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Cycloheximide Produces Adult-Like Retention Deficits of Prior Learning in Infant Mice[1,2]

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NAGELBERG, D. B. AND Z. M. NAGY. *Cycloheximide produces adult-like retention deficits of prior learning in infant mice.* PHARMAC. BIOCHEM. BEHAV. 7(5) 435–441, 1977. Utilizing a dosage of cycloheximide which was found to inhibit cerebral protein synthesis by almost 90% after injection, separate groups of 13-day-old mice received either cycloheximide or saline followed by 0 (control), 15, or 25 training trials in a discriminated shock-escape T-maze. Twenty-four hr later, each mouse was treated with cycloheximide or saline and tested for retention by an additional 25 trials in the T-maze. As reflected by correct choice-point turns, the results suggest that whereas saline treated mice demonstrated reliable retention of prior learning, cycloheximide treated mice exhibited memory impairment; cycloheximide per se had no effect on performance during either original training or retest. A final experiment indicated that this memory impairment was not due to cycloheximide's general debilitating side effects at the time of retention testing. Taken together, these data suggest that protein synthesis inhibition during training impaired consolidation and/or retrieval processes involved in memory. The biochemical and behavioral effects following cycloheximide injection in 13–14-day-old mice in the present study parallel those reported with adult animals and lend indirect support to the hypothesis that the 24-hr memory capacity exhibited by these young mice reflects the early functioning of those processes involved in adult long-term memory.

Cycloheximide Protein synthesis inhibition Mice Infant memory Amnesia T-maze shock escape

YOUNG ANIMALS typically display poorer retention of prior learning than do adults. This relationship between age and memory capacities has been demonstrated on a variety of instrumental learning tasks even when rates or asymptotic levels of learning are equated between age groups [10]. As both behavioral and physiological variables may account for the poorer memory found in infancy, the presence of a long-term memory capacity may be at least partly dependent upon the maturational state of the central nervous system (CNS) at the time of original learning [10]. Numerous biochemical changes take place in the CNS of most altricial rodent species during the time that long-term memory increases most rapidly [9]. In the mouse and rat, a period of most rapid postnatal brain growth and development occurs between 5–15 days of age, with many biochemical and physiological processes approaching adult levels by the end of this period [17]. Presumably, the poorer retention abilities displayed by young rodents are due, in part, to the immaturity of neurological mechanisms involved with long-term memory [10].

At least two lines of research support this immaturity hypothesis. Across species, it has been demonstrated that young guinea pigs, which are relatively mature at birth compared to other rodents such as the rat and mouse, do not show age-related retention deficits [9]. Within species, namely the mouse, Nagy and his colleagues have demonstrated that the emergence and development of memory capacities are a function of both age and complexity of learning task. For example, in a straight-alley shock-escape task, mice do not appear capable of remembering prior escape training over a 24-hr period until they reach 9 days of age, even though they show improvement in escape behavior within a training session as early as 5 days of age [19, 22, 23]. In contrast, a 24-hr retention of discriminated shock-escape T-maze learning does not reach functional maturity until 11 days of age [24,26]. Nine-day-old mice failed to display evidence of 24-hr retention of T-maze training in spite of extended original training [25] or higher drive (shock) level [20]. These data suggest that memory of more complex types of learning requires further maturation of the CNS than is necessary for memory of simpler responses and are consistent with the notion that learning and retention capacities will behaviorally emerge with the development of neuro-physiological and/or biochemical processes underlying these capacities.

If the onset of 24-hr memory is indeed reflecting the functional maturation of underlying CNS processes which are involved in adult long-term memory, then relevant physiological manipulations should have parallel effects on learning and retention in both young and adult animals.

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This hypothesis has been supported in the demonstration that 24-hr retention of a learning task can be impaired by posttraining hypothermia in infant mice [21] as has been reported with adult mice [7]. Another manipulation frequently employed in the study of adult memory is the inhibition of protein synthesis with antibiotic drugs (e.g., puromycin, cycloheximide, acetoxycycloheximide). It is well established that the administration of these drugs in animals before or shortly after a learning session, produces an impairment in memory (amnesia) on subsequent recall tests [6]. Until recently, the most widely accepted interpretation of this memory deficit has been in terms of storage failure; the dearth of proteins prevents the consolidation of the memory trace into permanent long-term memory [1, 2, 3, 5]. However, recent behavioral and biochemical evidence suggests that protein synthesis inhibitors may be suppressing later retrieval from storage rather than preventing the development of long-term memory [6, 18, 27, 28]. Whichever interpretation is correct, the behavioral retention deficit induced by protein synthesis inhibitors has been consistent in adult animals.

