elements (*2, 7, 9-11*). It seems to be large enough to support separation of calcium isotopes on a practical scale by the solvent flow technique.

An attempt to reduce the solute concentration gradient by increasing solvent flow was only partly successful. On day 14 the flow rates were increased to the following values: solvent feed, 0.60 g/hour; 40 percent solution feed, 0.25 g/hour; and top outflow, 0.85 g/hour. As indicated in Fig. 1, the solute concentration gradient did decrease, but the results were somewhat out of line with those predicted. A detailed examination of the composition profiles calculated from theory showed that a clearly defined positive concentration gradient would develop during the transition period following the change in conditions. This would be expected to lead to at least partial remixing of the column contents. As expected under these conditions, the isotope separation dropped somewhat. The results are equivalent to an average separation factor of 1.16 for the ^{40}Ca-^{48}Ca pair, as opposed to the value of 1.26 obtained during the first 14 days of the experiment.

Further development of this technique may lead to a practical process for separating isotopes in solution and thus to improved availability of calcium and other isotopes to the scientific community. It is already established that the thermal diffusion method is well suited to moderate-scale enrichment of noble gas isotopes and to enrichment of the isotopes of elements, such as sulfur and chlorine, that form simple stable liquid compounds over an appropriate temperature range.

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22 September 1980; revised 5 December 1980

Phase Shifting Circadian Rhythms Produces Retrograde Amnesia

Abstract. *Phase shifting circadian rhythms in rats shortly after passive avoidance training impaired their performance on retention tests. The amnesia was not due to simple performance deficits accompanying the "jet lag" effects of phase shifting or to differences in lighting or circadian phase at training and at testing. Amnesia was associated with specific rhythm reentrainment patterns. These data indicate that disrupting circadian organization can produce retrograde amnesia in rats.*

Circadian organization may be an important factor in memory processes. Performance on retention tests in a variety of appetitive and aversive tasks fluctuates rhythmically, with optimal retention occurring 24 hours after training (*1, 2*). These fluctuations in retention appear to depend on the integrity of circadian rhythms (*3*). Many processes that influence memory (brain protein synthesis, neural activity, synaptic excitability, neurotransmitter synthesis, and hormone secretion) display circadian oscillations (*4*). It is likely that the rhythms governing such processes account for periodic fluctuations in retention and for the circadian variations in the effectiveness of treatments, such as electroconvulsive shock (*5*), that affect memory.

It is difficult to evaluate the importance of rhythmic organization for memory processing, since prior research has been purely correlational, implicating internal rhythms indirectly or inferentially. Retention fluctuations may reflect widespread rhythms in processes that influence memory. But rhythmic organization may be a prerequisite for normal memory. Assessment of the relative importance of circadian organization in normal memory requires that retention be examined after some direct manipulation of circadian rhythms. The most effective way to alter circadian rhythms is to change, or phase-shift, the light cycle that entrains them.

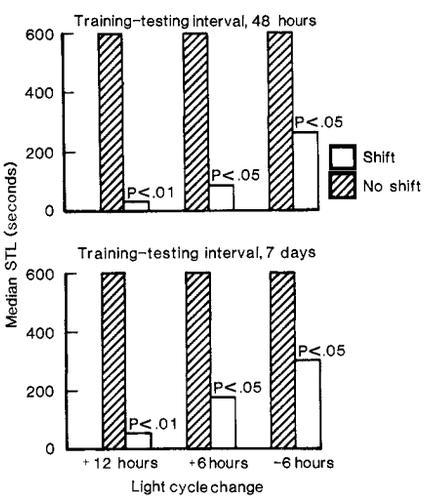

Fig. 1. Retention performance of rats in a one-trial passive avoidance task (maximum retention score, 600 seconds). No two groups, experimental or control, differed significantly in their training STL's, which were almost always less than 60 seconds. All animals were trained in light and placed in darkness immediately afterward. After training, light cycle changes were accomplished by varying the onset of the next light cycle. Positive phase shifts indicate phase advances; that is, the next light cycle began earlier than usual. The negative phase shift indicates a phase delay; the onset of the next light cycle began later than usual.

