Forgetting of a conditioned emotional response and its alleviation by pretest amphetamine

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Rats were given a single footshock after stepping through from a white to a black compartment. Retention was evaluated in terms of step-through latencies, darkness preference, and pattern of locomotor and exploratory activity, comparing behaviors of trained and nontrained animals 24 h after shock. Forgetting was seen at 25 days after training. Treatment with .5 mg/kg amphetamine before retention test alleviated the forgetting, but had no effect on darkness preferences or patterns of activity in either nontrained animals or animals tested at 24 h. In a second experiment, avoidance behavior was again facilitated by pretest amphetamine after a 25-day retention interval, and it was shown that this increased avoidance in drug-treated rats occurred only when shock had been associated with the testing environment; it was not seen in noncontingent-shock groups. The results show that reliable and robust forgetting of a well-learned conditioned emotional response can be demonstrated when other than step-through latencies are used as a performance measure. They further support the notion that retrieval processes are labile and that catecholamines may play a role in their modulation.

Studies of the biological bases of memory storage and retrieval processes have relied almost exclusively on animal "models" of amnesia using such treatments as electrolytic or chemical lesions, electroconvulsive shock, depletion of neurotransmitters, or inhibition of protein synthesis. Such approaches have the obvious disadvantage of using a preparation whose behavioral alteration could be due to a number of factors other than specific effects of the treatment on a particular function supposed to be involved in memory processes. We have been working with a behavioral paradigm in which we can demonstrate reliable, robust forgetting of a well-learned task in the intact, adult rat, thus providing a "model" with which to study memory retrieval processes. Using this model, we have been able to show that forgetting can be alleviated by a number of pretest treatment-reminders comprising elements of the training situation (contextual cues) (Deweere & Sara, 1981; Deweere, Sara, & Hars, 1980) and stimulation of the mesencephalic reticular formation (Sara, Deweere, & Hars, 1980). In more recent studies, it was shown that systemic injections of amphetamine (Sara & Deweere, 1982) or intraventricular injections of apomorphine (Sara, Grecksch, & Hongenaert, *in press*) just before the retention test can likewise alleviate forgetting. These results suggest that catecholamines are involved in some way in regulating memory retrieval processes. The present experiments are aimed

at lending support to this general hypothesis by examining the effect of amphetamine on retrieval in a task whose behavioral requirements are quite different from those of the spatially discriminated approach task.

While the passive avoidance procedure has been widely used in the study of memory in the rat, few studies have used this task independently of an amnestic treatment, probably because it is difficult to demonstrate reliable and robust learning in rats tested at 24 h, when training parameters are fixed to yield reliable forgetting at longer intervals. However, when the dependent variable is extended from the usual step-through latency to include a wider sample of the animal's behavioral repertoire at the retention test, such as total time spent in each compartment, one can see differences in behavior patterns which could be attributed to posttrial events. This strategy has been used to detect retention of FS in rats receiving posttrial amnestic treatment and pretest reminders where simple step-through latencies would have suggested persistent amnesia (Sara, 1973a, 1973b). In the present experiment, we used the same strategy to examine effects of long training-to-test intervals on retention and the effects of pretest amphetamine on alleviation of forgetting.

If memory retrieval could be facilitated by pretest amphetamine treatment in a one-trial shock-motivated inhibitory task, as it was in the multitrial food-reinforced approach task, it would lend support to the notion that forgetting, in general, was due to retrieval failure (Spear, 1971) and, at the same time, would provide a basis for generalization concerning the role of catecholamines in regulating memory retrieval processes.

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EXPERIMENT 1

Pilot studies were carried out to establish the training parameters, retention interval, and behavioral measures with which robust learning at 24 h and reliable forgetting at longer intervals could be obtained. These studies showed that footshock (FS) parameters of .5-mA intensity for 2 sec were the minimum necessary to produce avoidance in rats tested at 24 h. Forgetting was sometimes evidenced at 17 days, but was reliable only after a training-to-test interval of 25 days. The performance measure that was most sensitive to the effect of an extended retention interval was total time spent in the dark (shock) compartment, during a 300-sec test. Rearing and locomotor activity were lower in shocked than in nonshocked rats 24 h later, but this difference decreased when the animals were observed at longer intervals between training and testing, suggesting that these behaviors might be useful indices of memory for the association of FS with the experimental environment. Step-through latency did not reliably distinguish between shocked and pseudotrained rats, except at the 24-h interval.

