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Variable Decay of Memory and Its Recovery in Cycloheximide-Treated Mice

(amnesia/protein synthesis/discrimination task)

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ABSTRACT **Mice were trained in an automated visual-discrimination task. When injected with cycloheximide before training, mice showed: (*a*) some impairment of memory within minutes after the beginning of training, (*b*) gradual development of total amnesia in less than 3 hr or in more than 6 hr after training, depending on the extent of training, and (*c*) spontaneous recovery of memory within 3 days after training. The results are consistent with the operation of three processes of memory storage: (*i*) a short-term process that is independent of protein synthesis, (*ii*) a long-term process that is dependent on protein synthesis, and (*iii*) a slow-developing memory storage that becomes apparent during the days after training.**

Previous studies on effects of cycloheximide and acetoxycycloheximide on learning and memory in mice given brief training have repeatedly demonstrated normal acquisition in the presence of these potent inhibitors of cerebral protein synthesis, normal retention for about 3 hr, and then marked and permanent amnesia by 6 hr after training (1, 2). These experiments suggested a simple view of learning and memory in mice. Since acquisition and retention for at least 3 hr were normal during marked inhibition of cerebral protein synthesis, it seemed reasonable to postulate a "short-term" memory storage process that is independent of protein synthesis, with a fixed lifetime of 3–6 hr. Since long-term retention was impaired only if the drug were given shortly before or very shortly after training, a "long-term" memory storage process that is dependent on protein synthesis was also postulated that is normally initiated during training or within minutes thereafter. With decline of the proposed "short-term" process after 3 hr, absence of the "long-term" process is detectable and amnesia is observed.

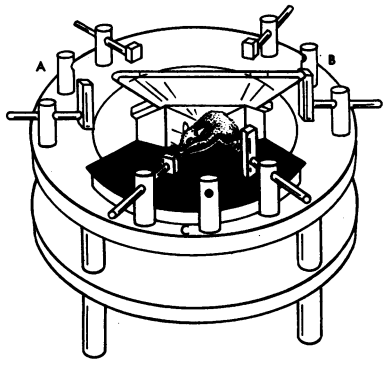

FIG. 1. The Deutsch Carousel for training a discrimination habit in mice. Mice are placed in the apparatus while facing position *A*. The mouse is then rotated to position *B* for trial one, and then to adjacent positions for subsequent trials. At each position, the mouse must touch the small object to escape shock. Once the mouse has been secured in the apparatus, the entire training procedure is controlled by a PDP/8L computer.

Recent experiments in a multiple-trial, automated discrimination task and the work of others suggest that the situation is somewhat more complex. In the present report, we describe studies indicating that (*a*) cycloheximide impairs the gradual acquisition of a discrimination task when training is continued beyond 20–30 trials, although it does not affect acquisition up to this point, (*b*) memory is detectable for less than 3 hr after training when cycloheximide-treated mice are given brief training, but considerable memory is detectable for more than 6 hr after training when cycloheximide-treated mice are given more extended training, and (*c*) once amnesia has developed, it may persist for only several days, followed by complete, spontaneous recovery of memory. We will present an interpretation of these results that is consistent with the existence of at least three processes in memory.

MATERIALS AND METHODS

Female mice (F_1 hybrids of 3CH/St male and BALB/c female inbred strains, bred in our laboratory) were housed 8 to a cage, and were trained when they reached 8 weeks of age (about 22 g each). Training was conducted in the newly developed, automated Deutsch Carousel (Fig. 1) controlled by a PDP/8L computer. The mouse learned a 2-choice discrimination habit by being rotated sequentially to three positions, each marked by two discriminating features: one large and one small stainless steel bar. A 0.4-mA shock delivered to the tail and feet of the mouse 5 sec after arrival at a new position could be terminated by touching the small object. 5 Sec later, the mouse was rotated to the next position. Rotation of the mouse between positions required 10 sec, so that the total intertrial interval, including delay time and rotation time, was 15 sec. A correct trial was recorded when the mouse touched the small object before touching the large object. An incorrect trial was recorded when the mouse touched the large object before touching the small object. For each consecutive trial, mice were always rotated to an adjacent position. Rotation of the mouse through the three positions was so programmed that positions where the correct object was to the mouse's left were visited 43% of the time and positions where the correct object was to the mouse's right were visited 57% of the time. This asymmetry was preferred to the alternative procedure, which would have required that mice not always be rotated to adjacent positions and that the intertrial interval be irregular. Mice were trained by presenting them with a fixed number of trials and later were

