Sleep
12(1):60–67, Raven Press, Ltd., New York

Sleep Deprivation in the Rat: IX. Recovery

Carol A. Everson, Marcia A. Gilliland, Clete A. Kushida, June J. Pilcher, *Victor S. Fang, †·*Samuel Refetoff, Bernard M. Bergmann, and Allan Rechtschaffen

*Sleep Research Laboratory, Departments of Psychiatry and Behavioral Sciences, Departments of *Medicine and †Pediatrics, University of Chicago, Chicago, Illinois, U.S.A.*

Summary: Eight rats were subjected to total sleep deprivation, paradoxical sleep deprivation, or high amplitude sleep deprivation until they showed major deprivation-induced changes. Then they were allowed to sleep ad lib. Three rats that had shown the largest temperature declines died within two to six recovery days. During the first 15 days of ad lib sleep, surviving rats showed complete or almost complete reversal of the following deprivation-induced changes: debilitated appearance, lesions on the paws and tail, high energy expenditure, large decreases in peritoneal temperature, high plasma epinephrine and norepinephrine levels, and low thyroxine levels. The most prominent features of recovery sleep in all rats were immediate and large rebounds of paradoxical sleep to far above baseline levels, followed by lesser temporally extended rebounds. Rebounds of high amplitude non-rapid eye movement (NREM) sleep occurred only in some rats and were smaller and less immediate. **Key Words:** Sleep deprivation—Recovery from sleep deprivation—Sleep rebounds—Catecholamines—Thyroxine—Metabolism.

Previous articles in this series reported the effects of total sleep deprivation (TSD) (1), paradoxical sleep deprivation (PSD) (2), and high electroencephalogram (EEG) amplitude sleep deprivation (HS2D) (3) on rats. This study reports on the reversibility of deprivation-induced effects when sleep was permitted ad lib.

METHODS

Surgical, deprivation, and measurement procedures were described previously (4). In the current study, two PSD, one HS2D, and five TSD rats were deprived for 29, 15, 45, 19, 19, 18, 22, and 12 days respectively. Individual rats will be designated by the type and number of days of deprivation, e.g., PSD-15, TSD-19a, TSD-19b. Deprivation was continued until the rats showed characteristic deprivation-induced changes. All developed increases in energy expenditure and lesions on paws and tails. All but the TSD-18

Accepted for publication August 1988.
Address correspondence and reprint requests to Dr. A. Rechtschaffen at the Sleep Research Laboratory, 5743 S. Drexel Avenue, Chicago, IL 60637, U.S.A.

rat developed a debilitated appearance with photograph ratings of 3 or lower. In six rats, deprivation was continued until peritoneal temperature decreased to at least 1.0°C below the individual deprivation mean. Edema of the paws developed in six rats. Deprivation was halted by inactivating disk rotation, thereby permitting ad lib sleep. Results will be reported for the first 15 days of recovery. The significance of differences between successive stages of the experiment (mean of baseline days, last deprivation day, first recovery day, and mean of recovery days 2 through 15) were evaluated for epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), corticosteroids, and thyroxine. An analysis of variance for repeated measures yielded a mean square error (MSE term), which was then used to compute the critical value for the Tukey *hsd* test. Yoked control rats were recorded with the PSD-15 and TSD-19a rats.

RESULTS

Despite a small number of animals, some results emerged with a strength and uniformity that indicate reliability. Other results are more tentative. Several evaluations of recovery combined results from PSD, HS2D, and TSD rats. The combination seemed reasonable since paradoxical sleep (PS), HS2, and total sleep (TS) were all reduced in each procedure, changes during sleep deprivation were similar, and—except for sleep parameters—recovery patterns were similar, i.e., similar processes appeared to be operating in the three conditions.

