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The Timing of an Injection Procedure Affects Pharmacological Actions on Memory

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BENLOUCIF, S., R. B. MORTIMER, E. L. BENNETT AND M. R. ROSENZWEIG. *The timing of an injection procedure affects pharmacological actions on memory*. PHARMACOL BIOCHEM BEHAV **37**(2) 295–298, 1990.—In a series of experiments examining the effects of the protein synthesis inhibitor anisomycin on memory for a novel active avoidance task in mice, we found that the timing of administering the drug (pretraining or posttraining) affected its amnestic potency. Anisomycin injected after training was more effective than when injected before training. Adding a saline injection, such that all groups received both pre- and posttraining injections, resulted in greater amnesia with anisomycin given before rather than after training. These results indicate that the procedure of drug administration alters the effectiveness of amnestic agents.

Anisomycin Active avoidance Memory Amnesia

THE use of drugs as tools to reveal the processes of memory consolidation is a common practice. Administration of transmitters and hormones demonstrates that naturally occurring chemical messengers can modulate the consolidation process (13) and protein synthesis inhibitors are used to demonstrate that protein synthesis is an essential step in the formation of long-term memory (5) (see the Discussion section for alternative interpretations of this work).

One working hypothesis that has evolved from this type of study is that there is a set number of biochemical events that take place during memory formation and that modulating treatments affect the strength or duration of these biochemical events (12,21). Stimulants given during the posttraining period reduce amnesia caused by a protein synthesis inhibitor (PSI), but do not affect the level of protein synthesis, indicating that stimulants act on a stage of memory formation that occurs prior to the protein synthesis dependent stage (1,8). Reductions in PSI-induced amnesia also occur with behavioral treatments such as shock that increase levels of circulating hormones (1). The improved retention that accompanies higher shock levels can be overcome by a longer duration of protein synthesis inhibition, suggesting that such treatments affect the strength or duration of the consolidation process (9).

We report here that amnesia induced by the protein synthesis inhibitor anisomycin can also be affected by the timing of the injection procedure itself. We present evidence that subcutaneous injections prior to active avoidance training cause less impairment of retention than injections following training. This effect on retention is demonstrated by an interaction of the severity of amnesia induced by anisomycin and the timing of the injection itself.

METHOD

Subjects

Male CD1 mice (30 days old) were obtained from Charles River Laboratories. They were housed 5 per cage until training, which began at 50 to 90 days of age. The mice were individually housed 2 days before experimentation. Food and water were provided ad lib and lights were on L/D 12/12 beginning at 7:00 a.m.

Apparatus and Training

The active avoidance training apparatus, the Wall Jump, is a two compartment Plexiglas box (10 by 32 by 21 cm) with a shock grid floor and wire mesh on the three walls of the escape compartment. Mice received 3 training trials each, consisting of a 10-sec period in the start compartment, then simultaneous opening of the door and start of a buzzer, followed 5 sec later by mild footshock (0.30 mA) in both compartments. The buzzer was turned off when the mouse climbed or jumped onto the mesh wall, signalling the end of a trial. The intertrial interval was 15 seconds. Ten test trials were given at 1, 4, or 7 days after training. Results were scored by the number of avoidances (climbing onto the mesh

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TABLE 1

COMPARISON OF PRE- AND POSTTRAINING ANISOMYCIN ADMINISTRATION

Pretraining Injections Avoidances ± S.E.M. (n)			Posttraining Injections Avoidances ± S.E.M. (n)	
Saline	Anisomycin	Test Day	Saline	Anisomycin
5.9 ± 0.5 (11)	5.2 ± 0.5 (12)	1	5.9 ± 0.5 (15)	4.1 ± 0.4 (15)
6.4 ± 0.5 (14)	4.7 ± 0.6 (13)	4	6.3 ± 0.5 (15)	4.3 ± 0.5 (15)
4.7 ± 0.7 (15)	5.0 ± 0.4 (13)	7	5.8 ± 0.5 (16)	3.9 ± 0.5 (15)
4.1 ± 0.6 (15)	3.8 ± 0.7 (15)	7	4.5 ± 0.6 (15)	2.9 ± 0.5 (14)
5.3 ± 0.6	4.7 ± 0.6†	Mean	5.6 ± 0.5	3.8 ± 0.5*

Injection of 120 mg/kg anisomycin reduced the number of avoidances at test for both pre- and posttraining injections ($p<0.0001$), but the time of injection altered the effectiveness of ANI ($p<0.05$). Mice receiving pretraining ANI injections were less amnesic than those receiving posttraining ANI injections (*$p<0.0001$; †$p<0.14$).

before the shock started) out of the 10 test trials.

