bandwidths of 6 to 12 nm. This was used for photopigment bleaching prior to dark-adaptation experiments (Fig. 1), or as an adapting field in light-adaptation experiments (Fig. 2). Flickering stimuli as well as the fixation target were provided by channels with separate light-emitting diode sources. These were either red-orange (General Instruments type MV5152), yellow (General Instruments type MV5352), or green (Stanley Electric type EBG5504) with respective peak wavelengths of 630, 585, and 540 nm and with half-bandwidths of about 40 nm.

11. Three indices were used to assess sensitivity to flicker. That used in the experiments shown in Figs. 1 and 2, the illuminance of a stimulus which causes it to appear to just flicker, is inversely proportional to sensitivity. We also fixed the time-averaged illuminance of a flickering stimulus, and the observer either varied its modulation depth for threshold or varied its frequency to achieve critical flicker frequency (CFF). Modulation depth varied inversely with sensitivity, whereas an increased CFF corresponded to an increase in sensitivity.
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Physiological Correlates of Prolonged Sleep Deprivation in Rats

Abstract. *The issue of whether sleep is physiologically necessary has been unresolved because experiments that reported deleterious effects of sleep deprivation did not control for the stimuli used to prevent sleep. In this experiment, however, experimental and control rats received the same relatively mild physical stimuli, but stimulus presentations were timed to reduce sleep severely in experimental rats but not in controls. Experimental rats suffered severe pathology and death; control rats did not.*

If sleep serves an important physiological function, sleep deprivation should produce severe physiological impairment. Literature reviews (*1*) have emphasized the lack of such impairment, however. Older animal studies (*2*) that reported pathological changes or death following prolonged sleep deprivation have been either neglected or discounted for their failure to control for stimulus effects. When sleep is blocked by unrelenting, noxious stimulation, such as continuous enforced locomotion, it is unclear whether subsequent pathology is mediated by sleep loss or by other effects of the stimulation, such as stress or fatigue. Our procedure delivered the same relatively mild physical stimuli to experimental and control rats, but timed their delivery to limit sleep severely in experimental rats but not in controls. The result was severe debilitation and death in experimental but not in control rats.

A deprived rat and a yoked control rat were housed in separate clear plastic cages; a single fiber glass disk formed a partial floor for both cages (*3*). Beneath each side of the disk and extending beyond it to the walls of each cage was a tray containing water to a depth of 3 cm (Fig. 1). Whenever the disk was rotated, both rats had to walk in the direction opposite disk rotation to avoid being forced into the water.

Each rat's electroencephalogram (EEG), electromyogram (EMG), and theta activity were continuously recorded and later scored by computer for wakefulness (W), high-amplitude non-REM sleep (HS), low-amplitude non-REM sleep (LS), paradoxical sleep (PS), and total sleep (TS) (*4*). Upon recognizing sleep onset in the deprived rat (usually within 5 seconds), a microcomputer activated disk rotation at a moderate speed of 3.5 rev/min until the deprived rat had been awake for 6 seconds. (Rats did not sleep in the water.) Direction of rotation was varied randomly. Thus, both rats were subject to the same environment and disk rotation, but deprived rats could not accumulate much sleep, whereas control rats could sleep whenever deprived rats were spontaneously awake.

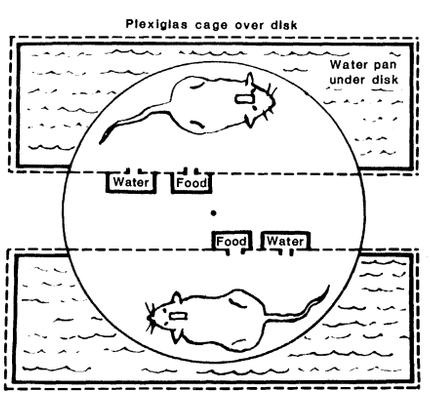

Fig. 1. Schematic diagram (top view) of the experimental apparatus.

