Pharmacology Biochemistry & Behavior, Vol. 4, pp. 13–16.

Inhibition of Cerebral Protein Synthesis: Performance at Different Times After Passive Avoidance Training[1]

HASKER P. DAVIS[2], CURT W. SPANIS AND LARRY R. SQUIRE

Veterans Administration Hospital and the Department of Psychiatry
University of California, School of Medicine, San Diego, CA 92161

(Received 13 June 1975)

DAVIS, H. P., C. SPANIS AND L. R. SQUIRE. *Inhibition of cerebral protein synthesis: retention at different times after training.* PHARMAC. BIOCHEM. BEHAV. 4(1) 13–16, 1976. – Inhibition of cerebral protein synthesis impairs long-term memory in a variety of species and tasks. Recently it was reported that subcutaneous injection of the protein synthesis inhibitor cycloheximide impaired short-term retention, measured 10 min after training in a passive avoidance task. To examine the possibility that inhibition of cerebral protein synthesis may sometimes disrupt short-term memory, mice were injected subcutaneously with cycloheximide (120 mg/kg) or anisomycin (150 mg/kg), or bitemporally with cycloheximide or anisomycin (100 μg/side) and given one training trial in a passive avoidance box. Subcutaneously injected cycloheximide reduced step-through latencies 10 min after training as reported previously, but anisomycin or bitemporally injected cycloheximide did not. All 4 drug groups exhibited impaired long-term memory. Since the results obtained at short intervals after training varied depending on the drug and route of injection, the impairment produced by subcutaneous cycloheximide at 10 min after training cannot be attributed to inhibition of cerebral protein synthesis. It is suggested that performance at short intervals after training reflects drug side effects on step-through behavior. By contrast, the impairment obtained at long intervals after training is consistent with the hypothesis that cerebral protein synthesis is required for formation of long-term memory.

Memory Inhibition of cerebral protein synthesis Passive avoidance Cycloheximide Anisomycin

INHIBITION of cerebral protein synthesis shortly before or after training markedly impairs long-term memory in a variety of species and tasks [1, 2, 11]. In the case of discrimination tasks, for example, learning during brief training is normal but amnesia develops gradually during the hours after training. This conclusion has been based on results with intracerebral or subcutaneous injection of three classes of protein synthesis inhibitors – represented by puromycin, cycloheximide, and anisomycin.

It has been reported previously that mice given cycloheximide subcutaneously before training in a one-trial, step-through passive avoidance task, exhibited a marked retention deficit 10 min after training [4, 7, 8]. This finding raised the possibility that inhibition of cerebral protein synthesis can sometimes disrupt short-term as well as long-term memory. We have therefore compared the effects of intracerebral or subcutaneous injections of two inhibitors of protein synthesis, cycloheximide and anisomycin, on short- and long-term retention of the passive avoidance habit. We found that inhibition of cerebral protein synthesis established by either of these drugs, regardless of the route of administration, produced a marked deficit in long-term memory in agreement with all previous studies. Yet, only subcutaneous injection of cycloheximide impaired retention at short training-retention intervals. Establishment of protein synthesis inhibition by anisomycin or by intracerebrally-injected cycloheximide had either no effect on performance or facilitated performance at short intervals after training.

Accordingly, the impairment in short-term retention produced by subcutaneously injected cycloheximide cannot be attributed to inhibition of protein synthesis. It is suggested that altered step-through latencies at short intervals after passive avoidance training may reflect specific effects of drugs on step-through behavior and need not reflect changes in short-term memory. However, the consistent impairment obtained with both anisomycin and cycloheximide 1–5 days after training supports the hypothesis that cerebral protein synthesis is required for the formation of long-term memory.

[1] We thank Carl Becker for technical assistance. Supported by NIMH Grant MH 24600 and by a Clinical Investigatorship (8084–C) from the Veterans Administration to Dr. Squire.
[2] Now at the Department of Psychology, University of California, Berkeley, California.

METHOD

Animals

Male Swiss albino mice were obtained from Simonsen Laboratories (Gilroy, California) at 10 weeks of age and trained 2–7 days later. Animals were housed 10 to a cage and given free access to food and water.

Apparatus and Procedure

Mice were given one-trial passive avoidance training in a standard step-through apparatus [5]. A black Plexiglas panel with a small hole at its base separated a clear Plexiglas start box (7.5 cm long x 3 cm wide at the base x 10 cm wide at the top) from a black Plexiglas shock compartment (23 cm long x 3 cm wide at the base x 10 cm wide at the top). The floor of the start box and shock compartment consisted of metal plates. The experimental room was dark except for a 25 W Tensor lamp that illuminated the start box.

