mately 24-hour duration have also been noted, and such phenomena as heart rate, body temperature and overall metabolic rate have been shown to have daily maxima which coincide with the active phase of the locomotor rhythm (*1*). These physiological oscillations led us to assume that the time required to detoxify certain drugs might also vary with respect to the phase of the animal's activity rhythm.

To explore this possibility, we administered intraperitoneal injections of Nembutal (0.1 mg per gram body weight) to seven male deer mice (*Peromyscus maniculatus rufinus*) at each of six predetermined times in their activity rhythms. The mice had previously been kept in cages where their activity was recorded, and from these records we chose the six times to represent different levels of activity in the daily cycle (*2*). (Three of the six times occurred during the active phase and three during the inactive phase of each animal's locomotor rhythm.) During the 4-week experimental period, each mouse received a total of 18 injections—that is, three injections at each of the six times being studied. Care was taken to keep injections at least 12 hours apart to insure that consecutive readings for any one mouse would be independent of one another. Precautions were also taken to prevent drifting of the activity rhythms. At the conclusion of the study, the mice were returned to the cages where their activity was recorded, and we found that no significant phase shifting occurred.

Several measures of the duration of the anesthetic effects of Nembutal were used. Of these, the time elapsed from the moment the animal first lost the ability to right itself until this ability was regained proved to be the most reliable as well as the least subjective and consequently this measure was used in the data analysis.

The results of the study demonstrate that the quantitative response of the deer mice to Nembutal was not constant throughout the daily locomotor cycle. The rate of recovery from anesthesia was 10 to 20 percent more rapid during the active phase than during the inactive part of the cycle.

When a two-way analysis of variance was carried out on the data (Table 1) a highly significant difference ($p << .005$) was found to exist between the rates of recovery at the different injection times. Upon subdividing this "time" factor into its component parts, it can be observed that the recovery rate during the active phase differed significantly from that during the inactive phase of the rhythm, but that within either phase no such differences occurred. This indicates that variance can be greatly reduced when comparisons of recovery rate are limited to tests on animals in similar circadian states.

Table 1. Analysis of variance for rates of recovery of *Peromyscus* from injections of Nembutal. (The raw data used in the analyses were the reciprocals of the recovery times, in minutes, × 100.)

Source	Degrees of freedom	Sums of squares	Mean square	*F* ratio	*p*
Times	5	301.37	60.27	8.70	⟨⟨.005
Between active and inactive phase	1	239.22	239.22	34.51	⟨⟨⟨.005
Within active phase	2	30.02	15.01	2.17	⟩.10
Within inactive phase	2	32.13	16.07	2.32	⟩.10
Mice	6	749.13	124.86	18.01	⟨⟨⟨.005
Interaction	30	292.81	9.76	1.41	⟩.10
Error	80	554.54	6.93		
Total	121	1897.84			

Whether a more detailed segregation of data would be profitable in further decreasing the amount of variance in the results would depend upon the variability of the circadian locomotor rhythms in the species in question.

The conclusion that the circadian rhythm has considerable influence on the rate of recovery from certain drugs is in accordance with the results of Halberg (*3*) and more recently of Davis (*4*) who showed a day-night periodicity in the response of *Mus musculus* to pentobarbital.

The implications of these types of studies should have far reaching importance in the fields of physiological and pharmaceutical research where various drugs are being tested for their effects on experimental animals. The role of circadian rhythms in studies of drugs has only recently received widespread attention and consequently, precautions are often not taken to control this source of variance. Animals kept in rooms which are not exposed to regular light-dark cycles can easily have their activity rhythms disrupted. The same is true of animals kept under conditions of constant light. If several animals, whose rhythms were greatly out of phase with each other, were tested as a group to note the effects of a drug or chemical, errors could ensue; proper results could be obscured by the high degree of variance due simply to differences in the times of locomotor phases of the experimental subjects.

We suggest, therefore, that experimental animals be kept under conditions of normal light-dark cycles (without interruptions), and that the phase of the activity rhythm during which a drug is administered be noted (*5*).

