychological import were found in all the studies reported here suggests the systems (ultrasonic activity and EEG) are sufficiently reliable for variables of the magnitude used.

The experiments reported here also have allowed the description of the parameters of sleep behavior in the rat. These are the first sleep studies in the rat of sufficient length to allow a description of the normal sleep cycle (see especially the normative sleep cycle data from Experiment II, and also the pre-deprivation measures from Experiment V shown in Table 11 and Figures 16 to 23). These data apply only to a limited subject population but are, at least, a beginning at a description of rat sleep.

The dextro-amphetamine technique was successful in almost completely eliminating sleep during the deprivation period. The two major findings of this procedure are: (a) that the drug induced sleep deprivation resulted in a temporary compensatory increase in sleep time following drug withdrawal, and (b) that increasing deprivation from 24 to 72 to 120 hours did not produce any significant change in the amount of compensatory sleep. The most exciting conclusion suggested by these data is that increased sleep deprivation over 24 hours up to 120 hours does not result in an increased sleep need over that present in the 24-hour sleep deprivation group. Two possible alternatives to this conclusion have been discussed earlier. First, although the amount of sleep during deprivation was small compared to the normal level, sleep was present. It may be that sleep during a heightened need is "more valuable" in

relieving the deficit than under conditions of normal drive. The data from Experiments III and V would seem to confirm this hypothesis, since in Experiment III the Ss obtained sleep in smaller than normal amounts on the treadmill, and in Experiment V this amount of sleep was sufficient to eliminate the need for post-deprivation slow wave sleep compensation. Further experiments which (a) are more successful in completely eliminating sleep, and (b) study the effect of small amounts of sleep inserted at various times during deprivation, will help to answer this question. Also, the dextro-amphetamine anorexigenia may interact with the sleep need and change the shape of the recovery function. Stomach loading during deprivation may control for this effect. However, the author prefers the interpretation that the lack of increased compensatory sleep with increased sleep deprivation from 1 to 5 days reflects the relationship between deprivation and need. This finding may be very significant if confirmed. It is conceivable that similar findings would be found in other species; of particular interest are the possibilities and implications for humans in acute behavioral requirement situations.

There was no cultural restraint on the sleeping time of the rats in this study. We can assume that they received all of the additional sleep they required. The amount of

compensatory sleep should reflect the biological deficit and sleep requirement produced by prolonged dextro-amphetamine induced sleep deprivation.

Dextro-amphetamine is a sleep depriver that requires no effort to stay awake by the subject; thus, perhaps leaving S free to perform tasks that an individual intent on remaining awake could not perform. These possible effects may have important applications in behavioral situations such as may be found in military and aerospace programs.

One conceivable fault of the dextro-amphetamine technique is that sleep loss effects are compounded by muscle fatigue, but it seems that all methods of producing sleep deprivation require movement and work on the part of the subject. At present it is not possible to keep an inactive S awake. Kleitman (1963) found that the only way he could keep human Ss awake was to have them engage in some sort of muscular activity. Whether the beneficial effects of dextro-amphetamine outweigh the toxic ones in particular behavioral situations remains an open question for further study. Further study of behavioral capacity during and following dextro-amphetamine administration in doses sufficient to maintain prolonged wakefulness in animals, and particularly in humans, would be of considerable importance. Of at least peripheral interest is

a review article by Weiss and Laties (1962), who have examined the effects of dextro-amphetamine on performance, concluding that physical endurance, capacity, and motor coordination are enhanced. Dextro-amphetamine seems to hasten conditioning, to improve discrimination learning in sleepy Ss, and increase the rate of motor learning. Dextro-amphetamine apparently does not lead to improved intellectual performance except when normal functioning is degraded by fatigue or boredom. Weiss and Laties conclude that there is no convincing evidence that a psychological or physiological price is paid for the enhanced performance.

The curious finding that sleep time does not increase or increases very little during the daylight but that large amounts of compensation occur at night following sleep deprivation is of some interest (see Figures 6 and 14). The suggestion that the rat sleeps maximally during the day, in the same sense that a human sleeping in bed from midnight to 7:00 A.M. sleeps maximally, should be tested.