Male albino rats weighing between 200 and 400 g were housed individually in clear plastic cages, put on a 12-hour light-dark cycle (lights on from 0800 to 2000 hours), and given unrestricted access to food and water. Activity was recorded when a rat broke either of two photocell beams (*6*) that traversed its cage longitudinally. Activity counts were accumulated and recorded every 10 minutes. Circadian activity rhythms were used to monitor the progress of the phase shift (*7*).

The rats were trained between 0830 and 1030 hours in a one-trial passive avoidance task (*8*). The trough-shaped apparatus consisted of a brightly lit start box and a larger, unlit shock box. The rat was placed in the start box facing away from a circular door to the shock box. When it stepped into the far end of the shock box, a shock generator-scrambler (BRS SGS-001) delivered current (0.1 W for 5 seconds) through steel plates in the shock-box floor. After stepping into the shock box, receiving shock, and escaping back into the start box, the rat was returned to its home cage. Testing was identical to training except that no shock was delivered. Passive avoidance was indexed by the length of time each rat took to step into the shock box. This step-through latency (STL) and the shock-box escape latency were recorded automatically by electronic timers. If a rat did not enter the shock box within 600 seconds an arbitrary STL of 600 seconds was recorded. Low testing STL's were interpreted as poor retention. We examined eight phase shift conditions, each of which was paired with a control (no shift) condition ($N = 8$ rats per group).

Circadian rhythms were phase-shifted by turning off the home-cage lights immediately after training. The rats were

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left in the dark until the time of light onset in the new cycle. The time difference in light onset for the old and new cycles was set at 6 or 12 hours. Thus, for a rat completing training at 1030 hours and receiving a 12-hour phase shift, the schedule was as follows: lights on from 0800 to 1030 hours, lights off from 1030 to 2000 hours, then the 12-hour light-dark cycle, with lights on from 2000 hours to 0800 hours.

The results of these experiments are shown in Fig. 1. At testing, all the control groups had median STL's at or near the maximum allowed. All the phase-shifted groups had dramatically reduced STL's, indicative of poor retention. It seems, then, that disrupting circadian organization in rats can produce a lasting loss of memory (*9*).

All phase shift conditions disrupted circadian activity rhythms. However, the retention deficits were not due to temporary impairments in performance, such as humans experience during jet lag (*10*). Phase shift amnesia persisted even after circadian activity rhythms were completely synchronized or reentrained to the new light cycle 7 days after training (Fig. 1) (*11*). Further, testing in the midst of circadian disorganization (48 hours after the phase shift) did not reveal impaired retention when the phase shift was delayed for 5 days after training (median STL for controls, 599.6 seconds; for rats phase-shifted 12 hours, 592 seconds). But like the effects of other amnestic treatments, the amnestic effects of phase shifting were time-dependent—that is, effective shortly after training but not 5 days after training.

The retention deficits were not due to training in one lighting condition and testing in another, since they persisted when rats were both trained and tested during light (Fig. 1). Nor were the deficits due to training and testing in different phases of the circadian activity rhythm. Amnesia induced by phase shifting was still present when rats were trained and tested in the same circadian phase and in the same lighting condition, as when they were tested 7 days and 12 hours after training, with a 12-hour shift immediately after training (median STL for controls, 600 seconds, for rats phase-shifted 12 hours, 42.8 seconds; (two-tailed Mann-Whitney U test, $P < .01$). Unshifted controls were not impaired when tested 12 hours out of phase with training (*12*).

Some phase-shifted rats exhibited good retention and specific patterns of reentrainment of their activity rhythm to the new cycle (Fig. 2A). For these animals, α, the portion of each 24-hour period devoted to activity (*13*), remained relatively constant during the steady, linear progress from the old to the new light-dark cycle. Except for the shifting of activity onset across days, these animals resembled the controls, for whom both the time of onset and the duration of the daily activity periods were regular and stable (Fig. 2B).

