THE EFFECT OF PREDICTABLE VERSUS UNPREDICTABLE SHOCK ON RAPID EYE MOVEMENT SLEEP IN RATS

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I hereby declare that this dissertation is my own work and that I have not submitted it for a Master's Degree to any other University.

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CONTENTS

ABSTRACT

The influence of predictable versus unpredictable electric shock on the distribution of sleep stages was examined with special reference to rapid eye movement (REM) sleep in rats. A five hour electric shock stress period indicated that both predictable and unpredictable shock were equally effective in decreasing REM as a percentage of total sleep time, although there was no evidence for an associated decrement in REM sleep as a percentage of total recording time. Slow wave sleep time and total sleep time were unaffected. In contrast to these findings a five hour total sleep deprivation period increased both indices of REM sleep. The effects of electric shock on sleep were assessed in terms of the possible confounding influence of sleep deprivation, and were discussed in terms of a neurohumoral model for the mediation of the stress effect on sleep.

During the past decade there has been an increasing concern with the effects of stress on human, feline and rodent sleep patterns. Investigators have tended to utilise a variety of stressful conditions involving appetitive deprivation, physical restraint, surgical intervention, forced exercise and intense, threatening or novel external stimulation. Stressful conditions which have been investigated in relation to sleep patterns in rats include exercise (Boland and Dewsbury, 1971; Matsumoto, Nishisho, Suto, Sadahiro and Miyoshi, 1968), temperature extremes (Schmidek, Hoshino, Schmidek and Timo-Iaria, 1972; Valatx, Roussel and Cure, 1973), thirst (Gibson and Broughton, 1969), combined hunger and thirst (Iwahara, Yang, Yamazaki and Sugimura, 1970), high intensity white noise (Van Twyver, Levitt and Dunn, 1966), restraint (Altman, Whitehead and Rechtschaffen, 1972; Bugbee, Duncan and Snyder, 1974) and predatory threat (Broughton and Gibson, 1972; Gibson and Broughton, 1969). Cats have been used to investigate the effects of exercise (Hobson, 1968a) and temperature extremes (Hobson, 1968b; Parmeggiani and Rabini, 1967; Parmeggiani and Rabini, 1970; Parmeggiani, Rabini and Cattalani, 1969) on sleep, while rabbits have been used to investigate the effects of high intensity white noise (Khazan and Sawyer, 1963) and restraint (Kawakami, Negoro and Teraswa, 1965) on sleep.

Human sleep patterns have been examined in relation to strenuous exercise (Baekeland and Lasky, 1966; Desjardins, Healey and Broughton, 1974; Hauri, 1968), temperature extremes (Otto, Kramer and Brauer, 1971; Putkonen, Elomaa and Kotilainen, 1973; Schmidt-Kessen and Kendel, 1973; Williams, Karacan and Hursch, 1969), thirst (Koulack, 1970), hunger (MacFadyen, Oswald and Lewis, 1973), intense noise

(Globus, Friedmann and Cohen, 1973; Townsend, Johnson and Muzet, 1973; Roth, Kramer, Trinder, Sandler, Riechers and Fishbein, 1970; Scott, 1972), the first night spent in a sleep laboratory (Agnew, Webb and Williams, 1966; Bernstein, Emde and Campos, 1973; Dement, Kahn and Roffwarg, 1965;Rechtschaffen and Verdone, 1964), the viewing of a stressful movie (Baekeland, Koulack and Lasky, 1968) and a variety of more naturalistic stressors such as the stress of an examination period (Holdstock and Verschoor, 1974; Lester, Burch and Dossett, 1967) and the stress of circumcision in neonates (Anders and Chalemian, 1974; Emde, Harmon, Metcalf, Koenig and Wagonfeld, 1971).

Despite this accumulation of evidence it is difficult to draw any general conclusions as to the effects of stress on sleep since both rapid eye movement (REM) sleep and slow wave sleep (SWS) have been shown to either increase, decrease, or remain unchanged in response to the action of various stressors. This is particularly true of the data concerning SWS. While some experimenters have demonstrated increments in SWS in response to the stressors of exercise (Baekeland and Lasky, 1966; Boland and Dewsbury, 1971; Hobson, 1968a; Matsumoto et al., 1968), temperature (Hobson, 1968b; Putkonen et al., 1973; Valatx et al., 1973), circumcision (Emde et al., 1971) and hunger (MacFadyen et al., 1973), other experimenters have reported SWS to be uninfluenced by exercise (Desjardins et al., 1974; Hauri, 1968), temperature extremes (Schmidek et al., 1972) and circumcision (Anders and Chalemian, 1974), as well as by the stressors of noise (Khazan and Sawyer, 1963; Scott, 1972; Townsend et al., 1973; Van Twyver et al., 1966), an examination period (Holdstock and Verschoor, 1974) and restraint (Altman et al., 1972; Bugbee et al.,

1974). In contrast to the work demonstrating stress-induced increments in SWS, a number of authors have reported reductions in SWS as a result of temperature extremes (Otto et al., 1971; Parmeggiani and Rabini, 1967; Parmeggiani and Rabini, 1970; Parmeggiani, Rabini and Cattalani, 1969; Schmidt-Kessen and Kendel, 1973), disruptive noise (Roth et al., 1970; Globus et al., 1973), an examination period (Lester et al., 1967), food and water deprivation (Iwahara et al., 1970) and predatory threat (Gibson and Broughton, 1969; Broughton and Gibson, 1972).

