Influence of Early Environment on the Circadian Period of the Tau-Mutant Hamster

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The role of the period of the environmental cycle during gestation and infancy on the circadian period of adult hamsters was studied. Tau-mutant hamsters of all three genotypes (+/+, 24-h circadian period; +/tau, 22-h period; tau/tau, 20-h period) were conceived and raised under either a 20-h light-dark cycle or a 24-h cycle. The circadian period in constant darkness was determined at 2 months of age by inspection of records of running-wheel activity. Differences in circadian period of up to 1.2 h were observed. However, changes of the same magnitude were also observed in animals conceived and raised under a 24-h cycle and exposed to a 20-h cycle at 8 months of age. Therefore, it is concluded that the aftereffects of entrainment can account for the apparent influence of the early environment. The free-running period of the circadian pacemaker seems to be under complete genetic control and not to be influenced by the period of the environmental cycle under which the animal is raised.

KEY WORDS: Circadian rhythms; golden hamster; development; tau gene.

INTRODUCTION

The life of most organisms is affected by the changes that occur in the environment as the earth rotates on its axis. Early in the evolutionary process, daily rhythmicity in the environment favored the evolution of endogenous biological clocks that allowed organisms to adjust behavioral and physiological functions in anticipation of environmental changes (Pittendrigh, 1981). Mammals, and perhaps other vertebrates as well, possess a circadian pacemaker located in the suprachiasmatic nuclei of the hypothalamus (Menaker et al., 1997).

The period of the circadian pacemaker, as determined by the analysis of behavioral or physiological rhythms monitored in a constant environment, deviates slightly—but in a species-specific manner—from 24 h. In invertebrates—mainly the fruitfly, Drosophila melanogaster, and the bread mold, Neurospora crassa—single-gene mutations have been found to shorten or lengthen the period of the circadian pacemaker by several hours (Hall, 1990). Great progress has been recently made in understanding the molecular mechanisms of circadian rhythmicity in these organisms (Crosthwaite et al., 1997; Sehgal et al., 1995). In mammals, at least three single-gene mutations have been described: the tau mutation in the golden hamster (Ralph and Menaker, 1988), the Clock mutation in the mouse (Vitaterna et al., 1994), and the Wheels mutation in the mouse (Pickard et al., 1995). While the Clock mutation is the best understood in terms of molecular mechanisms (King et al., 1997), the tau mutation is the best understood in terms of phenotypic expression. The tau mutation acts on circadian timing through the suprachiasmatic nuclei (Ralph et al., 1990) and affects the pacemaker’s circadian period.

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and resetting lability (Biello and Mrosovsky, 1996; Shimomura and Menaker, 1994) without affecting other parameters of circadian rhythms. The mutation has been shown to shorten the circadian period of the rhythms of locomotor activity (Ralph and Menaker, 1988), body temperature (Refinetti and Menaker, 1992b), and metabolic heat production (Refinetti and Menaker, 1997) and to cause a proportional increase in the frequency of ultradian oscillations in locomotor activity and body temperature (Refinetti, 1996). The specificity of the mutation is revealed by the absence of concomitant effects on heart rate (Refinetti and Menaker, 1993) and the duration of the estrous cycle (Refinetti and Menaker, 1992a). From breeding experiments, it is known that the tau gene is autosomal with semidominant alleles: wild-type hamsters (+/+) have an endogenous period of 24 h, heterozygous mutants (+/tau) have a period of 22 h, and homozygous mutants (tau/tau) have a period of 20 h (Menaker and Refinetti, 1993).

Although much is known about how environmental factors affect the operation of the circadian pacemaker in adult animals, very little is known about how the early environment of an animal interacts with its genetic makeup to determine the adult phenotype (Turek and Van Reeth, 1996). The present series of experiments investigates the role of the period of the environmental cycle during gestation and infancy on the circadian period of adult hamsters with different genetic makeup.

**EXPERIMENT 1**

**Methods**

The animals used were descendants (third generation) from original tau-mutant hamsters obtained from Dr. Michael Menaker's colony at the University of Virginia and bred with wild-type golden hamsters purchased from Charles River Laboratories (Wilmington, MA). Nine heterozygous females (+/tau) were randomly chosen to mate with heterozygous males (+/tau) to produce phenotypically diverse offspring (one-fourth +/+ , one-half +/tau, one-fourth tau/tau). Six of the females were placed under a 20-h light–dark cycle (LD, 12:8; 300:0 lux) and three were placed under a 24-h cycle (LD, 14:10; 300:0 lux) for the duration of the experiment. Before breeding, entrainment of the dams to the light–dark cycle was ascertained by analysis of records of running wheel activity.