The generality of this memory deficit extends to mice, rats, quail, and goldfish tested in various instrumental learning paradigms such as discrimination, active avoidance, and passive avoidance [6, 14, 30]. Although this effect has been repeatedly demonstrated with adult animals, it has not been investigated in the young. The purpose of the present investigation was to determine whether protein synthesis inhibition would result in similar amnesic effects in infant mice (13–14 days old). It is at this developmental stage that long-term (24-hr) memory is clearly displayed in a discriminated shock-escape T-maze. Results consistent with those reported with adult mice would provide further indirect support that the 24-hr memory capacity exhibited by these young mice reflects the early functioning of those processes involved in adult long-term memory.

A series of experiments is reported here which examined the effects of cycloheximide (CXM) on cerebral protein synthesis in 9, 11, and 13-day-old mice, and retention of T-maze learning in mice aged 13–14 days.

EXPERIMENT 1

The purpose of this experiment was to determine CXM's effect upon cerebral protein synthesis in young mice. On the basis of a drug dosage pilot study, 10 mg/kg CXM was administered to three groups of mice, aged 9, 11, and 13 days. The extent of protein synthesis inhibition was determined 40 min and 24 hr after drug injection. These times were chosen to coincide with the behavioral parameters of this study (Experiments 2 and 3) in which T-maze training was completed about 40 min after CXM injection, while retention testing followed 24 hr later.

METHOD

Animals

The animals were 104 Swiss-Webster (S–W) mice (*mus musculus*) of both sexes aged 9, 11, and 13 days, born and reared in 30.4 × 18 × 12.8 cm polyethylene cages with wire-grid tops and wood chips on the floor. The mothers had free access to food and water; the colony and test rooms were maintained at 24° ± 1°C on a normal 12 hr light-dark cycle.

Procedure

At each age (9, 11, and 13 days), separate groups of mice were injected subcutaneously on the back with either 10 mg/kg CXM (Sigma Chemical Co.) or an equivalent volume of physiological saline and received subcutaneous ^{14}C-leucine (10 μg/animal; New England Nuclear Corp.) 30 min or 24 hr later. Mice which did not receive the ^{14}C-leucine until 24 hr after CXM treatment were earmarked for identification and returned to their home cages during the interim.

The determination of protein synthesis inhibition was similar to the methods followed by Barondes and Cohen [4] and Flood, Rosenzweig, Bennett, and Orme [12]. After a 10 min incorporation period of the ^{14}C-leucine into protein, the animals were decapitated and the heads were placed into ice. Several min later, the brain was dissected and cortex was sampled posterior to the olfactory bulbs. The brain sample was immediately weighed, homogenized in 0.5 ml demineralized water, and washed out with an additional 0.5 ml demineralized water. To precipitate the protein, 0.2 ml of 30% trichloracetic acid (TCA) was added. The samples were centrifuged for 10 min and the supernatant was decanted into a counting vial. The remaining protein pellet was washed with 0.5 ml of 5% TCA and then ground. Two further centrifugations and decantations followed. NCS tissue solubilizer (0.4 ml; Amersham/Searle) was added to the protein sample, stirred, and left standing until fully dissolved. At this time, 4.0 ml of scintillation cocktail (Beckman Scintillation Toluene and Tolu Scint II) was added and 6 hr later, radioactivity was counted by a scintillation counter (Beckman LS-100C Liquid Scintillation System). The supernatants were heated (70°C) until almost dry; 0.4 ml NCS was added followed by 4.0 ml scintillation cocktail 6 hr later. Radioactivity was counted after 6 more hr. Appropriate corrections for counting efficiencies were made.