Amnesia induced by phase shifting was accompanied by different patterns of reentrainment of activity rhythms. Rats with poor retention performance had a marked change in α (Fig. 2C). Often, α became progressively shorter over several cycles, then lengthened abruptly with reentrainment to the new cycle (*14*). Clearly, the rhythm reentrainment patterns of rats with poor retention differed from those of controls and phase-shifted rats with good retention (*15*). It is not clear why some rats exhibited one pattern of activity rhythm reentrainment while others exhibited another, although individual differences are often seen in these studies (*10, 14, 16*).

Retention performance was impaired in rats whose circadian organization was clearly disturbed during reentrainment to a new light cycle (*17*). Normal circadian organization is characterized by fixed phase relations among different circadian rhythms that are running with the same period. The two peaks in rodent activity rhythms (early dark and late dark) reflect separate circadian oscillators (*13, 14*); the length of α is controlled by the phase angle or time between the two peaks. Changes in the phase relation or period of these activity peaks produced changes in length of α in phase-shifted rats with poor retention. In contrast, phase-shifted rats with no impairment exhibited neither of these alterations in circadian organization. In these rats, the two activity peaks continued to show the same time and phase relations throughout reentrainment to the new light-dark cycle.

Disturbances of circadian organization can have a variety of harmful consequences (*18*); how such disturbances affect memory remains unclear. De-

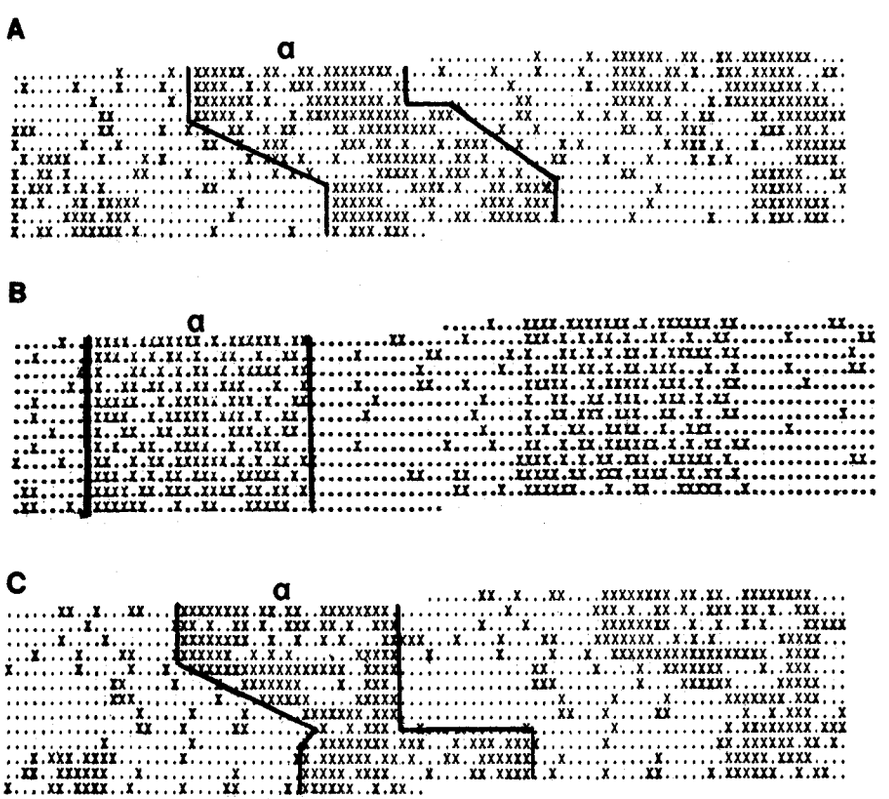

Fig. 2. Activity plots of phase-shifted and control rats. The half-row of data beginning each block (on the right) represents 24 hours and is repeated in the next row (on the left), which then gives activity data for the succeeding 24 hours. This pattern is repeated throughout each block. (A) Activity plot for a rat phase-shifted 12 hours and having good retention (STL, 600 seconds) 7 days after training. X's indicate the time when the rat was active (activity above the series mean) and the dots represent the time when the rat was resting (activity below the series mean). Heavy lines indicate the extent of α. Note that the length of α remains relatively constant as the onset of the rat's activity rhythm smoothly shifts to the new cycle. (B) Activity plot for a control (nonshifted) rat 7 days after training (testing STL, 600 seconds). This rat exhibits both a constant phase for activity onset and a very uniform α width from day to day. (C) Activity plot for a rat phase-shifted 12 hours and having poor retention (STL, 12.9 seconds) 7 days after training. Note the reduction in α across days followed by an abrupt increase as the animal adjusted to the new cycle.