Survival and pathology

Apparently, deprivation can progress beyond a point of no return, because the PSD-29, TSD-12, and TSD-22 rats died within two to six days of starting recovery. These rats had suffered the greatest temperature declines from baseline (3.3, 2.8, and 3.2°C) before recovery started. During their "recovery" periods, these rats did not sleep as much or have as many long sleep bouts as the rats that survived. When they did have occasional long sleep bouts, temperature often rose, only to fall again if sustained wakefulness followed. It was not clear whether these temperature changes were caused by sleep–wake variations or whether both reflected the waxing and waning of morbid processes.

The five rats that survived appeared healthier as recovery continued. Their mean appearance rating improved from 3.6 ± 1.4 SD on the last day of deprivation to 1.7 ± 0.6 on recovery day 15. Edema of the paws subsided within four days. Tail and paw lesions showed early scab formation on recovery day 2, well-formed scabs by day 4, and, except for one lesion on the heel of the HS2D rat, almost complete healing by day 15. Except for some residual browning, the fur appeared normal by recovery day 15. After 5 to 11 months, these rats still appeared normal.

Sleep parameters

Table 1 shows values for sleep, energy, temperature, and hormone measurements for the mean of 5 to 14 baseline days, the mean of sleep deprivation days, the last deprivation day, the first 4 h of recovery, the first day of recovery (including the first 4 h), and the mean of days 2 through 15 of recovery. Figure 1 shows sleep values on each recovery day.

All 5 rats showed immediate, large PS rebounds—3 to 11 times baseline amounts in the first 4 h of recovery and 2.5 to 8.5 times baseline amounts during the first day of

TABLE 1. *Baseline, deprivation, and recovery values for TSD, PSD, and HS2D*[a]

	Baseline, mean	Deprivation		Recovery		
		Mean	Last day	First 4 h	First day	Days 2–15
TSD (mean = 18.7 days), n = 3						
Total sleep (% total time)	53.0	6.2	4.2	75.9	61.3	59.8
	(6.1)	(2.9)	(0.6)	(7.7)	(4.0)	(2.1)
HS2 (% total time)	19.6	0.13	0.07	3.2	2.1	14.2
	(2.3)	(0.15)	(0.12)	(3.2)	(1.1)	(8.5)
PS (% total time)	5.7	0.53	0.07	54.1	32.3	10.5
	(0.6)	(0.49)	(0.12)	(11.0)	(8.8)	(1.9)
Energy expenditure (kcal/day)	79.2	155.2	158.1		76.8	60.6
	(21.9)	(50.5)	(66.0)		(30.4)	(4.9)
Temperature (°C)	37.5	37.9	36.9	37.6	37.5	37.2
	(0.6)	(0.3)	(0.6)	(0.5)	(0.2)	(0.6)
Epinephrine (pg/ml)	50.3	228.9	236.6	253.9	139.7	49.2
	(30.6)	(45.9)	(58.2)	(162.5)	(61.0)	(38.3)
Norepinephrine (pg/ml)	126.5	826.8	1210.9	702.5	597.6	203.2
	(30.4)	(221.2)	(18.1)	(166.3)	(184.3)	(30.8)
ACTH (pg/ml)	124.3	133.0	125.2	100.5	59.9	107.7
	(86.8)	(57.9)	(59.3)	(112.5)	(42.5)	(104.8)
Corticosteroids (μg/dl)	5.8	4.1	2.9	2.4	1.8	2.9
	(2.0)	(0.4)	(0.9)	(2.9)	(0.7)	(1.2)
Thyroxine (μg/dl)	3.1	1.3	0.9[b]		1.7[c]	2.2[d]
	(1.0)	(0.2)	(0.1)		(0.4)	(0.4)
PSD (15 days), n = 1						
Total sleep	56.5	38.2	33.1	84.0	71.7	68.4
HS2	15.6	6.6	1.3	11.1	19.0	19.9
PS	6.4	0.02	0.00	65.0	25.2	13.7
Energy expenditure	89.8	90.0	118.8		73.3	49.0
Temperature	38.3	37.7	35.9	38.5	38.2	38.0
HS2D (45 days), n = 1						
Total sleep	57.4	43.3	44.2	76.1	67.7	61.2
HS2	21.8	0.9	1.3	29.8	25.3	24.0
PS	8.7	3.0	2.2	27.7	22.1	11.1
Energy expenditure	74.4	97.4	110.3		78.5	56.7
Temperature	37.3	37.6	37.5	38.1	37.2	37.4

