Sleep Deprivation in the Rat: IV. Paradoxical Sleep Deprivation

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Summary: Twelve rats were subjected to paradoxical sleep deprivation (PSD) by the disk apparatus. All PSD rats died or were sacrificed when death seemed imminent within 16–54 days. No anatomical cause of death was identified. All PSD rats showed a debilitated appearance, lesions on their tails and paws, and weight loss in spite of increased food intake. Their yoked control (PSC) rats remained healthy. Since dehydration was ruled out and several measures indicated normal or accelerated use of nutrients, the food-weight changes in PSD rats were attributed to increased energy expenditure (EE). The measurement of EE, based upon caloric value of food, weight, and wastes, indicated that all PSD rats increased EE, with mean levels reaching more than twice baseline values. All of these changes had been observed in rats deprived totally of sleep; the major difference was that they developed more slowly in PSD rats. Key Words: Paradoxical sleep deprivation—Debilitation—Skin lesions—Food and weight—Energy expenditure.

Dement (1) first reported rebounds of paradoxical sleep (PS) following its selective deprivation, thereby indicating that it had a special functional significance. The first total sleep deprivation (TSD) study in our laboratory that used the disk apparatus (2) reported that, although deprived rats had very little PS overall (x = 0.2% of total time), deprived rats with the most PS survived the longest (r = 0.94). This result suggested a vital role for PS and prompted the present study of selective PS deprivation with the disk apparatus. Another impetus was our pilot data, which indicated a strong PS rebound priority following prolonged TSD.

METHOD

The procedures and nomenclature were described in Part II in this series (3). This report will generally parallel the report on TSD rats in Part III (4). Of 12 PSD-PSC pairs studied, eight had intracardiac cannulas.
RESULTS AND DISCUSSION

Sleep data

In the 12 paradoxical sleep deprivation (PSD) rats, mean baseline sleep quotas, as a percentage of total time, were as follows: total sleep (TS) 53.4 ± 5.5 SD; PS 5.4 ± 0.9; non-rapid eye movement (NREM) 48.0 ± 5.0; high EEG amplitude sleep, the fraction of which has EEG amplitude below the HS modal EEG amplitude (HS1) 19.6 ± 1.2; the fraction of HS with EEG amplitude above the HS mode (HS2) 21.8 ± 3.1; low-amplitude sleep (LS) 6.7 ± 3.7. Percentages of baseline values obtained during deprivation were the following: TS 81.8 ± 12.4; PS 0.8 ± 0.4; NREM 91.1 ± 13.7; HS1 140.5 ± 24.7; HS2 42.6 ± 16.9; LS 126.2 ± 98.0. Thus, PS loss was almost complete, while NREM was well preserved. Within NREM, there was a shift from HS2 to HS1, an observation we will return to later. To maintain PS deprivation required disk rotation for only 5.45 ± 1.86% of total time. Means and standard deviations of percent rotation times for successive quarters of the deprivation period were 4.8 ± 3.8, 7.0 ± 3.2, 6.4 ± 3.7, and 5.0 ± 3.2. After the first 1–2 weeks of deprivation, sleep onset PS periods were very frequent. For the yoked controls of PSD (PSC) rats, baseline values were the following: TS 58.1 ± 3.1; PS 6.2 ± 1.0; NREM 51.9 ± 2.8; HS1 20.4 ± 2.0; HS2 23.2 ± 2.4; LS 8.4 ± 2.4. Percentages of baseline obtained during the experimental period were as follows: TS 99.6 ± 7.0; PS 97.3 ± 13.4; NREM 99.7 ± 6.9; HS1 106.1 ± 14.7; HS2 92.1 ± 11.9; LS 116.4 ± 33.1. Thus, all sleep stages were well preserved in PSC rats.

Survival results

All four noncannulated PSD rats, which were studied first, were deprived of PS until they died in order to document the lethal effect. Deaths occurred after 33, 27, 43, and 54 days. The eight cannulated rats were sacrificed when death appeared imminent (after 16, 32, 33, 36, 37, 39, 40, and 49 days) to obtain viable tissue samples. Mean survival for the 12 PSD rats was 36.6 ± 9.9 days.

