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Time Variables Affecting the Permanence of Amnesia Produced by Combined Cycloheximide and Electroconvulsive Shock Treatments

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ANDRY, M. L. AND M. W. LUTTGES. *Time variables affecting the permanence of amnesia produced by combined cycloheximide and electroconvulsive shock treatments.* PHARMAC. BIOCHEM. BEHAV. 1(3) 301–306, 1973.–Mice pretreated with saline or cycloheximide were trained in a multiple-trial avoidance apparatus. Electroconvulsive shock (ECS) was administered at various intervals following training. The animals were retrained either one day or about one week later. Both transient and permanent memory deficits were observed. Presence and type of memory deficit were dependent upon time of ECS treatment, time of retraining and whether the animals received saline or cycloheximide prior to original training. A possible relationship between the results observed and reported effects of training on macromolecular synthesis in the brain is discussed.

Amnesia Cycloheximide Electroconvulsive shock Memory Protein synthesis inhibitors

MANY antibiotic inhibitors of protein synthesis have been employed successfully to disrupt memory storage in experimental animals [1, 2, 3, 7, 10, 12, 28, 34]. Early studies with cycloheximide, for example, demonstrated that mice could learn simple tasks while under the influence of cycloheximide but unlike untreated mice would forget the task within about three hours after training [5]. Appropriate treatments can apparently protect or restore memory from losses produced by antibiotics [5, 11, 31]. In several studies, the memory losses produced by various protein inhibiting antibiotics appear to be temporary [30,32]. A dominant factor for whether the amnesic consequences of antibiotics are permanent or transient appears related to the number of trials, amount of time, or both, required for training [4, 12, 32, 34].

We have recently shown that mice treated with cycloheximide and electroconvulsive shock (ECS) appear amnesic for training on a single-trial, passive avoidance task [2]. Although ECS treatments produced amnesia only when given at short intervals following the training of saline treated mice, the ECS produced amnesic effects when given an hour after training of cycloheximide mice. Mice given cycloheximide without ECS exhibited good retention within the same time periods. The combined cycloheximide-ECS treatments, thus, produced amnesia that neither treatment was capable of producing when used alone. Direct treatment effects on performance or retrieval could not account for the amnesic effects which were shown to persist for at least three days. It remains possible, however, that longer training periods or more training trials might produce a more stable form of memory which would not be susceptible to combined cycloheximide-ECS disruption.

The following study demonstrates that combined cycloheximide and ECS treatments are capable of producing both transient and permanent effects on performance associated with memory. The time of ECS treatment is crucial in both saline and cycloheximide mice if relatively permanent memory deficits are to be produced. As in the simple avoidance study, combined ECS and cycloheximide treatments appear to produce amnesic consequences which neither treatment produces alone.

METHOD

Approximately 270 HS (25) mice were between 60 and 120 days of age when used. During experimental use, the mice were housed four to each cage and were allowed free access to food and water in the home cage. All experimental groups were balanced in regard to numbers of males and females.

The mice were trained and retrained in automated avoidance apparatuses similar to those described earlier [21]. Four matched training units are separately housed in acoustically dampened chambers. Each training unit is essentially a square Plexiglas box (≈22 cm in height, width, and depth) with a grid (3 mm dia. stainless steel rod) floor and an acoustic speaker (8 Ω, 6-1/2 cm dia.) mounted on the top. The top and front of each unit are made of clear Plexiglas and the remainder, of opaque white Plexiglas. In the front, two side by side cylinders or wheels (each 9 cm long, 7 cm dia.) extend halfway into the training unit about 2 cm above the grid floor. The unit was programmed to begin each trial with an 80 db (re: 0.0002 dynes/cm^2), 600 Hz signal from the overhead speaker. Approximately three sec later the grid floor was activated (1 ma, constant current; pulsed and scrambled). To terminate both the footshock and sound either front-mounted wheel had to be turned at least 1/4 turn. All trials were automatically terminated after 12 sec. The interval between trials was programmed to average 22 sec (14–28 sec). The number of avoidances (turning a wheel before the onset of footshock) and number of wheel-turns during 50 trials were recorded on counters and a paralleled event recorder. All apparatus variables were the same for original training and retraining.