The data on REM sleep yields somewhat greater agreement, since the majority of studies favor stress-induced decrements in REM sleep. While some authors have found REM sleep to be unaffected by exercise (Baekeland and Lasky, 1966; Boland and Dewsbury, 1971; Hauri, 1968; Matsumoto et al., 1968), food and water deprivation (Iwahara et al., 1970), noise (Townsend et al., 1973), restraint (Bugbee et al., 1974), circumcision (Anders and Chalemian, 1974) and examination stress (Holdstock and Verschoor, 1974; Lester et al., 1967), others have found increments in REM sleep during temperature increases (Hobson, 1968b; Valatx et al., 1973). However, reductions in REM sleep have been reported following exercise (Desjardins et al., 1974; Hobson, 1968a), temperature extremes (Otto et al., 1971; Parmeggiani and Rabini, 1967; Parmeggiani and Rabini, 1970; Parmeggiani, Rabini and Cattalani, 1969; Schmidek et al., 1972; Schmidt-Kessen and Kendel, 1973; Williams et al., 1969), water deprivation (Broughton and Gibson, 1972; Koulack, 1970), food deprivation (MacFadyen et al., 1973), restraint (Altman et al., 1972; Kawakami et al., 1965), circumcision (Emde et al., 1971), stressful noise (Globus et al., 1973; Khazan and Sawyer, 1963; Roth et al., 1970; Scott, 1972; Van Twyver et al., 1966),

the viewing of a stressful movie (Baekeland et al., 1968), the first night spent in a sleep laboratory (Agnew et al., 1966; Bernstein et al., 1973; Dement et al., 1965; Rechtschaffen and Verdone, 1964) and predatory threat (Broughton and Gibson, 1972; Gibson and Broughton, 1969). These findings tend to support Hartmann's (1967) suggestion that REM sleep suppression is the most frequently occurring sleep alteration in response to stress.

Although electric shock stress has been commonly used as a stressor in animal experimentation, to the knowledge of the present author, work on the relationship between electric shock stress and sleep has been restricted to control procedures in a study designed to investigate the influence of active-avoidance learning on sleep (Leconte, Hennevin and Bloch, 1973). The results of this study (Leconte et al., 1973) provided some evidence for a shock-induced decrement in REM sleep with associated increments in SWS.

In view of the dearth of literature on the relationship between electric shock stress and sleep the aim of the present study was to examine the influence of predictable and unpredictable shock stress on the distribution of sleep stages. This paradigm was chosen for a number of reasons. Firstly, shock predictability and unpredictability have been differentiated in terms of their influence on stress reactions such as adrenocortical activation (Bassett and Cairncross, 1973), adrenomedullary activation (Mason, Mangan, Brady, Conrad and McK.Rioch, 1961), weight loss (Brady, Thornton and De Fisher, 1962; Friedman and Ader, 1965; Pare, 1964; Price, 1972), and gastric ulceration (Pare, 1964; Price, 1972; Weiss, 1970) but have not been investigated with regard to stress-induced changes in sleep. Secondly, several lines of neuroendocrinological evidence suggest the possibility that predictable

and unpredictable shock differentially influence REM sleep. It has been demonstrated that predictable shock leads to a greater corticosteroid elevation than does unpredictable shock (Bassett and Cairncross, 1973). In addition a dose dependent (Gillin, Jacobs, Fram and Snyder, 1972) inverted relationship (Gillin, Jacobs, Fram and Snyder, 1972; Gillin, Jacobs, Snyder and Henkin, 1972) between corticosteroid level and amount of REM sleep has been demonstrated.

METHOD

Animals

Eighteen experimentally naive, male Long Evans rats were used. At the time of surgery they were at least 90 days old and weighed between 300 and 400 grams. For the duration of the experiment, the animals were housed singly under conditions of constant light and temperature.

Surgery

The animals were chronically implanted with electrodes for recording electroencephalographic (EEG) and electromyographic (EMG) activity. Surgery was performed using Nembutal induced anaesthesia (50 mg/kg intraperitoneally). Atropine was administered subcutaneously (0,05 mg/kg) to ease respiration during the operation.

The recording electrodes used were unipolar Amphenol type 27-9 connectors that had been modified to serve a bipolar function according to the technique described by Holdstock and Franks (1971). The EEG electrode was implanted in the transverse plane such that one pole rested over the cortex of the right cerebral hemisphere at a point 2 mm anterior to the bregma and 3 mm lateral to the midline. The other pole rested over the cortex of the left cerebral hemisphere in an equivalent position. These coordinates were standard for all animals. Stainless steel wires were hooked into the dorsal neck muscles bilaterally for recording the EMG. Both EEG and EMG electrodes were firmly affixed to the skull using stainless steel anchoring screws and dental acrylic. Following surgery each animal received 75,000 units of penicillin injected subcutaneously (Compropen Vet, Glaxo-Allenbury).

Apparatus

The stress and recording procedures were carried out in different cages.

The cages required for the stress procedures had metal sides with a perspex front, back and top and measured 23 x 23 x 27 cm. The inside walls were lightly sprayed with matt black paint except for the front which was left clear to allow for observation. The top, which served as an entry lid, was lightly sprayed with a white spray paint, leaving it translucent. A 10 x 15 cm loudspeaker was mounted at the back of each cage for tone deliveries. The floor was constructed of 0,5 cm brass rods spaced 1cm apart to form a grid.

In two of the cages the grids were connected in parallel to a Layfayette A615C Constant Current Shock Supplier with a neon-scrambled output. The grid of another cage was left unconnected for use in the stress control condition.

