Figure 1, a semilogarithmic plot of the inactivation kinetics of β-glucuronidase activity, shows that the enzyme of N2 is more heat labile than that of Py-1. When N2 and Py-1 decay curves were compared with those of β-glucuronidase in C3H and Swiss mouse-liver homogenates, respectively, the inactivation kinetics proved to be similar, indicating that the properties of enzyme in each of the parental cell lines reflect its mouse strain of origin. Figure 1 shows, moreover, that the activity-decay curve of the somatic hybrid M109 closely approximates that of a mixture of the two parental types in the proportion of two parts N2 to one part Py-1 (*12*).

Presence of both parental activities has also been detected in liver homogenates from F_1 mice heterozygous for the mutant and wild-type β-glucuronidase alleles (*13*). Thus the expression of alleles in the heteroploid somatic hybrid cells appears to resemble qualitatively that in liver tissue of analogous murine heterozygotes.

The fact that the inactivation kinetics of the Py-1 and N2 activities were not linear (Fig. 1) suggests the presence of more than one component in each. A similar phenomenon was observed with enzyme activities from wild-type and mutant mouse-liver homogenates (*13*). However, the nature of the multicomponent kinetics is not understood, since only one enzyme band is observed after electrophoresis of wild-type enzyme under various conditions (*14*), and the Michaelis constants of wild-type enzyme activity surviving after 0 and 12 minutes at 71°C did not differ significantly. On the other hand, the inactivation kinetics were not artifacts of the heating procedure, since enzyme that had been heated for 30 minutes, cooled, and then reheated did not exhibit the initial rapid inactivation.

Although the decay rates of each parental enzyme activity are not linear, the similarity of the M109 inactivation kinetics with those of an N2–Py-1 mixture indicates that both parental activities are coexistent in the hybrid-cell population. This finding is confirmation at the enzymic level that genetic material derived from both parental cell types is expressed in somatic hybrids obtained by cell fusion.

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14. Disc electrophoresis, with the standard 7-percent gel at *p*H 9.5, and starch-gel electrophoresis, with several buffer systems ranging widely in *p*H, have been performed on wild-type liver glucuronidase; always only one activity band was detectable.
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Sleep Deprivation in the Rat

Abstract. *Sleep deprivation, induced by injections of dextroamphetamine or by forced treadmill activity, resulted in a temporary increase in daily sleep time. However, increasing the period of sleep deprivation above 24 hours to 72 or 120 hours did not result in increased recovery sleep above that present in the 24-hour group.*

A recent book has stressed the motivational characteristics of sleep (*1*), but there is a paucity of data demonstrating that subjects respond to sleep deprivation as if sleep were a drive. Webb (*2*) found that rats deprived of sleep had shorter sleep latencies (time to fall asleep) than control animals. Kleitman (*3*) has reported an increase in sleep time in humans on the first night after prolonged deprivation of sleep. A number of studies have reported changes in the stage characteristics of sleep after deprivation (*4*); however, in these studies, sleep was recorded on only one or, at the most, two recovery nights.

In my experiments, sleep cycles were monitored for prolonged periods before and after varying periods of sleep deprivation. Two methods of producing sleep deprivation were used, injection of dextroamphetamine and a slowly revolving treadmill. Svorad and Novikova (*5*) have induced up to 7 days of sleep deprivation in rats with periodic injections of dextroamphetamine. Sleep deprivation induced by forcing rats to walk a slowly revolving water-immersed treadmill has also been reported (*2, 6*).

Twelve 110- to 120-day-old male Long-Evans rats were subjected to dextroamphetamine-induced sleep deprivation in the first experiment. In addition to recording sleep cycles, food and water intake and body weight were also measured, because of the known anorexic action of dextroamphetamine. An ultrasonic movement-sensing device capable of differentiating the sleeping and waking states in rats has been developed (*7*) and correlated with electroencephalographic (EEG) records (*8*). The correlation of the results of the two methods for differentiating sleep-waking periods is .97, and the movement-sensing system is amenable to recording prolonged sleep cycles. Although this method does not require surgery or the presence of an experimenter during recording, neither does it permit the discrimination of sleep stages; thus, total sleep time during a prolonged period is the dependent variable in these studies.