[a] Values in parentheses are standard deviations.
[b] The last samples assayed for thyroxine during deprivation were drawn on days 15 (of 19), and 17 (of 19), and 17 (of 18) days of deprivation, rather than on the last day.
[c] The earliest recovery samples available for the assay of thyroxine were actually for the second or third day of recovery, rather than for the first day, as indicated by the column heading.
[d] The values are actually for days 4–15. The intent was to separate thyroxine levels early in recovery from thyroxine levels later in recovery.

recovery. A lesser PS rebound was sustained for the remainder of recovery. PS during the most stable portion of the baseline period, i.e., the last 5 days, was compared with PS after the large initial PS rebounds had subsided, i.e., during the last 10 recovery days; the baseline mean was 6.56 ± 1.25% of total time versus 10.39 ± 1.89% for recovery [$t(8) = 3.77$, $p < 0.01$]. The mean of the last 10 recovery days was also higher than the parallel means for the TSC-19a rat (6.92%), the PSC-15 rat (8.32%), and the baseline (8.0%) of 7 rats recorded in cages without disks and scored by the same methods (5). Evidently, chronic PSD, whether it is relatively selective or combined with deprivation of other stages, results in a large initial rebound followed by a lesser rebound that persists for a combined duration of at least 15 days.

During the first 4 h and the first day of recovery, all deprived rats showed lower than