At termination, the rats that died had a mean appearance rating of 5.6 ± 0.4. Appearance ratings prior to death available for five of the eight sacrificed rats averaged 5.6 ± 0.8. Of the four rats that died, temperature measures were available for three and showed declines of at least 15 SDs below their individual baseline values during the 24 h before death. Within 24 h of sacrifice, rats showed temperature declines of at least 4 SDs below their individual baseline values. Since death could occur shortly after temperature started to decline rapidly, we did not wait for the maximal temperature decline before sacrifice. Rats that were allowed to die showed a mean preterminal decline of 56.4 ± 8.8% from peak food intake; for sacrificed rats, the mean decline was 69.8 ± 25.4%. Of the rats that died, three showed severe and one moderate edema of the paws; of the sacrificed rats, edema was rated severe in three, moderate in three, and mild in two. All PSD rats showed motor weakness and/or ataxia. All of the rats that died and five of those sacrificed showed a notable decline in EEG amplitude. Preterminal signs in PSD rats generally resembled those of total sleep deprivation (TSD) rats (2). No PSC rat ever showed any of the above signs or indication that it could not have continued to live under the experimental conditions.

Appearance

All PSC rats appeared healthy throughout. PSD rats looked healthy during the first quarter and then looked progressively more debilitated (Fig. 1). The group × time
interaction ($F_{1,274} = 194.7$) was significant at $p < 0.001$. Rats in advanced PSD looked scrawny. Ulcerative and keratotic lesions appeared on the tails and plantar surfaces. The fur turned brownish yellow and aggregated into spiky clumps that resembled porcupine quills. The fur on the entire ventral surface and near the whiskers and rims of the eyes and ears came to appear dirty and brown. Small, brown, unidentified granules were scattered throughout the fur. Irregular 3–5-cm diameter patches of fur loss occasionally appeared on the back. The appearances of rats in advanced PSD and TSD were generally indistinguishable.

It is unlikely that the debilitated appearance of PSD rats resulted from a failure to groom. Based on extrapolations from videotapes taken at regular intervals during the experimental period, two PSD rats were estimated to groom 2.96 and 4.05 h daily compared to 3.16 and 3.96 h for their respective controls. Whether grooming in PSD rats was deficient in some other respect could not be determined. As noted previously (4), the appearance changes in sleep-deprived rats are not intrinsic to terminal processes, since food-deprived rats did not show any of these changes before they died.

**Necropsy and histology**

With only incidental exceptions, the internal organs of all PSD rats appeared normal. All 12 PSD rats showed a virtual absence of observable body fat, a reduction of connective tissues to thin membranes, enlarged adrenal glands, and pinpoint erosions of the stomach lining, but none showed large, hemorrhaging ulcers like those reported in stressed rats (e.g., refs. 5 and 6) or observed by us in food-deprived rats. Otherwise, we observed no anatomical changes that were uniform across PSD rats or sufficiently severe to explain their imminent or actual deaths. Other abnormalities seen in some PSD rats generally resembled those reported for TSD rats (4).

Organ weight data will be reported only for the eight runs in which PSD rats were sacrificed; in the rats that died, organ weights could be affected by changes related to death. There were no significant differences between PSD and PSC rats in the weights of spleen, liver, heart, or brain. Kidneys were significantly ($p < 0.05$) heavier in PSD rats ($\bar{x} = 1.73 \pm 0.16$ g) than in PSC rats ($\bar{x} = 1.46 \pm 0.14$ g). Lungs were significantly ($p < 0.05$) heavier in PSD rats ($\bar{x} = 2.73 \pm 1.49$ g) than in PSC rats ($\bar{x} = 1.70 \pm 0.23$ g). Adrenal weights (mean of both adrenals) were significantly ($p < 0.001$) heavier in PSD rats ($\bar{x} = 42.4 \pm 7.9$ mg) than in PSC rats ($\bar{x} = 28.5 \pm 5.8$ mg). The increased adrenal weights in PSD rats may have resulted partly from near-terminal stress. Four
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PSD rats, not included with previous results, were sacrificed at 15, 17, 19, and 21 days, after they showed definite deprivation effects (increased food intake, weight loss, debilitated appearance, foot and tail lesions, increased heart rate), but well before death seemed imminent. These four rats had mean adrenal weights of $32.5 \pm 4.2 \text{ mg}$ compared to $26.6 \pm 6.0 \text{ mg}$ for their yoked controls and $42.5 \pm 7.9 \text{ mg}$ for the sacrificed rats. Evidently, the appearance of deprivation signs was not contingent upon greatly enlarged adrenal glands.