Ultrasonic sound (nonaudible) is emitted into a test area containing the rat. Movement by the animal produces disturbances in the received portion of the wave, which activates a pulse. The record is read out on an Esterline-Angus operation recorder, model AW. Scoring rules, calibration procedures, and specifications for cages and test-area enclosures have been described (*8*). Briefly, each minute is scored for activity level on a 20-point scale based on the number of pen deflections during that minute. A minute with a score of 0 to 3 denotes sleep and 4 to 20, awake. The calibrator consists of a pendulum placed in the test area. The pendulum is started by moving it a specified distance from the vertical; calibration setting is the time from starting motion until the movement-sensing device stops responding to decreasing pendular swings.

There were four rats at each of the three deprivation levels (24, 72, and 120 hours). The sleep-waking cycle was recorded for 4 days before and 8 days after the period of sleep deprivation, in addition to recording during the sleep-deprivation period itself. The

lights were on for 12 hours and off for 12 hours each day. Recordings were made daily during 20 hours divided into 10 "lights-on" and 10 "lights-off" hours. The total minutes of sleep per 10-hour period (600 min) was the measure used for statistical analysis. During the 2-hour nonrecording periods each morning and night, the movement-sensing units were tested and calibrated, and food, water, and body weight measures were taken.

Doses and routes of administration were explored in a preliminary study. The dose in this experiment was 10 mg of dextroamphetamine sulfate per kilogram of body weight. The time between injections was determined by the animal's response; when the animal first showed signs of sleep (by lying down), it received another injection. In this study there were two separate groups of six animals each (two at each level of deprivation). Replications were 6 months apart, and results of both were essentially identical; therefore, the data will be presented in grouped form only.

There are two major effects of sleep deprivation to be reported: (i) Sleep deprivation did increase sleep time above the predeprivation level (Fig. 1). However, this effect was statistically significant only during the first 4 days after deprivation (P .001). By the fifth day after deprivation sleep time was essentially at its predeprivation level. (ii) There was no difference in total compensatory sleep caused by duration of deprivation (24, 72, or 120 hours). This can be seen in Fig. 1, where there is no divergence in sleep time between the three deprivation groups. Sleep time was considerably elevated on the first day after deprivation and then gradually returned to the predeprivation level. The 24- and 120-hour deprivation groups returned to a level of total sleep within their predeprivation ranges on the seventh day after deprivation, and the 72-hour group did this on the sixth day. Statistical analyses confirmed the conclusion that the duration of deprivation failed to significantly influence the amount of compensatory sleep. There was, of course, a large difference in the percentage of the accumulated "sleep debt" that each group repaid (24-hour, 100 percent; 72-hour, 18 percent; 120-hour, 14 percent). It is as if the need for sleep became asymptotic at 24 hours of deprivation.

Rats averaged about 25 minutes of sleep per day during the deprivation phase of the experiment (compared with 811 minutes of sleep per day during the control days). This is about 3 percent of their normal daily sleep time. The sleep occurred when effect of the drug wore off and an observer failed to note this immediately and give another injection. The daily amount of sleep during the deprivation phase did not change significantly as the period of deprivation lengthened. There was a significant decrease in length of drug-induced sleep deprivation per injection over the 5 days ($P < .01$; day 1, 360 minutes; day 5, 220 minutes). This effect may be due either to drug tolerance or to an increased sleep-need produced by the increasing deprivation. Drug treatment strongly reduced food and water intake, and body weight also decreased. These measures all returned to normal shortly after deprivation ended (3 to 7 days).