During the experimental sessions the onset and offset of the various shock-tone combinations were controlled by Grayson-Stadler electronic timers (types E1100 and E4430). These timers were automatically activated by pre-programmed pulses on a continuous loop magnetic tape running on a Sony TC 200 stereo tape recorder.

Each of these cages was housed in a ventilated, sound attenuating chamber with internal neon-fluorescent lighting and white noise at a level between 70 and 75 db.

The recording cages had the same basic dimensions and design as the stress cages. However, certain differences were implemented in an attempt to minimise the transfer of conditioned situational cues from the stress to the recording situations. These differences included unpainted inside walls instead of black walls, a square mesh floor

instead of a grid, and no loudspeaker. In addition, a 3 cm diameter hole was drilled in the center of the lid of each cage to provide for the passage of the recording cables. The cables were suspended from an overhead rod by means of elastic bands, thereby reducing the weight of each cable, and allowing the animals fairly unrestricted movement.

All the recording cages were housed side by side in a sound attenuating Faraday room with a one-way glass front. The ambient noise level in this room was 70 db, while temperature was maintained at 23 degrees C and humidity at 50 percent. The cages were illuminated by two 100 watt incandescent light bulbs.

All recordings were taken on a Beckman Type R Dynograph at a chart speed of 1 cm/sec.

Procedure

The animals were allowed a recovery period of at least one week following surgery. They were then randomly divided into three groups of six animals each: an unpredictable shock group, a predictable shock group, and a no-shock control group. The animals were tested three at a time, one from each group.

The experiment took place over a period of three consecutive days.

The first day served purely to adapt the animals to the experimental and recording environments. On the adaptation day the animals were placed in the stress cages for five hours (07h00 to 12h00), but received no treatment. This was followed by a six hour session (12h00 to 18h00) in the recording cages with the recording leads attached, but no recordings were taken. The animals were returned to their home cages overnight.

The purpose of the second day was to obtain baseline recordings. Thus the animals were again placed in the stress cages from 07h00 to 12h00 without receiving treatment. They were then removed to the recording cages and a six hour baseline recording was taken. During this time the animals were continuously observed through the one-way mirror and notations of their behavioral state were made on the EEG paper to facilitate scoring.

On the third day the animals received the group appropriate treatment between 07h00 and 12h00. Both the unpredictable and the predictable stress groups were subjected to a 24 minute variable interval schedule of shocks which was repeated every 24 minutes. Every 24 minute period contained twelve shocks. The shock duration was three seconds and the intensity was 0,5 ma.

In the predictable shock group each shock was preceded by a five second tone of 1000 Hz. The unpredictable shock group received the same tone schedule (with respect to the number of tones and intertone intervals), but the phase relationship between the tone schedule and the shock schedule was displaced by twelve minutes. Thus for the unpredictable shock group the tones were effectively unrelated to the shock delivery. The control group received the tone schedule but no shock.

The experimental session was immediately followed by a six hour recording session. The animals were then sacrificed by means of a 1 cc intraperitoneal injection of triple strength barbiturate (Euthanaze).

During the adaptation, stress and recording sessions only water was available. Both food and water were available ad libitum when the animals were returned to the home cages overnight.

The records were scored for wakefulness, slow wave sleep and REM

sleep according to the criteria specified by Holdstock and Verschoor (1972). Awake was defined as a predominance of theta in conjunction with tonic EMG activity, SWS by the presence of irregular slow waves of large amplitude and spindles in the EEG; and REM sleep by synchronous theta lasting at least 20 seconds in the presence of EMG suppression.

RESULTS*

The pretest-posttest control group design has been frequently analyzed by means of the repeated measures analysis of variance (Huck and McLean, 1975) in spite of the fact that the model underlying the repeated measures analysis of variance is inappropriate to this application (Huck and McLean, 1975). Since the between groups variance on the pretest measures is, by definition,uninfluenced by the action of the independent variable, the total between groups variance is reduced, rendering the main effect conservative and the interaction effect meaningless (Huck and McLena, 1975). As a viable alternative these authors recommend the use of analysis of covariance which is not only statistically correct but has the additional advantage of adjusting for possible differences in the pretest group means.

Thus data from the present experiment was analyzed using a single factor analysis of covariance (Edwards, 1968). When a significant F ratio was found individual group comparisons were made using the t-test for adjusted means (Edwards, 1968). The following data sets were subjected to the analysis (Appendix A):

(i) REM sleep as a percentage of total sleep (REM percent). (Appendix A, Table 1a);

(ii) REM sleep as a percentage of total recording time (REM time). (Appendix A, Table 2);

(iii) SWS as a percentage of total sleep (SWS percent). (Appendix A, Table 3);

(iv) SWS as a percentage of total recording time (SWS time). (Appendix A, Table 4);

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* Please see Appendix D.

(v) Total sleep as a percentage of total recording time (Total Sleep Time). (Appendix A, Table 5);

(vi) The number of REM periods. (Appendix A, Table 6);

(vii) The number of SWS periods. (Appendix A, Table 7);

(viii) The number of sleep periods. (Appendix A, Table 8);

(ix) The mean duration of each REM period. (Appendix A, Table 9);

(x) The mean duration of each SWS period. (Appendix A, Table 10);

(xi) The mean duration of each sleep episode. (Appendix A, Table 11);

(xii) The time elapsed between the start of recording and the first appearance of SWS (SWS latency). (Appendix A, Table 12);

(xiii) The time elapsed between the first appearance of SWS and the first appearance of REM (REM latency). (Appendix A, Table 13).

