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Investigation of the Reported Protective Effect of Cycloheximide on Memory[1]

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DAVIS, H. P., M. R. ROSENZWEIG, E. A. GROVE AND E. L. BENNETT. *Investigation of the reported protective effect of cycloheximide on memory*. PHARMACOL BIOCHEM BEHAV 20(3) 405–413, 1984.—Many findings support the hypothesis that formation of long-term memory requires synthesis of proteins in the nervous system close to the time of learning. This hypothesis has been challenged recently by reports that the protein synthesis inhibitor cycloheximide (CYC) injected 2 hr prior to passive avoidance training in mice or rats attenuated the memory impairment induced by a usually amnestic dose of CYC administered 30 min pretraining. To investigate the reports of a "protective" effect of the prior injection, we attempted to replicate them and test their generality. For replication we administered either paired injections of CYC—120 mg/kg 2 hr prior to training and 30 mg/kg 30 min prior to training—or single injections of CYC (either 120 mg/kg or 30 mg/kg) 30 min pretraining and tested for retention of the passive avoidance habit either 1 or 7 days later. No attenuation of amnesia was observed at 1 day tests. Attenuation of amnesia following the double injection of CYC was observed at 7 day tests. When another protein synthesis inhibitor, anisomycin, was used in the same experimental design, there was no "protective" effect; two injections of anisomycin produced greater memory impairment for the passive avoidance habit than did the single low dose. Also, for active avoidance training, two successive injections of CYC caused significantly greater amnesia than did a single dose; this is the opposite of a "protective" effect. We suggest that the reported "protective" effect of CYC on memory is an as yet unexplained phenomenon that does not generalize to other antiobiotic drugs and is specific to the passive avoidance task.

Active avoidance Amnesia Anisomycin Cycloheximide Inhibition of cerebral protein synthesis Memory Passive avoidance

ANTIBIOTIC drugs that inhibit cerebral protein synthesis during or shortly after training impair long-term memory formation in a variety of species and for a variety of tasks [2, 3, 25], although acquisition and short-term memory are normal [7,22]. These findings have been taken as support for the idea that one of the required steps in formation of long-term memory is brain protein synthesis at or near the time of training, and that antibiotic drugs induce amnesia by inhibiting the synthesis of proteins specifically required for long-term memory formation. Alternative hypotheses for the amnestic action of these drugs such as electrical disturbances, altered locomotor activity, sickness, or decreased catecholamine synthesis have been repeatedly considered and dissociated from effects on memory [6, 19, 26].

Recently Rainbow, Hoffman and Flexner [18] reported that a 120 mg/kg dose of the protein synthesis inhibitor cycloheximide (CYC) injected 2 hr prior to one-trial passive avoidance training in mice blocked the normally amnestic effect of a 30 mg/kg dose of CYC administered 0.5 hr prior to training. Single injections of CYC that produced less inhibi-

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tion of brain protein synthesis at the time of training than this combined dose were amnestic. It was proposed that the initial injection of CYC in the combined treatment provided a "protective" effect against amnesia by altering brain metabolism in some unspecified way. Further, it was suggested that CYC-induced amnesia following a single injection might be due to some effect other than inhibition of cerebral protein synthesis, since amnesia did not correlate with degree of protein synthesis inhibition. Similarly, amnesia for the passive avoidance habit is reported to be attenuated in rats given CYC (2.5 mg/kg) injections at 2 hr and 0.5 hr prior to training [15]. CYC also provided "protection" in rats against an ordinarily amnestic treatment of electroconvulsive shock (ECS). ECS 18 hr prior to training was also observed to attenuate the amnestic action of both CYC and ECS treatments. These findings were interpreted as support for the idea that ECS and CYC induce amnesia via a common mode of action [15].

These two studies seriously challenge the hypothesis developed in over a hundred experimental reports that antibiotic drugs induce amnesia by inhibiting the synthesis of proteins specifically required for the formation of long-term memory. Rainbow *et al.* [18] found that combined CYC treatments produced more inhibition of protein synthesis at training time than a single amnestic treatment, yet was not amnestic. Kasprow *et al.* [15] suggest a common amnestic mechanism for CYC and ECS, yet ECS does not inhibit protein synthesis to the extent required for inducing amnesia [11]. Accordingly, we have investigated in mice the claimed "protective" effect of antibiotic inhibitors of protein synthesis by examining the effects on memory of two antibiotic drugs in two behavioral tasks.

EXPERIMENT 1

This experiment was designed to demonstrate the reported protective effect of cycloheximide on retention of the one-trial passive avoidance habit [18]. Mice received the same dosages and dosage schedule as reported by Rainbow *et al.* [18]. The rationale for using mice as subjects rather than rats as used by Kasprow *et al.* [15] was the following: (1) We have found that mice given a single injection of CYC reliably demonstrate a permanent amnesia of the passive avoidance habit [8,12]. (2) There is very little data on inhibition of brain protein synthesis in rats by intraperitoneal injection of CYC, the method of injection used in the study that reported CYC attenuated amnesia in rats. There is, however, extensive data on CYC-induced inhibition of brain protein synthesis in mice [12,18]. (3) The toxicity of CYC is markedly less for mice than for rats. Thus, the use of subjects in which toxicity is lower reduces the likelihood of retention being confounded by some nonspecific effect of drug treatment.