For training, a mouse was placed in the start box facing towards the dark compartment. When the mouse touched the rear plates in the dark compartment (8.4 cm from the entrance), a 0.20 mA footshock was delivered through the plates until the mouse escaped back to the start box. The mouse was then returned to its home cage. Step-through latency, the time from placement in the start box to shock onset, was recorded automatically. Retest conditions were identical to those of training except that no shock was delivered. For retest, animals not entering the shock compartment within 600 sec were removed and given a score of 600. Step-through latencies at different training-retest intervals were compared with a Mann-Whitney U Test [10].

Subcutaneous injections of saline, anisomycin (150 mg/kg), or cycloheximide (120 mg/kg) in a volume of 0.30 ml/25 g body weight were given 10 min before training. It has been demonstrated previously that brain protein synthesis is inhibited by greater than 85 percent within 10 min after subcutaneous injection of cycloheximide or anisomycin [3,15]. Intracerebral injections of saline, cycloheximide (100 μg/side), or anisomycin (100 μg/side) were given bitemporally [13] in a volume of 10 μl/side 4 hr before training. Intracerebral injection of cycloheximide inhibits brain protein synthesis by about 70 percent 4 hr after injection and impairs long-term memory for discrimination training [13].

To determine the extent of inhibition 4 hr after intracerebral injection of anisomycin, 8 mice received subcutaneous injections of 4 Ci of L(^{14}C) 1-leucine (55.5 mCi/mmole, Int. Chem. Nuclear Corp.) 3-3/4 hr after intracerebral anisomycin (100 μg/side) or intracerebral saline and were killed 30 min later. Incorporation into TCA-precipitable material was determined as described previously [16].

RESULTS

Mice receiving subcutaneous injections of cycloheximide, anisomycin, or saline before training exhibited similar step-through latencies during the training trial. The mean step-through latency for these groups was 9.8, 7.4, and 7.9, respectively; and the difference between groups [6] fell just short of significance, $F(2,317) = 2.7$, $p>0.05$. The mean step-through latency for mice receiving intracerebral injections of cycloheximide, anisomycin, or saline was 11.3, 5.2, and 7.5, respectively. The effect of intracerebral injections on step-through latencies during the training trial was significant, $F(2,213) = 8.9$, $p<0.01$.

Step-through latencies of subcutaneously injected mice at different times after training are presented in Fig. 1. Mice given cycloheximide exhibited significantly lower step-through latencies than saline-treated mice at 10 min, 3 hr, and 24 hr after training ($p<0.02$). Anisomycin-treated mice exhibited either normal or significantly elevated step-through latencies at short training-test intervals (10 min to 3 hr). At 24 hr after training anisomycin-treated mice had significantly lower latencies than control mice ($p<0.01$). The median step-through latencies observed for control mice were typical for mice trained with 0.2 mA footshock [4].

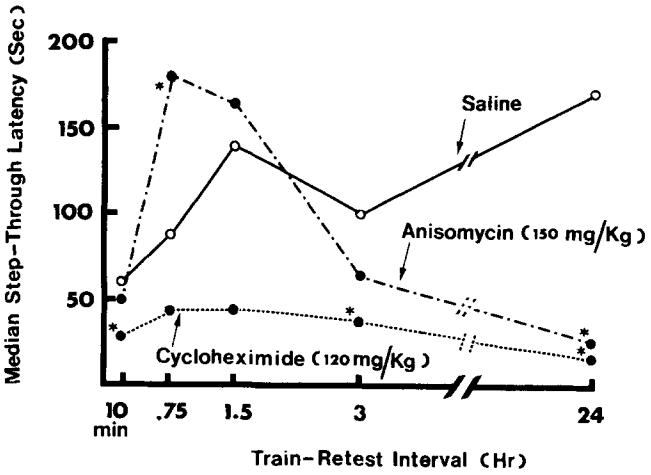

FIG. 1. Median step-through latencies at various times after one-trial passive avoidance training. Mice were subcutaneously injected with saline, cycloheximide (120 mg/kg), or anisomycin (150 mg/kg) 10 min before training. The number of mice in each group ranged from 19 to 26. Asterisks denote scores significantly different from those of the corresponding control group.