In addition, the baseline values of all the above variables were subjected to a single factor analysis of variance (Appendix B, Tables 1 to 13).

The unpredictable shock group (Group UP), the predictable shock group (Group P), and the control group (Group C) failed to differ in terms of their pre-stress values on REM percent ($F = 1,24$; $df = 2/15$), REM time ($F = 1,93$; $df = 2/15$), SWS percent ($F = 1,24$; $df = 2/15$), SWS time ($F = 1,18$; $df = 2/15$), total sleep time ($F = 1,39$; $df = 2/15$), the number of REM periods ($F = 2,32$; $df = 2/15$), the number of SWS periods ($F = 2,36$; $df = 2/15$), the number of sleep periods ($F = 2,79$; $df = 2/15$), the mean duration of each REM period ($F = 0,29$; $df = 2/15$), the mean duration of each SWS period ($F = 3,14$; $df = 2/15$), the mean duration of each sleep episode ($F = 3,39$; $df = 2/15$), SWS latency

(F = 0,34; df = 2/15), and REM latency (F = 0,12; df = 2/15).

The only variable found to be affected by the stress treatments was REM percent (F = 5,49; df = 2/14; p<0,05). Unfortunately, however, this particular data set failed to meet the re .irement of homogeneity of regression (F = 5,01; df = 2/12; p<0,05). In line with the recommendation of Edwards (1968) the pretest measures were transformed by means of the square transformation (Davis, 1957; Lindquist, 1956) resulting in a more acceptable level of homogeneity (F = 3,70; df = 2/12). An analysis of covariance on the transformed data (Appendix A, Table 1b) once again revealed the existence of a treatment effect (F = 7,89; df = 2/14; p<0,01). Comparisons made on the adjusted means revealed no difference between Group P and Group UP (t = 0,03; df = 14). However, as shown in Fig. 1, REM percent was depressed in Group P (t = 3,36; df = 14; p<0,005) and in Group UP (t = 3,33; df = 14; p<0,005) when compared with Group C.

In order to determine whether the depression in REM percent exhibited in the stress groups was distributed across the entire recording period, a comparison was made between the amount of REM percent in the first half and the amount of REM percent in the second half of the posttest recording session (Appendix C). This was done by means of an analysis of covariance for repeated measures (Winer, 1970) with the amounts of REM percent in the first half and second half of the pretest session acting as the covariates for the amounts of REM percent in the first half and the second half of the posttest session. The adjustment procedures followed were those used when the between group and within group regression coefficients are not assumed to be homogenous (Winer, 1970).