Experiment III
EEG and Behavioral Observation on Rats Walking the Water-Immersed Treadmill

The finding of micro-sleep in rats walking the sleep-deprivation treadmill is of considerable interest. This

finding effects the interpretation of a number of experiments which have studied the effects of sleep deprivation produced by a water-immersed treadmill or similar apparatus on later behavior. These Ss were not completely deprived of sleep, to say the least. This experiment is also of interest, since the micro-sleep phenomenon may be considered to have the properties of an instrumentally conditioned response motivated by the sleep need.

Experiment IV
The Effect of Treadmill Induced Sleep Deprivation on the Sleep Cycle (Activity Measure)

The results of this experiment suggest that, although rats do experience a "sleep-like" state on the treadmill, some type of deprivation condition is produced. The major findings confirm the dextro-amphetamine experiment: (a) deprivation did result in increased sleep, and (b) increasing the deprivation level from 24 to 72 hours did not significantly increase the amount of compensatory sleep. This finding would suggest that the similar observation in Experiment II was not due to food deprivation factors. However, these Ss also obtained larger amounts of sleep (micro-sleep) during increasing deprivation (see Figure 12), and this problem has not been resolved.

Experiment V

Dextro-Amphetamine or Treadmill Induced Sleep Deprivation and EEG Measured Sleep Cycle

The normative data produced by this experiment have been discussed above.

The most interesting finding is that dextro-amphetamine deprivation results in a compensatory increase in both slow wave and paradoxical sleep (confirming Experiment II), while treadmill deprivation produces a compensatory increase only in paradoxical sleep (suggesting that the compensation in Experiment IV was only for paradoxical sleep). The treadmill results confirm the finding of micro-sleep in Experiment III and suggest that this phenomenon is analogous to slow wave sleep. However, dextro-amphetamine deprivation also increased paradoxical sleep more than slow wave sleep. This finding that paradoxical sleep occupies a higher percentage of total sleep suggests a function in specifically remedying a high sleep need state. Certain specific psychological and/or physiological activities may occur during paradoxical, but not slow wave sleep. These activities apparently have a high need priority above those occurring during slow wave sleep. The nature of these activities is not known, although the psychological functions may be related to the dream state.

This finding of a high priority for paradoxical sleep compensation following deprivation has been confirmed by

Svorad for the rat (Webb, personal communication) and Ferguson and Dement (1965) for the cat. Berger and Oswald (1962) and Williams, et al. (1963) have also confirmed these findings in the human. However, the human studies have found a stage 4 compensation to occur before REM sleep can increase. Since stages within slow wave sleep have not been differentiated in the rat, these studies are not directly comparable. These studies would seem to indicate a high "need" for both paradoxical and stage 4 sleep. These results, showing specific compensatory changes in stage 4 and REM sleep to follow sleep deprivation, are in conflict with Dement's (1965) hypothesis that physiological variations within NREM sleep do not warrant further subdivision. The results of Berger and Oswald (1962) and Williams, et al. (1963) suggest a functional differentiation of stages within NREM sleep, since stage 4 responds differently than other NREM sleep to deprivation.

A further analysis of the paradoxical sleep effect indicated a specific activation of this state, which was similar for both dextro-amphetamine and treadmill deprivation procedures. Following deprivation paradoxical sleep occupied a significantly higher percentage of total sleep, and bursts of paradoxical sleep occurred more frequently during a sleep epoch. Also, a nonsignificant increase in the length of each burst and an earlier

appearance of the first paradoxical sleep burst (also not statistically significant) confirm that sleep deprivation specifically activates paradoxical sleep.

The finding that Ss awoke significantly more frequently from paradoxical than from slow wave sleep than would be expected suggests the occurrence of a disruptive phenomenon (perceptual dreams?) during paradoxical sleep.

Rechtschaffen and Maron (1964) have reported that dextro-amphetamine administered to human Ss prior to sleep resulted in a decrease in the percentage of sleep time spent in REM periods as compared with control nights. A compensatory increase in percentage REM followed 3 or 4 nights of this partial REM deprivation. These findings of Rechtschaffen and Maron are consistent with the results of dextro-amphetamine sleep deprivation reported in this dissertation. Rechtschaffen and Maron state that, "This REM reduction indicates that relative to other sleep stages, REMPS do not represent states of arousal in the sense in which arousal is used to describe waking behavior" (1964, p. 444).