The total drug time was defined as the duration from CXM injection to decapitation. The degree of incorporation was calculated by determining for each sample the ratio of ^{14}C-leucine incorporated into the protein precipitate to the total activity of ^{14}C-leucine found in the sample, that is,

$$\%\ p/p + s = \frac{\text{precipitate dpm (p)}}{\text{precipitate dpm (p)} + \text{supernatant dpm (s)}} \times 100$$

Values obtained from saline controls injected with ^{14}C-leucine represented 100% incorporation. Thus,

$$\%\ \text{inhibition} = 100 \times 1 - \frac{\text{Experimental Incorporation } \%\ p/p + s}{\text{Control Incorporation } \%\ p/p + s}$$

RESULTS

Table 1 presents the mean percent of protein inhibition for each age group 40 min and 24 hr after cycloheximide was administered. (The numbers in brackets represent the number of CXM- and saline-treated mice, respectively, used to determine protein inhibition levels for each group). As the table indicates, protein synthesis was reduced approximately 90% 40 min after the administration of 10 mg/kg CXM; this was consistently demonstrated in all three groups of mice, aged 9, 11, and 13 days. Protein inhibition was more variable when analyzed 24 hr after CXM treatment. Whereas protein synthesis was inhibited by about 28% in

TABLE 1

MEAN PERCENT PROTEIN INHIBITION AFTER CYCLOHEXIMIDE

Age Group	Male	Female	Total
40 min after CXM			
9	91.91(4,2)	89.68(4,3)	90.80
11	86.59(4,2)	92.90(4,3)	89.75
13	92.82(4,2)	85.87(4,2)	89.35
24 hr after CXM			
9	41.26(7,4)	14.82(7,4)	28.04
11	41.27(7,4)	14.59(7,4)	27.93
13	5.80(7,4)	18.02(7,4)	11.41

Numbers in parentheses represent the number of cycloheximide- and saline-treated mice, respectively, used to determine inhibition levels.

10- and 12-day-old mice (41% for males and only 15% for females), the 14-day-old group showed far greater recovery with only about 11.5% residual inhibition (5.8% for males and 18% for females).

EXPERIMENT 2

When adult animals are trained at a time when ongoing cerebral protein synthesis is inhibited by greater than 80%, subsequent retention deficits, contingent upon a number of training parameters, are often observed [2, 3, 6]. The results of Experiment 1 clearly demonstrated that the dosage of CXM used (10 mg/kg) was sufficient to suppress protein synthesis by almost 90% 40 min following injection of mice 9, 11, and 13 days of age. The purpose of Experiment 2 was to examine the effects of CXM upon learning and 24-hr retention of a discriminated shock-escape T-maze task in 13-14-day-old mice. By this age, S-W mice readily display 24-hr retention effects of prior training in a T-maze [24,26].

METHOD

Animals

The animals were 160 13-day-old S-W mice of both sexes born and reared under conditions described in Experiment 1. The mothers remained with the young at all times except during the testing sessions.

Apparatus

The apparatus was a Plexiglas T-maze, 6.2 cm high and 3.4 cm wide throughout. The stem was 18.8 cm long, with a removable door placed 5 cm from the closed end to form a start box. Each arm of the maze was 9.2 cm long. The maze was placed upon a grid floor, composed of 1 mm stainless steel rods spaced 3 mm apart, such that the grids were parallel to the maze stem and perpendicular to the length of the arms. A scrambled AC shock source (Harvard Instrument Co., Model 3121) delivered 0.2 mA constant current to the grid floor.

Procedure

At 13 days of age, each mouse was weighed, injected subcutaneously on the back with CXM (10 mg/kg) or saline, and began receiving 0, 15, or 25 training trials 15 min later. Between injection and training, each mouse was isolated in a holding cage. The training procedure began by placing each mouse in the start box facing the choice point. The door was removed as shock was initiated and a running time meter was started. Turn preference was established on the first trial; shock was terminated when the animal entered either arm of the maze. Shock offset occurred to the goal opposite each mouse's preference trial choice on all subsequent training trials. A maximum latency trial was 180 sec; any mouse which failed to reach the correct goal within that time was gently forced to it, shock was terminated, and a maximum latency score of 180 sec was assigned. A goal was arbitrarily assigned in the event that neither arm was reached within 180 sec on the preference trial. A 45 sec intertrial interval was used, during which time the mouse was held in the experimenter's closed hand. Following the session, each mouse was earpunched for identification and returned to its home cage.

Using a split-litter design, group assignment was determined within training drug and sex by the number of correct choice-point turns made during training, so that performance during the session was approximately equal within each group. On this basis, each mouse received either 15 or 25 training trials and was assigned to one of four drug groups; each of the resultant 8 groups consisted of 8 males and 8 females. Twenty-four hr after original training, these animals were weighed and injected with CXM or saline, isolated in a holding cage for 15 min, and retested for retention by an additional 26 trials to the previously reinforced goal. The drug groups are designated according to the treatment received, cycloheximide (CXM) or saline (S), prior to the training and retest sessions respectively, resulting in CXM-CXM, CXM-S, S-S, and S-CXM groups.