As demonstrated in Fig. 2, the suppression effect exhibited in the

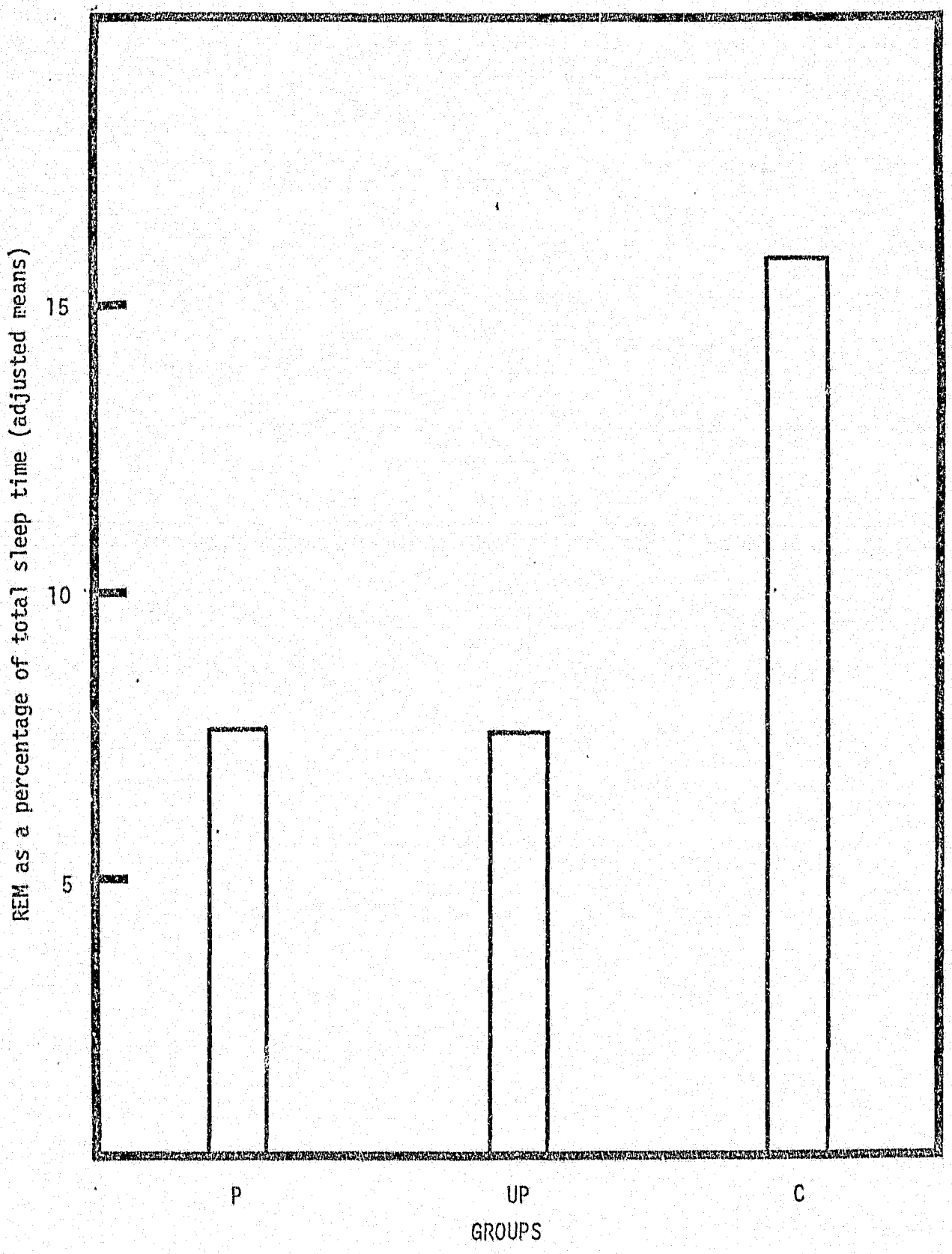

Fig 1. Adjusted mean percentage of sleep time spent in REM during the entire recording session for Groups C, P and UP.

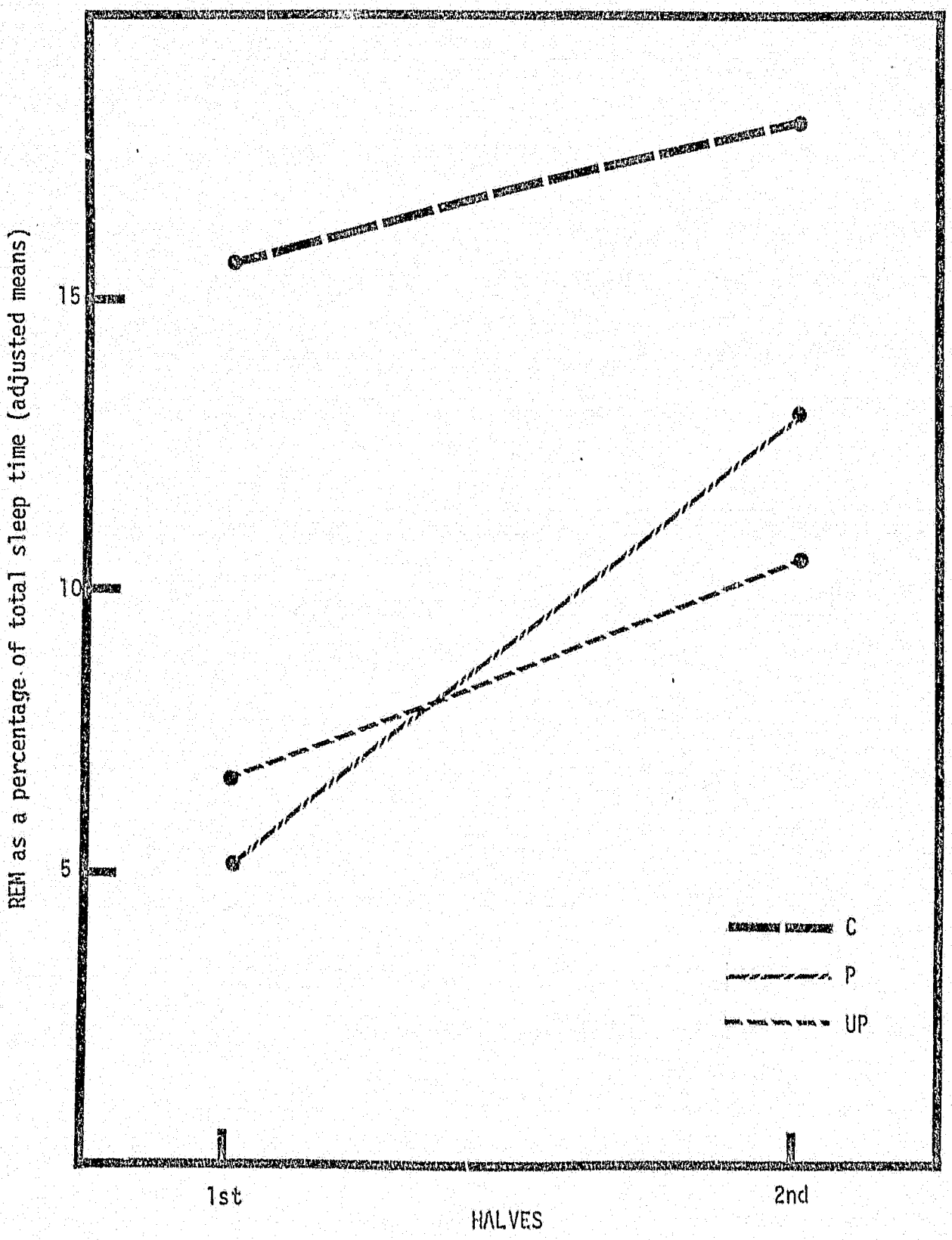

Fig 2. Adjusted mean percentage of sleep time spent in REM during each half of the recording session for Groups C, P and UP.

stress groups was not confined to a particular half of the recording period (Groups x Halves: $F = 1,85$; $df = 2/14$). Although both the stress groups and the control group showed an increased amount of REM sleep between the first and second halves of the posttest session (Halves: $F = 8,23$; $df = 1/14$; $p<0,025$), the stress groups were consistently suppressed in terms of REM percent relative to the control group (Groups: $F = 5,75$; $df = 2/14$; $p<0,025$).