Drugs and Injections

The protein synthesis inhibitor (PSI) anisomycin (ANI) was purchased from Pfizer Pharmaceutical Company (now obtainable from Warner-Lambert, General Diagnostics Division). It was dissolved in saline by adding equal molar amounts of 3 N HCl and adjusted to pH 6–7 with NaOH. The dose was 120 mg/kg (0.01 ml/g body wt.) given subcutaneously (SC). This dose decreases protein synthesis by 80% for at least two hours and results in amnesia for a passive avoidance task in mice (19).

Statistical Analysis

Experiments were analyzed by multifactor analysis of variance (ANOVA, by Statview 512+) for drug treatment, time of injection and test day. Subanalyses of main effects and pairwise comparisons were conducted as indicated. Since the number of avoidances made by saline controls fluctuated over the course of experiments, results of Experiment 3 are presented as percent of control avoidances.

Experiment 1

Initial experiments with the Wall Jump Task indicated that mice injected with ANI 15 minutes after training tended to have fewer avoidances at test than mice injected with ANI 15 minutes before training. This contradicted our own experience with protein synthesis inhibitors, as we had often found that protein synthesis inhibition must occur either before or very soon after training in order to impair memory processing in other tasks. The finding that posttraining administration of the drug led to even a slightly greater decrement in retention than pretraining injections was therefore quite surprising. In Experiment 1 we compared the effect of 5-minute pre- and 15-minute posttraining injections at three different training-test intervals (1, 4, and 7 days). A 7-day training-test interval was repeated to verify the replicability of results.

The results of Experiment 1 (Table 1) showed that ANI impaired test performance overall, $F(1,225)=19.6$, $p<0.0001$. Both pre- and posttraining injections impaired retention (no significant difference for the time of injection), but the time of injection did interact significantly with the effectiveness of ANI, $F(1,212)=4.7$, $p<0.05$. This interaction was examined by ANOVA of the effect of pretraining ANI for the three days and the effect of posttraining ANI for the three days. Posttraining injection of ANI significantly impaired test performance, $F(1,112)=25.3$, $p<0.0001$, but pretraining ANI did not when analyzed alone, $F(1,100)=2.2$, $p<0.14$. Average avoidances differed significantly over the course of testing, $F(3,212)=7.0$, $p<0.001$, but did not alter the effectiveness of ANI. These results showed that in this task, ANI given 15 minutes before training does impair retention, but is less effective than ANI given 15 minutes after training.

Experiment 2

Experiment 2 tested whether ANI affected consolidation of the memory trace or had nonspecific effects on recall or some other process. Since it is commonly assumed that agents that act specifically on consolidation affect memory only when given close to training, we administered ANI at times further from training than the times in Experiment 1. Injections were given at 45 or 30 min before training, or 30 or 45 minutes after training. Mice were tested one day after training. {Mean avoidances for mice injected with ANI at −45, −30, +30, and +45 minutes [± S.E.M. (n)] were 3.7 ± 0.6 (15), 3.6 ± 0.5 (15), 3.2 ± 0.5 (17), 4.2 ± 0.6 (15), respectively. Mean avoidances for saline control groups at these injection times were 4.3 ± 0.7 (11), 4.3 ± 0.5 (15), 3.6 ± 0.5 (15), 5.0 ± 0.6 (15).} ANI treatment did not significantly reduce avoidances with these injection times, indicating that the retention deficits in Experiment 1 were due to impaired memory consolidation rather than to some nonspecific effect.

Experiment 3

Experiment 3 (Fig. 1) examined whether the different effectiveness of pre- and posttraining ANI was due to the timing of the injection rather than the time of the drug administration. Effects of the injection procedure were controlled by giving both pre- and posttraining injections to all experimental groups. Thus, mice injected with ANI 15 minutes before training also received an SC injection of saline 15 minutes after training (ANI-Sal). The posttraining ANI group also received an injection of saline before training (Sal-ANI). The control group received saline injections both before and after training (Sal-Sal). A control group that received only posttraining saline injections (/Sal) was added after the 1-day test was completed. Mice were tested at 1, 4, or 7 days.

Overall analysis showed a difference between treatment groups, $F(2,153)=10.18$, $p<0.001$. When analyzed over the 3 test days, both Sal-ANI and ANI-Sal scores were lower than the Sal-Sal