Tissue samples from the following organs and glands of seven PSD-PSC pairs were preserved in 10% formalin and then embedded in paraffin, sectioned at seven intervals, stained with hematoxylin and eosin, mounted, and examined without knowledge of experimental condition by a pathologist (Dr. Lester Wold of the Mayo Clinic); spleen, liver, stomach, duodenum, small intestine, mesenteric nodes, adrenals, kidneys, heart, thymus, lungs, thyroid, and parathyroid were examined. As in TSD rats (4), no important or consistent abnormalities were found in PSD or PSC rats. In spite of the damp environment and occasional immersion in the water, none of the rats showed evidence of pneumococcal infection.

**Ion balance, hematology and urine measures**

Plasma potassium and sodium levels, evaluated weekly in eight runs, remained near baseline levels for PSD and PSC rats. Hematologic parameters were evaluated bi-weekly in six runs. White cell counts remained near baseline for PSC rats (unlike TSC rats, which showed increases). As in TSD rats, white cell counts rose progressively in PSD rats; they reached a fourth quarter mean that was 75% above baseline ($17.31 \times 10^3/\text{ul}$), though still within normal limits for rats (7). The group × time interaction ($F_{1,75} = 17.2$) was significant at $p < 0.001$. Differential counts in two pairs indicated that lymphocytes, eosinophils, and basophils were within normal ranges, but polymorphonuclear neutrophils increased 87% from baseline levels in PSD rats. Unlike TSD rats, which showed signs of anemia (4), mean red cell counts in PSD rats remained within 2% of baseline. PSC rats also remained near baseline. Neither were there significant interactions, notable group differences, or changes from baseline in hemoglobin, hematocrit, or reticulocyte count. There was a significant ($p < 0.02$) group × time interaction in mean cell hemoglobin that resulted mostly from an unexplained rise in the fourth quarter mean of PSC rats; the maximal change from baseline was a clinically insignificant decrease of only 5% in both PSD and PSC rats. Mean cell volume also showed a significant ($p < 0.001$) group × time interaction, which resulted more from desynchronization of the groups over time than from systematic group differences or systematic changes over time; maximal deviations from baseline were clinically insignificant changes of about 3%.

Urinates were evaluated every second day in 11 runs. During their last four days, PSD rats showed lowered pH, suggestive of metabolic acidosis, and large amounts of blood, indicating urinary tract damage. There were no clinically meaningful deviations from baselines or significant group × time interactions in urobilinogen, bilirubin, glucose, ketone, specific gravity, or protein.

**Food, weight, and energy expenditure**

All PSD rats increased food intake (Fig. 2). The group × time interaction ($F_{1,726} = 56.0$) was significant at $p < 0.001$. All PSD rats progressively lost weight (Fig. 3). The group × time interaction ($F_{1,726} = 198.6$) was significant at $p < 0.001$. Mean percentage
weight loss between baseline and the last PSD day was 21.6 ± 11.3% versus a mean weight gain of 0.7 ± 8.6% in PSC rats. For PSC rats, mean food intake and weight remained slightly above baseline throughout. (Two early runs in which weight was not measured on a daily basis were excluded from the above analyses.) Weight loss alone could not account for the deaths of PSD rats, since food-deprived rats survived much greater weight losses (4).

