Pharmacology Biochemistry and Behavior, Vol. 1, pp. 197–202. ANKHO International Inc., 1973. Printed in the U.S.A.

Facilitation and Disruption of the Long-Term Store of Memory with Neural Excitants[1]

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(Received 6 November 1972)

CRABBE, J. C. AND H. P. ALPERN. *Facilitation and disruption of the long-term store of memory with neural excitants.* PHARMAC. BIOCHEM. BEHAV. 1(2) 197–202, 1973. –Male hybrid mice (C57BL/6J x DBA/2J) were trained for 2 days in a 6-unit brightness discrimination maze. Beginning 24 hr after training, mice were administered daily injections of strychnine sulphate, Metrazol, *d*-amphetamine sulphate, caffeine citrate, nicotine alkaloid, or saline for 5 days. Forty-eight hr after the injection series was completed, mice were trained to criterion in the maze. Mice administered strychnine sulphate or Metrazol showed significantly better retention than those administered saline while mice administered *d*-amphetamine sulphate were significantly poorer. Nicotine alkaloid produced a trend toward facilitation, while caffeine citrate had no effect. The observed facilitation and disruption were not due to enhancement or impairment of learning ability and could not be attributed to effects upon the consolidation process.

Memory Long-term memory store Analeptics Memory facilitation Memory disruption
Strychnine Metrazol Pentylenetetrazol *d*-Amphetamine

THE POWER of pharmacological manipulation in the investigation of memory processes has been well established [13, 14, 19, 26, 27, 30]. Drugs have become useful tools for the dissection of specific aspects of memory. Specifically, the consolidation, or time-dependency, notion of memory has been well supported by this avenue of research in studies employing treatments administered after training which block or attenuate consolidation [2, 11, 24, 29, 33] as well as treatments which enhance consolidation [4, 20, 21, 28, 34]. The important feature of these studies is that the treatments were applied after the learning experience had occurred. Consequently, an effect on memory storage processes is implied, since these agents could not have been affecting other performance variables, such as perception, motivation, attention, etc., during the learning experience.

Recently, Alpern and Crabbe [1] reported that a series of low dosages of strychnine sulphate administered after two initial training trials in a maze, but not begun until 24 hr after that training experience, produced a facilitation of the information assumed to be stored in long-term memory. This facilitation was not due to proactive effects of the drug (e.g., enhancement of learning, hypersensitization to stimuli, or increased motivation), because injected animals that had not received the preliminary training were not facilitated. Further, a single injection either 24 or 120 hr after preliminary training did not enhance performance; thus a consolidation interpretration of the data was not supported. The major goal of the present study (Experiment 1) was to examine the generality of this susceptibility of the long-term store of memory to other neural excitants. Moreover, in Experiment 2, we undertook to investigate possible proactive facilitative effects of these compounds [5, 12, 18].

EXPERIMENT 1

Method

Animals. The animals were 96 male F_1 hybrid mice, obtained from crossings between the two highly inbred strains C57BL/6J and DBA/2J. Mice which ranged in age from 85–100 days were given access to mouse chow ad lib throughout the study.

Apparatus. The apparatus has been described in detail elsewhere [1]. The maze consisted of six discrimination units, a start box, and a goal box. The discrimination units had two parallel alleys, one painted flat black, the other flat white. The black alleys were obstructed at the distal end by a transparent window of 1/8 in. standard clear vinyl. The

[1]This investigation was supported by National Institute of Mental Health Grant MH 11167 and National Institute of General Medical Sciences Grant GM 14547.

[2]We wish to express our appreciation to Smith, Kline and French for supplying the *d*-amphetamine sulphate employed in this study.

white alleys, considered correct, appeared on the left side in units 1, 4, and 5, eliminating solution of the brightness discrimination problem with either a position or an alternation preference. Each discrimination unit, and the goal box, was preceded by an entryway, painted flat grey. The goal box, painted flat white, had a 1/4-teaspoon mounted at the far end, in which the reinforcing solution of 0.3% saccharin in tap water was placed. The start box, painted flat grey, had a rectangular funnel attached to the top, into which a mouse could be dropped. All other maze parts were covered with 1/8 in. clear Plexiglas.