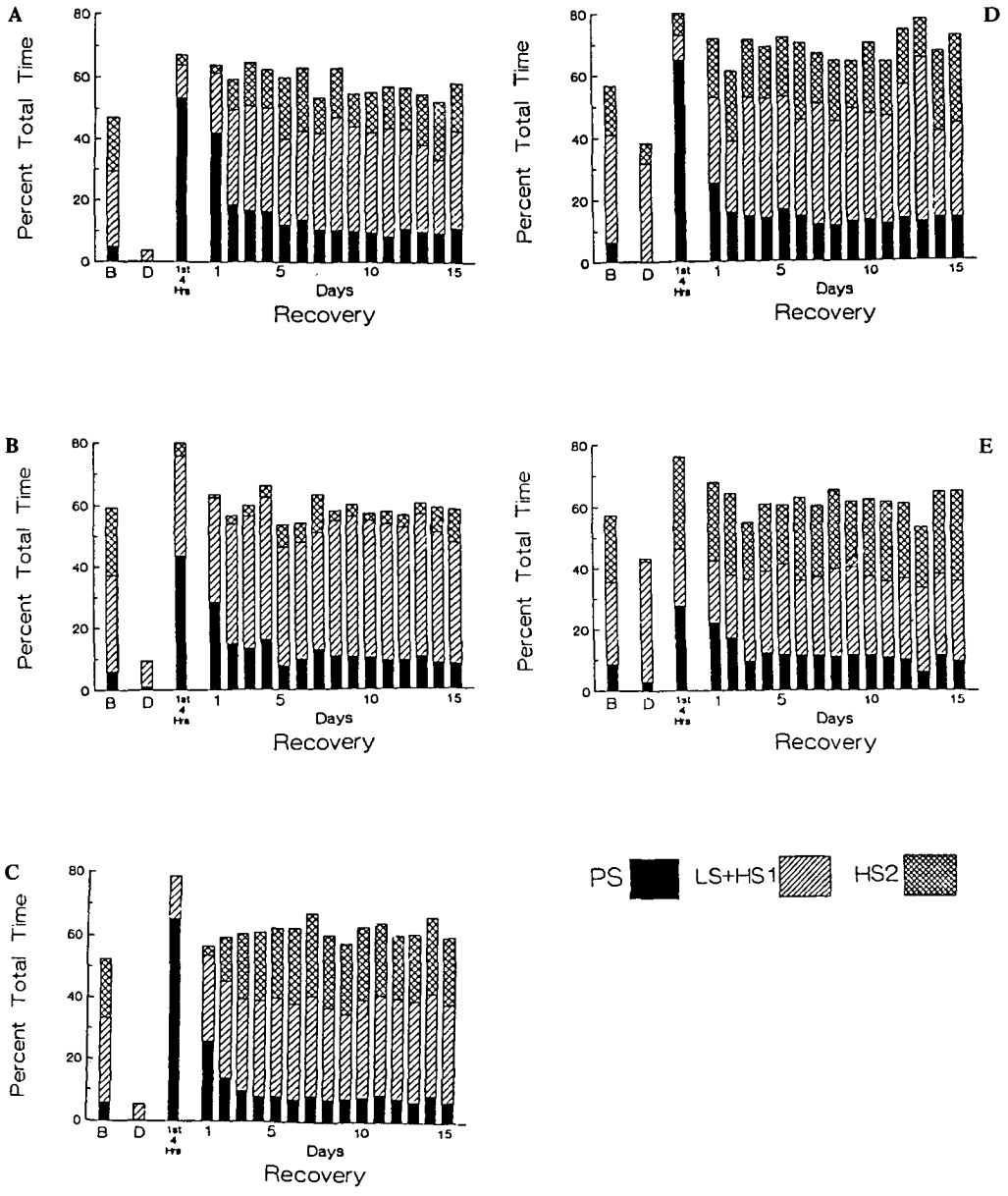

FIG. 1. Sleep stage values as percentage of total time for the mean of baseline days (B), the mean of deprivation days (D), the first 4 h of recovery, and each of the first 15 recovery days. NREM sleep is separated into two components: high EEG amplitude (HS2); low (LS) and moderate (HS1) EEG amplitude combined. A: TSD—19 days. B: TSD—19 days. C: TSD—18 days. D: PSD—15 days. E: HS2D—45 days.

baseline NREM amounts—at least in part the result of the large amount of time occupied by PS. Except for the TSD-19b rat, subsequent NREM sleep was modestly higher than baseline levels on most recovery days (Fig. 1). However, mean NREM% of all 5 rats during the last 10 days of recovery (51.27 ± 3.89%) was not significantly higher than during baseline (48.63 ± 3.90%) (t = 1.07, NS). Therefore, although all 5 rats had

lost NREM sleep for extended periods, there was no firm evidence of a reliable early or late NREM rebound.

As shown in Fig. 1, none of the deprived rats showed a very substantial HS2 rebound. All three TSD rats showed below baseline amounts of HS2 for at least the first two days of recovery. In the TSD-19a rat, HS2 subsequently recovered to near baseline levels. In the TSD-19b rat, recovery was characterized by an apparently chronic inability to generate high-amplitude waves. In the TSD-18 rat, subsequent recovery showed a modest, sustained increase in HS2. Only the HS2D rat showed an early as well as sustained HS2 rebound. Nevertheless, even these rebounds were relatively modest. In the first two days of recovery, the percentage and absolute HS2 rebound was smaller than the PS rebound. Data from additional chronically deprived animals are needed to assess the consistency of HS2 and NREM rebounds.

During the first 4 h and first day of recovery, all deprived rats showed an increase in TS that obviously resulted from the large PS rebound (Fig. 1). A TS rebound was sustained later in recovery. Mean TS% during the last 10 days of recovery (61.66 ± 4.35) was significantly higher than during the last five days of baseline (55.19 ± 4.37) ($t = 2.35$, $p < 0.05$). On most recovery days, increased PS accounted for more than half of the TS increase.

