*AROUSAL AND THE CONVERSION OF "SHORT-TERM" TO "LONG-TERM" MEMORY**

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We have reported[1-3] that mice trained while their cerebral protein synthesis was markedly inhibited by acetoxycycloheximide learned normally and remembered normally for more than three hours after training but had markedly impaired "long-term" memory.[4] These studies suggest that learning and "short-term" memory are not dependent on cerebral protein synthesis, but that it is necessary for the establishment of "long-term" memory. Acetoxycycloheximide was found to be maximally effective in impairing the formation of "long-term" memory if it was given prior to training so that inhibition of cerebral protein synthesis was established during training.[1-3] The drug was slightly effective if given immediately after training[3] and completely ineffective if given 30 minutes later. This suggests that the cerebral protein synthesis apparently required for "long-term" memory normally occurs during training or within minutes after training, or both.

In the previous experiments, it was not possible to determine whether "short-term" memory could lead to the formation of "long-term" memory at times later than a few minutes after training. Although "short-term" memory persisted for hours after training, inhibition of cerebral protein synthesis by acetoxycycloheximide also persisted for this period of time. Therefore, "conversion" of "short-term" memory to "long-term" memory hours after training would presumably be blocked by the persistent action of the inhibitor of cerebral protein synthesis. Recently, we have found that cycloheximide inhibits cerebral protein synthesis as intensely but more transiently than acetoxycycloheximide, and shares with it the ability to prevent the formation of "long-term" memory.[5] In the present experiments, the doses of cycloheximide that were used inhibited approximately 95 per cent of cerebral protein synthesis during training but permitted very marked recovery within three hours after training. Despite the extensive recovery of the capacity of the brain to synthesize protein at a time when "short-term" memory still remained, "long-term" memory was not spontaneously established. However, the introduction of manipulations that generate "arousal" led to the development of "long-term" memory if introduced at a time when "short-term" memory persisted (i.e., 3 hr after training). The reestablishment of marked inhibition of cerebral protein synthesis prior to the onset of the "arousal"-producing manipulation blocked the establishment of "long-term" memory.

Materials and Methods.—Male Swiss albino mice weighing approximately 30 gm were obtained from the Charles River Breeding Corp. They were trained to escape shock by choosing the lighted limb of a T-maze to a criterion of five out of six consecutive correct responses as described previously.[2] In other experiments, they were trained to choose the left limb of a T-maze for water reinforcement to a criterion of three out of four consecutive

correct responses as described previously.[5] From 12 to 15 mice were used for each time point in the behavioral studies. Savings were calculated as described previously.[1] Statistical analysis of the data was made with the Mann-Whitney U test.

The drugs used were obtained from commercial sources, except that acetoxycycloheximide was the gift of Dr. T. J. McBride, John L. Smith Memorial for Cancer Research, Charles Pfizer and Co., Maywood, N.J. (supported by contract PH-43-64-50). Drugs were dissolved in 0.15 *M* saline and injected subcutaneously on the back. Dextroamphetamine was used where indicated and is referred to as amphetamine. The corticosteroid mixture contained 5 mg each of hydrocortisone and corticosterone per ml of dimethylsulfoxide. Incorporation of valine-1-C^{14} into protein and inhibition of incorporation by cycloheximide were determined as described previously.[1, 3] At least three separate determinations were made at each time point. The degree of inhibition of protein synthesis is somewhat overestimated by this method of calculation as indicated previously,[3] and this is particularly true when the calculated degree of inhibition is small.

Results.—Administration of 0.12 gm/kg cycloheximide subcutaneously inhibited approximately 95 per cent of cerebral protein synthesis within the first 30 minutes after injection. With the dose used, inhibition subsided fairly rapidly. In the interval between 3 and $3^1/_2$ hours after injection, only 19 per cent of cerebral protein synthesis was inhibited and in the subsequent 30-minute interval, an average of 9 per cent inhibition was found. Mice injected with this dose of cycloheximide 30 minutes before training learned normally and remembered normally 3 hours after training but had markedly impaired memory 6 hours after training and thereafter (Table 1). Therefore, "long-term" memory was not formed despite marked recovery of the capacity for cerebral protein synthesis at a time when "short-term" memory remained. However, the slight inhibition of cerebral protein synthesis that remained 3 hours after training (in the range of 9–19% inhibition) might be sufficient to prevent the formation of "long-term" memory, although inhibition of at least 90 per cent of cerebral protein synthesis during training was previously found to be necessary for significant interference with the establishment of "long-term" memory.[1]

