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Response of Mice to Repeated Photoperiod Shifts: Susceptibility to Stress and Barbiturates (38877)

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The susceptibility of animals to various drugs, infectious agents, and other stress stimuli has been shown to vary in a circadian fashion (1–4). It has, therefore, been suggested that desynchronization of biologic rhythms by altering the photoperiod may lead to changes in the response to stress that may be detrimental to the animal (5). Genetically different strains of mice have been reported to respond to stress in varying degrees (6–8), and hence, it was anticipated that they may also show differential responses to circadian rhythm desynchronization. The three strains of inbred mice selected (BABL/cJ, CBA/J, and C57BL/10J) showed grossly different behavioral and physiological characteristics. The BALB/cJ mice exhibit spontaneous fighting behavior while the CGA/J animals were found to be particularly sensitive to cold stress. In addition, it has been reported (9) that these strains show genetic differences in adrenal catecholamine synthesis in response to stressful stimuli and pituitary–adrenal activation. Therefore, the effect of frequent phase-shifting on the survival, body weight, and gross behavior of these three strains of mice was determined. The effect of phase-shifting on their subsequent response to a cold stress or to the induction of sleep by secobarbital was also assessed.

Materials and Methods. Male mice of inbred strains BALB/cJ, CBA/J, and C57BL/10J were purchased from the Jackson Laboratory, Bar Harbor, ME. They were kept in a controlled environment of 12L:12D, 21° room temperature, and 50% relative humidity. They were housed 10 to a cage with free access to Wayne Lab chow and water. They were 7 wk old at the time of the study and weighed 20.75 ± 0.36, 23.13 g ± 0.37, and 24.43 g ± 0.42, respectively. Sixty mice of each strain were used. After a 10-day period of equilibration, each strain was divided into two groups, (30/group), one of which remained in that environment and served as controls while the experimental group was transferred to a similar chamber. The cycle of the latter chamber was also 12L:12D, but 180° phase shifts were effected by extending the light or dark period by 12 hr on a random basis. For the first 76 days, light reversals occurred on an average of once every 4 days (Cycle I). The frequency of the changes was accelerated in the later part of the experiment, when phase shifting was produced, on the average of once every 2 days (Cycle II) for the next 54 days. Thus, a total of 24 reversals occurred during Cycle I, 12 by extending the light period by 12 hr and 12 by extending the dark period by 12 hr. Cycle II included 36 reversals, 18 effected by extending the light period by 12 hr and 18 by extending the dark period by 12 hr.

Body weights were recorded at 4- to 5-day intervals. During the dark cycle, red lights were used when changing the cages. At the end of the experimental period, each strain of mice was subdivided into three groups, with 5–10 animals per group. Group I was sacrificed by decapitation, the blood collected in heparinized containers, the plasma separated by centrifugation, and stored frozen for corticosterone determinations. Group II was placed in a cold chamber at 4° for 24 hr. The mice were then decapitated and the plasma similarly collected for corticosterone determination. The experiment was designed so that all animals were killed between 3:00 and 4:15 PM. Plasma corticosterone was determined by the method of Vernikos-Danellis *et al.* (10). Group III was tested for sleeping time in response to secobarbital. Since differential susceptibility of the three strains to the barbiturate was anticipated, preliminary testing was necessary to establish satisfactory working doses of

the drug. The doses that produced a sleeping time of 60–80 min in the controls and which were subsequently used to test all groups of animals were as follows: BALB/cJ, 35 mg/kg; CBA/J, 50 mg/kg; and C57BL/10J, 55 mg/kg. Sleeping time was recorded as the time the mouse lost its righting reflex to the time it regained it. Secobarbital was dissolved in pyrogen-free saline and injected intraperitoneally in a volume of 0.15 ml/10 g body wt.

Results. The variation in the light schedule had little effect on the growth and body weight of the mice. By the end of the first cycle, there were no significant differences between control and experimental animals ($P > 0.05$). At the end of the second cycle, the experimental animals appeared to be gaining more weight than the controls. A total of nine deaths occurred in the BALB/cJ mice as a result of spontaneous fighting; eight of these were in the control group and one in the experimental. Gross observation of the fighting behavior of this strain appeared to indicate reduced fighting in the experimental group.

Table I compares the plasma corticosterone concentrations in control and experimental mice before and after exposure to cold for 24 hr. Plasma corticosterone ranged from 32.4 to 37.4 μg/100 ml in the three strains of control mice. Frequent changes in the photoperiod resulted in significant ($P < 0.05$) increases in the circulating steroid in all strains. Exposure of the control mice to the cold for 24 hr resulted in no change in the CBA/J mice, an increase in the C57BL/10J mice ($P < 0.05$), and a marked decrease ($P < 0.05$) in the BALB/cJ mice. In contrast, the experimental mice showed no further response to the cold, and the BALB/cJ strain showed no decrease in response to the cold ($P > 0.05$).

The sleeping times in response to the administration of secobarbital for the three strains of mice are shown in Table II. It can be seen that frequent changes in the photoperiod reduced the sleeping time significantly in all three strains.