Food and weight changes were similar for PSD rats and the TSD rats previously reported (2). The major difference was that, considering that survival was almost twice as long in PSD rats, the changes occurred more rapidly in chronological time in TSD rats. Therefore, NREM loss, of itself or in combination with PS loss, must have a functional significance similar in some respects to PS loss. PSC rats showed small weight gains, whereas TSC rats had shown substantial weight losses. The difference is most likely due to the loss of 27.6% of baseline NREM and 44.6% of baseline PS in TSC rats, whereas PSC rats suffered almost no loss from baseline sleep.

PSC rats remained near baseline in water intake (from bottles), whereas PSD rats increased water intake, especially during the third and fourth quarters, when it reached levels 44.9 ± 22.6% and 47.7 ± 28.1% above baseline means. The group × time interaction \( (F_{1,842} = 8.0) \) was significant at \( p < 0.005 \). Nevertheless, water intake did
not reach levels expected from the increase in food intake (8,9). Part of the discrepancy may have resulted from the undetermined amount of water the rats drank from the pan, grooming wet fur, increased metabolic water, and reduced water need because of lower body mass. In any event, the PSD rats showed no evidence that their weight loss resulted from dehydration. They showed normal urine specific gravity, normal plasma ion concentration, and, as will be reported later (10) (Part V of this series), an increase in total body water as a proportion of total body mass.

The food-weight changes in PSD rats did not appear to result from abnormalities in intermediary metabolism. For some nutrients, there were indications of accelerated use. That dietary fats were absorbed is indicated by the lack of urinary ketones and the absence of fatty stools; accelerated use was suggested by the absence of body fat at necropsy. That glucose was absorbed was indicated by the absence of hyperglycemia and urinary glucose excretion. Unlike TSD rats (4), PSD rats did not show a significantly greater increase in glucose uptake than their controls; glucose uptake was similar in PSD and PSC rats. Mean plasma levels of total protein were maintained at slightly above baseline in both PSD and PSC rats; there was no significant group × time interaction. However, accelerated protein catabolism was indicated by increased plasma urea nitrogen (Fig. 4) in PSD rats. The group × time interaction ($F_{1,58} = 4.3$) was significant at $p < 0.05$. Plasma albumin was slightly depressed from baseline during the third and fourth quarters in both PSD and PSC rats, but there were no significant group or interaction effects. Plasma globulins remained at about 5–10% above baseline in PSC rats, but rose progressively in PSD rats to a mean of 45.9 ± 23.4% above baseline levels during the fourth quarter. The group × time interaction ($F_{1,58} = 6.3$) was significant at $p < .02$. This pattern of stable albumin and increased globulins differed from the TSD pattern of relatively stable globulins and decreased albumin. In both cases, however, the shift from albumin to globulins carries some suggestion of protein deficiency (11).

Since neither dehydration nor impaired intermediary metabolism can explain the loss of weight in PSD rats in spite of their increased food intake, increased energy expenditure (EE) remained as the most likely explanation for both changes. Accordingly, daily EE was calculated from a formula that included food intake, weight change, and estimated wastes (3). In PSC rats, mean EE remained stable at about 25% above the baseline mean. In PSD rats, EE increased progressively to 136.3 ± 17.1% above base-

**FIG 4.** Mean plasma urea nitrogen for 8 PSD–PSC pairs. PSD (—) baseline = 21.0 mg/dl (SD = 4.6); PSC (---) = 21.5 mg/dl (SD = 3.6).
line during the fourth quarter (Fig. 5). The group × time interaction \( F_{1,700} = 148.8 \) was significant at \( p < 0.001 \).

As expected from high EE, heart rate was elevated in PSD rats. For waking heart rate (Fig. 6A), the group × time interaction \( F_{1,231} = 17.9 \) was significant at \( p < 0.001 \). Heart rate during HS (Fig. 6B) showed even greater percentage increases than waking heart rate; the group × time interaction \( F_{1,230} = 74.6 \) was significant at \( p < 0.001 \).

FIG. 5. Mean energy expenditure of 12 PSD (---) and 12 PSC (---) pairs. PSD baseline = 74.5 kcal/day (SD = 17.6); PSC = 76.5 kcal/day (SD = 16.4).