Attempts were then made to determine whether or not "long-term" memory could be induced by various manipulations of cycloheximide-injected mice 3 hours after they were trained. The effect of the administration of a brief foot shock at this time was studied. Mice trained 30 minutes after injection of cycloheximide were removed from their home cage at several times after training, placed on an electric grid, and given a 0.5-ma shock of 0.5-second duration. If foot shock was administered 3 hours after training, the mice had an average of

TABLE 1. *Effect of cycloheximide on "short-term" and "long-term" memory.*

Injection	Time tested	Per cent savings
Saline	3 hr	75
	6 hr	73
	7 days	76
Cycloheximide	3 hr	73
	6 hr	28
	7 days	31

Mice were injected with 0.12 gm/kg cycloheximide 30 min before being trained to escape shock by choosing the lighted limb of a T-maze to a criterion of five out of six consecutive correct responses.[2, 3] Savings were calculated as described previously.[1] Groups of 15 mice were tested for retention at the indicated times. The cycloheximide-injected mice did not differ significantly from controls if tested 3 hr after training but differed significantly ($P < 0.01$) if tested at 6 hr or 7 days.

63 per cent savings when tested 7 days later and differed significantly ($P < 0.01$) from a cycloheximide-treated group that received no foot shock and that had 27 per cent savings 7 days after training. In contrast, administration of foot shock 6 hours after training, a time when "short-term" memory had terminated, did not significantly induce "long-term" memory. This group had an average of 22 per cent savings when tested 7 days after training. Therefore, a single foot shock without any additional discriminative training, if given while "short-term" memory persisted, could lead to the establishment of "long-term" memory.

The mechanism by which the foot shock led to the establishment of "long-term" memory could not be determined from this experiment. Several aspects of foot shock were considered. First, the foot shock resembled the shock received during training and might thereby act as a "reminder." It has been reported that a "reminder" shock, given prior to testing to animals which received electroconvulsive shock after training, improves retention,[6] but the experimental design differed so markedly from the one we used that this phenomenon may have little relationship to that reported here. A second possibility is that the foot shock aroused the animal and that this state of "arousal" might be necessary for the formation of "long-term" memory. To evaluate the possible role of "arousal," the effect of dextroamphetamine and corticosteroids, two drugs that produce "states of arousal,"[7] was studied. Controls were handled identically and injected with either 0.15 *M* saline or with dimethylsulfoxide.

Cycloheximide-treated mice injected with amphetamine 3 hours after training showed much greater memory than controls when tested either 3 hours or 7 days later (Table 2). Amphetamine injected 3 hours after training in 15 saline-injected mice had no effect on performance measured 7 days later. Therefore, this drug had no discernible action if "long-term" memory was established normally. Injection of amphetamine 6 hours after training in cycloheximide-injected mice had no significant effect on memory tested 3 hours or 7 days later (Table 2). Injections of amphetamine 3 hours before testing had no effect on memory at 7 days (Table 2). Likewise, injections of amphetamine 30 minutes before training along with the cycloheximide had no effect on memory when tested 7 days after training (Table 2). These experiments indicate that if amphetamine injections are given at a time when cerebral protein synthesis inhibition is slight and when "short-term" memory remains, "long-term" memory

TABLE 2. *Effect of amphetamine on memory in cycloheximide-treated mice.*

Time amphetamine given	Time tested	Per cent savings
——	7 days	31
3 hr	6 hr	54
3 hr	7 days	62
6 hr	9 hr	32
6 hr	7 days	39
7 days	7 days + 3 hr	28
−30 min	7 days	25

Mice (12 in each group) were injected with cycloheximide 30 min before training as indicated in the legend of Table 1. Dextroamphetamine (1 mg/kg) was injected subcutaneously at various times after training or 30 min before training as indicated. The group that received no amphetamine was injected with saline 3 hr after training. The mice were tested at the indicated times after training. Mice injected with amphetamine 3 hr after training had significantly more savings ($P < 0.01$) than any of the other groups.

is formed. Administration of the amphetamine after "short-term" memory has terminated (at 6 hours in this situation) is ineffective.