After 4 days of stable baseline recording with the disk stationary, eight yoked pairs of age-matched Sprague-Dawley male rats, 6 to 16 months old and adapted to constant light, were run for 5.7 to 33.4 days in constant light. Food and water were freely available. Cage air temperature was held near 29°C. Criteria for disk rotation provided by EEG, EMG, and theta were occasionally varied within a small range to maximize wakefulness in deprived rats and sleep in control rats. Extreme criteria for sleep onset could theoretically produce 100 percent sleep deprivation in deprived rats, but rotations might be so frequent as to severely limit sleep in control rats as well. The procedure produced a mean of 109 rotations per hour, but the disk rotated only 23 percent of total time. We estimate that the rats were forced to walk an average of 0.9 mile a day; rats may voluntarily run 30 miles a day on a wheel (*5*).

Percentages of total recording time spent in sleep states are shown in Table 1. From baseline to experiment, TS was reduced by 87.4 percent in deprived rats and 30.6 percent in control rats. Thus, this study is best viewed as a comparison between severe and moderate sleep deprivation. Brief sleep episodes in deprived rats resulted from apparatus failures and difficulty in blocking LS without markedly increasing rotation frequency (*6*).

Apart from modest weight loss and minor skin lesions, no control rat showed outward signs of pathology or any observable indication that it could not have continued in the experiment indefinitely. All control rats appeared groomed, motorically active, and responsive to stimuli.

At least two of the following pathological signs became obvious in each deprived rat: debilitated appearance, including yellowed and apparently ungroomed fur, various skin lesions, and swelling of the paws (eight rats); ataxia or severe motor weakness, manifest by difficulty in maintaining balance and staying on or remounting the disk (seven rats); loss of EEG amplitude to less than half of normal waking values (seven

rats). Three deprived rats died after 5, 13, and 33 days while being observed. In each case, EEG amplitude declined within 24 hours of death. Even when the disk was held still, EEG amplitude did not recover and there were no prolonged periods of HS, PS, or unambiguous behavioral sleep, suggesting impairment of brain function and sleep mechanisms. Within minutes of death, these animals were perfused with saline followed by 10 percent Formalin for necropsy and histology. Yoked rats were always killed together.

Four deprived rats were killed with Ketamine and perfused after 5, 13, 19, and 21 days, because death seemed imminent. All showed declines in EEG amplitude like those described above; three of the four had to be rescued from drowning because they had become too weak or ataxic to remount the disk after collapsing into the water. Typically, rats had little difficulty staying on the disk until the day before they died. An eighth deprived rat was killed during its eighth experimental day after the recording plug separated from its skull. This rat had become severely debilitated and ataxic, but had not yet shown an EEG decline.

Gross necropsies were performed on seven yoked pairs. Obvious pathological signs in deprived rats included: fluid in lungs and trachea (three rats), collapsed lung (one rat), stomach ulcers (three rats), internal hemorrhage (two rats), severe edema in limbs (two rats), testicles atrophied (one rat), severe scrotal damage (one rat), and much-enlarged bladder (one rat). Although each deprived rat showed at least one of the above signs, no immediately apparent cause of death was uniform across deprived rats. Control rats showed little comparable pathology; one showed stomach ulcers and another, scrotal lesions.

Considering the damp environment, the gross pulmonary findings at necropsy, the susceptibility of Sprague-Dawley rats to pulmonary infection, and the possible effects of stress and sleep loss on immune function (*7*), we had lung tissues examined with hematoxylin-eosin and silver chromate stains. No deprived or control rat showed significant pneumonic inflammatory infiltrate, pneumocystis pneumonia, or any fungal infection. A single control rat showed a small focus of early bronchopneumonia.

Organ weights were taken for six yoked pairs and six age-matched home-cage control rats (Table 2). Deprived rats had significantly lower liver and spleen weights than controls, and higher adrenal weights. Organ weights of control rats were usually intermediate between home-cage and deprived rats. It is unclear whether the pathological tendencies in control rats resulted from moderate sleep loss or from the experimental environment.