Two additional groups of 8 males and 8 females were randomly assigned to maturation control groups (0 training trials) on the training day. These mice were weighed and injected with CXM or saline, isolated in a holding cage for 15 min, and then earmarked for identification and returned to their home cages. Twenty-four hr later, each mouse was injected with the same treatment (CXM or saline) which was administered on the training day. Following 15 min in a holding cage, each mouse received 26 trials in the T-maze to the goal opposite its first trial choice-point turn. These two groups are designated as CXM-CXM and S-S controls.

Performance was measured during both the training and retest sessions by recording the goal arm entered upon first reaching the choice-point on each trial. Following the retest session, each mouse was returned to its home cage; arbitrarily selected animals were weighed at 25 days of age to determine the extent to which CXM may have produced a long-term detrimental effect as reflected by weight loss.

RESULTS AND DISCUSSION

The numbers of correct choice-point turns were separately totaled for each block of five trials during training and retention sessions, and submitted to a series of factorial analyses of variance with one repeated factor [41]; the initial preference and first retest trials were not included in the analyses. The main factors entered (where appropriate) were training drug, retention drug, amount of original training, sex, and trial blocks. As sex proved not to be reliable as a main effect and did not interact with any other

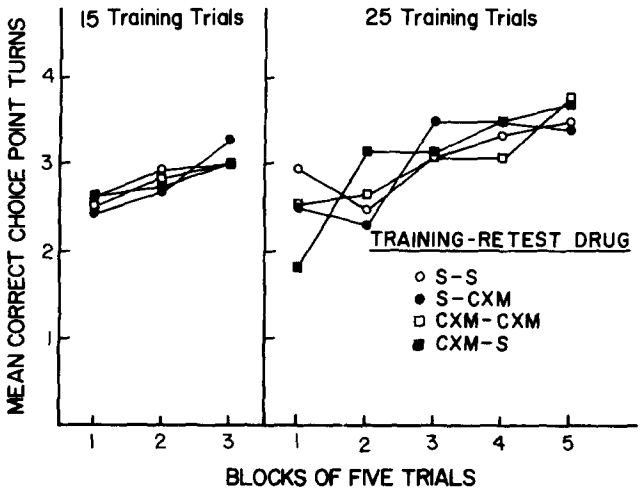

FIG. 1. Mean number of correct choice-point turns during original training as a function of the number of training trials, trial blocks, and drug injections prior to training and retest (S = saline; CXM = cycloheximide).

factors in any of the analyses, this factor has been combined for all comparisons.

Original Training

The mean numbers of correct choice-point turns made during training by the 15-trial (left panel) and 25-trial (right panel) groups are presented in Fig. 1 as a function of drug group and trial blocks. There were no significant differences among drug groups which received 25 training trials nor were the 15- and 25-trial groups significantly different from one another over the first three trial blocks. All groups demonstrated a reliable increase in correct turns over the first three trial blocks, $F(2,224) = 11.83$, $p<0.001$. Additional training (trials 16–25) did not result in further improvement in choice-point performance; Duncan's Multiple Range Tests failed to reveal reliable differences among the third, fourth, and fifth mean trial block scores of the 25-trial groups (all p's>0.05).

Overall, the training data indicate that the saline and CXM groups performed about the same during training with all groups demonstrating reliable increases in the number of correct turns over the first 15 trials and were comparable to those groups receiving 25 trials.

Retention

Figure 2 presents the mean numbers of correct choice-point turns made during the retention test as a function of the amount of original training, drug groups, and trial blocks. In an analysis incorporating only those groups with previous training (15 and 25 trials), although all groups showed increased performance over trial blocks during retest, $F(4,448) = 16.30$, $p<0.001$, those groups which received CXM prior to original training made fewer correct choice-point turns during retest than did saline treated groups, $F(1,112) = 35.63$, $p<0.001$. The retention drug was not a reliable effect suggesting that CXM did not impair performance when injected just prior to the retest session. In the absence of any significant interactions, it is reasonable to conclude that a pretraining injection of CXM produced an amnesic effect when retention was measured 24 hr later. This was true of both the 15- and 25-trial groups, which did not reliably differ from one another on the retention test.