In view of the findings on compensation for sleep deprivation in my first experiment, I attempted to replicate the results by use of a different method of inducing sleep deprivation. Ten 110- to 120-day-old male Long-Evans rats were deprived of sleep by being forced to walk a slowly revolving treadmill,

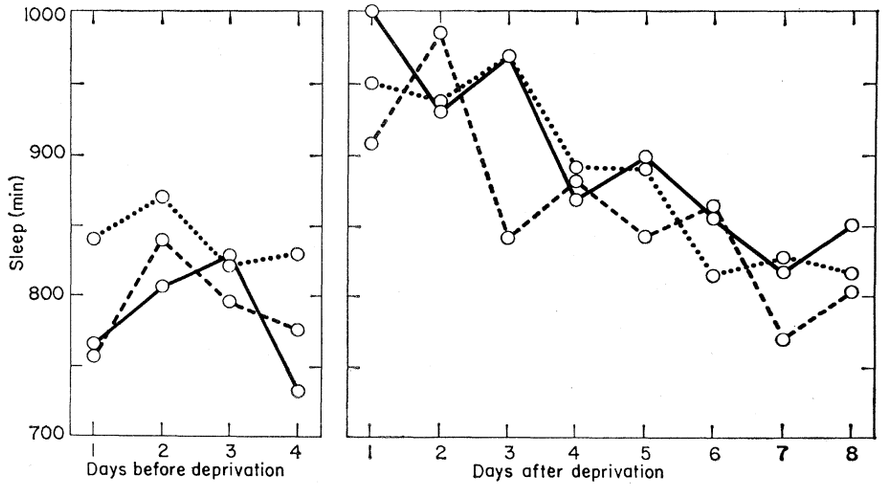

Fig. 1. Dextroamphetamine deprivation, daily sleep time (minutes of sleep out of the daily record of 1200 minutes). Solid line, 24 hours of deprivation; dotted line, 72 hours; and dashed line, 120 hours.

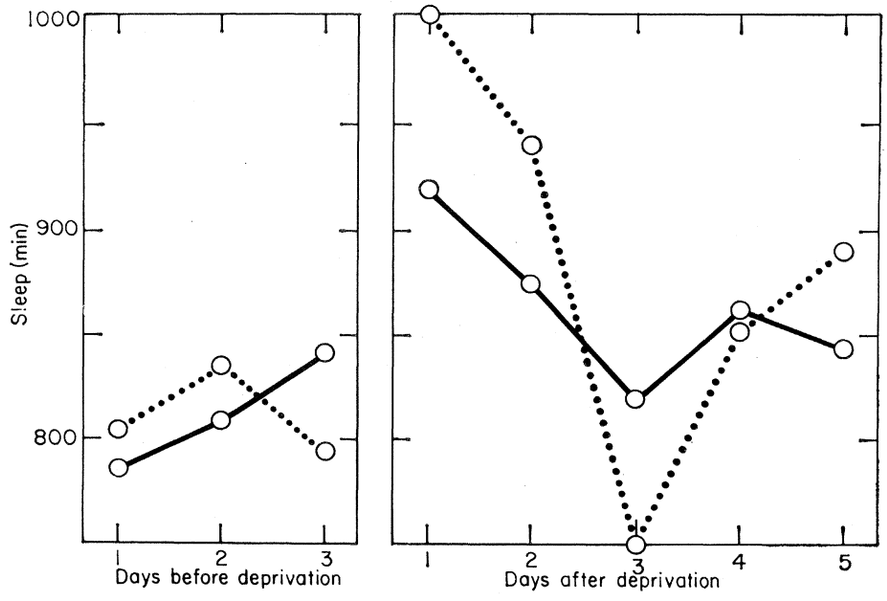

Fig. 2. Treadmill deprivation, daily sleep time (minutes of sleep out of the daily record of 1200 minutes). Solid line, 24 hours of deprivation; dotted line, 72 hours.

two-thirds submerged in water. This apparatus has been described (*2*, *6*). The rats were placed individually on separate wheels that rotated at a constant speed of 2 rev/min; the total distance traversed by an animal was approximately 0.7 mile (1.1 km) per 24-hour period. Sleep cycle was recorded with the same movement-sensing device that was used in the first experiment. Food and water were freely available in each cubicle.