Discussion. In contrast to what was anticipated, mice of all three strains showed no obvious detrimental effects of the frequent 180° phase shifting, whether it was imposed once every 4 days or once every 2 days. Weight gain was normal or improved and the fighting behavior of the BALB/cJ mice was not aggravated as had been expected but in fact appeared to be reduced. The higher circulating corticosterone levels at the end of the photoperiod shifts could be evidence of stress or possibly due to a shift in the corticosterone circadian rhythm so that a higher part of the cycle occurred earlier than that for the control group. Plasma steroid levels in all three strains of experimental animals showed no further increase in response to the 24-hr cold stress. This is probably due to the fact that the chronic exposure to phase shifts resulted in maximal adrenal corticosterone secretion which could not be raised further by an additional stress. Control mice showed differential responses to cold exposure, C57BL/10J mice showing an increase, CBA/J animals showing no response, and BALB/cJ mice showing a significant reduction. No obvious explanation for these varied responses in the control animals is available other than the possibility that these

TABLE I. EFFECT OF 180° PHASE SHIFTS IN PHOTOPERIOD ON PLASMA CORTICOSTERONE LEVELS BEFORE AND AFTER COLD STRESS.

	Plasma corticosterone[a] (μg/100 ml ± SE)			
	Control		Experimental	
Mouse strain	21°	4°	21°	4°
BALB/cJ	37.44 ± 2.45 (10)	14.52 ± 0.66 (4)[b]	45.12 ± 2.34 (10)[b]	48.26 ± 7.45 (8)
C57BL/10J	32.40 ± 3.60 (8)	47.43 ± 3.71 (8)[b]	39.68 ± 2.00 (10)[b]	44.74 ± 7.04 (8)
CBA/J	37.12 ± 3.73 (10)	39.93 ± 3.23 (10)	51.20 ± 6.79 (10)[b]	45.01 ± 3.71 (10)

[a] Numbers in parentheses represent number of animals per group.

[b] Significant from 21° control at $P < 0.05$.

TABLE II. EFFECT OF FREQUENT 180° PHASE SHIFTS IN PHOTOPERIOD ON SECOBARBITAL-INDUCED SLEEPING TIME.

Mouse strain	Sleeping time (minutes and seconds ± SE)		
	BALB/cJ	CBA/J	C57BL/10J
Concentration	35 mg/kg	50 mg/kg	55 mg/kg
Control	59 30 ± 9 48 (10)	72 22 ± 16 34 (8)	85 50 ± 4 8 (12)
Experimental	35 24 ± 5 06 (10)[a]	39 00 ± 13 16 (7)[a]	53 45 ± 7 44 (8)[b]

[a] Differs from control at $P < 0.05$.
[b] Differs from control at $P < 0.001$.

data reflect greatly different time courses in the adrenal response to cold stress in these three strains. Levine and Treiman (6) have, in fact, demonstrated that not only does the magnitude of the plasma corticosterone response to stress vary but the time course of this response differs greatly among genetic strains of mice. Similar genetic differences have been reported in the induction of the adrenal enzyme phenylethanolamine *N*-methyl transferase (PNMT) that synthesizes epinephrine and is under corticosteroid regulation (9). The half-life of PNMT was reported to be 3–4 hr in the C57BL/10J strain and 7 hr in the CBA/J strain (11).

The duration and intensity of the responses to many drugs are dependent upon the rate at which the drug is metabolized. Hepatic drug metabolism has been shown to vary in a circadian fashion (12). Thus, the duration of action of several barbiturates that are metabolized by the liver has been found to be shortest during the active period in a variety of species (12–14). In the present study, the sleeping time induced by secobarbital was found to be greatly reduced during the normally inactive period of these three strains of mice exposed to frequent phase shifting. Such treatment would have been expected to result in disturbance or internal desynchronization of their circadian rhythms (5, 15). It has been shown that steroid hormones influence drug metabolism since adrenalectomy decreases the activity of some oxidative drug-metabolizing enzymes in rats and mice (16). Radzialowski and Bousquet (12) have also reported that adrenalectomy or maintenance of elevated plasma corticosterone levels by administration of the steroid abolished the circadian rhythm in drug metabolism. In fact, Kuntzman *et al.* have suggested that the steroid hormones may be the natural substrates for the drug-metabolizing enzymes (17). Hence, it may be reasonable to suggest that the decreased sleeping time observed in all the strains of mice in this study may be the result of the elevated circulating corticosterone levels.

It would, therefore, appear that repeated random changes in the circadian photoperiod for 140 days is a stressful experience to which animals do not adapt, as evidenced by the high circulating corticosterone levels. Furthermore, the ability to respond to an additional stimulus, such as exposure to cold, or to metabolize drugs, is definitely affected.

Summary. Three inbred strains of mice selected for their spontaneous aggressive behavior and differential susceptibility to stress were exposed to a controlled environment where on an average of once every 4 days for 76 days and subsequently on an average of once every 2 days for an additional 55 days a 12L:12D photoperiod was reversed by 180°. This procedure did not affect the growth of the mice and appeared to reduce fighting. However, plasma corticosterone concentrations in all three strains of mice were high, and their response to a 24-hr cold stress was no longer evident. The most pronounced effect of the altered photoperiod was on the barbiturate-induced sleeping time which showed a 40% reduction in all strains in spite of differential suceptibility to the drug among strains. It is concluded that repeated random phase shifting by varying the photoperiod is a stressful experience to which animals do not adapt and

that the ability to respond to an additional stimulus or drugs may be greatly altered.

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