However, Rossi (1963) and Oswald, Berger, Jaramillo, Keddie, Olley, and Plunkett (1963) have shown that barbiturates also suppress REM sleep, indicating that neither are REMPs states of nonarousal relative to other sleep states. These results contribute to the conclusion that

REM or paradoxical sleep represents a neurophysiologically and psychologically unique stage of sleep not classifiable along a continuum of light to deep sleep (Dement, 1965; Jouvet, 1960).

APPENDICES

APPENDIX A

ANALYSES OF VARIANCE

TABLE 13

Dextro-Amphetamine Induced Deprivation and Sleep Cycle*

Source	Pre-deprivation (days 1-4)	Post-deprivation (days 1-4)	Post-deprivation (days 5-8)
Subjects	.05		
Circadian rhythm	.001	.001	.001
Days	.05	-	.05
S x C	-	-	.01
S x D	-	-	-
C x D	-	-	-

Source	Days 1-4 pre-deprivation vs. days 1-4 post-deprivation	Days 1-4 pre-deprivation vs. days 5-8 post-deprivation
Treatment	.001	-
Deprivation levels	-	-
Circadian rhythm	.001	.001
T x D	-	-
T x C	.001	.001
D x C	-	-
T x D x C	-	-

* Measured by ultrasonic activity units.

TABLE 14

Dextro-Amphetamine Deprivation and Food Intake

Source	Pre-deprivation (days 1-6)	Post-deprivation (days 1-6)	Post-deprivation (days 7-12)
Subjects	-	.01	-
Circadian rhythm	.001	.05	.01
Days	.001	.001	-
S x C	.001	.001	.001
S x D	-	-	-
C x D	.001	-	-

Source	Days 1-6 pre-deprivation vs. days 1-6 post-deprivation	Days 1-6 pre-deprivation vs. days 7-12 post-deprivation
Treatment	.001	-
Deprivation levels	.001	-
Circadian rhythm	.001	.001
T x D	-	.05
T x C	.001	.001
D x C	-	-
T x D x C	.01	.05

APPENDIX A - Continued

TABLE 15

Dextro-Amphetamine Deprivation and Water Intake

Source	Pre-deprivation (days 1-6)	Post-deprivation (days 1-6)	Post-deprivation (days 7-12)
Subjects	-	-	.01
Circadian rhythm	.01	.05	.01
Days	.05	.001	-
S x C	.001	.01	.001
S x D	-	-	-
C x D	-	-	-

Source	Days 1-6 pre-deprivation vs. days 1-6 post-deprivation	Days 1-6 pre-deprivation vs. days 7-12 post-deprivation
Treatment	-	.001
Deprivation levels	-	.05
Circadian rhythm	.001	.001
T x D	-	.01
T x C	.01	-
D x C	-	.05
T x D x C	-	.001

APPENDIX A - Continued

TABLE 16

Dextro-Amphetamine Deprivation and Body Weight

Source	Pre-deprivation (days 1-6)	Post-deprivation (days 1-6)	Post-deprivation (days 7-12)
Subjects	.001	.001	.001
Circadian rhythm	.01	.01	.01
Days	.001	.001	.001
S x C	.05	-	.001
S x D	.05	.001	.01
C x D	.05	.05	.001

Source	Days 1-6 pre-deprivation vs. days 1-6 post-deprivation	Days 1-6 pre-deprivation vs. days 7-12 post-deprivation
Treatment	.001	.05
Deprivation levels	.001	.001
Circadian rhythm	.01	.01
T x D	.001	.01
T x C	-	-
D x C	-	-
T x D x C	-	-

APPENDIX A - Continued

TABLE 17

Treadmill Induced Deprivation and Sleep Cycle*

Source	Pre-deprivation (days 1-3)	Post-deprivation (days 1-3)	Post-deprivation (days 3-5)
Subjects	-	-	-
Circadian rhythm	.001	.001	.001
Days	-	.001	.05
S x C	.001	-	.01
S x D	.001	-	-
C x D	.05	-	-

Source	Days 2-3 pre-deprivation vs. days 1-2 post-deprivation	Days 1-3 pre-deprivation vs. days 3-5 post-deprivation
Treatment	.001	-
Deprivation levels	-	-
Circadian rhythm	.001	.001
T x D	-	-
T x C	-	.05
D x C	-	-
T x D x C	-	-

* Measured by ultrasonic activity units.