Although REM percent was decreased in both the stress groups ($F = 7,89$; $df = 2/14$; $p<0,01$) and SWS percent was increased in both the stress groups (since SWS percent is the complement of REM percent) there were no changes in the absolute measures of REM time ($F = 1,55$; $df = 2/14$), SWS time ($F = 0,19$; $df = 2/14$) or total sleep time ($F = 0,03$; $df = 2/14$). The remaining variables which failed to discriminate between the three groups were the number of REM periods ($F = 3,43$; $df = 2/14$), the number of SWS periods ($F = 0,46$; $df = 2/14$), the number of sleep episodes ($F = 0,40$; $df = 2/14$), the mean duration of REM periods ($F = 0,04$; $df = 2/14$), the mean duration of SWS periods ($F = 0,42$; $df = 2/14$), the mean duration of sleep periods ($F = 0,20$; $df = 2/14$), SWS latency ($F = 0,01$; $df = 2/14$) and REM latency ($F = 1,66$; $df = 2/11$).

DISCUSSION

The results of the present study indicated that both predictable and unpredictable shock were equally effective in decreasing REM sleep as a percentage of total sleep time, although there was no evidence for an associated decrement in REM sleep as a percentage of total recording time.

The absence of a differential predictable-unpredictable shock effect on sleep may be related to the specific nature of shock unpredictability employed in the present study. Bassett and Cairncross, (1973) defined the unpredictable condition as an irregularly presented unsignalled shock. However the present study, employed an irregularly presented shock which was randomly related to the occurrence of a signal in an attempt to control for overall stimulation levels between the two stress groups. The predictable condition was defined as an irregularly presented signalled shock in both the present study and that of Bassett and Cairncross, (1973). It is therefore possible that the smaller overall differences existing between the predictable and unpredictable conditions in the present study were insufficient to provoke a differential adrenocortical response and ultimately differential effects on REM sleep.

The finding of a shock-induced decrement in REM percent with no change in REM time is consistent with the findings of Leconte et al., (1973), who reported REM percent suppression following the administration of 50 shocks delivered over a ninety minute period (representing a shock density of 0,55 shocks per minute). Although the stress period employed in the present study was three and a half hours longer than that used by Leconte et al., (1973), the shock density was comparable

(0,50 shocks per minute). The use of a lower shock density (0,33 shocks per minute delivered over a ninety minute period) was associated with a diminished REM suppression effect (Leconte et al., 1973) suggesting a relationship between the degree of REM suppression and the intensity of the stressor. However, data on the relative contributions of the length of the stress period and the shock density are lacking.

Decrements in REM percent have been reported in response to stressors such as intense noise (Khazan and Sawyer, 1963; Scott, 1972; Van Twyver et al., 1966), exercise (Desjardins et al., 1974; Hobson, 1968a), restraint (Altman et al., 1972), predatory threat (Broughton and Gibson, 1972), starvation (MacFadyen et al., 1973) and the first night spent in a sleep laboratory (Agnew et al., 1966; Bernstein et al., 1973; Dement et al., 1965). However the REM sleep suppression effect in response to stress in general is not necessarily limited to REM percent since decrements in REM time have also been reported in response to predatory threat (Broughton and Gibson, 1972), thirst (Koulack, 1970), hunger (MacFadyen et al., 1973), the first night spent in a sleep laboratory (Rechtschaffen and Verdone, 1964) and temperature extremes (Schmidek et al., 1972). It is difficult at present to relate the nature of the stressor employed to the specific index of REM sleep affected since few authors have utilised both REM percent and REM time as measures of REM suppression (Broughton and Gibson, 1972; Hobson, 1968b; MacFadyen et al., 1973). While Broughton and Gibson, (1972) and MacFadyen et al., (1973) found stress-induced decrements in both REM percent and REM time, Hobson (1968b) found decreases only in REM percent.

Since SWS percent is the compliment of REM percent, decrements

in REM percent in the present study were matched by corresponding increments in SWS percent. However, SWS as a percentage of total recording time was unaffected by the action of either predictable or unpredictable shock. In contradistinction to these findings Leconte et al., (1973) demonstrated increases in SWS time following the administration of high shock density stress (0,55 shocks per minute) as well as with a low shock density stress (0,33 shocks per minute) which was insufficiently intense to provoke a decrement in REM percent, suggesting that SWS was more readily altered by the stressor than was REM sleep. This is surprising since REM has been characterised as a fragile state of sleep (Hartmann, 1967), whereas SWS is considered particularly difficult to alter (Hartmann, 1973) and is primarily increased by conditions, such as exercise (Baekeland and Lasky, 1966; Boland and Dewsbury, 1971; Hobson, 1968a; Matsumoto et al., 1968), sexual activity (Boland and Dewsbury, 1971), starvation (MacFadyen et al., 1973), hyperthyroidism (Oswald, Dunleavy and Strong, 1972; Ross, Agnew, Williams and Webb, 1968), and exposure to extreme temperatures (Hobson, 1968b; Putkonen et al., 1973; Schmidek et al., 1972), influencing fundamental metabolic equilibria.