Procedure. Twenty-four hr before the first day of initial training, all mice are deprived of water in their home cages. Twenty-four hr later, each mouse was dropped into the start box and the number of initial errors (first entry into the black alley of any unit), total errors (first and all reentries into black alleys), and the latency (in sec) en route to the goal box were recorded. On the first day, animals were retained in the goal box until they had found the saccharin solution; no animal required more than one min. Each mouse was allowed to drink for 20 sec before being returned to its home cage. On the second day of initial training, and on all retention testing days, the same procedure was followed, except that the mice were removed from the goal box 20 sec after entry. One hour after the last animal had completed maze training, all animals were given water in their home cages for one hour. Following the second day of initial training, water was restored ad lib for the duration of the injection period.

On the basis of initial and total errors for the two initial training days, six nearly identical groups were formed. Twenty-four hr after the second initial training trial, each animal received the first of a series of five daily intraperitoneal injections. The drugs were dissolved in 0.9% saline and were adjusted for an injection volume of 1 cc/0.1 kg body weight. Sixteen mice received each of the following injections: (1) saline; (2) 0.2 mg/kg strychnine sulphate; (3) 1.0 mg/kg *d*-amphetamine sulphate; (4) 7.0 mg/kg pentylenetetrazol (Metrazol); (5) 5.0 mg/kg caffeine citrate, or (6) 1.0 mg/kg nicotine alkaloid. These dosages were selected because of their effectiveness in producing facilitation of learning and memory [20, 21, 25, 26, 28, 30, 32]. Comparisons of error and latency scores on the two initial training days for each of the drug groups with the saline group by Dunnett's *t* statistic revealed no significant differences in the level of pretraining.

Twenty-four hr after the fifth (last) injection day, all animals were again water deprived, and 24 hr after that they were tested for retention in the maze. All mice were trained, one trial per day, according to the procedure outlined above, until they had attained the learning criterion of no more than two initial errors summed over two consecutive days.