Energy expenditure and temperature

Daily EE (calculated from the caloric values of food intake, weight change, and wastes) showed increases during deprivation (Fig. 2) as described previously (1–3). The more gradual rise in the PSD rat was consistent with the earlier reports. All deprived rats showed lower EE on the first recovery day ($\bar{x} = 76.4 \pm 21.6$ kcal) than on the last deprivation day ($\bar{x} = 140.7 \pm 52.5$ kcal) ($t = 2.53$, $p < 0.05$); EE returned to baseline levels or below within one to three days. For the remainder of recovery, EE either remained near baseline levels or, in rats with relatively high baseline levels, remained mostly below those levels. All the rats ate less during recovery than during deprivation, but, in contrast to their weight loss during deprivation, they progressively gained weight.

Only two of the surviving rats had been deprived to the point of large temperature declines. The TSD-19a rat declined from a deprivation mean of 37.6°C to 36.4°C on the last day of deprivation. During the first 4 h of recovery, temperature rose to 37.8°C. The PSD rat declined from a deprivation mean of 37.7°C to 35.9°C on the last day of deprivation. During the first 4 h of recovery, temperature rose to 38.5°C. It is not clear whether the temperature increases during recovery resulted from a specific thermoregulatory effect of PS (which comprised most of sleep during the first 4 h of recovery) or from a more general reversal of morbid processes.

Hormones

Plasma hormone changes were evaluated only for TSD rats. Because of limited blood sample volume, the hormones were evaluated on a rotating basis at intervals of two to nine days. Catecholamines and corticosteroids were measured more frequently than thyroxine and at 4 to 12 h intervals during the first 2 days of recovery. ACTH showed no systematic changes across baseline, deprivation, and recovery (Table 1). Corticosteroids declined (nonsignificantly) from baseline and showed no recovery trends.

As in the earlier TSD study (6), epinephrine levels increased during deprivation (MSE = 514.4; $p < 0.01$). There was a significant decrease ($p < 0.01$) during the first