In addition, Leconte's (Leconte et al., 1973) finding of stress-induced increments in SWS time is less compatible with existing biochemical evidence than is the present finding of stress-induced decrements in REM percent without associated increments in SWS time. Firstly it has been demonstrated that electric shock causes a decrement in the absolute level of brain norepinephrine (Barchas, Ciaranello, Stock, Brodie and Hamburg, 1972; Paulsen and Hess, 1963), reflecting a stress-induced acceleration of norepinephrine catabolism (Paulsen and Hess, 1963; Putkonen and Putkonen, 1971). In addition, electric shock

increases the metabolic rate of serotonin (Bliss, Thatcher and Alion, 1972; Putkonen and Putkonen, 1971) with resulting increments in the level of its deaminated metabolite, 5 hydroxyindolacetic acid (Bliss et al., 1972). However the absolute level of serotonin remains unchanged after shock stress since, unlike the metabolism of norepinephrine, accelerated serotonin catabolism is paced by an acceleration in anabolism (Bliss et al., 1972; Radulovacki, 1973). Thus shock results in the selective depletion of absolute levels of norepinephrine leaving the absolute levels of serotonin unchanged. Jouvet (1975) has demonstrated that SWS and total sleep time are dependent upon the raphe serotonergic system. While the release of serotonin constitutes a necessary precondition for the appearance of REM sleep, the initiation of REM sleep depends upon a complex interaction between serotonergic, catecholamergic and cholinergic systems (Jouvet, 1969; Jouvet, 1975; Kostowski, 1975). The effects on sleep of selective norepinephrine depletion are therefore likely to be restricted to REM sleep, leaving absolute levels of SWS and total length of sleep time unaffected. In addition, increments in steroid levels consequent upon shock administration (Bassett and Cairncross, 1973) selectively depress REM (Gillin, Jacobs, Fram and Snyder, 1972; Gillin, Jacobs, Snyder and Henkin, 1972). However, the failure of shock to influence absolute levels of REM sleep (REM as a percentage of total recording time) cannot be explained in terms of the neurohumoral evidence mentioned above, although the possiblity remains that suppression in the absolute level of REM sleep is related to more extreme shock conditions than were employed in the present study and that of Leconte et al., (1973).

EXPERIMENT 2

The administration of shock stress in Experiment 1 was unavoidably associated with a five-hour period of total sleep deprivation since the average interval between shocks was only two seconds. On the other hand, the behavioral manifestations of sleep were observed in the control group after habituation to the tone delivery. Because the stress condition and total sleep deprivation were confounded in the first experiment, it was decided to assess the specific influence of five hours of total sleep deprivation, occurring between 07h00 and 12h00, on the subsequent distribution of sleep stages.

METHOD

Animals

Six experimentally naive, male Long Evans rats were used. At the time of surgery they were at least 90 days old and weighed 300 grams. For the duration of the experiment the animals were housed singly under conditions of constant light and temperature.

Surgery

All the animals were implanted for the recording of electroencephalographic and electromyographic activity according to the procedures employed in Experiment 1.

Apparatus

The sleep deprivation and the recording procedures were carried out in different cages. The cage required for the sleep deprivation procedure was identical to the stress cages used in Experiment 1, but was housed in an empty fish tank measuring 32 x 32 x 45 cm instead of a sound-attenuating box. The recording cage was the same as those used in Experiment 1, and was housed in the Faraday room described earlier.

Procedure

One animal was tested at a time. The experiment took place over a period of three consecutive days. On the first day the animal was adapted to the sleep deprivation cage for five hours (07h00 to 12h00) followed by six hours (12h00 to 18h00) in the recording cage where the recording leads were attached, but no recordings were taken.

On the second day the animal was again placed in the sleep deprivation cage from 07h00 to 12h00. During the six hours spent in the recording cage (12h00 to 18h00) a baseline recording was taken.

Prior to the sleep deprivation session on the third day (07h00 to 12h00) water was let into the fish-tank until it reached a level 0,5 cm above the grid floor of the sleep deprivation cage. This level was sufficient to induce total sleep deprivation without causing unnecessary discomfort. In an attempt to minimise cold stress, the water was thermostatically maintained at 32 degrees C.

Following the sleep deprivation session the animal was removed to the recording cage and a six-hour recording was taken.

RESULTS

A t-test for correlated samples (Ferguson, 1966) was used to compare the baseline and post-deprivation data with respect to the following dependent variables:

(i) REM as a percentage of total sleep;

(ii) REM as a percentage of total recording time;

(iii) SWS as a percentage of total sleep;

(iv) SWS as a percentage of total recording time;

(v) Total sleep as a percentage of total recording time;

(vi) The number of REM periods;

(vii) The number of SWS periods;

(viii) The number of sleep episodes;

(ix) The mean duration of each REM period;

(x) The mean duration of each SWS period;

(xi) The mean duration of each sleep episode;

(xii) The latency to the first appearance of SWS;

(xiii) The latency from the first appearance of SWS to the first appearance of REM.

Both REM percent ($t = 3,48$; $df = 5$; $p<0,02$; two-tailed) and REM time ($t = 3,30$; $df = 5$; $p<0,05$; two-tailed) increased between the baseline and post-deprivation recordings, indicating a deprivation-induced enhancement in REM sleep.

The increase in REM sleep is attributable to an increase in the number of REM periods ($t = 3,20$; $df = 5$; $p<0,05$; two-tailed) rather than increments in the average length of REM periods ($t = 0,67$; $df = 5$). In addition, a decrease in REM latency followed sleep deprivation ($t = 3,12$; $df = 5$; $p<0,05$; two-tailed).

Table 1 Mean time spent in SWS as a percentage of total recording time, mean time spent in sleep as a percentage of total recording time and the mean number of SWS periods before and after total sleep deprivation.