The cycloheximide (160 mg/kg) and saline control mice received injections 30 minutes prior to avoidance training. Injections were given intraperitoneally at a volume of 10^{-2} ml per gram of mouse body weight through 27 gauge needles. The cycloheximide treatments on brain protein metabolism were assessed using intracranial injections (1.0 μ Ci total) of ^{14}C-lysine. Thirty minutes after cycloheximide treatments (the time that training usually began), mice were injected bilaterally with 15 μl (0.5 μ Ci) of ^{14}C-lysine. Each injection was made at a depth of 3 mm beneath the skull either into or near the lateral ventricles. After 10 min, the brains were removed and homogenized in 0–4° C 0.15 M NaCl. Protein was subsequently precipitated with 5% (W/V) trichloroacetic acid. A sample of supernatant was removed and the precipitate was solubilized and reprecipitated two additional times. Samples of the precipitate were removed to assess radioactivity content [18] and protein content [19]. Compared to saline animals, those mice receiving cycloheximide exhibited > 82% less radioactivity in recovered brain proteins. Soluble radioactivity levels were comparable for both saline and cycloheximide animals.

Treatments with ECS were administered transcorneally (30 ma, 0.2 sec, 500 Hz, square wave) and all mice received brief periods of assisted respiration whether required or not. All of the mice exhibited nearly immediate, full tonic convulsions and only two mice died from the ECS treatments. Mice treated with saline received ECS immediately or 6 hours after the end of training. Mice treated with cycloheximide received ECS immediately, 1 hr or 6 hr after the end of training.

Three separate experiments were conducted. In the first, mice were retrained 24 hr after the original training session. Each group contained 16 mice, of 60–70 days of age. In the second experiment, the animals were retrained 8 days after original training. Each group in the second experiment also contained 16 animals of 60–70 days of age. The third experiment was a replication of the second, except retraining occurred 7 days after original training. Each group of mice contained 16 animals of 60–70 days of age, plus an additional 12 animals of 90–120 days of age. No statistical differences were found between matched groups of the second and third experiments; therefore, the data from these two experiments were combined.

RESULTS

Mice injected with saline 30 min before training averaged 20 avoidances during the first training session of 50 trials. Despite ECS treatments after training, saline mice scored between 24 and 36 avoidances during the 50 retraining trials. The increased avoidances indicated a significant ($p<0.05$) improvement during retraining. Such retention of original training effects was evident whether the saline mice were tested 24 hr or 7–8 days after training. These results are summarized in part of Fig. 1. Average avoidances made by saline mice in original training were treated as 100% levels. Average total avoidances of cycloheximide animals and of the saline animals in subsequent sessions were converted to percentage scores relative to the saline 100% levels.

Since all mice received ECS treatments and only the interval between training and ECS was varied, the ECS effects on retention were assessed for different training-ECS intervals. Saline mice given ECS immediately after training performed significantly ($p<0.05$) fewer avoidances during retraining than mice given ECS 6 hours after training. The disruption of retention produced by ECS given immediately after training appeared during retraining conducted either 24 hr or 7–8 days after training. However, both groups of saline mice, those given ECS immediately and those given ECS 6 hr after training, made significantly more avoidances in retraining than in training ($p<0.05$, both cases). As noted above, the results were essentially the same whether saline animals were retrained 24 hr or 7–8 days later.

Mice injected with cycloheximide 30 min prior to training averaged 14 avoidances during the 50 original training trials. Compared to saline mice during original training, the cycloheximide mice exhibited significantly fewer ($p<0.01$) avoidances. The amount of retention of avoidance training varied for the mice given cycloheximide. Both the interval between training and ECS, and the interval between training and retraining influenced the amount of retention.

When retraining was conducted 24 hr after original training, the cycloheximide mice, regardless of training-ECS intervals, did not perform any more avoidances than the naive saline mice during original training. Those cycloheximide mice which received ECS 6 hr after original training showed more ($p<0.02$) avoidances than they had during original training. In contrast, ECS administered immediately after training resulted in mice which exhibited the same number ($p>0.05$) of avoidances as naive cycloheximide mice during original training.

Avoidance scores of cycloheximide treated mice given ECS one hour after training were not evaluated statistically. However, in both 24 hr and 7–8 day retraining conditions, the performance of these mice was between that of