METHOD

Subjects

Male Swiss-Webster CD-1 mice, 60–90 days old, were obtained from Simonsen Laboratories (Gilroy, CA). Animals were housed individually 24 hr prior to training and remained so throughout the experiments. Ad lib access to food and water was provided.

Drugs

CYC was dissolved in saline (SAL). Subcutaneous injections of SAL or a SAL solution containing varying amounts of CYC (12 mg/ml or 3 mg/ml) were made on the backs of mice either 2 hr, 0.5 hr, or both 2 and 0.5 hr prior to training in a volume of 10 ml/kg, which translates to dosages of 120 mg/kg and 30 mg/kg, respectively. Extent and duration of inhibition of brain protein synthesis by CYC have been reported previously [12,18].

Apparatus and Procedure

The three drug conditions and two vehicle control conditions were the following: (1) CYC (120 mg/kg) 2 hr prior to training and CYC (30 mg/kg) 0.5 hr prior to training; (2) CYC (120 mg/kg) 0.5 hr prior to training; (3) CYC (30 mg/kg) 0.5 hr prior to training; (4) SAL 2 hr prior to training and 0.5 hr prior to training; and (5) SAL 0.5 hr prior to training. All solutions were coded so that the investigator injecting mice was unaware of their content, and a separate investigator training mice was unaware of the coded solution injected. After training and before test, mice cage numbers were recoded and the position of cages on the animal rack shifted.

Mice received one-trial passive avoidance training in a standard step-through apparatus [12]. A black Plexiglas start box (9 cm long × 10.2 cm wide × 12.5 cm high) was separated from a white Plexiglas shock compartment (35 cm long) by a black panel with a 3.8 cm diameter hole at its base. Illumination of the apparatus was by a 1.8 W bulb situated behind a white translucent Plexiglas panel at the end of the shock compartment. Entry into the shock compartment was controlled by a guillotine door of white translucent Plexiglas. A 0.30 mA footshock was delivered through 2.4 mm diameter brass rods in the shock compartment by a constant current 18-pole shock scrambler.

For training, a mouse was placed in the start box and after 10 sec the light illuminating the apparatus was turned on. Approximately 10 sec later the guillotine door blocking access to the shock compartment was removed when the animal was oriented away from the entrace. The step-through-latency (STL) was measured as the time from the mouse's first orientation to the entrance until the point at which it had all four paws on the grid of the shock compartment. Five sec after the mouse entered the shock compartment, a continuous 0.30 mA footshock was delivered through the grid until the mouse escaped back into the start box. The guillotine door was replaced and the light turned off. After 5 sec the mouse was returned to its home cage. Mice with training STLs greater than 20 sec or escape latencies over 12 sec were eliminated from the experiment (total of 17 animals in Experiments 1 and 4 eliminated out of 339 trained under the conditions described for Experiment 1).

All animals were given an initial retention test (designated T_1) either 1 day after training or 7 days after training. Approximately half of the mice tested at 1 day and all of the mice tested at 7 days were given three additional retention tests on the subsequent three days (designated as T_2, T_3, and T_4). Four tests were given because it previously has been shown that multiple tests are useful in assessing the degree of memory impairment and are sensitive to differences between groups that do not show up on the initial retention test [5]. Testing was identical to training except that no footshock was delivered, and mice that entered the shock compartment were forced back into the start box after 5 sec by gentle touching of their hindquarters with the hand. Animals not entering the shock compartment within 300 sec were given a test score of 300. Training STLs and escape latencies demonstrate a normal distribution and were analysed by

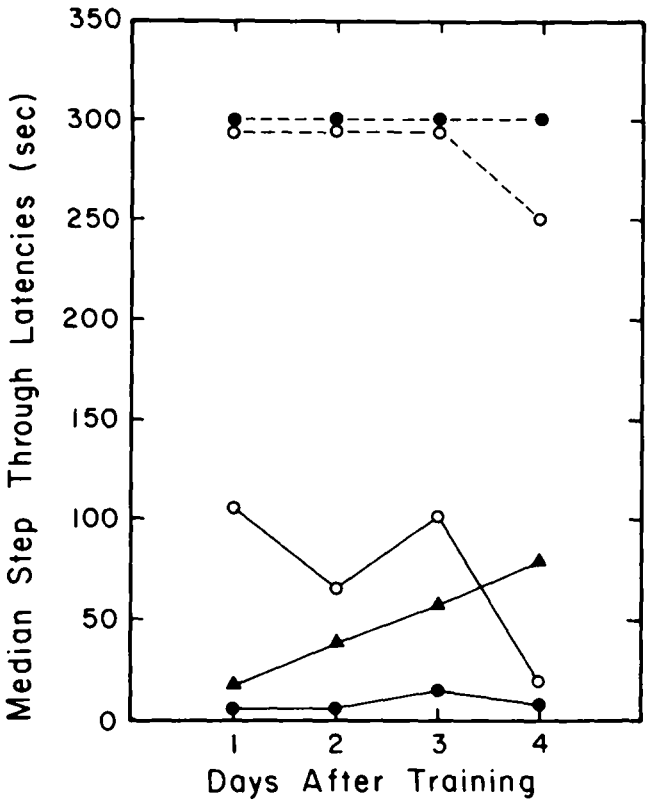

FIG. 1. Median step-through latencies for mice first tested at 1 day after training and then approximately one-half of the mice were given a single retention test on each of the 3 following days. The different groups are represented as follows: CYC 120 mg/kg + 30 mg/kg ○—○; CYC 120 mg/kg ●—●; CYC 30 mg/kg ▲—▲; SAL + SAL ○— — —○; SAL ●— — —●. The N per group ranged between 33 and 45.