Step-through latencies of intracerebrally injected mice at different times after training are presented in Fig. 2. Anisomycin inhibited brain protein synthesis by 88 percent at 4 hr after injection. Neither cycloheximide (100 μg/side) nor anisomycin (100 μg/side) affected performance at 10 min after training. The latency for cycloheximide-injected mice was considerably higher than the latency for saline- or anisomycin-injected mice, but this difference did not reach significance ($p<0.12$). Retest scores in the passive avoidance task often exhibit high variability because step-through scores at retest tend to be bimodally distributed. Both cycloheximide and anisomycin significantly impaired performance at longer intervals after training. At 1 day after training, the impairment was signficant only for anisomycin-injected mice ($p<0.01$). By 5 days after training, the step-through latencies of each drug group were significantly below those of the saline control group ($p<0.02$).

DISCUSSION

Inhibition of cerebral protein synthesis impaired

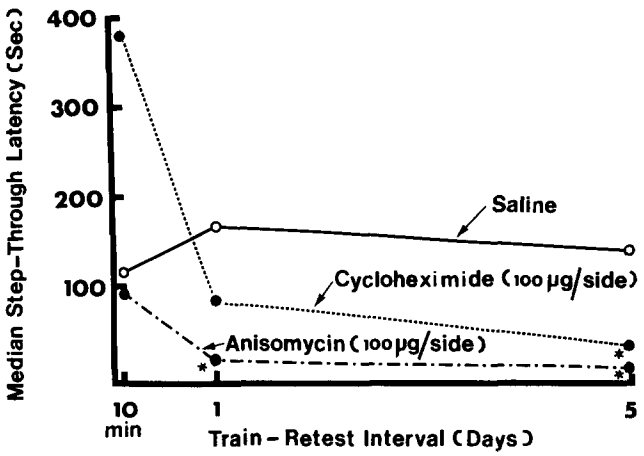

FIG. 2. Median step-through latencies at various times after one-trial passive avoidance training. Mice were given intracerebral injections of saline, cycloheximide (100 µg/side), anisomycin (100 µg/side) 4 hr before training. The number of mice in each group ranged from 20 to 30. Asterisks denote scores significantly different from those of the corresponding control group.

long-term retention of a passive avoidance habit 1 to 5 days after training. These results were obtained with two drugs, cycloheximide and anisomycin, and with two routes of injection, subcutaneous and intracerebral. The results at short training-retest intervals varied, depending on the drug and the route of injection. Subcutaneously injected cycloheximide impaired performance at most retention intervals tested, from 10 min to 1 day. Subcutaneously injected anisomycin had no effect on performance 10 min after training, but facilitated performance 45 min after training. Intracerebrally injected cycloheximide or anisomycin had no effect on performance 45 min after training. Intracerebrally injected cycloheximide or anisomycin had no effect on performance at short training-test intervals.

Since the results at short training-retest intervals depended on which drug and which route of injection was used to establish inhibition of cerebral protein synthesis, the results could not have been caused by inhibition of protein synthesis itself. It seems likely instead that both the short-term impairment produced by cycloheximide and the short-term facilitation produced by anisomycin are due to side effects of these drugs. For example, the effects observed at short training-retest intervals could be due to the fact that cycloheximide produces hyperactivity and anisomycin produces hypoactivity during the hour after subcutaneous injection [9, 14, 17].

Recently an attempt was made to dissociate the effects of cycloheximide on locomotor activity from its effect on short-term retention in the passive avoidance task [4]. Pargyline(75 mg/kg) caused hyperactivity like cycloheximide, but did not impair performance of the passive avoidance task 10 min after training. In view of this finding it is possible that side effects of these drugs other than effects on locomotor activity are responsible for altered performance at short training-retest intervals. However, the fact that two drugs produce similar effects on locomotor activity in normal mice does not necessarily mean that these drugs will produce similar locomotor effects after the mice have been stressed by footshock.

Whatever the explanation for the effects observed at short training-retest intervals, it seems clear that the passive avoidance task is not a favorable situation for investigating effects of drugs on short-term memory. Indeed, the observation that training step-through latencies, as well as retest latencies, were affected by the drug and the route of injection further emphasizes the difficulty of evaluating step-through behavior during the time when a drug is most active. In the passive avoidance task any effects of a drug on freezing, locomotor activity or the general health of the animal can affect response latency and thereby confound the measurement of performance.