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Retention in Rats: The Effect of Proactive Interference

Abstract. *Four groups of rats were trained to make a simple spatial discrimination. Two of the groups had previously been trained to make the reverse discriminations, the other two had no prior training. Two groups were tested 1 day after training, while two others were tested 44 days after training. There was no retention loss unless prior interference had been provided.*

It is generally believed that one of the major causes of forgetting is interference. The most recent version of interference theory was proposed by Underwood (*1*), who suggested that much of forgetting is due to the interfering effect of competing responses

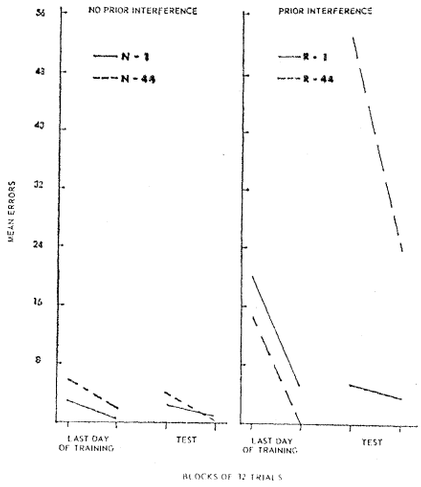

Fig. 1. Mean number of errors as a function of retention interval and proactive interference.

acquired before original learning (proactive inhibition). According to Underwood, such inappropriate responses are extinguished in the course of original learning but are recovered spontaneously during the retention period. A number of studies on verbal learning provide support for this hypothesis: of particular importance is the finding that forgetting increases with increasing degrees of prior learning (*1*) and that proactive inhibition increases with increasing time intervals between original learning and retention tests (*2*). To date, no corresponding confirmations have been reported at the level of animal behavior; in fact, there have been some negative findings (*3*, *4*). This presents a serious problem for modern interference theory which is couched to a large extent in the conceptual terms of animal learning and conditioning and might, therefore, be expected to apply at that level at least as well as it does to the verbal learning of humans. In this report we present some results indicating proactive inhibition of a simple spatial discrimination in rats.

Twenty adult male rats were trained in a modified Skinner-box with two pigeon-keys on one wall and a lever on the other. The animals were maintained at 85 percent of their satiated body weight, run on a 24-hour food deprivation schedule, and were rewarded with 45 mg of food pellets. Each session consisted of 64 discrete trials with a 15-second interval between each trial.

To start any trial, the subject first had to press the lever in order to light up the keys. The task then was to press the correct key ten times (not necessarily in succession). The trial was over and the reward was presented when ten correct key-presses had been accumulated. This procedure allowed us to utilize a more sensitive measure of discrimination than a dichotomous choice score—the number of false responses per trial.

Four groups of five animals were used. All the animals first learned to choose one key rather than the other. Training them to make this spatial discrimination continued until they reached a criterion of 58 or more errorless trials in any one daily session of 64 trials. Two of the groups (N-1 and N-44) were "nonreversal" groups: they received no further training but were tested either one day or 44 days following the criterion session. The retention test consisted of one relearning session, in which the procedure was identical to that used during training. The remaining two "reversal" groups (R-1 and R-44) were trained to make the spatial discrimination in the reverse of that which they had first mastered. They were trained until they reached the same criterion as before; retention was tested either 1 day or 44 days after they reached the required standard.

The mean number of sessions required to reach the criterion of discrimination by the nonreversal animals, and by the reversal animals was not significantly different for the four groups ($F<1$). The means were 2.8 and 4.0 for groups R-1 and R-44, respectively; for groups N-1 and N-44, both means were 3.2. However, as Fig. 1 shows, there was some difference in the number of errors on the last day of training indicating some negative transfer from the earlier discrimination for the reversal animals. The mean number of errors in the criterion session (based on 6 trials at the most per animal, since at least 58 trials had to be errorless) was somewhat greater for the reversal groups ($F = 8.93$, $df = 1/16$, $p< .01$).

The effect of prior reversal was most dramatic on the retention test. As Fig. 1 shows, there was no retention loss unless prior interference was provided. An analysis of variance was performed on the difference scores for errors on test and final training sessions, yielding a significant interaction between retention period and presence of prior interference ($F = 11.01$, $df = 1/16$, $p < .01$). There was rapid relearning, but a considerable retention loss was nevertheless still evident in the second half of the test session. The interaction was again significant ($F = 4.98$, $df = 1/16$, $p < .05$).

The results of this experiment are in agreement with modern interference theory: as predicted, proactive inhibition increases with time. Not all studies of retention in animals yield results as favorable to the theory. Gleitman and Steinman found no increased retention loss of runway performance in rats when final training was preceded by extinction (*3*); perhaps prior interference (for example, exploratory and fear responses) was already at a maximum, so that extinction training could add little more. Using pigeons, Kehoe did not obtain proactive inhibition in the retention of a visual discrimination (*4*). For a possible explanation, Kehoe points to her use of the noncorrection method (that is, following a false response the trial was ended without any chance for "correction") since by this procedure the old response would be quite thoroughly, and perhaps irrecoverably, extinguished. The results of our experiment in which the correction method was used, lend some credence to this suggestion (*5*).

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