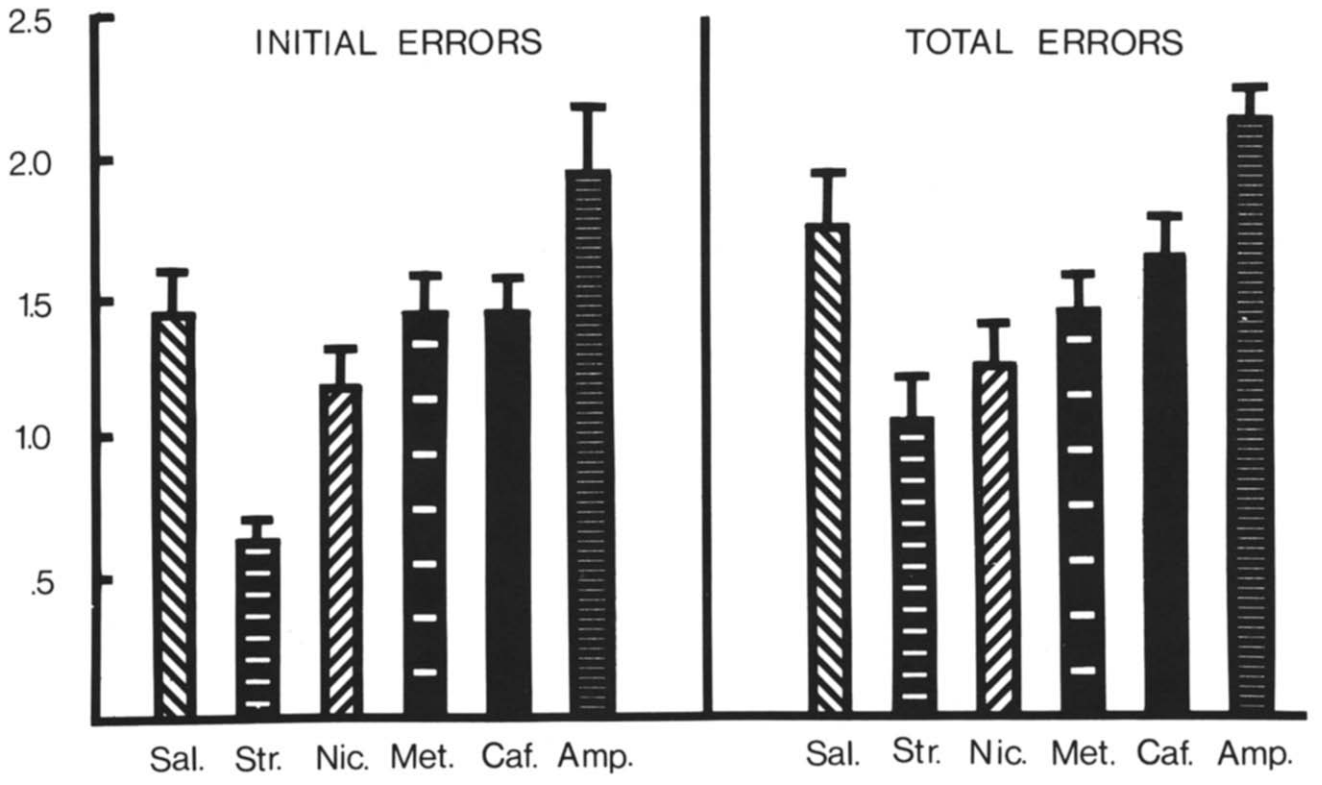

FIG. 1. Means and standard errors for initial and total errors on the first day of retention testing for each of the treatment groups. (Sal.-Saline, Str.-Strychnine, Nic.-Nicotine, Met.-Metrazol, Caf.-Caffeine, Amp.-Amphetamine.)

Results

Day 1 of retention testing. The principal finding on the first day of retesting was that three of the drugs (strychnine, Metrazol and nicotine) had a facilitating effect on memory and one compound (*d*-amphetamine) had a disruptive effect (see Fig. 1). Initial and total errors on Day 1 for each group were compared with the control group by Dunnett's *t* statistic (see Table 1). Mice receiving strychnine and nicotine made significantly fewer initial and total errors than control mice while mice receiving *d*-amphetamine made significantly more initial and total errors. In addition, the animals administered Metrazol made significantly fewer total errors than controls.

The mean latency for each group on the first day was also compared with the control group mean by Dunnett's *t*. Strychnine and Metrazol animals had lower latencies than controls, while the latencies of the other drug groups did not differ from control animals (see Table 1). Means (in sec) and standard errors for the latencies on Day 1 were: saline, 42.81 ± 3.15; strychnine, 33.69 ± 3.18; Metrazol, 37.75 ± 3.22; caffeine, 41.75 ± 4.08; nicotine, 43.00 ± 4.13; and *d*-amphetamine, 42.38 ± 3.34.

Criterion learning. The results for criterion learning, with the exception of nicotine, were similar to those for the first retention test day. Strychnine and Metrazol had an enhancing effect, and *d*-amphetamine had a disruptive effect. The other drugs produced no appreciable effects on criterion measures (see Fig. 2). Trials, initial errors, and total errors to criterion for each drug group were compared to the control group with Dunnett's *t* statistic. On all measures, strychnine- and Metrazol-treated animals had significantly lower scores than saline-treated controls, while *d*-amphetamine-treated animals had significantly higher scores (see Table 1). Although nicotine produced facilitation on the first day of retention testing, the effect was not reflected in the criterion measures.

Mean latency on the criterion trial for each experimental group was also compared with the control group mean latency by Dunnett's *t* statistic; no group differed significantly from the control group (see Table 1). Thus, the increase in running speeds seen on the first day of retention testing for the strychnine and Metrazol animals did not persist for the duration of testing.