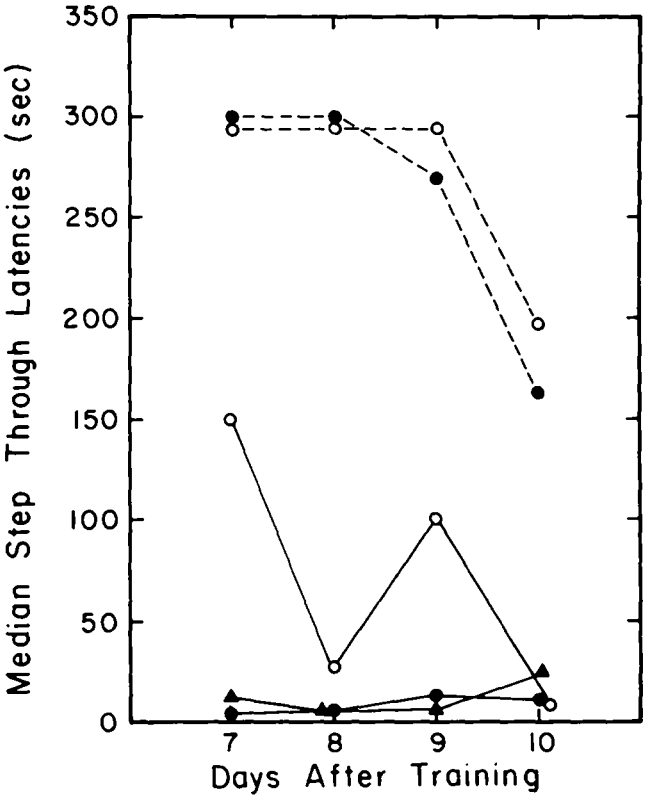

FIG. 2. Median step-through latencies for mice first tested at 7 days after training and then all mice were given a single retention test on each of the 3 following days. The different groups are represented as follows: CYC 120 mg/kg + 30 mg/kg ○—○; CYC 120 mg/kg ●—●; CYC 30 mg/kg ▲—▲; SAL + SAL ○— — —○; SAL ●— — —●. The N per group ranged between 21 and 27.

analysis of variance. The test STLs for passive avoidance are bimodally distributed, so for this measure different drug groups were compared with the Kolmogorov-Smirnov two-sample test.

RESULTS AND DISCUSSION

Training

The mean STLs (±SEM) at training for the groups of mice were the following: CYC 120 mg/kg + 30 mg/kg, 5.2±0.4 sec; CYC 120 mg/kg, 4.2±0.5; CYC 30 mg/kg, 5.9±0.5; SAL + SAL, 6.8±0.5; SAL, 6.1±0.5. A one-way analysis of variance revealed a significant effect of drug on STLs, $F(4,310)=4.08$, $p<0.05$, and application of the Tukey-HSD test at the 0.05 level indicated that this effect was due to the lower STLs by the mice injected with CYC (120 mg/kg) as compared to the STLs of either SAL injected group. Mice injected with CYC or SAL demonstrated similar escape latencies at training, $F(4,310)=2.13$, $p>0.05$. The mean escape latencies (±SEM) for the five conditions were the following: CYC 120 mg/kg + 30 mg/kg, 3.6±0.3 sec; CYC 120 mg/kg, 3.0±0.3; CYC 30 mg/kg, 3.6±0.3; SAL + SAL, 3.0±0.2; SAL, 3.0±0.3.

It is not likely that the low training STLs by the CYC (120 mg/kg) treated mice can account for the amnestic action of this agent. In general, CYC has not produced a significant effect on training STLs in past experiments in this laboratory (Berkeley), but this agent has consistently been found to impair passive avoidance retention by mice. Further, in an experiment by one of the authors, CYC treated mice tended to have higher training STLs than SAL treated mice (9.8 vs. 7.9 sec), yet CYC impaired retention [8]. Thus, the amnesia following CYC cannot be explained in terms of differing training strength based on training STLs.

Retention Tests

Median STLs of SAL- and CYC-treated mice on test series beginning either 1 or 7 days after training are shown in Figs. 1 and 2, respectively. p-Values for comparisons of test STLs starting at 1 or 7 days posttraining between CYC treated mice and their corresponding SAL controls, as well as for comparisons between the different CYC treatments, are presented in Table 1.