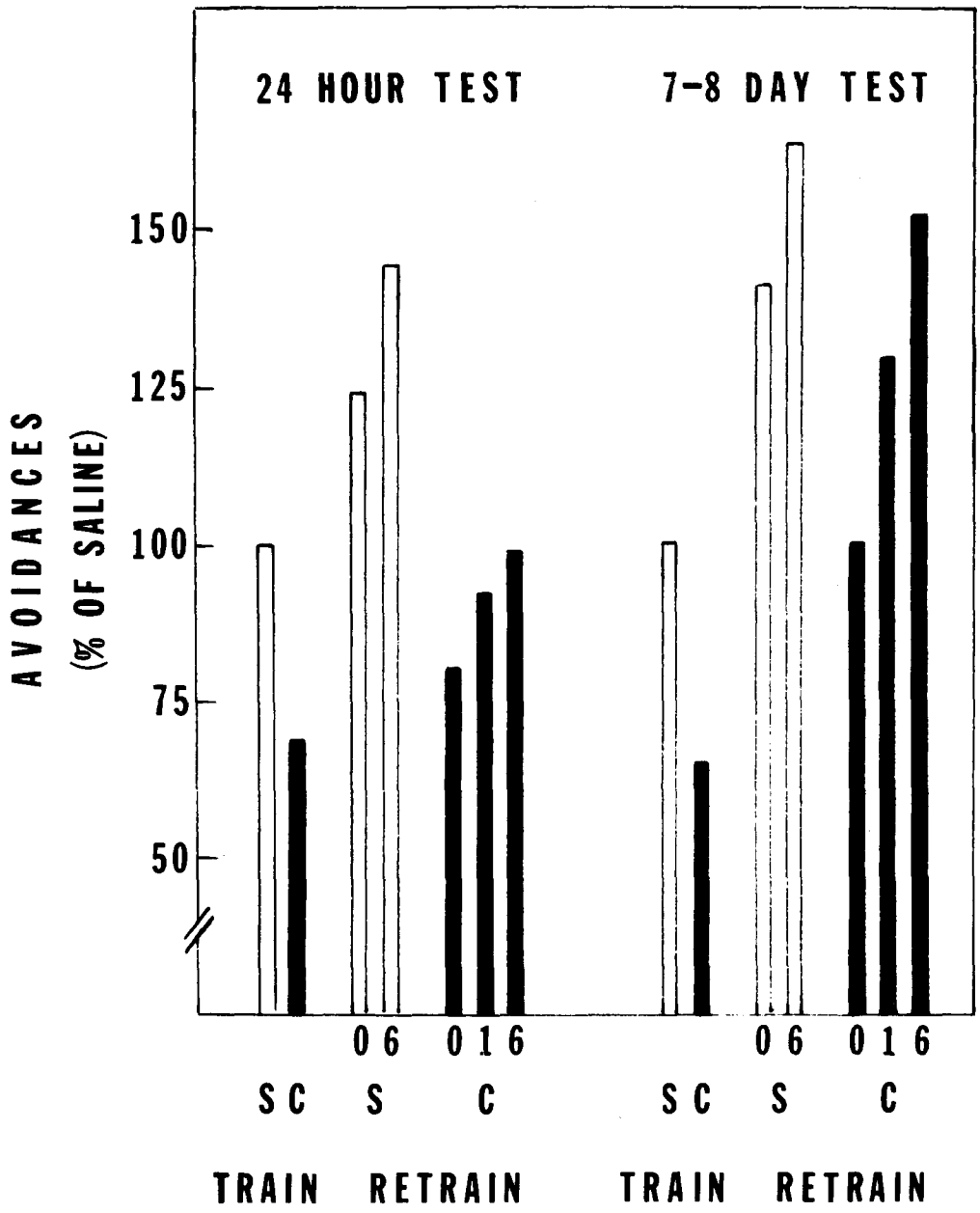

FIG. 1. Avoidances of saline (S) or cycloheximide (C) pretreated mice in original training (train) and in retraining (retrain) 24 hr or 7–8 days later. All mice received ECS, either immediately (0), 1(1), or 6(6) hr after original training. The average avoidances made by saline mice during original training are expressed as 100% control levels. All other scores are a percentage expression of this control level.

cycloheximide mice given ECS immediately and that of cycloheximide mice given ECS 6 hr after training.

Retraining delayed for 7–8 days after original training indicated that mice treated with cycloheximide and ECS often retained considerable benefit from original training. Although the cycloheximide mice had fewer avoidances during original training than saline mice, on retraining 7–8 days later the saline and cycloheximide mice which received ECS 6 hr after original training exhibited comparable numbers of avoidances ($p>0.05$). Those mice which received ECS immediately after original training and then tested 7–8 days later showed different amounts of avoidance depending on whether saline or cycloheximide was given prior to original training. The cycloheximide mice showed significantly ($p<0.02$) fewer avoidances than saline mice which also received immediate ECS. As might be expected, the mice given cycloheximide followed by immediate ECS showed significantly ($p<0.01$) fewer avoidances during retraining than cycloheximide mice given ECS delayed 6 hr after training. Similarly, the cycloheximide-immediate ECS mice did not show more ($p>0.05$) avoidances than the saline mice had during original training.