Similar results were found when a mixture of corticosteroids was used. Administration of 0.1 ml. of the corticosteroid mixture 3 hours after cycloheximide-injected mice were trained significantly improved "long-term" memory. The mice injected with cycloheximide 30 minutes before training and with corticosteroids 3 hours after training had 58 per cent savings when tested at 6 hours, and 60 per cent savings when tested at 7 days. Controls that received only dimethylsulfoxide 3 hours after training had 29 per cent savings 7 days later. However, cycloheximide-treated mice injected with corticosteroids 6 hours after training, a time when "short-term" memory had markedly declined, had 30 per cent savings when tested 9 hours after training and 22 per cent savings when tested at 7 days. The group injected with corticosteroids 3 hours after training differed significantly ($P < 0.01$) from the controls, whereas the group injected with corticosteroids at 6 hours had no significant enhancement of savings.

Administration of 0.12 gm/kg of cycloheximide or 8 mg/kg of acetoxycycloheximide $2^1/_2$ hours after training, so that 95 per cent inhibition of cerebral protein synthesis was again established when the amphetamine was administered, markedly antagonized the amphetamine effect (Fig. 1). Acetoxycyclo-

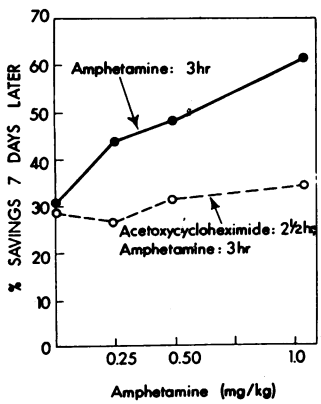

FIG. 1.—Antagonism of the amphetamine effect by injection of acetoxycycloheximide. All mice (each point represents a group of 12) were injected with 0.12 gm/kg of cycloheximide 30 min before training. Acetoxycycloheximide (8 mg/kg) was injected $2^1/_2$ hr after training, where indicated. In the ensuing 30 min, 93% of cerebral protein synthesis was inhibited. The indicated dose of dextroamphetamine was administered 3 hr after training. All mice were tested for retention 7 days later.

heximide was found to be somewhat more effective than cycloheximide. This may be due to its longer duration of action, whereby inhibition of cerebral protein synthesis persisted throughout the period when the arousing effect of amphetamine remained.

To evaluate the hypothesis that the amphetamine was exerting its effect through "arousal," the effect of administration of 40 mg/kg of pentobarbital concurrently with 1 mg/kg amphetamine was determined. This combination produced sedation. The 15 cycloheximide-treated mice injected 3 hours after training with a combination of amphetamine and pentobarbital had 34 per cent savings 7 days later. This was significantly less than the savings of the mice treated with amphetamine alone. Therefore, sedation obliterates the action of amphetamine. Neither cycloheximide nor acetoxycycloheximide administered subcutaneously has any sedative effects on mice, and indeed their behavior ap-

peared quite normal at a time when maximal inhibition of cerebral protein synthesis was achieved. Therefore, the inhibitors of protein synthesis do not antagonize the amphetamine effect by producing sedation.

Since large doses of protein synthesis inhibitors are known to produce illness,[1] the possibility that amphetamine or corticosterone antagonize this illness must be considered. This might be particularly true of the corticosteroids that have multiple, potentially therapeutic properties. However, the dose of cycloheximide used in the present studies produced no significant signs of illness. Furthermore, it would be difficult to ascribe such therapeutic properties to amphetamine or to foot shock. Nevertheless, this possibility cannot be excluded. A possible stimulation of cerebral protein-synthesizing capacity by amphetamine injections in cycloheximide-treated mice was also considered. Injection of amphetamide with cycloheximide had no significant effect on inhibition of cerebral protein synthesis observed in the ensuing hour. Injection of amphetamine 3 hours after cycloheximide injection did not significantly stimulate cerebral protein synthesis measured in the following 30-minute interval. However, the degree of inhibition was already small and varied between about 5 and 25 per cent. Therefore, a relatively small change might not be readily detectable.