Body weight decreased ($P < .001$) from baseline to termination in both control and deprived rats. However, the decrease was greater ($P < .05$) in deprived rats (Table 3). The weight losses could not be attributed to reduced food intake; both groups ate more during the experiment. Deprived rats increased food intake substantially more than control rats, but the group difference was not statistically significant because of large variances. Furthermore, the rats which lost the most weight ate the most. Correlations between percentage weight loss and percentage baseline food intake were: $r = .49$ (not significant) for deprived rats, $r = .70$ (not significant) for control rats, and $r = .61$ ($P < .05$) for the two groups combined. The greater weight loss in deprived rats, in spite of their increased food intake, suggests an increased ratio of catabolism to anabolism. Some weight loss may have been attributable to a significant decrease in water intake in both groups (Table 3), but substantial, albeit nonsignificant, correlations between percentage weight loss and percentage of baseline water intake ($r = .49$ for deprived rats; $r = .42$ for control rats) argue otherwise. These results do not rule out the possibility of weight loss resulting from impaired fluid retention.

The enlarged adrenals and higher incidence of stomach ulcers in deprived rats indicates a greater stress response that may have mediated subsequent debilitation. Since the physical stimuli for the two groups were the same, however, it is likely that the differentially greater stress was in response to the sleep deprivation or some other effect of it. The significant reduction from baseline of blood corticosterone levels in both groups (from 24.0 to 17.7 μg/dl in deprived rats and 23.3 to 13.2 μg/dl in control rats) deviates from the "classical" stress syndrome (Table 3).

Although deprived rats accumulated

Table 1. Percentages (means ± standard deviations) of total recording time spent in sleep states. For standard errors of the mean, divide the standard deviations by $\sqrt{N}$, where $N = 8$ per group.

Group	HS	LS	PS
Deprived			
Baseline	42.3 ± 4.6	7.1 ± 1.8	5.5 ± 0.8
Experiment	2.5 ± 1.3	4.2 ± 3.3	0.2 ± 0.2
Control			
Baseline	45.3 ± 3.2	6.6 ± 2.9	5.6 ± 0.9
Experiment	28.6 ± 7.4	9.5 ± 6.9	1.8 ± 1.0

Table 2. Organ weights (means ± standard deviations) of sleep-deprived (D), control (C), and home-caged (H) rats. Comparisons were made with two-tailed *t*-tests, with $\alpha = .05$ and $N = 6$ in each group. N.S., not significant.

Organ*	Organ weights (g)			*P*		
	D	C	H	D versus C	D versus H	C versus H
Adrenals	0.047 ± 0.013	0.031 ± 0.004	0.025 ± 0.003	.05	.005	N.S.
Liver	12.16 ± 1.98	15.48 ± 2.00	19.41 ± 1.30	.02	.001	.005
Lungs	4.00 ± 1.48	2.84 ± 0.51	3.03 ± 0.61	N.S.	N.S.	N.S.
Kidney	1.65 ± 0.28	1.93 ± 0.26	2.41 ± 0.33	N.S.	.002	.02
Spleen	0.29 ± 0.04	0.51 ± 0.14	0.89 ± 0.20	.005	.001	.005

*Values are for the average of both adrenals, both lungs combined, and one kidney.

Table 3. Percentages of baseline values (means ± standard deviations) for terminal body weight, average food intake during the experiment, average water intake during the experiment, and terminal serum corticosterone level. Comparisons were made with two-tailed *t*-tests for paired samples, with $\alpha = .05$.

Variable	Pairs (*N*)	Percentage of baseline value		*P*		
		D rats	C rats	D versus baseline	C versus baseline	D versus C
Body weight	7	80.0 ± 5.1	86.7 ± 5.3	.001	.001	.05
Food intake	6	148.4 ± 42.1	128.6 ± 19.5	.005	.02	N.S.
Water intake	6	73.8 ± 12.4	68.0 ± 18.0	.01	.01	N.S.
Corticosterone	6	75.0 ± 21.0	61.0 ± 35.0	.05	.05	N.S.

very little PS, there was a high correlation ($r = .94$, $P < .001$) between their PS (percentage of baseline) and survival time in the experiment. Survival time did not correlate highly with other sleep variables. The result suggests a vital role for PS, but it is so at variance with previous reports (*8*) that we believe it needs further confirmation.