For the recording of running wheel activity, the animals were housed individually in plastic cages (25 × 46 × 20 cm) lined with wood shavings. The cages were fitted with 16-cm-diameter running wheels and maintained inside a lighttight ventilated incubator at 24°C. Prolab rodent pellets and water were available ad libitum. Magnetic switches attached to the running wheels were connected to a data acquisition system (A-BUS; Alpha Products, Darien, CT) that recorded the number of wheel revolutions in 6-min bins.

After the occurrence of entrainment had been ascertained, the dams were transferred to identical cages without running wheels and mated. The offspring (usually delivered 16 days after conception) was kept with the dam until weaning (age, 20 days) and then transferred to sex-matched group cages under the same light–dark cycle. At 60 days of age, the animals were transferred to individual cages with running wheels (as described above) and maintained in constant darkness for 2 weeks. Circadian period was calculated by plotting the activity records as actograms and determining the plot modulo that resulted in the vertical alignment of the daily onsets of activity. This procedure is exemplified in Fig. 1.

**Results**

The six dams maintained under the 20-h LD cycle gave birth to 64 pups (average, 10.7 pups per litter). The three dams maintained under the 24-h LD cycle gave birth to 36 pups (average, 12.0 pups per litter). The difference in litter size was not significant [t(7) = 0.84, p > .10]. Due to infant death, equipment failure, or scheduling conflicts, reliable data were obtained from 47 animals raised under the 20-h LD cycle and 35 raised under the 24-h LD cycle. Males and females were equally represented.

Because short-term aftereffects of the light–dark cycle on the free-running rhythm of adult animals are known to occur (Aschoff, 1981), circadian period was calculated separately for the first and the second weeks after the animals were placed into constant darkness. The differences in period between the 2 weeks were rather small (about 0.2 h) compared to the differences in period due to genotype (up to 4 h) but were consistent with the expectation of aftereffects. For instance, heterozy-
Fig. 1. Illustration of the procedure used to calculate circadian period by plotting activity records as actograms and determining the plot modulo that results in the vertical alignment of the daily onsets of activity. A segment of the records of a typical animal is shown as plotted in modulo 22, 22.4, and 23 (i.e., each line corresponding to a “day” of 22, 22.4, or 23 h and successive lines corresponding to successive days). For this animal, which has a circadian period of 22.4 h, the onsets of activity show a progressive delay when plotted in modulo 22, a progressive advance when plotted in modulo 23, and a stable vertical alignment when plotted in modulo 22.4.

Fig. 2. Frequency histograms for the distribution of circadian period (determined during the second week in constant darkness at 2 months of age) of hamsters conceived and raised under either a 24-h (top) or 20-h (bottom) light–dark cycle. Different shadings are used to emphasize the clusters corresponding to tau/tau (hatched bars), +/tau (filled bars), and +/- (open bars) animals.

Gotes raised under the 24-h LD cycle showed a slightly longer mean period (±SE) of 22.7 ± 0.08 h during the first week than during the second week (22.6 ± 0.05 h)—a small but significant difference \( t(16) = 2.88, p = .01 \).

The distribution of circadian periods, as determined during the second week after the animals were placed into constant darkness, is shown in Fig. 2. A strong effect of the environment can be observed. The animals raised under the 24-h LD cycle are distributed in accordance with the expected 1:2:1 ratio, whereas those raised under the 20-h LD cycle are distributed at a 3:1 ratio. The 3:1 ratio is probably the result of a drastic reduction of the circadian period of +/-tau hamsters raised under the 20-h LD cycle. The circadian period of +/- and tau/tau hamsters seems to be only slightly shorter for animals raised under the 20-h than the 24-h LD cycle. Experiment 2 was conducted to evaluate the effect of the environment on the circadian period of heterozygotes.

**EXPERIMENT 2**

**Methods**

Full litters of heterozygotes were obtained by mating homozygous parents of opposite genotypes (as inferred from the phenotypic expression determined by records of running wheel activity). To ensure proper entrainment of the dams to the light–dark cycle, a female tau/tau was maintained under a 20-h LD cycle and mated with a male +/-, whereas a female +/- was maintained under a 24-h LD cycle and mated with a male tau/tau. The offspring were raised and tested as in Experiment 1, except that testing (in constant darkness) started at age 40 days.

**Results**

The female tau/tau gave birth to 10 pups; the female +/-, to 9 pups. Eight animals from each
litter were tested. Figure 3 shows the mean circadian period of the two litters during the first and second weeks after placement into constant darkness. The animals raised under a 24-h light–dark cycle had circadian periods slightly longer than 22 h, whereas those raised under a 20-h cycle had periods considerably shorter than 22 h. As in Experiment 1, there was a small but significant difference in period between the first and the second weeks. Analysis of variance revealed significant effects of the light–dark cycle \( F(1,14) = 485.95, p < .001 \), of week of recording \( F(1,14) = 5.46, p < .04 \), and of their interaction \( F(1,14) = 40.46, p < .001 \).