The single injections of CYC (120 mg/kg or 30 mg/kg) significantly impaired retention of the passive avoidance habit at all tests beginning either 1 or 7 days after training. The animals that received dual injections of CYC (120 mg/kg +30 mg/kg) demonstrated significantly poorer retention performance than corresponding control SAL mice on 3 of the 4 tests started 1 day posttraining (T_1, T_2, T_4), but they were not significantly impaired on any of the tests in the series ini-

TABLE 1
SIGNIFICANCE OF EFFECTS OF CYC ON MULTIPLE TESTS OF RETENTION

	T_1	T_2	T_3	T_4
Day 1 to Day 4 (Fig. 1)				
Sal + Sal vs. Cyc 120 mg/kg + 30 mg/kg	0.001	0.01	0.09	0.01
Sal vs. CYC 120 mg/kg	0.001	0.001	0.001	0.01
Sal vs. CYC 30 mg/kg	0.001	0.01	0.01	0.01
CYC 120 mg/kg + 30 mg/kg vs. CYC 120 mg/kg	0.001	0.025	0.18	0.25
CYC 120 mg/kg + 30 mg/kg vs. CYC 30 mg/kg	0.35	0.55	0.98	0.74
CYC 120 mg/kg vs. CYC 30 mg/kg	0.01	0.18	0.33	0.10
Day 7 to Day 10 (Fig. 2)				
Sal + Sal vs. CYC 120 mg/kg + 30 mg/kg	0.27	0.20	0.12	0.25
Sal vs. CYC 120 mg/kg	0.001	0.001	0.001	0.25
Sal vs. CYC 30 mg/kg	0.001	0.001	0.001	0.38
CYC 120 mg/kg + 30 mg/kg vs. CYC 120 mg/kg	0.025	0.11	0.10	0.38
CYC 120 mg/kg + 30 mg/kg vs. CYC 30 mg/kg	0.07	0.46	0.58	0.25
CYC 120 mg/kg vs. CYC 30 mg/kg	0.09	0.42	0.66	0.81

tiated 7 days after training. This nonsignificant difference despite rather large differences in median scores reflects the bimodal distribution of test STLs for the passive avoidance task. At the initial 7-day test (T_1), mice receiving the combined CYC treatment showed significantly superior retention performance as compared to mice receiving the single high dose of CYC ($p<0.025$), and they tended to have higher STLs than the animals injected with the low dose of CYC ($p<0.07$).

The results confirm our previous studies reporting that single injections of CYC shortly before training produce a permanent retention deficit for the passive avoidance habit [8,12]. Attenuation of amnesia was not apparent in animals given the combined CYC treatment and initially tested at 1 day despite an N of 42 as compared to Ns of 7 to 11 reported by Rainbow *et al.* [18]. However, mice receiving the combined CYC treatment did demonstrate intermediate retention on all tests starting at 7 days after training, and their differences from SAL controls were not statistically significant. Thus, the two hr pretraining dose of CYC (120 mg/kg) did tend to attenuate the normally amnesic effect of the low dose of CYC (30 mg/kg) injected 0.5 hr prior to training. In subsequent experiments we investigated the generality of this effect on memory and the effect of CYC on spontaneous locomotor activity.

EXPERIMENT 2

If attenuation of amnesia by CYC is related to its effects on protein synthesis, then similar attenuation should be observable following treatment with other antibiotic drugs that inhibit brain protein synthesis. This experiment examines the effect of anisomycin (ANI), a drug that reliably impairs long-term memory [5, 13, 24], on retention of the passive avoidance habit. Specifically, this experiment is designed to determine if the attenuation of amnesia by CYC generalizes to other inhibitors of brain protein synthesis.

METHOD

Subjects

Male Swiss-Webster CD-1 mice were used as in Experiment 1.

Drugs

ANI was dissolved in SAL by adding an approximately equal molar amount of 3N HCl and adjusting the pH to 6–7 with 0.1 NaOH. Subcutaneous injections of SAL or a SAL solution containing varying amounts of ANI (12 mg/ml or 3 mg/ml) were made on the backs of mice either 2 hr, 0.5 hr, or both 2 hr and 0.5 hr prior to training, in a volume or 10 ml/kg which translates to dosages of 120 mg/kg and 30 mg/kg, respectively. Extent and duration of protein synthesis inhibition by ANI has been reported previously by the authors [5, 6, 7].

Apparatus and Procedure

The apparatus, training, and testing were as described in Experiment 1. The three drug conditions and two vehicle conditions were the following: (1) ANI (120 mg/kg) 2 hr prior to training and ANI (30 mg/kg) 0.5 hr prior to training; (2) ANI (120 mg/kg) 2 hr prior to training; (3) ANI (30 mg/kg) 0.5 hr prior to training; (4) SAL 2 hr prior to training and 0.5 hr prior to training; (5) SAL 0.5 hr prior to training.

RESULTS AND DISCUSSION

Training

Mice injected with ANI or SAL demonstrated similar training STLs, $F(4,228)=2.1$, $p>0.05$. The mean STLs ($\pm$SEM) in seconds were the following: ANI 120 mg/kg + 30 mg/kg, 9.0$\pm$0.7 sec; ANI 120 mg/kg, 8.0$\pm$0.8; ANI 30 mg/kg, 9.6$\pm$0.7; SAL + SAL, 8.0$\pm$0.8; SAL, 6.9$\pm$0.7. The mean escape latencies ($\pm$SEM) for the groups of mice were the following: ANI 120 mg/kg +30 mg/kg, 2.4$\pm$0.4 sec; ANI 120 mg/kg, 1.8$\pm$0.2; ANI 30 mg/kg, 1.8$\pm$0.1; SAL + SAL, 1.2$\pm$0.2; SAL, 1.8$\pm$0.2. A one-way analysis of variance revealed a significant effect of drug on escape latencies, $F(4,228)=2.5$, $p<0.05$, and application of the Tukey-HSD test at the 0.05 level showed this was due to the difference between the double injected ANI mice and the double injected SAL mice. Since the ANI treated mice show higher escape latencies and thus received greater training, the amnestic effect of this drug cannot be attributed to differing training strengths.