FIG. 6. Mean heart rate during awake (A) and during HS sleep (B) in four PSD-PSC pairs. PSD mean baseline = 387.0 beats per min (bpm) (SD = 11.5) during awake and 355.1 bpm (SD = 11.4) during HS. For PSC, parallel values were 406.9 (SD = 23.4) and 352.2 (SD = 18.6).
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Behavior

After the first week of deprivation, PSD rats became extremely sensitive to tactile stimuli—often flinching, jumping, and squealing when lightly touched, as if in great pain. They also became very aggressive, attacking objects or hands that came near them. Their viciousness prompted the purchase of steel mesh gloves, which had not been necessary during many years of research with rats.

Control studies

The negligible contribution of NREM sleep loss to changes in PSD rats is highlighted by the fact that TSC rats (4) had slightly less NREM sleep than PSD rats, but did not show comparable changes. Nevertheless, two controls were in order. Although TS in PSD rats dropped only 19.2% from baseline, in some cases the loss continued for several weeks, so the results might be partly explained by chronic partial loss of TS, rather than by PS loss. Therefore, two chronic total sleep restrictions (CTSR) rats were run on a schedule that permitted sleep for 44.2% (corresponding to mean TS of PSD rats) of each 4-h block for 54 days (corresponding to maximal survival of PSD rats). Unfulfilled quotas of each block were added to the quotas of the next block. Actual TS of the CTSR rats averaged 44.3%; sleep loss was spread across all stages. Disk rotation averaged 12.6% of total time. Food intake, weight, EE, water intake, heart rate, appearance, and temperature all stayed near baseline. There was no indication that the rats could not continue to live under those experimental conditions. Necropsy evaluation revealed no pathology, no remarkable skin lesions, and no appreciable differences between CTSR rats and their yoked controls in organ weights. The changes in PSD rats cannot be explained by chronic partial TS loss.

Since HS2 was reduced by 57.4% in PSD rats, and, as will be shown in Part VIII (12), HS2 deprivation can have pathological and lethal effects, we ran three controls in which HS2 was restricted (HS2R) (within 4-h blocks) to quotas matched to the three PSD rats with the greatest HS2 loss for periods matched to the survival of PSD rats. Actual mean HS2 was 9.3 ± 0.4% total time, comparable to the target of 9.8%. Mean TS was increased to 60.4 ± 10.2%; PS was at 6.0 ± 0.4%. Mean disk rotation was 14.2 ± 5.0%. With the exception of an unexplained 36.1% increase in mean water intake, there were no large changes from baseline or differences from yoked controls in the parameters described above for CTSR rats. HS2R rats appeared healthy in every respect. These results indicate that HS2 loss alone could not explain the changes in PSD rats. They do not rule out the possibility that moderate HS2 loss interacted with the near total PS loss to contribute to the mortality and pathology in PSD rats.

In the two PSD rats evaluated, the mean number of partial immersions in the pan water per day for the fourth quarters were 111, 178, 164, and 129; full immersions were 11, 27, 19, and 52. Mean total time (min) spent in water per day for each quarter was as follows: partial = 10.7, 33.5, 22.3, and 15.5; full = 0.7, 1.2, 0.8, and 4.7. Two immersion control (IC) rats were matched to each of these two PSD rats by the procedure described earlier (3). None of the four IC rats showed any indication of impending death before they were sacrificed. They looked healthy; mean appearance rating before sacrifice was 1.33. Necropsy evaluations revealed no remarkable abnormalities. Organ weights were comparable to those of normally caged rats. There was some yellowing of the fur in the scrotal region, but no more so than in PSC rats. Two IC rats developed a lesion on the posterior plantar surface of one hindpaw, but the lesions were morphologically different from those of PSD rats. In one case, the lesion...
healed before the end of the experiment; paw lesions in PSD rats never healed. In the other case, the lesions was bloody and suppurative and showed some scar tissue formation that was never seen in the plantar lesions of PSD rats. Obviously, water immersion was not responsible for the major changes observed in PSD rats.

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