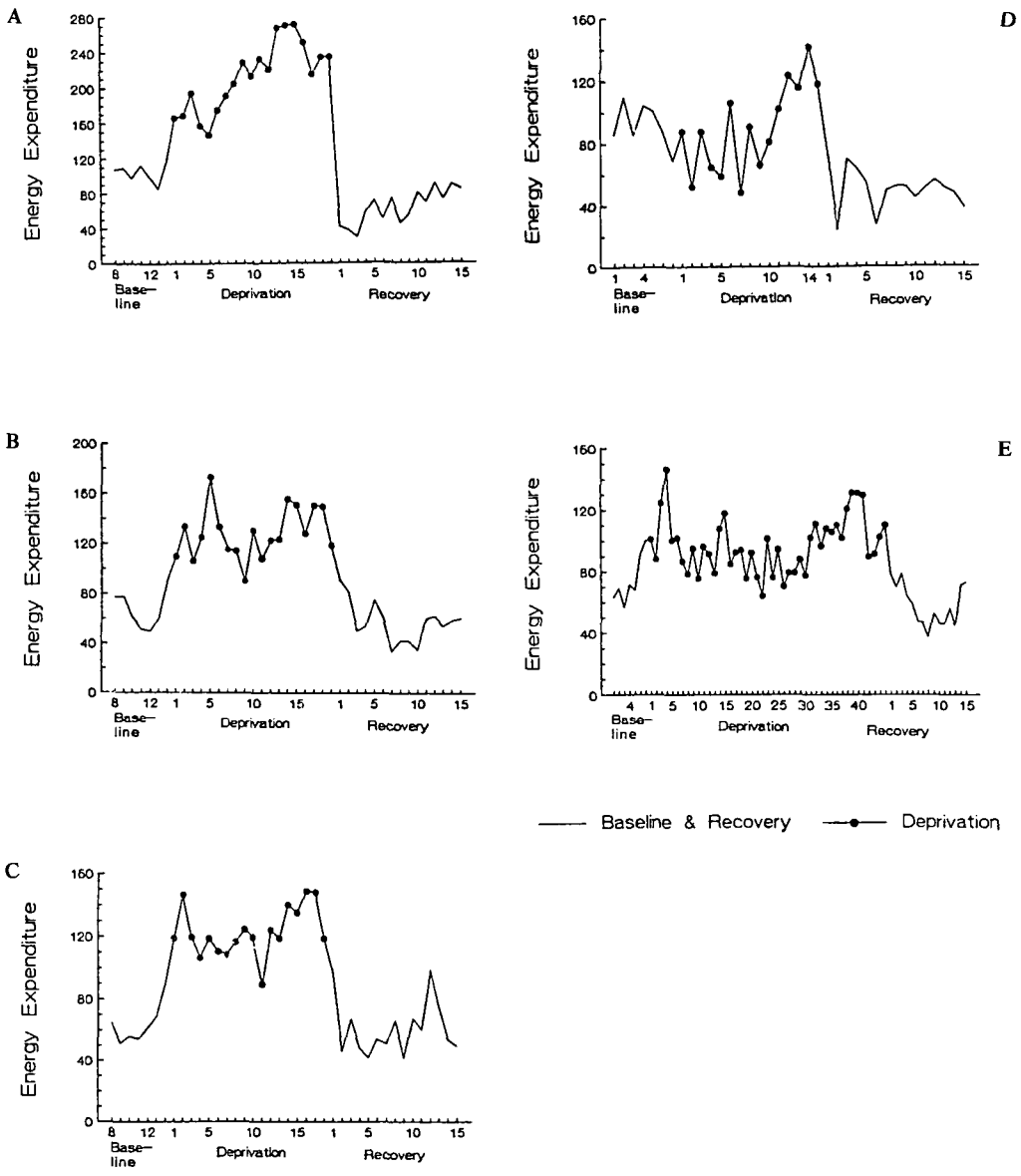

FIG. 2. Energy expenditure in kcal/day for each of the last seven baseline days (———), all deprivation days (●——●), and each of the first 15 recovery days (———). A: TSD—19 days. B: TSD—19 days. C: TSD—18 days. D: PSD—15 days. E: HS2D—45 days.

day of recovery. Mean epinephrine level decreased significantly from the last deprivation day to the baseline level during recovery days 2 through 15 ($p < 0.01$). As in the earlier TSD study (6), norepinephrine also increased during deprivation (MSE = 15,434.9; $p < 0.01$). Levels declined significantly during the first recovery day ($p < 0.01$) and then significantly further ($p < 0.05$) to approximately the baseline mean during recovery days 2 through 15. Epinephrine and norepinephrine assays for recovery days 23 or 24 all showed near baseline levels in all three rats.

As in the earlier TSD study (6), thyroxine had decreased by the end of deprivation (MSE = 0.21; $p < 0.01$). Plasma samples taken on recovery days 2 or 3 showed higher thyroxine levels than samples taken late in deprivation ($p < 0.05$). Thyroxine levels increased further during recovery days 4 through 15 to levels significantly higher than late in deprivation ($p < 0.05$). For TSD rat 19a, thyroxine also was assayed for recovery days 17, 26, and 43; values were 4.02, 4.89, and 4.53 μg/dl—all near the baseline mean of 4.22.

DISCUSSION

Earlier studies in this series had shown the following deprivation-induced effects: debilitated appearance, lesions on the paws and tails, increased EE, increased plasma epinephrine (in TSD rats) and norepinephrine, and decreased plasma thyroxine. All these changes were evident in the rats of the present study. In rats which survived when sleep was permitted ad lib, all these changes were reversed and baseline or near baseline levels were restored within 15 days. In rats that showed large temperature declines during deprivation, they too were reversed. None of the rats previously subjected to unrelenting total or selective sleep deprivation had ever shown a sustained spontaneous reversal of deprivation-related effects. Therefore, the reversal of effects in the present study is attributable to recovery sleep. Apparently, sleep serves or permits generative processes; i.e., progressive effects were not simply halted, they were reversed.