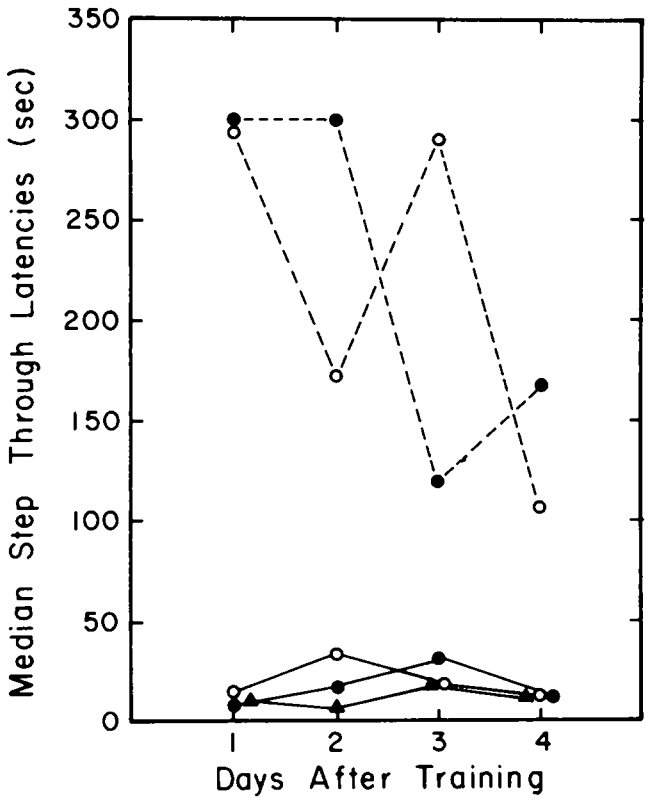

FIG. 3. Median step-through latencies for mice first tested at 1 day after training and then given a single retention test on each of the 3 following days. The different groups are represented as follows: ANI 120 mg/kg + 30 mg/kg ○—○; ANI 120 mg/kg ●—●; ANI 30 mg/kg ▲—▲; SAL + SAL ○- - -○; SAL ●— — —●. The N per group ranged between 24 and 30.

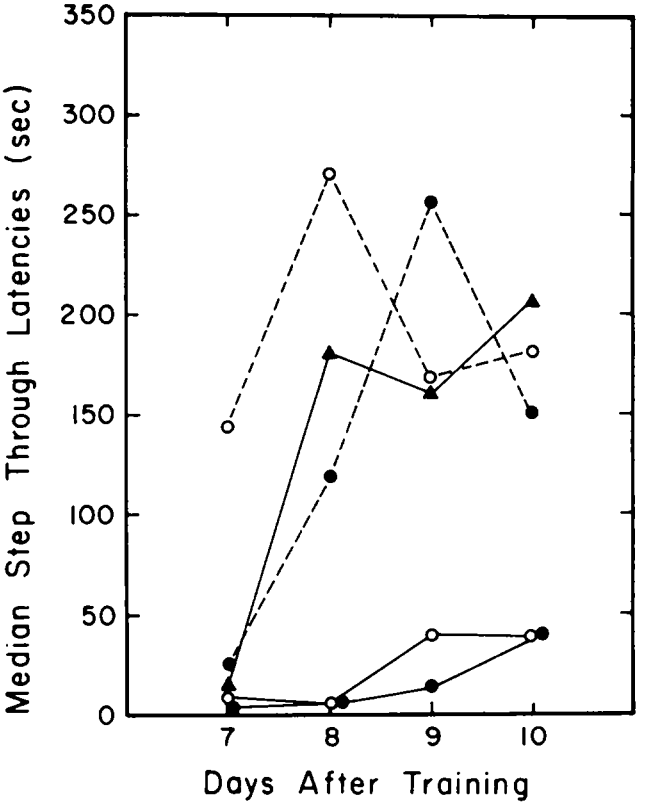

FIG. 4. Median step-through latencies for mice first tested at 7 days after training and then given a single retention test on each of the 3 following days. The different groups are represented as follows: ANI 120 mg/kg + 30 mg/kg ○—○; ANI 120 mg/kg ●—●; ANI 30 mg/kg ▲—▲; SAL + SAL ○— — —○; SAL ●— — —●. The N per group ranged between 18 and 20.

Retention Tests

The median STLs achieved by ANI- and SAL-treated mice on test series beginning either 1 or 7 days posttraining are shown in Figs. 3 and 4, respectively. The p-values for comparisons between ANI treated mice and corresponding SAL control mice for tests starting either 1 or 7 days after training are given in Table 2.