APPENDIX A - Continued

TABLE 18

Dextro-Amphetamine or Treadmill Induced Deprivation and Sleep Cycle*

Source	Waking	Paradoxical sleep	Normal sleep
	Dextro-amphetamine		
Deprivation	.001	.001	.01
Circadian rhythm	.05	-	.05
D x C	.01	-	.05
	Treadmill		
Deprivation	-	.001	-
Circadian rhythm	.001	.001	-
D x C	-	-	.05
	Dextro-amphetamine and treadmill deprivation compared		
Source	Waking	Paradoxical sleep	Normal sleep
Method	-	-	-
Treatment	.001	.001	.05
Circadian rhythm	.001	.01	.001
M x T	.01	-	.01
M x C	-	-	-
T x C	.01	-	.001
M x T x C	-	-	-

* Measured by EEG.

APPENDIX A - Continued

TABLE 19

Mean Length of Sleep Epoch as a Function of Sleep Deprivation

Source	
Method	-
Treatment	.001
Circadian rhythm	.01
M x T	-
M x C	-
T x C	-
M x T x C	-

APPENDIX A - Continued

TABLE 20

Length, Frequency, and Percentage of Paradoxical Sleep as a Function of Sleep Deprivation

Source	Length of paradoxical sleep bursts	Frequency of paradoxical sleep bursts	Per cent of total sleep time in paradoxical sleep
Method	-	-	-
Length	-	.05	.05
Treatment	-	.001	.001
M x L	-	-	-
M x T	-	-	-
L x T	-	-	-
M x T x L	-	-	-

APPENDIX B

ORIGINAL DATA FOR EXPERIMENT II
DEXTRO-AMPHETAMINE DEPRIVATION AND ULTRASONIC ACTIVITY*

		24-hr. group				72-hr. group				120-hr. group			
Subject		1	2	3	4	5	6	7	8	9	10	11	12
Control days													
1	Light	479	466	411	422	467	547	525	475	474	525	441	421
	Dark	381	339	281	289	272	449	301	336	299	397	279	196
2	L	469	442	429	536	485	590	472	522	525	531	521	517
	D	374	290	262	427	344	326	431	523	317	326	410	224
3	L	432	465	473	512	461	595	502	458	496	477	513	425
	D	333	412	392	301	350	269	288	368	276	433	295	278
4	L	480	469	401	490	434	579	523	485	451	468	456	454
	D	375	217	285	232	388	355	239	323	398	330	304	250

* Minutes of sleep per 600-minute recording period.

APPENDIX B - Continued

		24-hr. group				72-hr. group				120-hr. group			
Subject		1	2	3	4	5	6	7	8	9	10	11	12
Post-drug days													
1	L	475	520	558	502	575	500	525	484	512	465	456	426
	D	539	510	475	429	440	486	388	409	409	488	453	433
2	L	447	457	490	522	468	505	440	529	463	487	519	517
	D	385	378	544	514	508	422	392	488	454	570	439	503
3	L	482	545	565	509	487	535	490	516	396	447	504	537
	D	464	320	579	416	394	488	435	545	427	388	341	333
4	L	471	442	564	443	470	448	432	543	476	436	450	497
	D	379	337	498	341	448	366	398	468	307	456	495	411
Post-drug days													
5	L	498	526	486	474	486	452	463	553	464	493	461	456
	D	363	396	489	372	511	318	342	435	358	368	443	332
6	L	485	498	524	441	411	401	415	504	411	350	458	521
	D	347	340	495	298	440	284	338	461	423	409	530	356
7	L	439	486	551	440	469	421	421	483	346	412	495	482
	D	296	369	416	276	351	325	372	466	425	345	314	259
8	L	428	457	536	474	434	418	411	416	383	439	430	465
	D	420	261	460	359	372	326	409	480	420	434	395	247