EXPERIMENT 2

Method

Animals. The animals were 64 male mice from the same

TABLE 1

VALUES OF DUNNETT'S *t* FOR COMPARISONS OF EACH DRUG GROUP WITH CONTROL GROUP (N = 16/GROUP; *p* VALUES FOR TWO-TAILED TESTS) SIGN OF *t* INDICATES DIRECTION OF DEVIATION OF EXPERIMENTAL GROUP MEAN FROM CONTROL GROUP MEAN: (+) = GREATER THAN CONTROL; (−) = LESS THAN CONTROL

Group	Retention Measures on Day 1			Criterion Measures			
	Initial Errors	Total Errors	Latency	Trials	Initial Errors	Total Errors	Latency on Criterion Trial
			Groups Receiving Initial Training				
			(k = 6, *df* = 90)				
Strychnine	−8.65 *p*<0.01	−5.97 *p*<0.01	−7.02 *p*<0.01	4.81 *p*<0.01	−7.37 *p*<0.01	−5.99 *p*<0.01	−1.19 NS
Metrazol	0.00 NS	2.84 *p*<0.05	4.18 *p*<0.01	−4.01 *p*<0.01	−2.95 *p*<0.02	−4.17 *p*<0.01	−0.45 NS
Amphetamine	+5.92 *p*<0.01	+3.41 *p*<0.01	0.36 NS	+4.00 *p*<0.01	+5.01 *p*<0.01	+5.47 *p*<0.01	−0.64 NS
Nicotine	−2.66 *p*<0.05	−4.55 *p*<0.01	+0.15 NS	0.00 NS	−1.47 NS	−1.82 NS	+0.16 NS
Caffeine	0.00 NS	−1.14 NS	0.88 NS	0.00 NS	−0.29 NS	−1.04 NS	−1.17 NS
			Naive Groups				
			(k = 4, *df* = 60)				
Strychnine				−0.42 NS	0.00 NS	−0.18 NS	+0.51 NS
Metrazol				+0.99 NS	+0.33 NS	+0.14 NS	−0.39 NS
Amphetamine				−0.42 NS	−0.49 NS	−0.77 NS	−0.23 NS