A dose of 0.5 mg/kg of amphetamine was used in this experiment because our previous work had shown that this was the minimum dose that did not increase spontaneous activity in our conditions and also the dose that effectively alleviated forgetting in an appetitive maze task (Sara & Deweer, 1982).

Method

Subjects. Seventy-seven Sprague-Dawley rats served as subjects in this experiment. They had been used in a previous behavioral experiment involving long-term retention of a food-motivated spatial maze task. Thus, they had been handled extensively and had been on a food-deprivation schedule for 2 weeks approximately 4 weeks before the present experiment. They were feeding freely at the time of this experiment and weighed 350–420 g. From the time of arrival in the laboratory, they were housed in pairs in wire-mesh cages on a natural light-dark cycle.

Apparatus. The training cage consisted of a two-compartment box. The start section was painted white and had a Plexiglas floor; the other section was painted black and had a grid floor consisting of stainless steel rods, 4 mm in diameter with a 12-mm distance center to center. FS was delivered through a Foringer scrambler (see Sara, David-Remacle, & Lefevre, 1975, Experiment II for a detailed description). Photoelectric cells were located on the walls of both compartments to monitor locomotor activity. The Plexiglas floor of the startbox was hinged, so that when the rat left the compartment with all four feet, a microswitch was activated and the step-through latency was recorded. The photoelectric cells monitored horizontal and vertical activity, and the information was fed into a microprocessor.

Experimental design and procedure. The animal was placed in the white side of the box, facing a corner opposite the door. Three seconds later, the door was opened, the latency to step through was noted, the door was closed, and the rat was administered a FS of 2-sec duration and .5-mA intensity. The rat was then removed from the black side and returned to its home cage.

An additional group of animals received pseudotraining; the rat did not receive footshock upon stepping through (Group NFS sal, $n=6$, and Group NFS amph, $n=5$).

Twenty-eight of the FS group were tested 24 h later (FS sal, $n=14$, and FS amph, $n=14$). Twenty minutes before the retention test, the animals were injected with saline or amphetamine

(.5 mg/kg, sc). The retention test procedure was exactly like that of training: the rat was placed in the white (nonshock) side of the box, facing away from the door, which was opened 3 sec later. Total time in the dark, rearings, and horizontal displacements were automatically counted for the 300-sec duration of the test. The remaining 38 rats, 20 of which were treated with saline and 18 of which were treated with amphetamine before the retention test, were tested 25 days after training (Groups sal-25d and amp-25d). It should be noted that the photoelectric cell arrangement was such that even very slight rearing activity was registered.

Results

As indicated by the differences in dark preferences between FS and NFS groups tested at 24 h, there was robust learning. All 28 FS rats spent less time in the dark than the 10 NFS rats (Figure 1). FS also induced a significant decrease in rearing and horizontal activity [$t(36)=8.06$, $p<.001$ and $t(36)=3.98$, $p<.002$], confirming that these behaviors are a part of the conditioned emotional response to the environment associated with FS, and therefore useful measures in evaluating retention (Figure 2). There was no effect of .5 mg/kg amphetamine on the behavior of nonshocked rats in terms of dark preference or activity. The drug, likewise, had no effect on the behavior of Group FS, tested at 24 h, as shown in Figure 1.

Saline-treated rats tested 25 days after training spent significantly more time in the shock compartment than did those tested at 24 h, as shown in Figure 1 ($U=80$, $n=20.14$; $p<.02$), indicating robust

Figure 1. Median time in the dark during the 5-min retention test. White bars = saline, striped bars = .5 mg/kg amphetamine 20 min before retention test. Note robust avoidance learning in rats tested 24 h after training and the significant retention deficit in rats tested 25 days after training. This deficit is alleviated by pretest amphetamine. Amphetamine has no effect on avoidance in trained rats (Group 24 h) and no effect on dark preference in untrained rats (Group 25 days).

Figure 2. Mean ($\pm$ SEM) number of rearings and horizontal activity as measured by photoelectric cells in both compartments 24 h after FS. Note that there is no effect of amphetamine at .5 mg/kg on these activities in shocked or nonshocked rats (white bars = saline, striped bars = amphetamine 20 min before test). Data for rats tested 25 days after shock is not available.

forgetting. Animals treated with amphetamine before the test spent less time in the shock compartment. The difference between Group amph-25d and Group sal-25d was significant ($U = 78$, $n = 20.18$; $p < .01$) on this measure, although the differences in step-through latencies were not significant. Due to equipment failure, the rearing and activity scores were lost for animals tested at the 25-day retention interval. However, the amphetamine clearly alleviated the retention deficit when total time in the shock department, alone, is taken as the index of retention.