tested for retention by giving them the same number of trials with the same procedure. Retention was evidenced by an increase in the number of correct responses made during the second session, compared to the number of correct responses made during training.

Mice were subcutaneously injected with 120 mg of cycloheximide, or an equivalent volume of the saline solvent/kg of body weight, 30 min before initial training or in a few instances 30 min after training. Inhibition of incorporation of [^{3}H]leucine into cerebral protein by cycloheximide was also determined. Mice received a subcutaneous injection of 10 μCi of L-[4,5-^{3}H]leucine (56 Ci/mmol, New England Nuclear Corp.) 15 min after subcutaneous injection of the cycloheximide. The mice (4 injected with cycloheximide, 4 controls, injected with saline) were killed 30 min after injection with [^{3}H]leucine, and incorporation into cerebral protein in both groups was determined (3).

Appropriate *t*-tests were used to compare the retention and training scores of individual groups of mice and to evaluate differences between the retention scores of independent groups.

RESULTS

Inhibition of Protein Synthesis. Administration of 120 mg of cycloheximide/kg of body weight subcutaneously inhibited about 95% of cerebral protein synthesis in the period 15–45 min after injection of the drug. Thus, the extent of inhibition by cycloheximide observed in this group of mice is comparable to that observed previously in Swiss albino mice (4, 5, 7).

Initial Training. Cycloheximide did not affect acquisition of the discrimination task, when training was conducted for 15 or 21 trials. When training was continued for 21 trials, 130 mice that were injected with saline accumulated an average of 12.9 correct responses and 151 mice that were injected with cycloheximide accumulated an average of 12.6 correct responses. Each of the two groups of mice reached a final level of performance of about 65% correct responses, and the learning curves were similar. These results are in agreement with previous studies with inhibitors of protein synthesis, where training was typically completed in less than 20 trials and where the learning curves of experimental and control groups were superimposable (6). Further training for an additional 21 trials, however, revealed an impairment in the performance of cycloheximide-treated mice (see 10-sec retention interval for mice trained for 21 trials, Fig. 3*B*). Thus, with extended training, an effect of cycloheximide on acquisition can be demonstrated. This impairment has been explored in detail and cannot be explained by sickness or by known side effects of cycloheximide on activity (manuscript in preparation).

TABLE 1. *Effects of cycloheximide on memory after different levels of training*

Drug	Number of trials	N*	Trials correct training	Trials correct retention	Δ Trials
Saline	15	27	8.0	10.7†	2.7
Cycloheximide	15	27	9.1	9.2‡	0.1
Saline	21	30	12.7	15.8†	3.1
Cycloheximide	21	42	11.9	12.7‡	0.8
Saline	27	21	17.8	22.2†	4.1
Cycloheximide	27	19	17.4	20.3†	2.9

All mice were injected subcutaneously with 120 mg of cycloheximide/kg of body weight or saline 30 min before training for 15, 21, or 27 trials in the Deutsch Carousel. During a retention test 24 hr later, each mouse received the same number of trials it had received during training. * N, number of mice in each group; † significantly greater than training score, $P < 0.05$; ‡ significantly less than corresponding saline group, $P < 0.05$.