APPENDIX C

ORIGINAL DATA FOR EXPERIMENT IV
TREADMILL DEPRIVATION AND ULTRASONIC ACTIVITY*

Subject		24-hr. group 1	2	3	4	5	72-hr. group 6	7	8	9	10
Control days											
1	Light	458	443	507	459	478	487	387	402	427	505
	Dark	246	346	263	343	384	334	278	389	399	401
2	L	510	433	463	475	509	490	512	445	437	517
	D	380	304	338	349	276	327	429	422	221	380
3	L	481	443	472	497	497	512	437	454	474	512
	D	335	356	296	392	427	329	292	286	235	428
Post-deprivation days											
1	L	529	517	551	483	481	531	574	548	548	564
	D	516	252	454	354	438	402	551	466	398	401
2	L	525	494	565	550	419	526	544	483	514	548
	D	251	328	503	345	381	342	473	373	484	392

* Minutes of sleep per 600-minute recording period.

APPENDIX C - Continued

Subject		24-hr. group 1	2	3	4	5	72-hr. group 6	7	8	9	10
Post-deprivation days (cont.)											
3	L	485	460	550	514	433	394	494	472	498	474
	D	351	342	403	248	303	351	381	262	180	235
4	L	532	497	484	532	447	528	588	471	488	509
	D	352	338	327	362	436	459	417	257	206	318
5	L	521	456	504	515	475	533	593	564	560	575
	D	407	315	304	340	380	380	327	304	319	279

APPENDIX D

ORIGINAL DATA FOR EXPERIMENT V
DEXTRO-AMPHETAMINE OR TREADMILL DEPRIVATION AND EEG*

	Dextro-amphetamine			Treadmill		
Subject	1	2	3	4	5	6
	Pre-deprivation					
Time						
6:00 A.M. - noon						
W	292	240	243	194	217	264
L	3	13	16	7	21	4
S	65	107	101	159	122	92
noon - 6:00 P.M.						
W	190	68	150	140	91	65
L	30	11	17	26	35	31
S	140	281	193	194	234	264
6:00 P.M. - midnight						
W	113	107	179	108	79	109
L	21	8	33	18	43	32
S	226	245	148	234	238	219
midnight - 6:00 A.M.						
W	194	173	236	202	278	214
L	30	15	16	13	13	15
S	136	172	108	145	69	131

* Minutes of waking, LVFS, or slow wave sleep per 360-minute observation period; lights on 9:00 A.M. to 9:00 P.M.

APPENDIX D - Continued

Subject	Dextro-amphetamine 1	2	3	Treadmill 4	5	6
			Post-deprivation			
Time						
6:00 A.M. - noon						
W	66	102	22	129	228	189
L	57	25	52	50	22	15
S	237	233	286	181	110	156
noon - 6:00 P.M.						
W	62	88	55	113	83	68
L	44	35	44	61	68	46
S	254	237	261	186	209	246
6:00 P.M. - midnight						
W	115	127	104	159	98	156
L	63	32	59	64	72	46
S	182	201	197	137	190	158
midnight - 6:00 A.M.						
W	117	101	203	198	182	157
L	55	27	22	28	25	32
S	188	232	135	134	153	171

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BIOGRAPHICAL SKETCH

Robert Alan Levitt was born in Baltimore, Maryland, on November 9, 1938. His family moved to Miami, Florida, when he was a child. He attended Miami Senior High School. Upon graduating from high school he enlisted for six months' active duty in the U. S. Army. After his active duty, he spent one year at the University of Miami, after which he transferred to the University of Florida, receiving the B.S. degree (major in pharmacy) in June, 1961. In January, 1962, he re-entered the University of Florida for graduate studies in psychology, and received the degree of Master of Science in June, 1963. From September, 1963, until the present time he has pursued his work toward the degree of Doctor of Philosophy. During this period, he held a NASA Pre-doctoral Traineeship.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 14, 1965

Ernest H. Cox
Dean, College of Arts and Sciences

Dean, Graduate School

Supervisory Committee:

Chairman

W B Webb

Frederick A. King

Robert L. King

James A. Hood