Comparison of the S-S and CXM-CXM groups as a function of 0, 15, and 25 training trials provides further support for an amnesic effect of CXM. Both S-S groups with previous training made more correct turns than the S-S maturation control group, both F's$(1,84)>8.30$, p's<0.01, which suggests that the greater number of correct turns made on the retest day was the result of previous training rather than maturation. In contrast, none of the CXM-CXM groups differed from one another nor did they differ from the S-S maturation control group. Both S-S trained groups made more correct turns than their respective CXM-CXM groups (p's<0.005).

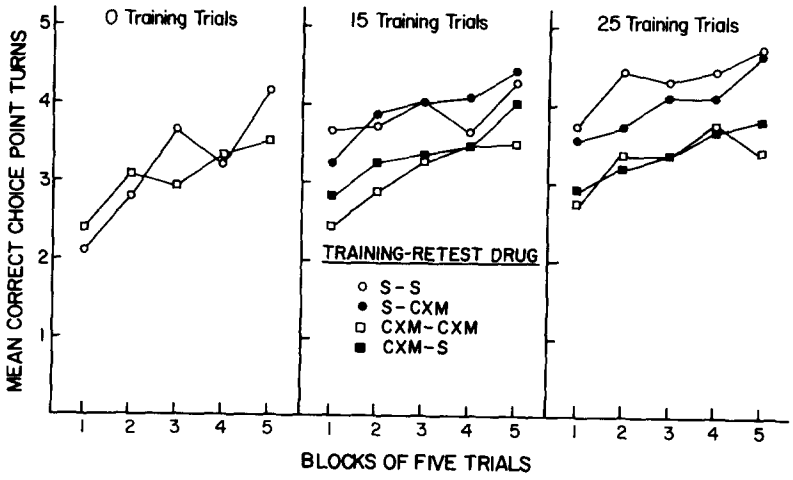

FIG. 2. Mean number of correct choice-point turns during 24 hr retention testing as a function of the number of original training trials, trial blocks, and drug injections prior to training and retest (S = saline; CXM = cycloheximide).

TABLE 2

MEAN BODY WEIGHT (g)

Training-Retest Drug Condition	Age 13	14	25*
S-S			
15 and 25	6.79(32)	7.11(32)	13.83(23)
Control	6.69(16)	7.01(16)	
CXM-CXM			
15 and 25	6.86(32)	6.62(32)	13.21(18)
Control	7.10(16)	6.83(16)	
S-CXM			
15 and 25	6.80(32)	7.06(32)	12.58(12)
CXM-S			
15 and 25	6.74(32)	6.59(32)	13.49(13)

Number in parentheses indicates *n* in each group.Key: S = saline; CXM = cycloheximide.

*The small *n* at 25 days is not the result of mortality; only some animals were sampled on this day.

Body Weight

CXM had an overall debilitating effect; at the time of retest, diarrhea and weight loss were commonly observed in mice injected with CXM on the previous day. Table 2 presents the mean body weights for each group at 13, 14, and 25 days of age. Although body weights were comparable between CXM and saline groups 15 min prior to original training, a slight loss of weight by CXM treated mice and a small gain by saline injected mice resulted in a significant weight difference prior to the retention test, $t(126) = 3.01$, $p<0.01$. A similar trend was noted in the S-S and CXM-CXM maturation control groups. In spite of about 10 subsequent fatalities in the CXM groups, the mean body weights of the remaining CXM groups were not significantly different from those of S-S mice at 25 days of age, indicating a temporary rather than permanent weight loss.

EXPERIMENT 3

The results of Experiment 2 suggest that CXM, when injected into 13-day-old mice prior to a learning session in a discriminated shock-escape T-maze, produced an amnesic effect when retention was measured 24 hr later. However, the interpretation of these results may be confounded by CXM's general debilitating side effects. Numerous studies with adult animals have reported that CXM-induced amnesia occurred only when the drug was administered prior to, and not after, training [6]. Squire and Barondes [34] argue therefore, that CXM's amnesic effect in adult animals cannot easily be attributed to sickness because 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 purpose of Experiment 3 was to determine whether the impaired retention performance of 13-day-old mice trained after CXM administration could be attributed to drug-induced side effects or to memory failure.