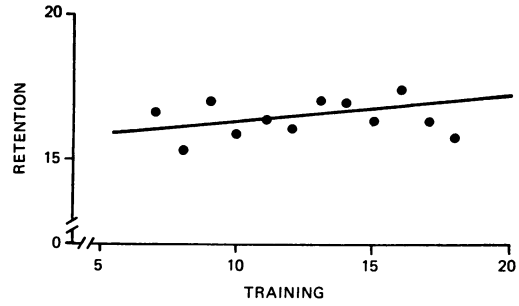

FIG. 2. The number of correct responses out of 21 trials obtained during retention for subgroups of mice that obtained the indicated number of correct responses during training. The data were obtained from saline-injected mice (N = 162) that were trained for 21 trials and retested by giving them another 21 trials 3 hr to 7 days later. The nonsignificant correlation coefficient '*r*' is 0.07.

Measurement of Retention. When training involves a fixed number of trials rather than the achievement of a learning criterion, percent saving scores (3) cannot be used to measure retention. For the "fixed-trials" procedure, we determined that a consistently reliable index of retention is the number of correct responses made during the second session. A regression analysis of training against retention scores indicated that the retention score was not significantly affected by observed variation in the training score (Fig. 2). Mice making very few correct responses during initial training performed as well in the retention test as mice that made many correct responses during initial training.

Effect of the Degree of Training on the amnesia induced by treatment with cycloheximide was examined by giving 15, 21, or 27 training trials. Mice were trained 30 min after injection of cycloheximide or saline and were tested again 24 hr later (Table 1). Mice given cycloheximide and trained for 15 or 21 trials were markedly amnesic 24 hr later, and performed significantly worse during retention than their respective saline-control groups. The amnesic effect of cycloheximide, when it occurred, was apparent on the first trial of the retention test. These results confirm the amnesic effects of cycloheximide that were observed after brief training in the T-maze (5, 7) and that have also been observed in the passive avoidance task (8) and with goldfish in the shuttlebox (9). When training in the Carousel was extended to 27 trials, mice given cycloheximide exhibited significant retention and were not measurably different from the saline-control group ($P > 0.1$, $t = 1.6$). Thus, extended training in this task protects mice from the amnesic effects of cycloheximide, just as extended training in a spatial task or a light–dark discrimination to a 9/10 or 15/16 criterion, respectively, protects mice from the amnesic effects of acetoxycycloheximide (6).

Rate of Development of Amnesia after Training. Having established that cycloheximide can produce amnesia for this discrimination task 24 hr after initial training, we next determined the time after initial training when amnesia for this task develops. Mice were given 15 or 21 trials in the Deutsch Carousel 30 min after injection of cycloheximide or saline. Mice given 15 trials were tested for retention by exposure to 15 additional trials after 10 sec (these mice received 30 consecutive trials), 15 min, 1 hr, 3 hr, or 24 hr (Fig. 3*A*). Mice trained for 21 trials were tested for retention by exposure to 21 additional trials after 10 sec (these mice received 42 consecutive trials), 1 hr, 3 hr, 6 hr, 12 hr, or 24 hr (Fig. 3*B*). Initial training for 15 or 21 trials was not affected by cycloheximide. Mice given 15 training trials developed complete amnesia within 3 hr. At this time, mice injected with cycloheximide performed significantly worse than the corresponding control mice that were injected with saline and did not score significantly better than their own initial training score. For mice given 21 training trials, considerably more time was required for memory to decay to a point where it was no longer detectable. Whereas with 15 trials amnesia appeared 3 hr after training, when 21 trials were given complete amnesia developed within 12 hr. Significant retention was observed 3 hr and even 6 hr after training.