Evaluation of STLs at the tests beginning 1 day after training indicates that all ANI treated groups performed significantly worse than their corresponding SAL controls (see Table 2). Memory impairment was observed in all ANI treated groups on at least three out of four test days, and was invariably present on the first day of testing. No significant difference was detected for any comparison between ANI treated groups at T_1–T_4. At tests starting seven days posttraining, mice receiving either the two injection of ANI or the high dose of ANI demonstrated impaired retention on two of the four tests. Mice receiving the 30 mg/kg dose of ANI were not significantly different from their SAL controls at any test. The unusual poor retention at 7 days (T_1) by SAL controls given one injection might account for the failure to detect a memory deficit in mice receiving a single injection of ANI. However, animals treated with the low dose of ANI performed significantly better on two retention tests as compared to mice given two injections of ANI, and significantly better on three tests as compared to mice injected with the 120 mg/kg dose of ANI.

These results clearly show that inhibition of brain protein synthesis, an effect shared by ANI and CYC, is not responsible for the attenuation of amnesia by an initial pretraining injection of CYC. In fact, far from protecting against amnesia, a large dose of ANI (120 mg/kg) given 90 minutes prior to a low dose of ANI (30 mg/kg) had a detrimental effect on retention of the passive avoidance habit.

EXPERIMENT 3

Since it has previously been shown that the passive avoidance task used alone is not ideal for evaluation of drug effects on behavior [8], and since Experiment 2 indicated that the protective effect of antibiotic drugs on memory might be specific to CYC, this experiment was designed to determine if CYC's attenuation of amnesia is restricted to the passive avoidance task. Specifically, CYC's effect on memory of an active avoidance position habit was assessed.

METHOD

Subjects

Male Swiss-Webster CD-1 mice 60–90 days of age were used.

Drug

CYC was prepared as per Experiment 1. The drug and vehicle conditions were also as per Experiment 1.

TABLE 2
SIGNIFICANCE OF EFFECTS OF ANI ON MULTIPLE TESTS OF RETENTION

	T_1	T_2	T_3	T_4
Day 1 to Day 4 (Fig. 3)				
Sal + Sal vs. ANI 120 mg/kg + 30 mg/kg	0.001	0.06	0.01	0.11
Sal vs. ANI 120 mg/kg	0.001	0.01	0.36	0.01
Sal vs. ANI 30 mg/kg	0.001	0.01	0.15	0.05
ANI 120 mg/kg + 30 mg/kg vs. ANI 120 mg/kg	0.95	0.78	0.94	0.90
ANI 120 mg/kg + 30 mg/kg vs. ANI 30 mg/kg	0.99	0.90	0.99	0.95
ANI 120 mg/kg vs. ANI 30 mg/kg	0.74	0.15	0.99	0.99
Day 7 to Day 10 (Fig. 4)				
Sal + Sal vs. ANI 120 mg/kg + ANI 30 mg/kg	0.05	0.025	0.39	0.22
Sal vs. ANI 120 mg/kg	0.09	0.001	0.001	0.08
Sal vs. ANI 30 mg/kg	0.99	0.99	0.63	0.99
ANI 120 mg/kg + 30 mg/kg vs. ANI 120 mg/kg	0.82	0.82	0.56	0.98
ANI 120 mg/kg + 30 mg/kg vs. ANI 30 mg/kg	0.24	0.05	0.40	0.05
ANI 120 mg/kg vs. ANI 30 mg/kg	0.06	0.001	0.025	0.05

Apparatus and Procedure

Mice received 6 training trials in a Plexiglas T-maze (12.5 cm high, 9.8 cm wide throughout, the stem being 46 cm long, and each arm 17.5 cm long) painted flat black except for a clear top. A guillotine door 11 cm from the closed end of the stem formed a start box. Each maze arm was lined with a removable clear Plexiglas container that extended below the shock grid and was used for removing animals from the maze after each trial. Footshock (0.30 mA) was delivered through 2.4 mm diameter brass rods by a constant current 18 pole shock scrambler.

For training a mouse was placed into the start box. Five sec later a door bell buzzer sounded and the guillotine door was removed. After 5 sec footshock was initiated and continued until the mouse entered the correct arm of the maze. On the first trial the arm initially entered was incorrect and the buzzer and shock continued until the mouse moved into the other arm. For all subsequent trials the first arm entered on trial 1 was considered incorrect and the opposite arm correct. When the mouse entered the correct alley prior to shock onset, the buzzer was turned off and an avoidance response was scored. If the mouse entered the correct alley after shock initiation, the buzzer and shock were terminated and an escape response was scored. Exit from the correct alley was blocked by lowering a guillotine door and after 10 sec the mouse was returned to its home cage for an inter-trial interval of approximately 30 sec. A mouse was discarded if it made no correct escape response (6 mice), if it made more than one avoidance response (1 mouse), or if it received more than 60 sec of shock on one trial (7 mice). A total of 14 animals, 2 or 3 from each experimental condition, were discarded out of 99 trained.

Seven days later mice were tested for retention by retraining a mouse until it made one correct avoidance response. Mice not avoiding within 10 trials were returned to their home cage and given a test score of 10. The apparatus was wiped clean with alcohol and allowed to dry between the training and testing of each mouse.