An impairment in performance produced by cycloheximide or anisomycin within a few minutes after the beginning of prolonged discrimination training has been reported previously [12, 13, 18]. This impairment, however, is quite small compared to the impairment that subsequently develops during the hours after training. Specifically, the deficit that appears during the second 20 or 40 massed training trials in a discrimination task can be considerably increased by lengthening the interval between the two blocks of 20 trials from 15 sec to 24 hours. The greater the training-retest interval, the greater the deficit during retest. As has been argued previously [13], the impairment observed during prolonged discrimination training may be early evidence of progressive impairment in a long-term memory process that is dependent on cerebral protein synthesis. Thus, proteins synthesized during training may sometimes be required within minutes after training for the normal expression of memory. The results previously obtained with discrimination training therefore provide further support for the notion that the short-term deficit in passive avoidance produced by cycloheximide is specific to this drug and to this task. Only in the passive avoidance task has a protein synthesis inhibitor been reported to cause as marked an impairment in short-term retention as in long-term retention.

By contrast, a consistent impairment in long-term retention was observed in the present study, in agreement with all previous studies. Results obtained 1 to 5 days after training, long after the capacity for brain protein synthesis has recovered, are difficult to explain by side effects of drugs on performance. These results support the hypothesis [1, 2, 11] that cerebral protein synthesis is required for formation of long-term memory.

REFERENCES

1. Agranoff, B. W. Effects of antibiotics on long-term memory formation in the goldfish. In: *Animal Behavior*, edited by W. K. Honig and P. H. R. James. New York: Academic Press, 1971, pp. 243–258.
2. Barondes, S. H. Protein synthesis-dependent and protein synthesis-independent memory storage processes. In: *Short-Term Memory*, edited by D. Deutsch and J. A. Deutsch. New York: Academic Press, 1975.
3. Flood, J. F., M. R. Rosenzweig, E. L. Bennett and A. E. Orme. Influence of training strength on amnesia induced by pretraining injections of cycloheximide. *Physiol. Behav.* **9**: 589–600, 1972.
4. Gutwein, B. M., D. Quartermain and B. S. McEwen. Dissociation of cycloheximide's effects on activity from its effects on memory. *Pharmac. Biochem. Behav.* **2**: 753–756, 1974.

5. Jarvik, M. E. and R. Kopp. An improved one-trial passive avoidance learning situation. *Psychol. Rep.* **21:** 221–222, 1967.
6. Keppel, G. *Design and Analysis.* Englewood Cliffs, New Jersey: Prentice Hall, Inc., 1973.
7. Quartermain, D. and B. S. McEwen. Temporal characteristics of amnesia produced by inhibition of protein synthesis. *Nature Lond* **228:** 667–678, 1970.
8. Randt, C. T., B. M. Barnett, B. S. McEwen and D. Quartermain. Amnesic effects of cycloheximide on two strains of mice with different memory characteristics. *Expl. Neurol.* **30:** 467–474, 1971.
9. Segal, D. S., L. R. Squire and S. H. Barondes. Cycloheximide: Its effects on activity are dissociable from its effects on memory. *Science* **172:** 82–84, 1971.
10. Siegel, S. *Nonparametric Statistics.* New York: McGraw-Hill, 1956.
11. Squire, L. R. and S. H. Barondes. Inhibitors of cerebral protein or RNA synthesis and memory. In: *Macromolecules and Behavior,* edited by J. Gaito, 2nd Ed. New York: Appleton-Century-Crofts, 1972, pp. 61–82.
12. Squire, L. R. and S. H. Barondes. Variable decay of memory and its recovery in cycloheximide-treated mice. *Proc. natn. Acad. Sci., U. S. A.* **69:** 1416–1420, 1972.
13. Squire, L. R. and S. H. Barondes. Memory impairment during prolonged training in mice given inhibitors of cerebral protein synthesis. *Brain Res.* **56:** 215–225, 1973.
14. Squire, L. R. and S. H. Barondes. Anisomycin, like other inhibitors of cerebral protein synthesis, impairs "long-term" memory of a discrimination task. *Brain Res.* **66:** 301–308,
15. Squire, L. R. and S. H. Barondes. Amnesic effects of cycloheximide not due to depletion of a constitutive brain protein with short half-life. *Brain Res.* 1976, in press. 1974.
16. Squire, L. R. and H. P. Davis. Cerebral protein synthesis inhibition and discrimination training: effects of extent and duration of inhibition. *Behav. Biol.* **13:** 49–57, 1975.
17. Squire, L. R., A. Geller and M. E. Jarvik. Habituation and activity as affected by cycloheximide. *Communs Behav. Biol.* **5:** 249–254, 1970.
18. Squire, L. R., G. A. Smith and S. H. Barondes. Cycloheximide can affect memory within minutes after the onset of training. *Nature* **242:** 201–202, 1973.