EXPERIMENT 2

Experiment 1 clearly showed that rats fail to avoid the compartment in which they were shocked 25 days previously. If they are treated with amphetamine before the test, they avoid the dark compartment associated with shock. The amphetamine had no effect on rearing activity or dark preference in rats that did not receive FS and were "tested" after pseudotraining. It is, however, always possible that amphetamine interacts with FS after a long time interval, producing behavioral responses such as changes in activity or dark preference, which are not a direct reflection of an association between training environment/step-through and shock reinforcement.

The purpose of Experiment 2 was to replicate the findings of the first experiment concerning the effect of amphetamine on time spent in the dark by trained animals and to control for nonspecific effects by using a noncontingent FS (NCFS control group).

A further purpose of this experiment was to collect rearing activity data (which was lost in Experiment 1) after a long retention interval.

Method

Subjects. Fifty-eight male Sprague-Dawley rats were used in this study. They were housed in pairs in wire-mesh cages and subjected to a 12-h light-dark cycle (8 a.m.-8 p.m. lights on). Behavioral observations were carried out between 11 a.m. and 2 p.m. The animals weighed about 200 g at the time of training. They were handled for 2 min/day for 3 days just before training. They were experimentally naive.

Apparatus. For training the FS group, the passive avoidance apparatus was the same as that used in Experiment 1. NCFS was delivered in a 22 $\times$ 22 cm box with a grid floor, the bars of which were brass, 5 mm in diameter, and 16 mm apart center to center.

Experimental design and procedure. Twenty-nine rats were trained as in Experiment 1. The remaining 29 received a NCFS of the same intensity and duration in another room in an apparatus that was distinctively different from the training/testing apparatus. Twenty-five days after training, all rats were tested in the passive avoidance apparatus. Rearing activity and total time spent in the dark were measured automatically, as in Experiment 1.

Results

Median total time spent in the shock compartment can be seen in Figure 3. There was no difference between the FS sal and NCFS sal groups ($U = 102$), suggesting forgetting of the FS experience. There were no differences in step-through latencies among the four groups (median latencies: FS sal = 46, FS amph = 38, NCFS sal = 39, NCFS amph = 24). Trained animals treated with amphetamine avoided the dark compartment; the difference between the FS sal and FS amph groups was significant ($U = 66$, $p < .05$). There was no effect of amphetamine on dark prefer-

Figure 3. Median time in dark during a 5-min retention test 25 days after FS. White bars = saline, striped bars = .5 mg/kg amphetamine 20 min before retention test. FS = trained rats, NCFS = rats receiving FS in another environment. Note that FS animals treated with saline fail to avoid, while those treated with amphetamine, show good avoidance behavior, thus replicating the results of Experiment 1. There is no effect of amphetamine on dark preference in the NCFS group.

ence in pseudotrained animals, that is, no difference between Groups NCFS sal and NCFS amph. Rearing activity and horizontal activity scores were not significantly affected by either the FS condition or amphetamine treatment. Mean rearing activity, as measured by the photoelectric cell arrangement, is shown in Figure 4.

Figure 4. Mean ($\pm$ SEM) number of vertical displacements as measured by photoelectric cells in both compartments. Note that there is no effect of amphetamine at .5 mg/kg on this activity in either trained rats (FS) or in rats shocked in another environment (NCFS) 25 days before the test. (White bars = saline, striped bars = .5 mg/kg amphetamine 20 min before the test).

DISCUSSION

After an extended retention interval, rats in the passive avoidance situation forget, as measured by total time in the shock compartment, more than do rats similarly trained and tested 24 h later. In the animals tested 24 h after training, the instrumental response of inhibiting step-through behavior is accompanied by a decrease in exploratory activity, including rearing (relative to that of nonshocked rats), this decrease being a manifestation of a conditioned emotional response to the testing situation. The deficit in rats tested after a long retention interval is seen in terms of failure to avoid the dark, but not in terms of rearing activity (see Figures 2 and 4). Thus, there is some residual effect of the training as evidenced by the fact that the behavior of this group is different from that of naive animals. But it should be noted that this residual effect of FS (as indicated by the fact that activity is lower in shocked than in nonshocked animals) is found in the NCFS group also, suggesting a memory for FS, and its association with handling, in both groups. The association of place (dark box) and FS and/or step-through response and FS is what appears to have been forgotten by the FS group.