	SWS TIME	TOTAL SLEEP	SWS PERIODS
PRE-DEP	40,16	45,87	67,17
POST-DEP	46,87	57,43	56,33

There was an increase in the average length of SWS periods ($t = 4,25$; $df = 5$; $p<0,01$; two-tailed) and a resultant increase in the average length of sleep periods ($t = 4,24$; $df = 5$; $p<0,01$; two-tailed). These changes were associated with non-significant tendencies for SWS time and total sleep time to increase, and a tendency for the number of SWS periods to decrease (Table 1).

DISCUSSION

It is evident from the findings of the present study that a five-hour total sleep deprivation period, occurring between 07h00 and 12h00, resulted in increased REM sleep as manifested by increments in REM time and REM percent, and decrements in REM latency. The fact that deprivation-induced changes in REM sleep occurred in the opposite direction to the stress-induced changes in REM sleep (Experiment 1) is unlikely to be attributable to baseline differences since the REM percent and the REM time baseline data obtained by the three groups employed in Experiment 1 and the animals in the present experiment are essentially comparable (Table 2).

Table 2 Mean time spent in REM as a percentage of total recording time and the mean time spent in REM as a percentage of total sleep before and after treatment for Groups P, UP and C, and the deprivation group (Group D).

GROUP	REM TIME		REM PERCENT	
	Baseline	Posttreatment	Baseline	Posttreatment
P (Exp. 1)	7,61	4,95	15,72	8,38
UP (Exp. 1)	4,82	4,09	12,32	6,98
C (Exp. 1)	6,42	7,50	12,76	15,54
D (Exp. 2)	5,71	10,56	11,01	17,96

The deprivation-induced increment in REM has some bearing on the stress-induced changes demonstrated in Experiment 1.

The deprivation-induced changes in REM sleep appear to have been more profound than those occurring after stress, in that a larger number of REM related variables were affected by deprivation than by stress.

Since deprivation increased REM it is extremely likely that REM decrements in response to stress were lessened by increments in response to deprivation. It is also possible that the absence of stress-induced decrements in REM time were attributable to this antagonizing effect. However this possibility should be assessed against Leconte's (Leconte et al., 1973) failure to find stress-induced changes in REM time after using a considerably shorter stress period, and therefore inducing less sleep deprivation, than in the present study. The difference in the total stress period between Leconte's (Leconte et al., 1973) study and the present study, together with the similarity in shock density between the two studies, strongly suggests that the failure to demonstrate stress-induced decrements in REM time was related to the intensity of the shock, rather than the length of the stress period, or the amount of sleep deprivation induced.

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APPENDIX A

TABLE 1a

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON REM SLEEP AS A PERCENTAGE OF TOTAL SLEEP

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	272,20	2	136,10	5,49	<0,05
Error	346,89	14	24,00		
Total	619,09				

TABLE 1 b

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON REM SLEEP AS A PERCENTAGE OF TOTAL SLEEP AFTER SUBJECTION TO A SQUARE TRANSFORMATION

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	134012,13	2	67006,06	7,89	<0,01
Error	118943,31	14	8495,95		
Total	252955,44				

TABLE 2

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON REM SLEEP AS A PERCENTAGE OF TOTAL RECORDING TIME

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	33,50	2	16,75	1,55	
Error	151,02	14	10,79		
Total	184,52				

TABLE 3

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON SWS AS A PERCENTAGE OF TOTAL SLEEP

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	272,08	2	136,04	5,49	
Error	347,03	14	24,79		
Total	619,11				

TABLE 4

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON SWS AS A PERCENTAGE OF TOTAL RECORDING TIME

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	93,91	2	46,96	0,19	
Error	3533,60	14	252,40		
Total	3627,51				

TABLE 5

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON TOTAL SLEEP AS A PERCENTAGE OF TOTAL RECORDING TIME

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	22,35	2	11,17	0,03	
Error	4856,68	14	346,91		
Total	4879,02				

TABLE 6

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE NUMBER OF REM PERIODS

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	331,54	2	165,77	3,43	
Error	676,03	14	48,29		
Total	1007,57				

TABLE 7

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE NUMBER OF SWS PERIODS

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	207,74	2	103,87	0,46	
Error	3146,64	14	224,76		
Total	3354,38				

TABLE 8

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE NUMBER OF SLEEP PERIODS

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	182,23	2	91,12	0,40	
Error	3219,25	14	229,95		
Total	3401,48				

TABLE 9

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE MEAN DURATION OF EACH REM PERIOD

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	130,32	2	65,16	0,04	
Error	21493,80	14	1535,27		
Total	21624,12				

TABLE 10

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE MEAN DURATION OF EACH SWS PERIOD

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	4603,31	2	2301,66	0,42	
Error	76529,50	14	5466,39		
Total	81132,81				

TABLE 11

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON THE MEAN DURATION OF EACH SLEEP EPISODE

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	3742,13	2	1871,06	0,20	
Error	130942,00	14	9353,00		
Total	134684,13				

TABLE 12

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON SWS LATENCY

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	32311,00	2	16155,50	0,01	
Error	16473349,00	14	1176667,00		
Total	16505660,00				

TABLE 13

SOURCE TABLE FOR THE ANALYSIS OF COVARIANCE ON REM LATENCY*

SOURCE OF VARIANCE	SS	DF	MS	F	P
Treatments	32607088,00	2	16303544,00	1,66	
Error	108328896,00	11	9848081,00		
Total	140935984,00				

* One animal in Group P and one animal in Group UP showed complete REM suppression throughout the entire post-stress recording period. Thus REM latency could not be computed for these animals, and the data was not included in this analysis. To maintain equal numbers in all three groups an animal was randomly selected from Group C and its data dropped from the analysis.