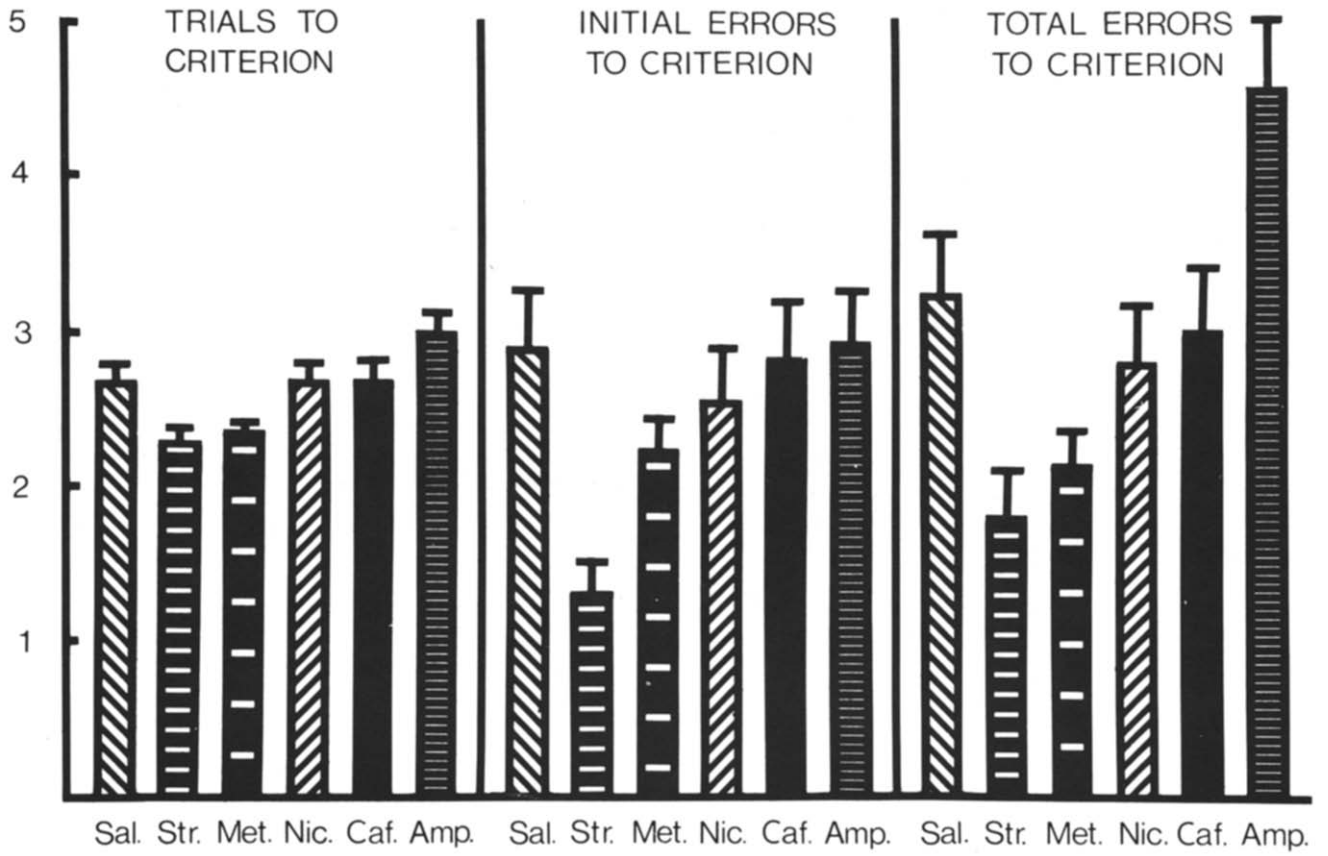

Fig. 2. Means and standard errors for trials to criterion, initial errors to criterion, and total errors to criterion for each of the treatment groups (Sal.-Saline, Str.-Strychnine, Met.-Metrazol, Nic.-Nicotine, Caf.-Caffeine, Amp.-Amphetamine.)

TABLE 2

MEANS AND STANDARD ERRORS FOR ERROR AND LATENCY MEASURES (IN SECONDS) IN NAIVE GROUP (N = 16/GROUP)

	Naive-Saline	Naive-Strychnine	Naive-Metrazol	Naive-Amphetamine
Trials to Criterion	3.63 ± 0.21	3.44 ± 0.28	4.06 ± 0.47	3.44 ± 0.28
Initial Errors to Criterion	5.81 ± 0.07	5.81 ± 0.76	6.19 ± 1.11	5.25 ± 0.71
Total Errors to Criterion	7.19 ± 0.85	6.94 ± 0.90	7.38 ± 1.34	6.13 ± 0.85
Latency on Criterion Trial	40.06 ± 3.78	43.44 ± 7.02	37.50 ± 3.91	38.56 ± 3.76

cross described in Experiment 1.

Apparatus. The apparatus was the same maze described in Experiment 1.

Procedure. To control for the possibility that these agents might act through some chronic proactive effect, four additional groups were tested. These animals were trained and treated exactly as were those described in Experiment 1 (including being water-deprived), with the important exception that the mice received no initial training in the maze. Animals in these groups received five daily injections of either saline strychnine sulphate, Metrazol, or *d*-amphetamine sulphate at identical dosages to those groups already described. Beginning 48 hr after the injection series, and being 24 hr water-deprived, these naive animals were trained to criterion in the maze as previously described.

Results

Examination of trials, initial errors, and total errors to criterion, and latency on the criterion trial using Dunnett's *t* statistic revealed that no naive drug group differed significantly from the naive control group (see Tables 1 and 2).

DISCUSSION

The results of these experiments support the notion that the long-term store of memory, previously demonstrated to be susceptible to pharmacologic manipulation by strychnine, is also susceptible to other neural excitants. However, the drugs employed in this study have been reported to affect memory consolidation processes [20, 21, 25, 26, 28, 30, 32] and to facilitate acquisition of a habit when administered shortly before training [4, 20, 21, 22, 24, 25, 26, 28, 32]. Consequently, it might be argued that the results of Experiment 1 could be attributed to: (a) retrograde effects on memory consolidation for the initial training experience; or (b) proactive effects on learning ability. A consolidation interpretation can be eliminated for two reasons. First, the phenomenon of retrograde influence on consolidation has been amply demonstrated to be time-dependent; that is, the further in time from the training experience that the treatment is applied, the weaker is its effect. The duration of susceptibility of a consolidating memory to these agents has generally been found to be less than 24 hr [20, 21, 25, 26, 28, 30]. Since in Experiment 1 the first injection was administered 24 hr after training, it is unlikely that the compound could have exerted a significant effect on the consolidation process, especially at the moderate dosages employed in this study. Further, in the initial report of this phenomenon, a single injection of the effective dosage of strychnine given 24 hr after initial training did not have a significant effect [1].