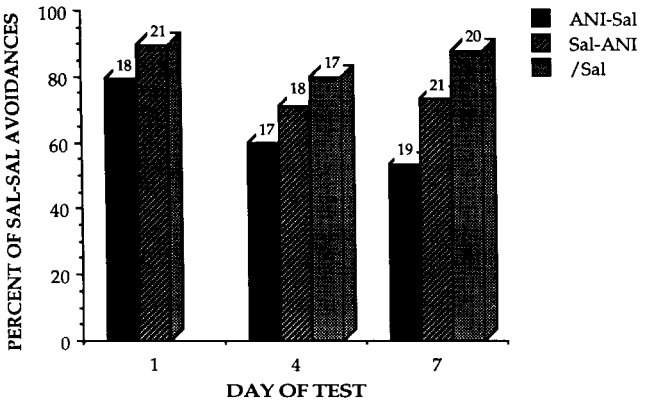

FIG. 1. Counterbalancing the pretraining anisomycin injection with a posttraining saline injection (ANI-Sal) resulted in significant reductions in test avoidances compared to groups injected with saline both before and after training ($p<0.001$). Groups that received saline before and anisomycin after training (Sal-ANI) had test scores that were in between ANI-Sal and Sal-Sal groups (different from Sal-Sal at $p<0.01$). Groups that received only a posttraining saline injection (/Sal) had nonsignificantly lower scores than Sal-Sal groups. The number of mice per group is noted at the top of each column. ANI-Sal scores for test days 1, 4, and 7 $\pm$S.E.M. were 3.9 ± 0.5, 3.4 ± 0.6, 2.6 ± 0.5 respectively. Sal-ANI scores for days 1, 4, and 7 were 4.4 ± 0.5, 3.9 ± 0.5, 3.6 ± 0.5. Sal-Sal scores for days 1, 4, and 7 were 4.9 ± 0.5, 5.5 ± 0.4, 4.9 ± 0.6. /Sal scores for days 4 and 7 were 4.4 ± 0.3 and 4.3 ± 0.6.

controls [Sal-ANI: $F(1,102)=7.51$, $p<0.01$; ANI-Sal: $F(1,102)=21.50$, $p<0.001$]. ANI-Sal scores were consistently but nonsignificantly lower that Sal-ANI group means. The reduction in avoidances by the /Sal group as compared to Sal-Sal group approached significance, $F(1,68)=2.9$, $p<0.09$.

While direct comparison across experiments was not feasible, the relative order of pre- and posttraining ANI groups in Experiments 1 and 3, and the increase in avoidances by Sal-Sal groups compared with /Sal mice in Experiment 3, suggested that a posttraining injection of saline increased the amnestic efficacy of ANI and that a pretraining injection of saline tended to decrease the amnestic efficacy of ANI.

DISCUSSION

In this paper we report that: 1) a single pretraining injection of ANI was less amnestic than was a posttraining injection, and 2) the administration of a posttraining saline injection enhanced the amnesia caused by ANI given before training, whereas saline administered before training reduced the effectiveness of ANI injected after training. These results indicate that minor changes in experimental protocol can modify the effects of pharmacological agents on memory.

PSI-induced amnesia can be attenuated by a variety of treatments. For example, Barondes and Cohen (1) reversed amnesia by administering either a reminder shock, amphetamine, or corticosteroids three hours after training. Similarly, Sara (22) reinstated retention by a contextual reminder cue, amphetamine, or an alpha-2 receptor antagonist given immediately prior to the 3-week retention test. Gold and Sternberg (14) blocked PSI-induced amnesia by the pretraining administration of an alpha-adrenergic antagonist, and others have reported that PSI-induced amnesia is attenuated by agents that stimulate the adrenergic system (7, 11, 20, 23).

To our knowledge, this is the first report of potentiation of PSI-induced amnesia by a posttraining procedure. A plausible explanation for this effect is that the posttraining injection procedure produced a retroactive interference, or a weakening of the memory trace, similar to the retroactive interference caused by a posttraining change in background lighting (3, 4, 15, 16).

Regarding pretraining administration of ANI, the level of stimulation (or arousal) at training can affect retention (13,24) and overcome PSI-induced amnesia (9,10). The minor decrement in retention (10 to 30% fewer avoidances at test than controls) and the relative ease of overcoming retention impairments in this report recalls the long standing controversy of whether the critical factor in retention deficits with PSIs is due to protein synthesis inhibition or another factor such as an alteration in catecholamines [e.g., see discussions of (8) and (14), and reviews (5, 13, 18)].

On the other hand, these results are consistent with the hypothesis that arousal level (or other types of treatments) primarily alters a stage of memory formation that occurs prior to a protein synthesis dependent stage, leading to secondary changes in the duration of protein synthesis (21). Flood and colleagues have shown that increasing the duration of protein synthesis inhibition can counteract both stimulant-induced reversal of PSI-induced amnesia (8) and strong training conditions such as those used here (6, 9, 10).

One possible mechanism underlying the effects reported here is stress-induced release of norepinephrine and other amines resulting in enhanced retention and resistence to memory-impairing drugs (17). Experiments in our laboratory with central administration of the serotonergic and noradrenergic antagonist propranolol support this hypothesis, since pretraining propranolol enhanced amnesia induced by both pre- and posttraining administration of ANI (2).

In conclusion, the unusual effectiveness of posttraining protein synthesis inhibition in comparison with pretraining inhibition was due to an effect of the timing of the injection procedure on memory strength. The most plausible explanation is that the injection procedure was arousing to the mice, thus enhancing retention when given before training and interfering with retention when given after training. Finally, the finding that minor changes in procedure can affect the apparent effectiveness of pharmacological agents is, in itself, an important point to consider in studies of this kind.

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