synchrony between rhythms might disrupt the timing or function of circadian systems involved in long-term memory processes (for example, protein synthesis). Alternatively, disturbed circadian organization might act as a nonspecific stress to produce memory changes. Recent evidence suggests that many treatments which alter memory act by producing or mimicking stress responses (*19*). Regardless of the mechanism, circadian organization appears to be an important factor in memory processes, and disturbing circadian organization can produce retrograde amnesia. Psychometric tests to detect memory disturbances in travelers suffering from jet lag would be enlightening.

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Sex-Biased Litter Reduction in Food-Restricted Wood Rats (*Neotoma floridana*)

Abstract. *Mothers of eastern wood rats* (Neotoma floridana) *normally invest their lactation energy equally in male and female offspring, but alter that investment when their food is severely restricted during lactation. The effect of the altered investment is a significant bias against males in both mortality and growth.*

A sexual bias in growth and mortality of nestling wood rats, *Neotoma floridana*, is induced by severely restricting the diet of their mothers. These observations bear on ideas about the effect of mating systems and maternal condition on litter or brood reduction.

Brood reduction by selective starvation of nestlings has been reported in many species of birds (*1*, *2*), but there are few reports of litter reduction in mammals (*3*). It has been argued that food-stressed parents would be expected to alter the sex ratio of their offspring to favor the less costly sex (*4*), or females, in polygynous species (*5*). Most studies of brood reduction, however, have not explicitly considered the sex of the starved nestlings. My results may be the first to demonstrate experimentally induced sex-biased litter reduction in mammals.

Laboratory-raised *Neotoma* were housed in 23 by 45 by 20 cm wire-topped plastic cages equipped with wood shavings and drain-tile shelters. The animals were given free access to food and water. Each female was mated to a compatible male, and they were caged together until the young were born. Cages were examined daily. When a birth occurred, mother and young were placed in a clean cage and were assigned either to control or food-restricted groups. Controls continued to have free access to food. Food-restricted rats had a measured daily ration calculated from the formula $F = 5.35 + 0.02\,M$, where F is the daily food ration (in grams) and M is the body mass of the rat (in grams) on the day of the measurement; this fairly severe restriction amounted to about 70 to 90 percent of the maintenance requirement of a nonreproductive female of equivalent body mass. As a measure of their condition the body mass of all individuals was determined daily. The length of the experiments was 20 days from the birth of the young unless the mother's body mass fell below 75 percent of her mass on the first day of the experiment; free access to food was then given for the rest of the experiment. Under normal conditions, young wood rats are thermally and nutritionally weaned by about 20 days of age

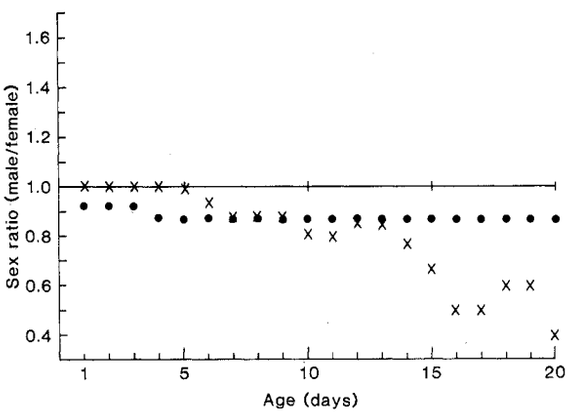

Fig. 1. Changing ratio of males to females in (●) control litters ($N = 29$) and (X) litters in which the mother's food was restricted during lactation ($N = 32$). N is the total number of young at birth.

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