It is interesting to note that the amphetamine treatment has its effect on the total time spent in the dark and has no effect whatsoever on step-through latency. We have shown in previous work that repeated testing after an amnestic treatment will gradually induce avoidance of the shock compartment. It was suggested in those experiments (Sara, 1973a) that repeated exposures to the stimuli comprising the shock compartment was an effective reminder, facilitating retrieval of the target memory. It is possible that, in the present experiments, amphetamine enhanced the reminder value of these stimuli by increasing selective attention (see discussion below), so that a single exposure was sufficient to facilitate memory retrieval. Such an analysis would predict no effect on step-through latencies, since the animal must have a minimal exposure to stimuli in order for the drug treatment to enhance attention to these stimuli.

The facilitation by amphetamine injected before the retention test lends support to the view that the "forgetting" was due to inability to retrieve the information concerning the association of a particular environment and the FS. The fact that the drug treatment had no effect on dark preference or exploratory/locomotor activity in nonshocked rats (Experiment 1) or in NCFS rats (Experiment 2) rules out an interpretation of facilitory effects after a long retention interval in terms of nonspecific effects on behavior. In order for this relatively small dose of amphetamine to have its behavioral effect of increasing dark avoidance, the animals must have experienced the FS and that FS must have occurred in the apparatus in which the test takes place. Furthermore, it is unlikely that the behavioral change is due to fear en-

hanced by the drug alone. If the amphetamine increased the conditioned fear response, there would be an increase in fear-related behaviors such as defecation, freezing, etc. This was not observed, and, moreover, there was no enhanced inhibition of exploratory activity in drug-treated rats tested at 24 h (compare Group 24 h sal and Group 24 h amph, Figure 2).

The present results permit a generalization of the conclusions of our previous studies of alleviation of spontaneous forgetting by pretest treatment with amphetamine. Those studies used a multitrial appetitive spatial discrimination task, requiring an active motor response. The present task involved a single-trial shock-reinforced task requiring behavioral inhibition. Pretest amphetamine facilitates the behavior in both situations when control groups have forgotten after the long retention interval. In either task, there was no effect of the drug on the performance of well-trained animals that had a 24-h training-to-test interval, thus supporting the interpretation in terms of facilitation of retrieval processes by amphetamine.

Whether the effect of amphetamine is dopaminergic or noradrenergic, or both, still remains an open question. Both systems have been implicated in memory processes (Quartermain, Judge, & Friedman, 1983; Stein, Belluzzi, & Wise, 1975). Clinical studies have shown that patients suffering from memory disorders associated with Korsakoff's disease have an abnormally low level of MHPG, the major metabolite of noradrenalin, and the amount of the reduction is correlated with the extent of the memory loss; there were no differences in the levels of metabolites of other biogenic amines (McEntee & Mair, 1978). It has been suggested that such clinical amnesia might be due to dysfunction of retrieval processes and be related to deficits in attentional mechanisms, particularly attention to context (O'Keefe & Nadel, 1979; Winocur & Kinsbourne, 1978). Our previous studies, which showed the striking effectiveness of pretest reminders in the form of contextual cues, have led us to emphasize the importance of attention, particularly to the context or background stimuli, in the memory retrieval process (Deweere et al., 1980; see also O'Keefe & Nadel, 1979; Tulving, 1974). There is a large literature based on both behavioral (Mason & Iversen, 1978; Roberts, Price, & Fibiger, 1976) and electrophysiological (Woodward, Moises, Waterhouse, Hoffer, & Freedman, 1979; Segal and Bloom, 1976) studies which implicate the noradrenergic system in selective attention or enhancement of signal-to-noise ratio. But there is also some suggestion that the striatal dopamine system is involved in regulation of attention (Marshall, Berrios, & Sawyer, 1980).

The experiments reported here show that spontaneous forgetting, even of a simple task, can be demonstrated in the rat and is a useful tool in the study of memory-retrieval processes, since it does not re-

quire traumatic posttrial treatments or preparations that are biologically deficient or pathological. They further emphasize the lability of memory retrieval in the normal, intact rat which has a "lapse" after a long retention interval. The results lend support to our previous conclusions that memory retrieval depends not only on the strength of the stored information, but also on the perceptual and information-processing activities occurring prior to and during the retention test. Increasing attention to relevant stimuli by making these stimuli more salient (Sara, 1977) or by increasing the level of arousal and attention either electrically by brain stimulation (Sara et al., 1980) or chemically (Sara & Deweer, 1982) will increase the probability of the animal's responding adaptively at the retention trial.

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