RESULTS AND DISCUSSION

Training

Mice receiving subcutaneous injections of CYC (120 mg/kg + 30 mg/kg, 120 mg/kg, or 30 mg/kg) or SAL (SAL + SAL or SAL) did not differ in the total amount of time exposed to shock over the 6 training trials. The means (±SEM) for shock exposure time were 36.6±2.1, 31.8±3.6, 30.0±2.4, 31.5±2.7, and 25.2±2.6 sec, respectively, and a one-way analysis of variance revealed no overall effect of drug on shock exposure time, $F(4,80)=2.26$, $p>0.05$. Similarly, there was no significant effect of drug on the number of correct escape responses during training, $F(4,80)=0.54$, $p>0.70$. The mean number of correct responses (±SEM) for the groups of mice were the following: CYC 120 mg/kg + 30 mg/kg, 3.1±0.3; CYC 120 mg/kg, 3.1±0.3; CYC 30 mg/kg, 3.4±0.3; SAL + SAL, 2.8±0.3; SAL, 3.2±0.3.

Retention Test

The mean number of trials (±SEM) required to make a correct avoidance response at a 7 day test for CYC- and SAL-treated mice are shown in Fig. 5. A one-way analysis of variance revealed a significant effect of drug treatment on retraining performance, $F(4,80)=4.1$, $p<0.025$. Application of the Tukey-HSD test at the 0.05 level indicated this effect was due to the greater number of retraining trials required by the doubly injected CYC mice as compared to the mice receiving two injections of SAL, a single injection of SAL or a 30 mg/kg injection of CYC.

This experiment clearly shows that a large dose of CYC (120 mg/kg) results in amnesia when given 90 minutes prior to what is normally a subamnestic dose for active avoidance. Thus, the opposite of a "protective" effect by CYC emerges when an active avoidance task is used.

EXPERIMENT 4

Since CYC showed indications of a "protective" effect in

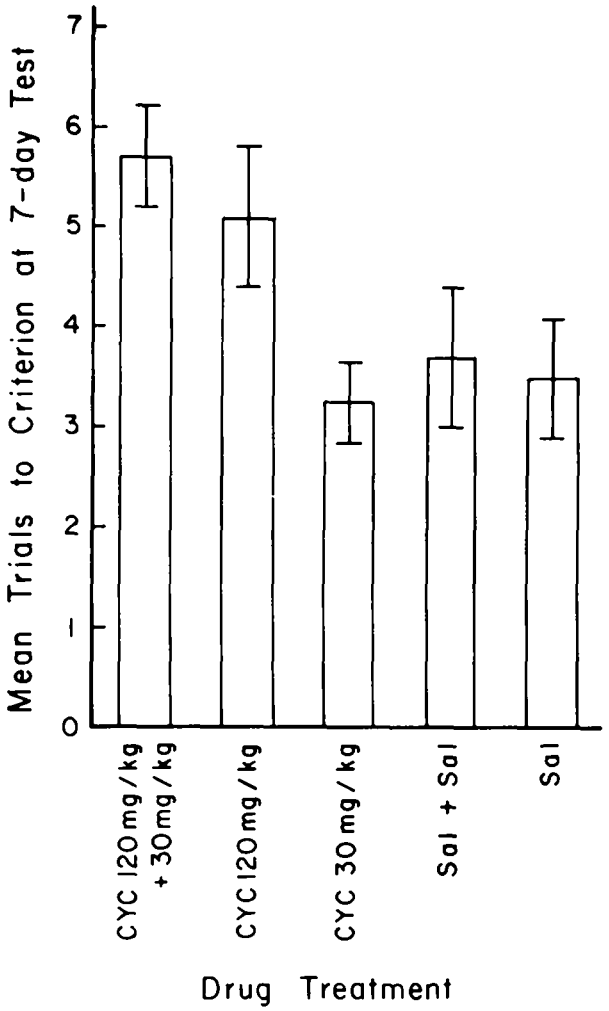

FIG. 5. Mean number of trials to make one correct avoidance response 7 days after training. The N per group ranged between 15 and 20, and the standard error of the means are shown by the vertical bars.

the passive avoidance task that was not found with ANI, we looked further into ways in which CYC differs from other inhibitors of protein synthesis. An obvious difference is in toxicity. All deaths in this study occurred in the group of mice that received two successive injections of CYC. Since CYC alters spontaneous locomotor activity and general health in mice, these side-effects might have effects on STLs in the passive avoidance task [20]. Thus, in this experiment we examine the effects of CYC on spontaneous locomotor activity to determine if it might account for the reported protective effect of CYC.

METHOD

Subjects

Male Swiss-Webster CD-1 mice as per Experiment 1 were used.

Apparatus and Procedure

Mice in three drug groups (CYC 120 mg/kg + CYC 30 mg/kg; CYC 120 mg/kg; CYC 30 mg/kg) and two vehicle control groups (SAL + SAL; SAL) were treated and trained in precisely the same manner as described in Experiment 1 except that 1 day after training instead of being tested for retention of the passive avoidance habit, each mouse was placed into an activity box for 10 min. The activity box (30.5 cm square × 15.5 cm high) was painted flat black and divided into quadrants by photocells. The number of crossings/min were automatically recorded during the 10 min session. The N per group was 12 or 13 animals.