The results of Experiment 2 do not support the notion that treatment with these compounds altered learning ability. Although animals in Experiment 2 were treated and drugged exactly as were those in Experiment 1, with the sole exception that they did not receive the initial training trials, no naive-drugged group displayed facilitation or disruption. Moreover, previous investigations of proactive facilitation with these compounds have reported time-dependency similar to that discussed for the retrograde facilitation phenomenon [20, 21, 25, 26, 28, 30]. Again, susceptibility to proactive facilitation seems to be limited to less than 24 hr; yet, in this study, the interval between the last drug administration and the first retention trial was 48 hr. However, behavioral manifestations of a proactive effect of strychnine (facilitated acquisition) were reported for a single high dosage of strychnine both 24 and 72 hr later [12]. This is not an unequivocal finding, for Greenough and McGaugh [18] were unable to confirm this effect. Nevertheless, with *d*-amphetamine sulphate, Bauer and Duncan [5] have reported that a series of five or ten, but not two, daily administrations facilitated acquisition of a habit when training was begun 24 hr after the last injection. Although most available data support the contention that the compounds employed in our study are completely metabolized within 24 hr [3, 6, 9, 10, 15, 17, 23, 26, 38], it is possible that some biochemical effects of amphetamine persist beyond 24 hr [8,31]. Lack of certainty about the rate of metabolism of *d*-amphetamine, however, is not critical to the interpretation of its effects in our study. If small residual amounts of *d*-amphetamine accumulated during the injection series and were present during retention testing, facilitation of memory would have been the most probable result [5,21]. Nevertheless, amphetamine significantly disrupted memory for the initial training trials.

The observed effects of amphetamine in this study were somewhat surprising. In naive animals, the dosage employed produced a trend toward facilitation of acquisition, supporting the results of Bauer and Duncan [5]. However, the same dosage of amphetamine significantly disrupted retention in animals that had received prior maze training. These oppositive effects of a single dosage of *d*-amphetamine could indicate that this drug is influencing different facets of the learning process (e.g., registration of information and storage or retrieval). An analysis of the effects of amphetamine employing dosages up to 2.0 mg/kg has confirmed these findings. In naive mice, all dosages tended to facilitate acquisition, while in animals that had received initial training prior to drug administration, all dosages tended to produce disruption of memory (Crabbe and Alpern, manuscript in preparation). The only other drug for which more than one dosage has been examined is strychnine sulphate; even at 1.0 mg/kg, no disruption was displayed. It is conceivable that some of these compounds will generally disrupt the long-term store of memory while others will be facilitatory. This could provide an important clue to the effective mechanisms of action of neural excitants with respect to stored memory.

One additional point concerns the behavioral measures used to assess maze performance. Error measures and latency measures have been found to index different behavioral attributes of mice in previous research using a similar apparatus and task (Crabbe and Alpern, 1972, submitted for publication). We have interpreted error scores (and the criterion measures) to indicate the strength of the specific retrievable memory trace, while we believe that latency reflects the transient performance capabilities of the animals as well. Hence, the initial reduction in latencies caused by two drugs (strychnine and Metrazol) is interpreted by us as an index of these drugs' effects on performance rather than on memory. Such an interpretation is consistent with the further findings that: (a) nicotine-treated mice showed no effect on latency on Day 1 when their memory for the pretraining was significantly stronger than control mice; (b) amphetamine-treated mice did not differ from control mice in latency, while their memory for the task was much poorer; and (c) all differences in latency had disappeared by the criterion trial.

In summary, the results of this study confirm the previously reported susceptibility of the long-term memory store to treatment with strychnine and extend the demonstrated range of that susceptibility to two other compounds, Metrazol and amphetamine. Although the initial facilitation of nicotine did not appear in the criterion measures and caffeine had no effect, it would be unwise to conclude that they are inefficacious in this situation, for only one dose level was employed. Since marked strain differences in dose-response relationships for these compounds are known to occur [7, 16, 20, 35, 37], caffeine or nicotine might prove effective at some other dosage. Further research will clarify the dose-response characteristics of these phenomena.

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