Although the results of this experiment, as well as those of the preceding one, suggest that the aftereffects of the light–dark cycle are rather small compared to the effects of the early environment, the experimental design did not fully allow the effects of early environment to be distinguished from the aftereffects of the light–dark cycle. Experiment 3 was conducted to investigate the specific mechanism of aftereffects in adult heterozygous animals.

**EXPERIMENT 3**

**Methods**

Eight male heterozygotes, conceived and raised under a 24-h LD cycle by heterozygous parents, were entrained to a 20-h LD cycle starting at 8 months of age. After a month under the LD cycle, the animals were placed into constant darkness and observed for 40 days.

**Results**

The results are shown in Fig. 4. The data point for the first 5-day block shows that, at 8 months of age, the mean circadian period (±SE) of these +/-Iau hamsters was 21.8 ± 0.2 h, which is the expected phenotypic expression for this genotype but is approximately 40 min shorter than the period of similar animals tested a week after their first exposure to constant darkness at two months of age (see Fig. 3). This suggests that the apparently permanent effect of the early photic environment observed in the two previous experiments was actually a transient aftereffect of the light–dark cycle that vanished during the 6 months that the animals spent in constant darkness.

When exposed to a 20-h light–dark cycle, the animals gradually entrained to the zeitgeber, so that their circadian period was exactly 20 h by the sixth 5-day block (Fig. 4). After transfer to constant darkness, the circadian period lengthened rapidly to 21 h but remained below the original period (21.8 h) for the duration of the experiment (40 days). During the eight 5-day blocks in constant darkness,
the period seemed to lengthen slightly but at a very slow rate. Linear interpolation by the least-squares method yielded a slope of 0.024 h per 5-day block, so that a period of 21.8 h would be reached only after 6 months (or a quarter of the life span of the animals) in constant darkness. Thus, the aftereffects of the 20-h light–dark cycle on the circadian period of these +/tau hamsters were very persistent and comparable in magnitude to the effects observed in younger animals in Experiments 1 and 2.

**DISCUSSION**

Experiment 1 showed that hamsters conceived and raised under a 20-h light–dark cycle had shorter circadian periods in constant darkness than hamsters conceived and raised under a 24-h light–dark cycle. Although this was true for all three genotypes, the effect of the early environment was especially pronounced in the heterozygotes (+/tau). This is most likely explained by the fact that, while +/tau hamsters can easily entrain to both a 20- and a 24-h light–dark cycle, the other two genotypes are less labile (+/+ hamsters are incapable of entraining to a 20-h cycle and not all tau/tau hamsters can entrain to a 24-h cycle). It is to be expected that animals that do not entrain to the environmental cycle will be less affected by it than those that entrain.

Experiment 2 provided results consistent with those of Experiment 1. It also showed that the shortening of circadian period caused by the 20-h cycle is much greater than the lengthening caused by the 24-h cycle. Since both cycles were equally spaced from the genotypic period (22 h), the differential effects must be due to a differential sensitivity to environmental cycles. Since hamsters raised under a 20-h cycle had to phase advance 2 h each “day,” whereas hamsters raised under a 24-h cycle had to phase delay 2 h each day, the differential effects on circadian period may be due to differential mechanisms of phase advance and phase delay. It is known that the phase-delay region of the tau-mutant hamster’s phase–response curve is much greater than its phase-advance region (Menaker and Refinetti, 1993), although it is not evident how this difference could lead to the observed differences in circadian period.

Experiment 3 showed that adult +/tau hamsters raised under a 24-h light–dark cycle but maintained under a 20-h cycle for a month at 8 months of age experienced a shortening of circadian period in constant darkness comparable to that of +/tau hamsters raised under a 20-h cycle. This suggests that the period changes observed in Experiments 1 and 2 were the result not of a permanent alteration induced by the early environment but of a transient aftereffect of the light–dark cycle. A similar conclusion was reached by Davis and Menaker (1981) in their study of wild-type mice raised under cycles of 20 and 28 h and by Possidente and Stephan (1988) in their comparison of inbred strains of mice, although the magnitude of the aftereffects was much smaller in their studies than in the present one. The present results suggest that the aftereffects may persist for up to 6 months in the absence of further photic stimulation. Persistence of aftereffects in adult rodents for longer than 3 months has been reported previously (Pittendrigh and Daan, 1976).

Because the aftereffects of entrainment on the subsequent free-running period were found to be of the same magnitude and duration in adult hamsters as in hamsters exposed to the same conditions since conception, it seems legitimate to conclude that the early environment has no specific effect on the circadian period of the animals. Thus, the free-running period of the circadian pacemaker seems to be under complete genetic control and not to be influenced by the period of the environmental cycle under which the animal is raised.

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