Effects of five hours of restraint stress on subsequent sleep in the rat*

JACK L. ALTMAN, WILLIAM E. WHITEHEAD
and ALLAN RECHTSCHAFFEN†
University of Chicago, Chicago, Ill. 60606

Rats subjected to 5-h restraint stress showed no significant difference from sleep-deprived controls in amount of slow sleep obtained during any part of a 12-h poststress recording period. This result contrasts with reports of increased slow sleep in rats and cats following stress and with anecdotal reports of stress-induced insomnia in humans. Stressed animals did show less paradoxical sleep than controls during the first 6 h after treatment.

Emde, Hamon, Metcalf, Koenig, & Wagonfeld (1970) reported that circumcision without anesthesia in human neonates produced large and significant increases in slow sleep (SS) but left paradoxical sleep (PS, corresponding to rapid-eye-movement sleep) unchanged. Emde et al attributed this effect to the stress of circumcision and believed it to be independent of exercise because the Es observed long periods of wakefulness with vigorous crying which were not followed by major increases in SS. This was the first report which claimed to have increased SS by a stress procedure other than exercise, although Hobson's (1968b) report on the effects of heating cats to raise their body temperatures may be interpreted as a stress study. Hobson found that the heating increased SS and left PS unchanged. This evidence for stress-induced increases in SS contrasts with the anecdotal evidence that stress can produce insomnia in adult humans.

The present study was undertaken to expand the available data on stress and sleep by using another species, the rat, and a different stressor, restraint. Five-hour chronic restraint was selected as the stressor because Curzon & Green (1969) had shown that restraint produced a significant decrease in brain levels of serotonin (5HT) and a significant increase in 5-hydroxy-indole-acetic acid (5HIAA), a metabolite of 5HT, and changes in brain 5HT (Jouvet, 1969) and brain 5HIAA (Rechtschaffen, Ledecy-Janecek, & Lovell, 1968) have been independently related to sleep.

METHOD

Eight adult male Sprague-Dawley

rats were implanted with epidural screw electrodes for recording the electroencephalogram (EEG) and silver plates placed subcutaneously over the neck muscles for recording the electromyogram (EMG). Leads from all electrodes were soldered to a miniature connector plug cemented to the skull.

At least 3 weeks following surgery, Ss were placed in sound-attenuated temperature-regulated recording cages for 4 days of habituation prior to the experimental manipulation. Cables counterbalanced for weight connected Ss to recording equipment through slip-ring commutators, which permitted the cables to rotate freely. Ss were left undisturbed, except for a ½-h period each day when they were fed and cages were cleaned. Food and water were continuously available. Cage lights were on from 8 a.m. to 8 p.m.

On Day 5, the eight Ss were removed from the recording cages and assigned randomly to the stress group or control group. Stress-group Ss had to be anesthetized briefly to facilitate placing them into restraint. Consequently, both groups were etherized at the beginning of the treatment period. The four Ss of the stress group were restrained from 9 a.m. to 2 p.m. by placing them astride a 2.5-in.-diam steel pipe, taping their backs down, and taping their feet together underneath. Control Ss were placed in their regular colony cages near the stressed animals so that E could sleep-deprive both groups for the 5 h. Because the stress procedure was certain to produce sleep loss and

because it would be difficult to deprive the control group of equivalent amounts of the same types of sleep, we deprived both groups of sleep entirely. Gentle nudging at the first behavioral signs of sleep—lying still, eyelids beginning to close—was sufficient to keep all Ss awake.

At the end of the 5 h, all Ss were returned to the recording cages and EEG and EMG were recorded for 12 consecutive hours after the experimental treatment. Polygraph records were scored for SS, PS, and waking according to conventional EEG and EMG criteria of demonstrated reliability (Rechtschaffen, Dates, Tobias, & Whitehead, 1969).