The shock escape task that we used would be expected to cause the release of large amounts of catecholamines and corticosteroids during training. The injection of amphetamine or corticosteroids might, therefore, produce a state reminiscent of training. Because of this, it seemed of interest to determine the effects of injections of amphetamine on memory in cycloheximide-treated mice trained in an appetitive task. Mice were injected with cycloheximide or saline 30 minutes before being trained to choose the left limb of a maze for water reinforcement, as described previously.[5] The saline-injected mice had 73 per cent savings when tested 7 days later, whereas the cycloheximide-injected mice had 33 per cent savings. Therefore, cycloheximide had an amnesic effect in this appetitive task, as observed previously.[5] Cycloheximide-treated mice injected with 1 mg/kg amphetamine 3 hours after training had 56 per cent savings 7 days later and differed significantly ($P < 0.05$) from controls. Cycloheximide-treated mice injected with amphetamine 6 hours after training had only 31 per cent savings. Therefore, the effectiveness of amphetamine is not confined to a shock-motivated task but is quite similar in a task in which water reinforcement is employed. Nevertheless, there is "arousal" during training in this appetitive task, and the amphetamine may be acting as a "reminder" in this situation as well. The "reminder," if this is what it is, is effective only if administered while "short-term" memory persists.

Discussion.—The mechanisms for "short-term" and "long-term" memory are not known. There is evidence, from studies in which disruptive manipulations are made at different times after training, that "long-term" memory is "consolidated" during a time period of seconds to hours after training.[8] A number of drugs, particularly analeptics, enhance "long-term" memory if given within minutes after training,[8] presumably by facilitating "consolidation." Although amphetamine injections have usually produced no significant effect on memory in normal animals, some enhancement has been observed in multiple-trial

avoidance training when the drug was given within 4 hours after each training session.[9] The fact that the enhancing effects of drugs and the disruptive effects of electroconvulsive shock may, under certain circumstances, occur when these manipulations are made within several hours of training has suggested that "short-term" memory may be available for "consolidation" for this relatively prolonged period. There is also evidence that the time when such "consolidation" begins can be influenced by the environment and presumably the state of the organism.[10]

The present studies have permitted a dissection of "short-term" memory and "long-term" memory by producing conditions in which "short-term" memory is established and persists for hours without the ultimate development of "long-term" memory. These studies suggest that if the establishment of "long-term" memory is prevented by inhibitors of cerebral protein synthesis administered prior to training, it will not subsequently develop spontaneously, despite persistence of the information in "short-term" memory concurrent with the marked recovery of the capacity for cerebral protein synthesis. However, manipulations that are thought to act by producing "arousal" will lead to the development of "long-term" memory. The view which emerges from these studies is that both the persistence of the cognitive information acquired from training and an intact cerebral protein synthesis capacity are insufficient for the production of "long-term" memory. An appropriate state of "arousal," which appears to specifically direct the establishment of "long-term" memory, also seems necessary. Livingston has speculated on the possible role of "arousal" in ordering the memory stage system to "Now print!"[11]

Summary.—Mice whose cerebral protein synthesis was markedly inhibited by cycloheximide during training learned normally, remembered normally for the following 3 hours, but had markedly impaired "long-term" memory. Impaired "long-term" memory was observed even though "short-term" memory persisted through the time when the capacity to synthesize cerebral protein had largely returned. Brief foot shock, amphetamine, or corticosteroids given 3 hours after training induced the formation of "long-term" memory, but this effect was blocked by resumption of inhibition of cerebral protein synthesis. Cognitive information, an intact capacity for cerebral protein synthesis, and an appropriate degree of "arousal" are all apparently necessary for the establishment of "long-term" memory.

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[4] Since mice that learned while cerebral protein synthesis was markedly inhibited remember normally for at least 3 hr but less than 6 hr, we have for convenience chosen to refer to memory for 3–6 hr as "short-term" memory and memory for 6 hr or more as "long-term" memory.

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