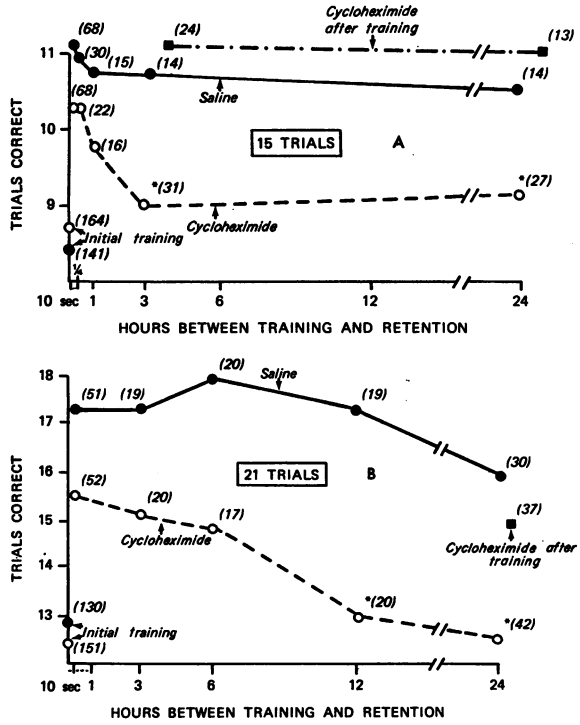

FIG. 3. Effects of cycloheximide on memory of mice tested for retention at different times after training. Mice were injected with cycloheximide (120 mg/kg of body weight), and 30 min later were given 15 or 21 training trials in the Deutsch Carousel. Retention was tested at various times later by giving an additional 15 or 21 trials. Numbers in parentheses indicate the number of mice in each group. (*A*) For mice given 15 training trials, two additional groups of mice were given cycloheximide 30 min after training and tested either 4 or 25 hr after training. Retention in the cycloheximide-treated mice tested 3 hr after training was significantly poorer than in the corresponding group that was injected with saline ($P < 0.05$, $t = 2.2$), or in the corresponding group that was injected with cycloheximide after training ($P < 0.05$, $t = 2.6$). Retention in the cycloheximide-treated group tested 24 hr after training was also significantly poorer than in the corresponding group that was injected with cycloheximide after training ($P < 0.05$, $t = 2.2$). Difference between the cycloheximide-treated and saline-treated groups tested 24 hr after training were not significant ($P > 0.1$, $t = 1.6$), although significance was obtained for this comparison with a larger N (Fig. 4*A*). (*B*) For mice given 21 training trials, an additional group of mice was given cycloheximide 30 min after training and tested 25 hr after training. The cycloheximide-treated group tested 24 hr after training was significantly different from the group injected with cycloheximide after training ($P < 0.01$, $t = 3.0$). Every group given cycloheximide before training is significantly different during the retention test from the corresponding saline-treated group ($P < 0.05$).

* Not significantly greater than the initial training score of this group.

Amnesia Is Not Due to Sickness during Retest. To test for the possibility that amnesia observed after training might have been due to sickness that impaired performance, we assessed performance when cycloheximide was injected shortly after training. Mice were given cycloheximide 30 min after 15 trials of training and were tested for retention 4 or 25 hr after training. Significant retention was exhibited by both groups given cycloheximide after training (Fig. 3*A*). A third group given cycloheximide 30 min after training for 21 trials, and retested 25 hr later, also exhibited significant retention. (Fig. 3*B*). All the groups injected with cycloheximide after training performed significantly better than the corresponding groups injected with cycloheximide 30 min before training.

Recovery of Memory. Since inhibition of cerebral protein synthesis is not complete at the time of training, the potential conceivably exists for expression of memory at a later time. The duration of amnesia produced by cycloheximide was examined by training mice for 15 or 21 trials 30 min after injection and retesting them 1–7 days later. In contrast to previous findings in discrimination (3–6), passive avoidance (8), and shuttlebox (9) tasks, where amnesic effects were permanent within the limits of testing, the amnesia found in the present experiments was spontaneously reversible (Fig. 4). Recovery appeared to be complete after 3 days, since cycloheximide-treated mice were not distinguishable from saline controls after this time.