A preliminary analysis of the data from these eight Ss indicated that there were no group differences in SS but that the difference in percent PS (PS expressed as a percent of total sleep obtained) during the first 6 h of recording only marginally missed significance. Because the samples were small, the experiment was repeated with eight new Ss using identical experimental procedures. The results of the replication experiment were very similar to the results of the original experiment. Consequently, the data from the two experiments were combined. One of the control Ss in the replication experiment died of an overdose of ether, so all statistical analyses were based on eight experimentals and seven controls.

RESULTS

As Table 1 indicates, there was a tendency for stressed animals to have more SS than controls during the early part of the recovery period, but this difference did not approach significance. The tendency for more SS in the stressed animals was not reflected in the values for total sleep (TS) because of an opposite tendency for PS to be reduced more in stressed animals than in controls during the first half of the recovery period.

As shown in Fig. 1, percent PS was relatively depressed in both groups during the first part of the recording period, this depression being greatest in the stress group. Analysis of variance of the 12-h recording period in 2-h blocks indicated that the overall effect due to conditions was not significant, but the trials effect was

Table 1

	First 6 Hours		Second 6 Hours	
	Experimentals	Controls	Experimentals	Controls
SS Percent	41.3	38.4	36.0	35.4
TS Percent	48.3	49.9	48.5	48.3
PS Percent	14.1	22.3	25.2	26.1

Note—SS percent = mean slow sleep percent of recording time, TS percent = mean total sleep (PS plus SS) percent of recording time, PS percent = paradoxical sleep percent of TS.

*This study was supported in part by Grant MH-4151 and by Research Scientist Award MH-K3-18,428 from the National Institute of Mental Health, U.S. Public Health Service to Allan Rechtschaffen.

†Requests for reprints should be addressed to Allan Rechtschaffen, University of Chicago Sleep Laboratory, 5741 South Drexel Ave., Chicago, Ill. 60637.

Fig. 1. Percentage of total sleep time spent in PS during consecutive 2-h blocks following stress and control procedures.

highly significant ($p < .001$) and the Trials by Conditions interaction effect was moderately significant ($p < .05$). The implication of the significant interaction term, that the suppression of PS was greater in the stress group during part of the recording period, was confirmed by the finding of a significant difference between groups in percent PS in the first 2-h block ($p < .05$) and by the finding of a greater latency from beginning of recording to the first PS episode in stressed Ss (59.8 min) than in controls (36.2 min; $p < .06$).

DISCUSSION

The observed tendency of stressed rats to have more SS than controls was not significant, but in view of the stress-induced enhancement of SS in neonatal humans (Emde et al, 1970) and cats (Hobson, 1968b), this lack of statistical significance should not close the book on the possible soporific effects of stress on sleep.

The significantly greater reduction in PS in stressed rats compared to controls is consistent with the finding of reduced PS by Matsumoto, Nishisho, Suto, Sadahiro, & Miyoshi (1968) following exercise in the rat and by Hobson (1968a) following exercise in the cat. Decreases in PS were not found by Emde et al (1970) following circumcision in neonatal humans or by Hobson (1968b) following hyperthermia in cats. The rats in the present study were observed to struggle for long periods when restrained, and exercise may have

contributed to the reduction in PS.

Although the suppression of PS in the early part of the recording period was greater in stressed animals than in controls, PS was suppressed in both groups in the early part of the recording period. This suppression of PS is possibly attributable to the novelty of the presleep experimental and control procedures. As such, the suppression may be analogous to the "first night effect" in humans (Rechtschaffen & Verdone, 1964; Agnew, Webb, & Williams, 1966), whereby PS, more commonly called "rapid-eye-movement sleep" in humans, is suppressed in the first night of laboratory sleep. The differentially greater suppression of PS in the stressed rats suggests an exaggeration of the phenomenon in this group.