Mice treated with saline made no more wheel turns during retraining than they had during original training ($p>0.05$), whether the retraining occurred at 24 hr or 7–8 days. Table 1 summarizes wheel turn performance for saline and cycloheximide animals under original training, 24-hr and delayed (7–8 day) retraining conditions.

TABLE 1

WHEEL TURN PERFORMANCE OF SALINE OR CYCLOHEXIMIDE PRETREATED MICE. MICE WERE GIVEN ECS EITHER IMMEDIATELY (ECS-0), 1(ECS-1) OR 6(ECS-6) HOURS AFTER ORIGINAL TRAINING. THE AVERAGE NUMBER OF WHEEL TURNS BY SALINE MICE DURING ORIGINAL TRAINING ARE EXPRESSED AS THE 100% CONTROL LEVEL. ALL OTHER SCORES ARE A PERCENTAGE OF THIS CONTROL LEVEL

Group	Session		
	Training	Retraining	
		24 Hours	7–8 Days
Saline	100%		
ECS-0		91.5%	107.5%
ECS-6		98.7%	97.9%
Cyclo	76.9%		
ECS-0		60.1%	80.5%
ECS-1		63.1%	87.8%
ECS-6		90.8%	100.8%

Mice treated with cycloheximide performed significantly fewer wheel turns in the training session than saline mice ($p<0.01$). When retrained 24 hr later, cycloheximide mice made no more wheel turns than in original training ($p>0.05$). When retrained 7–8 days after original training they made significantly more wheel turns than in original training ($p<0.05$), and there was no difference between the wheel turn performance of saline and cycloheximide mice in the 7–8 day retraining session ($p>0.05$).

Prior to these reported experiments several pilot studies were conducted in which approximately 200 HS mice were used to examine various dosages of cycloheximide and different parameters of ECS. In some of the studies in which mice were injected with cyclohexmide (160 mg/kg) as many as 30% of the animals died within a few days after injection. All deaths which occurred during the three experiments reported here were recorded. Out of 64 cycloheximide-induced fatalities, 29 occurred during the first 24 hr after injection, 25 during the second, 8 during the third, and 1 each during the fourth and fifth 24-hr periods after injection. The largest number of fatalities thus occurred during the first and second days after the animals were injected.

To assess the previously reported effects of cycloheximide alone on long-term retention [32], a final study was done following these reported experiments. Two groups, each of 12 female and 12 male HS mice received either saline or cycloheximide (160 mg/kg) 30 min prior to the 50-trial training session. Injection procedures were as already described. No ECS treatments were administered. Seven days later both groups underwent a second 50-trial session. In the initial training, cycloheximide mice made significantly fewer avoidances than did saline mice ($p<0.05$). When retrained 7 days later, both groups made more avoidances than in original training ($p<0.05$) and there was no difference between the avoidance scores of the two groups ($p>0.05$). Thus, as reported in the literature, no long-term effects of cycloheximide on retention of a complex task were found.

DISCUSSION

Mice trained on a multiple trial, active avoidance task were resistant to the amnesic effects usually produced by cycloheximide alone, cycloheximide paired with delayed ECS, or ECS used separately. ECS treatments given to saline treated mice immediatley after training allowed a significant amount of memory to remain intact, yet there were still significant memory deficits when compared to ECS treatments given six hours after training. The memory deficits appeared stable over a 7–8 day period, unlike the transient effects reported by other investigators [6, 17, 37] but similar to permanent ECS deficits reported previously [15,20). In pilot studies we corroborated the common observation [14, 26, 27] that a long delay between the end of training and the adminsitration of ECS results in no memory deficits. It appears, therefore, that the present training task produces learning which is especially stable and particularly immune to amnesic effects usually associated with ECS treatments.

Those mice given cycloheximide prior to training and delayed ECS treatments after training exhibited variable amounts of memory depending on the amount of time elapsing between training and testing. Even though the cycloheximide mice appeared to learn significantly less than saline mice during training,those which received delayed ECS and which were tested 7–8 days after training exhibited levels of memory comparable to saline-delayed ECS mice. In effect, these cycloheximide mice actually showed more improvement between training and test than shown by saline mice. With additional training trials, saline mice can typically attain better than 47–48 correct avoidances during a 50-trial training session. Therefore, it is unlikely that a ceiling effect could explain these data. Unless it is postulated that cycloheximide facilitates memory, it appears that cycloheximide mice learned as much as saline mice during original training. In this case, the performance of cycloheximide mice during original training was clearly dissociated from the amount of learning which occurred. Performance and activity effects of cycloheximide in mice have been reported earlier [29,33] but the present findings seem an especially fortuitous separation of direct performance effects and memory effects.