METHOD

Animals

Thirty-two 13-day-old S-W mice, half of each sex, born and reared as described in Experiment 1, were used.

Apparatus

The apparatus was identical to that used and described in Experiment 2.

Procedure

At 13 days of age, separate groups of 8 males and 8 females were randomly assigned to one of two groups according to a split-litter design. Each mouse was weighed, injected subcutaneously on the back with CXM (10 mg/kg) or saline, and placed in a holding cage for 15 min. Training in the T-maze consisted of a preference trial followed by 25 training trials to the goal opposite each mouse's initial trial preference. At the end of training, each mouse was earpunched for identification and returned to its home cage. One hr later, each mouse received the treatment not administered prior to training. The two groups are designated as S/CXM-S and CXM/S-S representing the treatment (CXM or saline) administered 15 min before training, 1 hr after training, and 15 min prior to retention testing, respectively. Each mouse was immediately returned to its home cage following the second injection.

Twenty-three hr later, each mouse was weighed, injected with saline, and placed in a holding cage for 15 min. Retention testing consisted of 26 trials to the previously correct goal. All other training and testing procedures were the same as described in Experiment 2.

RESULTS AND DISCUSSION

For purposes of comparison, the training scores for the S-S and CXM-S 25-trial groups from Experiment 2, as well as the retest scores for these groups and the S-S maturation control group, were included in the analyses. If the retention deficits observed in Experiment 2 were due to the amnesic effect of CXM injected prior to original training rather than to the debilitating effects noted prior to the retention test, then the S/CXM-S and S-S groups would be expected to perform comparably during retention testing and both groups should make more correct turns than either the CXM/S-S and CXM-S groups with prior training or the S-S maturation control group without prior training. On the other hand, if the retention deficits were due to the debilitating side effects of CXM, then the S/CXM-S group would be expected to perform as poorly during retest as the CXM/S-S and CXM-S trained groups and the S-S maturation control group.

Original Training

The mean numbers of correct choice-point turns made during training are shown in the left panel of Fig. 3 as a function of drug groups and trial blocks. As suggested by examination of the data, none of the groups differed among themselves, and all demonstrated reliable increase in the number of correct turns over the training session, $F(4,224) = 19.44$, $p<0.001$.

Retention

As indicated in the right panel of Fig. 3, CXM, when

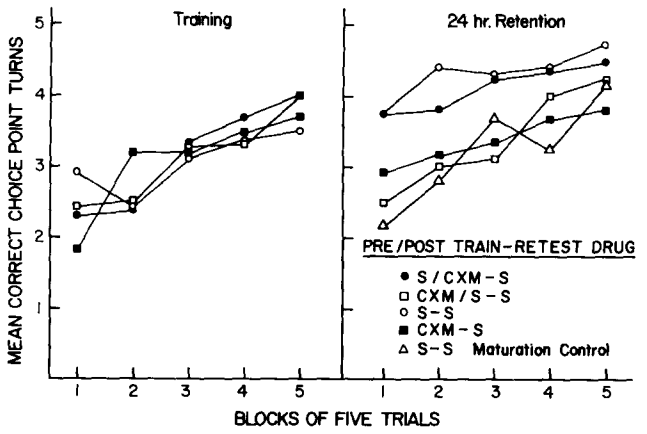

FIG. 3. Mean number of correct choice-point turns during training and 24 hr retention testing as a function of trial blocks and drug injections preceding and following original training (S = saline; CXM = cycloheximide. S-S maturation controls received no original training.) All groups received S injection prior to retention testing.

injected before but not after training, had an amnesic effect 24 hr later on the retention test. The S/CXM-S group made more correct turns than did the CXM/S-S group, $F(1,56) = 41.65$, $p<0.001$, but did not differ from the S-S group with prior training. In addition, the S/CXM-S group made more correct turns than the S-S maturation control group, $t(30) = 5.03$, $p<0.001$. In other words, retention effects were observed in spite of the debilitating side effects produced by a posttraining CXM injection. In contrast, the CXM/S-S group was not significantly different from either the CXM-S trained group or the S-S maturation control group ($p>0.05$).