To evaluate whether the observed effects of stress on sleep might have been mediated by stress-induced changes in biogenic amines, 20 new rats, age-matched to those in the sleep study, were divided into experimental and control groups and subjected to stress and control procedures identical to the sleep experiment. Immediately afterwards, they were decapitated and their brains assayed¹ by the method of Jonssen & Lewander (1970). Despite the use of an assay method known to be highly sensitive to levels of 5HT and 5HIAA, we found no differences in whole brain levels of these compounds. The discrepancy between our assay results and previously reported data is not unique, since

other investigators have also failed to observe changes in 5HT and 5HIAA following stress treatment (Tagliamonte, Tagliamonte, Perez-Cruet, Stern, & Gessa, 1971). Curzon and Green reported that their 5-h restraint procedure produced a 70% decrease in brain 5HT level. This difference in results might be attributable to procedural differences. Curzon and Green placed the rats' legs through wire mesh and taped them underneath, whereas we taped the legs underneath a pipe to avoid the deep abrasions and loss of blood which we found to be produced by the rats' struggling against the wire mesh. This might suggest that our stress procedure was less stressful than theirs and, in turn, that perhaps it was not stressful enough to produce the enhancement of SS observed in other species. However, the struggling and squealing observed in our restrained animals indicates that the procedure definitely was stressful for them. Certainly, the effects on PS indicate that our restraint was not an innocuous procedure.

The present findings of no change in TS or SS consequent to stress and the findings of others that stress produces an increase in SS contrast with anecdotal reports of stress-induced insomnia in adult humans. This contrast suggests a lack of correspondence between the operational definitions of stress used in the physiological research literature and the experience which leads to insomnia in humans. Perhaps it is only the prolonged "worry" associated with stress that produces insomnia in humans rather than the stress experience per se.

REFERENCES

- AGNEW, H. W., WEBB, W. B., & WILLIAMS, R. L. The first night effect: An EEG study of sleep. *Psychophysiology*, 1966, 2, 263-266.
- CURZON, G., & GREEN, A. R. Effects of immobilization on rat liver tryptophan pyrrolase and brain 5-hydroxytryptamine metabolism. *British Journal of Pharmacology*, 1969, 37, 689-697.
- EMDE, R. M., HAMON, R. J., METCALF, D. R., KOENIG, K. L., & WAGONFELD, S. Effects of stress on neonatal sleep. *Psychophysiology*, 1970, 7, 340.
- HOBSON, J. A. Sleep after exercise. *Science*, 1968a, 162, 1503-1505.
- HOBSON, J. A. Sleep as a response. II. Temperature as a possible agent of fatigue-induced somnolence. *Psychophysiology*, 1968b, 5, 225.
- JONSSON, J., & LEWANDER, T. A method for the simultaneous determination of 5-hydroxy-3-indole-acetic acid (5HIAA) and 5-hydroxytryptamine (5HT) in brain tissue and cerebrospinal fluid. *Acta Physiologica Scandinavica*, 1970, 78, 43-51.
- JOUVET, M. Biogenic amines and the states of sleep. *Science*, 1969, 163, 32-41.
- MATSUMOTO, J., NISHISHO, J., SUTO, J., SADAHIRO, J., & MIYOSHI, M. Influence of fatigue on sleep. *Nature*,

- 1968, 218, 177-178.
- RECHTSCHAFFEN, A., DATES, R., TOBIAS, M., & WHITEHEAD, W. E. The effect of lights-off stimulation on the distribution of paradoxical sleep in the rat. *Communications in Behavioral Biology*, 1969, 3, 93-99.
- RECHTSCHAFFEN, A., LEDECKY-JANECEK, S., & LOVELL, R. The effects of 5-hydroxyindoleacetic acid (5HIAA) on sleep in the cat. *Psychophysiology*, 1968, 5, 211.
- RECHTSCHAFFEN, A., & VERDONE, P. Amount of dreaming: Effect of incentive, adaptation to laboratory and individual differences. *Perceptual & Motor Skills*, 1964, 19, 947-958.
- TAGLIAMONTE, A., TAGLIAMONTE, P., PEREZ-CRUET, J., STERN, S., & GESSA, G. L. Effect of psychotropic drugs on tryptophan concentration in the rat brain. *Journal of Pharmacology & Experimental Therapeutics*, 1971, 177, 475-480.

NOTE

1. Brain assays were performed by Dr. William O. Boggan and Barbara Collins of the Department of Psychiatry, University of Chicago.