A surprising result was the high priority, in sequence and amount, for PS rebound and an absence of an early HS2 rebound in TSD rats. This result contrasts with the rebounds of high-amplitude, slow-wave sleep—sometimes concomitant with PS rebounds—in studies with shorter deprivation durations (5,7–15), although one study of 72 h of TSD in the cat (16) did show a strong PS rebound priority. Perhaps the pressure for PS does not become greater than the pressure for NREM stages until PS loss has been severe and prolonged. The HS2D rat, which suffered a smaller PS loss during deprivation than the other rats, showed a smaller PS rebound combined with an early HS2 rebound.

Acknowledgment: This work was supported by National Institute of Mental Health grants MH4151 and MH18428 and by National Institutes of Health grant DK 17050.

REFERENCES

1. Everson CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: III. Total sleep deprivation. *Sleep* 1989;12(1):13–21.
2. Kushida CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: IV. Paradoxical sleep deprivation. *Sleep* 1989;12(1):22–30.
3. Gilliland MA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: VIII. High EEG amplitude sleep. *Sleep* 1989;12(1):53–9.
4. Bergmann BM, Kushida CA, Everson CA, Gilliland MA, Obermeyer W, Rechtschaffen A. Sleep deprivation in the rat: II. Methodology. *Sleep* 1989;12(1):5–12.
5. Mistlberger RE, Bergmann BM, Waldenar W, Rechtschaffen A. Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 1983;6(3):217–33.
6. Bergmann BM, Everson CA, Kushida CA, Fang VS, Leitch CA, Schoeller DA, Refetoff S, Rechtschaffen A. Sleep deprivation in the rat: V. Energy use and mediation. *Sleep* 1989;12(1):000–000.
7. Berger RJ, Oswald I. Effects of sleep deprivation on behaviour, subsequent sleep, and dreaming. *J Ment Sci* 1962;108:457–65.
8. Williams HL, Hammack J, Daly R, Dement WC, Lubin A. Responses to auditory stimulation, sleep loss and the EEG stages of sleep. *Electroencephalogr Clin Neurophysiol* 1964;16:269–79.

9. Gulevich G, Dement W, Johnson L. Psychiatric and EEG observation on a case of prolonged (264 hours) wakefulness. *Arch Gen Psychiatry* 1966;2:29–35.
10. Vimont-Vicary P, Jouvet-Mounier D, Delorme F. Effets EEG et comportementaux des privations de sommeil paradoxal chez le chat. *Electroencephalogr Clin Neurophysiol* 1966;20:439–49.
11. Kales A, Tan T-L, Kollar EJ, Naitoh P, Preston TA, Malmstrom EJ. Sleep patterns following 205 h of sleep deprivation. *Psychosom Med* 1970;32(2):189–200.
12. Ursin R. Differential effect of sleep deprivation on the two slow wave sleep stages in the cat. *Acta Physiol Scand* 1971;83:352–61.
13. Takahashi Y, Ebihara S, Nakamura Y, Takahashi K. Temporal distributions of delta wave sleep and REM sleep during recovery sleep after 12 h forced wakefulness in dogs; similarity to human sleep. *Neurosci Lett* 1978;10:329–34.
14. Nakazawa Y, Kotorii M, Ohshima M, Kotorii T, Hasuzawa H. Changes in sleep pattern after sleep deprivation. *Folia Psychiatr Neurol Jpn* 1978;32(1):85–93.
15. Borbély AA, Neuhaus HU. Sleep-deprivation: effects on sleep and EEG in the rat. *J Comp Physiol* 1979;133:71–87.
16. Kiyono S, Kawamoto T, Sakakiera H, Iwama K. Effects of sleep deprivation upon the paradoxical phase of sleep in cats. *Electroencephalogr Clin Neurophysiol* 1965;19:34–40.