DISCUSSION

Previous studies, in which memory in cycloheximide-treated mice lasted for several hours and then declined irreversibly, could readily be interpreted in terms of two processes: a "short-term" process that is independent of protein synthesis and that persists for at least 3 hr after training, and a second "long-term" process that is dependent on protein synthesis. The present experiments indicate that the situation is more complex. We believe that the data can be best interpreted in terms of three processes of memory storage: (*a*) a short-lasting process that is independent of protein synthesis and that is fully established for only minutes after training, though it may persist in partial form for some period thereafter; (*b*) a process that is dependent on protein synthesis and that is normally required within minutes after training for full expression of memory, and (*c*) a slow-developing, memory storage process that is manifested during the days after training.

Process 1: Cycloheximide-Insensitive Process That Lasts for Minutes and Possibly Hours at Partial Strength. As with mice trained in the T-maze, cycloheximide-treated mice trained in the Deutsch Carousel for 21 trials showed normal acquisition. A striking finding of the present experiments is that cycloheximide exerted a measurable amnesic effect when an additional 21 trials were given immediately thereafter (see Fig. 3*B*, 10-sec retention group). It is thus reasonable to suppose that memory required for normal performance during the first few minutes of training can be mediated by a "short-term" process that is independent of protein synthesis. As training continues, impairment of a cycloheximide-sensitive process apparently attenuates further improvement. Thus, the first process is postulated to be at full strength for only a few minutes.

Process 2: Cycloheximide-Sensitive Process, Active within Minutes after Training, and Persists for At Least Several Days. The impairment in cycloheximide-treated mice that can be observed almost immediately after training is taken as evidence that within minutes after the start of training, protein synthesis is required for complete expression of memory. This impairment is at first small, but within a few hours develops into total amnesia that lasts for 2 days. Therefore, it is postulated that under normal circumstances, a memory process that is independent of protein synthesis is very quickly succeeded by a memory process that lasts for a relatively long duration, and that depends on cerebral protein synthesis.

The basis for survival of partial memory for hours after training cannot be determined from the present experiments. The earlier "short-term" process that is independent of protein synthesis could persist for a variable number of hours, depending on the degree of training and on the nature of the task. Alternatively, partial memory that persists for hours after training could reflect an attenuated form of the postulated later process that is sensitive to cycloheximide. Since a small amount of protein synthesis always escapes inhibition after treatment with cycloheximide (1, 2), abnormally rapid loss of memory during the hours after training might reflect the gradual loss of a weakly established process that depends on cerebral protein synthesis. Hence, it need not reflect decay of a "short-term" memory process that is independent of protein synthesis and that has a fixed lifetime of several hours as we and others (10) have previously argued. Recent studies with electroconvulsive shock have also reported that the time required for the retention score to decrease to asymptote is variable, and depends on the time after training when the electroconvulsive shock is administered (11, 12) or on the magnitude of the shock (13).

Process 3: A Slow Developing, Memory Storage Process That Becomes Apparent within Days after Training. In our previous experiments with the T-maze (1, 2) and in a "passive-avoidance task" (8), the amnesia that developed after training did not disappear, although retention was tested in some instances as long as 6 weeks later. In the present experimental situation, amnesia persists for less than 3 days. Recovery from cycloheximide-induced amnesia has also been reported recently for the "passive-avoidance task" when mice were trained with a high-intensity shock to the feet, although recovery was not observed when a weaker shock was used during training (14). Likewise, rats given discrimination training after treatment with acetoxycycloheximide were amnesic after 1 day but exhibited significant retention 7 days after training (15, 16). Whereas it is easy to explain permanent amnesia in our earlier experiments by postulating that the cerebral protein synthesis required for memory is blocked during training, the finding of recovery is more difficult to interpret.