Because the only difference in the treatment of the rats was that disk rotation was linked to sleep onset in deprived but not control rats, the pathological changes and mortality in deprived rats must be attributed to the disruption of sleep or a related process. Since the observed physiological impairments were not uniform across deprived rats, the nature of the functional deficit remains unclear. Nevertheless, these results support the view that sleep does serve a vital physiological function.

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Environmental Component of Morphological Differentiation in Birds

Abstract. *Geographic character variation in birds is usually attributed to natural selection for phenotypes that reflect locally adapted genetic differences. However, experimental transplants of red-winged blackbird eggs between nests in northern and southern Florida, and from Colorado to Minnesota, show that in this species a significant proportion of the regional differences in nestling development is nongenetic. If natural selection is maintaining the clines of character variation that are observed in adult phenotypes, the genetic and nongenetic components of phenotypic variation must covary.*

Many species of vertebrates exhibit parallel patterns of geographic variation in external morphology (*1*). In birds, regional trends of size variation change gradually in a way that may reflect topographic features (*2*). The mechanism that maintains such clines of character variation is traditionally assumed to be natural selection for polygenic traits that represent adaptations to local conditions (*3*). Inferences about the agents of selection are usually based on correlative methods and are necessarily weak. Nevertheless, the thermoregulatory arguments of Bergmann (*2, 4, 5*) are supported by very high correlations between size variation and climatic factors, especially when all avenues of heat exchange are considered (*2, 6, 7*). Other arguments proposed for mammals involve the energetics of procuring food (*8*), predator-prey relationships (*9*), and competition with related sympatric species (*10*). There has been little attention paid to the significance of shape variation that is allometrically associated with size variation or to the extent to which clinal variation is environmentally induced.

I report here the results of transplant experiments designed to estimate the magnitude and direction of direct environmental effects on regional morphological differentiation in the red-winged blackbird (*Agelaius phoeniceus*). In the United States the smallest adult redwings are the slender-billed population in southeastern Florida. Breeding adult males there weigh about 47 g and have a bill length of approximately 23 mm. An example of large birds is the stocky conical-billed population in central Colorado, where adult males weigh about 70 g and have a bill length of approximately 21 mm (*11*). These regional intraspecific differences are similar to generic differences in the blackbird family Icteridae (for example, between orioles, *Icterus*, and cowbirds, *Molothrus*), but they are smaller (*12*). Despite massive winter movements of red-winged blackbirds (*13*), relatively stable clines of character variation are reestablished across the continent every breeding season.

Regional variation in the shape of adult redwings in Florida can be detected in the development of nestlings. For example, nestlings (*14*) and adults (*15*) at a study site in the northern part of the state (Tallahassee, Leon County) have higher ratios of bill depth to tarsus, and lower ratios of bill length to tarsus and toe to tarsus than do nestlings in the southern part (Everglades, Dade County). Similarly, nestlings in Clearwater County, in northern central Minnesota, have higher ratios of wing length to tarsus and toe to tarsus than do those at Fort Collins, Larimer County, in central Colorado, and differences in small samples of adults appear to be in the same direction. The fact that the shape characters that best distinguish the populations in Florida are different from the ones that best distinguish the Colorado from the Minnesota populations is evidence of the complex nature of geographic character variation.

In 1980 and 1981, eggs were transported between study sites by car. Clutches of eggs were carried in an incubator connected through an inverter to the car battery and then placed in foster nests (*16*). For comparison control eggs were held in an incubator for 2 days and then placed in other local nests. The results are presented as (i) differences between the control and transplanted nestlings (Table 1) and (ii) differences between these groups plotted along the axis that best separates the normal (unmanipulated) nestling populations (Fig. 1) (*17*). In a reciprocal transplant of eggs between northern and southern Florida, and in a transplant from Colorado to Minnesota, the transplanted nestlings demonstrated a shift from the phenotype of the controls toward the phenotype of nestlings in normal unmanipulated nests of the foster population (*18*). Discriminant analyses and univariate F tests between the control and transplant groups (Table 1) show which characters have the largest component of environmental plasticity. In the reciprocal transplant across Florida the ratio of bill length to tarsus shifted most and was in the direction