When cycloheximide mice are retrained 24 hr after original training, they appear to exhibit direct performance deficits. In the present study, direct performance alterations appear to account for many of the cycloheximide induced deficits in 24 hr retention. There is little doubt that cycloheximide animals simply do not perform as well as saline animals. In fact, no cycloheximide animals perform better during 24 hr retention tests than did the naive saline mice during original training. The time course of mortality from the toxic systemic effects of cycloheximide also appears to corroborate the possibility of severe performance deficits during 24 hr tests.

When retention of cycloheximide mice is evaluated against the original training scores of only cycloheximide mice an interesting result is evident. Mice given delayed ECS treatments and tested 24 hr later show significant improvement over original training. The mice which received immediate ECS, however, showed no improvement. Since all cycloheximide mice received ECS, a direct performance effect of the ECS treatments seems untenable. A typical ECS time gradient [26,27] of amnesic effectiveness seems better able to account for these findings. In addition, such findings and interpretations are more consistent with the results obtained for 7–8 day tests. In delayed tests the direct performance effects of cycloheximide appeared to have dissipated and only those cycloheximide mice given immediate ECS showed amnesia. Although other cycloheximide mice received delayed ECS and showed memory comparable to that exhibited by saline mice, the cyclohexmidie-immediate ECS mice showed performance which was indistinguishable from that shown by naive saline mice during original training. It is important to notice that cycloheximide pretreatment plus ECS immediately after training results in what appears to be complete amnesia. These combined treatments appear to produce virtually complete amnesia, although the training is especially resistant to amnesic effects produced by cycloheximide alone, cyclohexmide paired with delayed ECS, or ECS used separately.

The mechanisms of cycloheximide and ECS actions which relate to their amnesic consequences remain unclear. The combined effectiveness of these two treatments, however, may suggest some possible modes of action. Both cycloheximide and ECS produce disaggregation of brain polysomes [23,24] and both treatments inhibit protein synthesis in the brain [2, 5, 8, 9]. When cycloheximide is administered together with ECS, the ECS treatments produce no more polysomal disaggregation than observed with cycloheximide alone [24]. The combined effects of these two treatments on brain protein systhesis as well as other macromolecular events remains to be studied. When, however, cycloheximide is paired with ECS, resulting electrical activity of the brain [22] resembles an enhancement of that activity which is usually associated with ECS-produced amnesia [16,38]. On these bases it appears that only the disaggregation of polysomes can be ruled out as contributing directly to the pronounced amnesic effectiveness of cycloheximide and ECS used together.

When trained with a single trial, animals treated with either ECS or cycloheximide develop rather complete amnesia. The time relationships between training and treatment, however, usually impose severe constraints on the amnesic effectiveness of ECS or cycloheximide. Paired cycloheximide-ECS treatments relax these temporal constraints for animals trained on a single trial task [2]. Multiple trial training restores the temporal constraints for amnesic effects even for combined cycloheximide-ECS treatments. As observed in the present study, complete amnesia is produced only when the mice are pretreated with cycloheximide and given ECS immediately after training. It seems that multiple training trials yield a capacity for some neural alteration capable of preserving memory. Immediate ECS treatments seem able to prevent the full realization of this capacity. If, however, ECS is delayed, the capacity is expressed in a form which is not perturbed by either ECS or cycloheximide and behavioral memory survives. A single trial apparently does not depend upon such capacity or does not evoke the prerequisite conditions for establishing such capacity, thus cycloheximide-ECS treatments produce complete amnesia.

To date, only one line of research seems consistent with the time course of events noted in our behavioral studies. Training is followed by increased RNA metabolism in the brain, and this increase reaches maximal levels at 15 min after training [13, 35, 36]. Although observed increases in brain RNA metabolism do not occur during training, the capacity for such increments apparently does. Such changes in RNA metabolism related to training appear to satisfy the requirements of the processes delineated by behavioral studies with cycloheximide and ECS. Several corroborating experiments seem necessary before a scheme can be formalized but, at present, RNA seems a good candidate for the fleeting intermediate memory trace often suggested previously [5,26]. Accordingly, the intermediate memory trace may only become functionally significant in multiple trial training tasks and this hypothetical trace might only be evident when other associated processes are disabled.

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