Five animals were used at each of two deprivation levels (24 and 72 hours). The sleep-waking cycle was recorded for 3 days before and 5 days after the sleep-deprivation period. During the periods before and after deprivation the rats were in the same cages and enclosures that had been used in the first experiment; during the deprivation period they were on the treadmill. Deprivation on the treadmill was not maintained as long as deprivation from dextroamphetamine since previous studies have shown that animals, at the ages used, became exhausted between 72 and 120 hours on the treadmill (*6*).

The major findings of this experiment are identical with those of the first experiment. Sleep time temporarily increased after treadmill deprivation ($P < .001$ for a comparison of days 2 and 3 before deprivation with days 1 and 2 after deprivation) and had returned to normal by the end of the study (no significant difference between sleep time on days 1 to 3 before deprivation and days 3 to 5 after deprivation). Also, the duration of deprivation failed to significantly influence the amount of compensatory sleep obtained (Fig. 2). However, the amount of compensatory sleep obtained after treadmill deprivation is considerably less than that obtained after dextroamphetamine deprivation (21 percent of the debt incurred for the 24-hour group and 13 percent of the debt for the 72-hour group; see above for the comparable figures on drug deprivation).

The use of dextroamphetamine succeeded in almost completely eliminating sleep during the deprivation period. The major findings of this experiment are: (i) The drug-induced sleep deprivation resulted in a temporary compensatory increase in sleep time after discontinuing the drug; and (ii) increased sleep deprivation from 24 hours to 72 to 120 hours did not produce any significant change in the amount of compensatory sleep. These data suggest the conclusion that increasing sleep deprivation over 24 hours up to 120 hours does not result in an increased need for sleep above that present in the 24-hour sleep-deprivation group. A possible alternative to this conclusion is that, although the amount of sleep during deprivation is very small compared with the normal level, the sleep during a state of heightened need was "more valuable" in relieving that state than it is under normal drive conditions. An experiment that investigates the effect of small amounts of sleep, at various times during deprivation, should help to answer this question.

The results of treadmill-induced sleep deprivation are in agreement with results obtained with dextroamphetamine-induced deprivation. Deprivation resulted in a temporary increase in sleep, and the duration of deprivation failed to significantly influence the amount of compensatory sleep obtained.

The two major findings of this study are: (i) Sleep does act as a need state (compensation for lost sleep does occur); and (ii) increasing deprivation from 1 up to 5 days does not increase the total amount of compensatory sleep. The second finding may suggest that, within the deprivation range utilized, the sleep need became asymptotic at 24 hours and is consistent with anecdotal reports by humans that prolonged sleeplessness can be recovered from with minimal additional sleep.

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Qualitative versus Directional Cues in Two Forms of Differentiation

Abstract. *Dogs given opportunities to base their instrumental conditioned responses in differentiation learning on either the quality of the auditory conditioned stimulus (for example, metronome versus buzzer) or the direction of its source (in front or behind) choose different cues in different tasks. In* $S_1 \rightarrow R_1$, $S_2 \rightarrow R_2$ *(left leg–right leg) differentiation they exclusively use directional cues and are almost unable to learn this task when only quality cues are available. When confronted with Pavlovian* $S+ \rightarrow R$, $S- \rightarrow$ *no R (go–no go) differentiation, however, they generally learn on the basis of quality cues, although some animals also attend to the directional cues. Thus an animal's success or failure in a given differentiation procedure depends not only on its ability to discriminate the stimuli but also on the task with which it is confronted.*

Lawicka (*1*) has shown that, in a free-moving situation, success or failure of training in go left–go right differentiation or go–no go differentiation depends on the character of auditory cues used for the particular task. While for go left–go right differentiation the adequate cues are provided by auditory stimuli presented from *different directions*, for go–no go differentiation they are provided by stimuli of *different quality*. We have now further investigated the same problem, using a different technique.

We used 29 dogs in a Pavlovian soundproof conditioned-reflex (CR) chamber. An animal, placed on a stand, was given food by remote control from a feeder situated before him. An instrumental CR consisted in placing the left or right foreleg on the feeder in response to a conditioned stimulus (CS); intertrial intervals were about 1 minute.

In experiment 1, 11 dogs were trained to place their right forelegs on the feeder in response to the sound of a metronome situated in front of