RESULTS AND DISCUSSION

Animals receiving CYC or SAL demonstrated similar activity levels 1 day after treatment and training. The mean number of quadrant crossings (±SEM) in the activity box during the 10 min session were the following: CYC 120 mg/kg + 30 mg/kg, 225±15; CYC 120 mg/kg, 210±13; CYC 30 mg/kg, 235±8; SAL + SAL, 236±11; SAL, 223±11. A one-way analysis of variance with repeated measures on the number of crossings per minute revealed no measurable effect of drug on activity, $F(4,56)=1.31$, $p>0.25$. Thus, proactive effects on activity at one day retention tests cannot account for the reported protective effect of CYC or for the trend toward attenuation of amnesia observed in Experiment 1. This lack of effect occurred despite the fact that two injections of CYC is a near fatal dosage (7 mice of the 339 trained in Experiments 1 and 4 died, all in the group of 59 mice that received two successive injections of CYC).

GENERAL DISCUSSION

The results of this series of experiments do not support the idea that a prior injection of CYC affords protection against amnesia. Instead, it appears that the attenuation of amnesia by an injection of CYC 90 min prior to a normally amnestic dose of CYC is as yet an unexplained artifact specific to CYC and the passive avoidance task. Contrary to a protective effect on memory, ANI given to animals trained in the passive avoidance task and CYC given to animals trained in an active avoidance task resulted in greater impairment of memory.

The suggestion by Rainbow *et al.* [18] that some effect other than inhibition of brain protein synthesis might be responsible for CYC's amnestic action was based on the observation that extent of inhibition did not correlate with degree of amnesia. However, if the apparently artifactual double injection of CYC is not considered in the Rainbow *et al.* [18] study, then the single injections of CYC given 30 minutes prior to training do not provide support for this idea since all produced amnesia. There are a number of studies showing a fairly good correlation between the degree of protein synthesis inhibition during training and the severity of amnesia. For example, Quinton and Kramarcy [17] reported that the level of inhibition during passive avoidance training correlated with the extent of amnesia. Similarly, Squire and Davis [24] reported that inhibition of cerebral protein synthesis during training by ANI (210 mg/kg) produced considerably more amnesia for object discrimination training than did ANI (30 mg/kg). Both dosages produced a profound inhibition of cerebral protein synthesis 15–45 minutes after injection; the high dose of ANI (210 mg/kg) produced 98% inhibition and the low dose of ANI (30 mg/kg) produced 96% inhibition. A criticism of this view is that while a significant difference in the degree of amnesia is

produced by these two doses, the small difference (2%) in inhibition of protein synthesis is not significant. However, if what is important in determining whether an animal will or will not remember a training experience is the slight capacity for protein synthesis after treatment with an antibiotic drug, then the remaining residual capacity for protein synthesis during training is what should be examined. In the study of Squire and Davis [24] just mentioned, the residual capacity of cerebral protein synthesis during training was calculated to be about twice as great after the low dose (30 mg/kg; 4% capacity) as after the high dose (210 mg/kg; 2% capacity). These differences are not, however, significantly different and this points up *one* of the difficulties associated with obtaining dose response curves for protein synthesis inhibiting drugs. That is, the biochemical assay is not sufficiently sensitive to detect small differences in the percentage of protein synthesis inhibition that may result in a large relative difference in the capacity of an organism to form long-term memory. However, if the large number of studies that have used several different dosages of protein synthesis inhibitors are considered (for reviews see [4], and Davis and Squire, Manuscript submitted), then evidence is consistent with an inverse relationship between inhibition of brain protein synthesis and level of retention. Lack of information about recovery of synthesis for different proteins, cell types, and brain areas precludes a more definitive statement on this relationship.

Kasprow *et al.* [15] suggest that CYC and electroconvulsive shock have a common mode of action for affecting memory since either treatment can attenuate the memory impairment following normally amnestic treatments of either CYC or electroconvulsive shock. This would imply that the antibiotic drugs are affecting memory by some mechanism other than inhibition of brain protein synthesis, since ECS does not inhibit protein synthesis to the extent (80–90%) required for inducing impairment of memory. However, from the finding that two treatments disrupt memory, or that one treatment disrupts memory and another treatment improves memory, it cannot logically be assumed that both treatments influence memory by the same mechanism. This is demonstrated by the following two examples. Two lines of evidence that have been cited as support for the idea that antibiotic drugs induce amnesia via effects on catecholamines are that (1) adrenergic agonists can attenuate CYC or ANI induced amnesia, and (2) adrenergic antagonists produce amnesia as do antibiotic inhibitors of protein synthesis. However, several studies have dissociated the effects of protein synthesis inhibiting drugs on catecholamines from their effects on memory [14, 21, 26]. Quinn and colleagues [1, 9, 10] have reported that three different mutants of *Drosophila* demonstrate impaired ability to learn an odor discrimination task, yet the single-gene mutations underlying this behavioral impairment are different for the three mutants. There are numerous biochemical [16,25] and neuroanatomical systems [23] that affect memory and agents that act on multistep biochemical pathways in these systems may alter an organism's retention of an experience. It does not follow that such agents share a common mode of action.

Alternative hypotheses that propose that inhibitors of brain protein synthesis induce amnesia via an effect other than on protein synthesis have been previously considered in detail ([19,25], and Davis and Squire, Manuscript submitted). The results of this study are consistent with the hypothesis that cerebral protein synthesis during or shortly after training is one of the necessary biochemical steps in the formation of long-term memory.

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