Body Weight

The mean body weights for the S/CXM-S and CXM/S-S groups were comparable prior to training and did not reliably differ from the groups in Experiment 2. On the retest day, both the S/CXM-S and CXM/S-S groups showed a slight loss of weight from the previous day (from a mean weight of 6.58 to 6.45 g) and weighed significantly less than the S-S and S-C groups on the retest day, $t(94) = 2.86$, $p<0.01$. These data demonstrate that S/CXM-S group experienced the same level of debilitating side effects from the CXM injection at the time of retest as did the CXM/S-S, CXM-CXM, and CXM-S groups, yet did not evidence any deficit in retention of prior training.

GENERAL DISCUSSION

The results of this study indicate that 13-day-old mice showed impaired 24-hr memory of prior T-maze learning when inhibition of protein synthesis by CXM was initiated shortly before training. In addition, it was clearly demonstrated that this impairment was not due to CXM's debilitating side effects. These findings in young mice are in agreement with numerous reports of CXM-induced amnesia in adult animals [6]. The similar biochemical and behavioral effects which CXM produces in adult and 13-day-old mice lend support, albeit indirect, to the hypothesis that the 24-hr memory capacity exhibited by the young mice reflects the early functioning of those processes involved in adult long-term memory [24, 25, 26].

While the memory processes underlying the 24-hr memory of 13-day-old mice in the present study may be the same as those involved in adult long-term memory, several lines of evidence suggest that they are yet immature in comparison to adult memory processes. First, a memory capacity for training of this task has become functionally mature only several days earlier in ontogeny and is still rapidly increasing. Whereas 13-day-old S-S groups in the present study demonstrated reliable 24-hr retention after only 15 training trials, previous research with the same mouse strain has shown that 24 training trials are necessary for 24-hr retention at 11 days of age, while 9-day-old mice fail to show any evidence of similar retention even after 40 training trials on this task [25].

Second, memory processing at this early age appears highly susceptible to disruption. Whereas the majority of work with adult mice employed CXM dosages ranging from 50 to 150 mg/kg [34,40] to obtain amnesia, 10 mg/kg was sufficient to obtain 90% protein synthesis inhibition and subsequent amnesia in the present study. This finding may reflect the fact that young mice simply have lower levels of brain protein than do adult mice; Folch-Pi [15] has reported that there is a steady increase in the protein content in the mouse brain from birth until 25 days of age, when the levels approach 94% of adult values. However, this interpretation must be viewed with caution as a recent study has reported amnesic effects in adult rats with only 2.5 mg/kg CXM injections [31], which resulted in only 76% protein synthesis inhibition [32].

Third, overtraining effects were not demonstrated with the levels of training utilized in the present experiments. Flood *et al.* [12] defined overtraining as the amount or strength of training that brings control animals to their asymptote of performance and found that CXM was most effective as an amnesic agent when training was terminated just prior to the asymptote of the acquisition function. Although 13-day-old mice appeared to achieve asymptotic performance within 15 training trials, CXM produced amnesia even after 25 training trials. Several possible reasons may account for this finding. First, memory consolidation processes might proceed more slowly in young mice due to immaturity of the CNS and thereby account for the greater susceptibility to memory impairment by CXM. A similar rationale was used to account for the finding that posttraining hypothermia could result in retention deficits in young mice even after multiple training trials [21], a finding uncommon with adult animals. Second, while adult mice generally recover from protein inhibition several hr after CXM injection, the relatively high residual inhibition 24 hr after injection found in 9- and 11-day-old mice in the present study suggests that the duration of inhibition may be longer in 13-day-old mice than in adults. Increasing the duration of protein synthesis inhibition in adult mice has been reported to counteract the effects of overtraining [11,13]. In addition, a number of training parameters, including amount of original training [4,27], intertrial interval [27], footshock intensity and duration [12,29], and training-retest interval [8,28], have all been shown to affect the amnesic effect of CXM in adult animals and may be contributing factors to the results of the present study.

Whether or not protein synthesis inhibition was directly responsible for CXM-induced amnesias is yet another

concern. Indeed, one of the current controversies between the consolidation and retrieval hypotheses lies not in the authenticity of CXM's ability to inhibit protein synthesis, resulting in the blockage of long-term memory, but rather the interpretation of such findings. This issue has been considered in detail elsewhere [6, 16, 33–39].

In summary, the present study further supports the hypothesis that the emergence of a memory capacity in mice is largely a function of maturational (physiological) processes in the CNS. The present finding that CXM produced memory impairment in young mice parallels those reported with adult animals and suggests that the retention displayed by 13-day-old mice reflects the functional maturation of neurophysiological and biochemical processes necessary for adult long-term memory.

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