The amnesia that is observed cannot easily be attributed to sickness or to a general deficit in "retrieval", as others have suggested (14, 16). Such an effect of the drug should be equivalent 1 day after training, whether the drug is given just before or 30 min after training. The fact that the deficit occurs only when the drug is given before training strongly suggests that temporary amnesia is due to specific impairment of the *memory storage process*.

The finding that memory recovers after a period of sustained amnesia raises, in our view, the necessity to postulate a third process in memory storage that develops gradually during the days after training. Our argument for such a slowly developing process rests heavily on earlier findings that memory is differentially susceptible to amnesic treatment during this period. For example, puromycin (17) or actinomycin-D (18) causes amnesia when injected into the temporal regions of the mouse brain 1 day after training, but

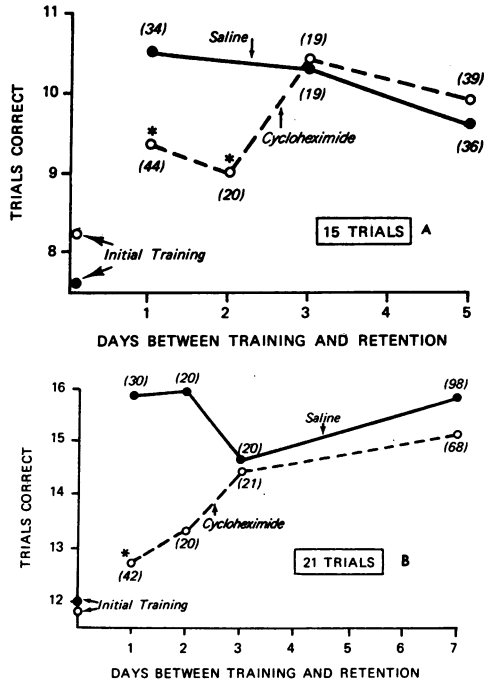

FIG. 4. Mice were given 15 or 21 trials 30 min after injection of cycloheximide (120 mg/kg) and were given the same number of trials 1–7 days later. Numbers in parentheses indicate the number of mice in each group. (*A*, 15 trials) Mice given saline performed significantly better than mice given cycloheximide 1 day after training ($P < 0.05$, $t = 2.2$). Cycloheximide-treated mice and saline-treated mice were indistinguishable at later times. (*B*, 21 trials) Saline-treated mice performed significantly better than cycloheximide-treated mice 1 day ($P < 0.001$, $t = 4.1$) and 2 days ($P < 0.05$, $t = 2.4$) after training. The group given cycloheximide and tested 7 days after training exhibited significant savings ($P < 0.001$, $t = 4.7$) and was significantly different from cycloheximide-treated mice tested 1 day ($P < 0.001$, $t = 4.2$) or 2 days ($P < 0.01$, $t = 2.5$) after training.

* Not significantly greater than the initial training score of this group.

these drugs are without effect when injected 7 days after training. Likewise, experiments with cholinergic drugs suggest that normal memory changes between 1 and 7 days after training (19).

It seems reasonable that this slow-developing process, that appears to operate under normal circumstances, is the basis for recovery of memory in cycloheximide-treated mice. Recovery of memory within 3 days is not peculiar to this hybrid variety of mice, since cycloheximide exerted marked amnesia in this same variety 3 days after training in the T-maze (20). The present experiments further suggest that this slow process in memory can be dissected from earlier stages of memory formation. If the slow process underlying gradual changes in memory during the days after training were simply the direct continuation of processes that mediate memory for a few hours, impairment of retention soon after training should be followed by an equally severe impairment of memory days after training. We therefore suggest that recovery depends on a *discrete process*, which may appear gradually even when prior processes have been impaired. It seems likely that the development of this process, too, is ultimately dependent on cerebral protein synthesis, since in maze studies cycloheximide administration before training can permanently abolish it. The possible relationship of this recovery phenomenon to other known slow biological processes has been discussed elsewhere (21).

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