Pharmacology Biochemistry and Behavior, Vol. 3, pp. 1–5. ANKHO International Inc., 1975. Printed in the U.S.A.

Reversal of ECS-induced Amnesia by Post-ECS Injections of Amphetamine

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(Received 4 March 1974)

MAH, C. J. AND D. J. ALBERT. *Reversal of ECS-induced amnesia by post-ECS injections of amphetamine.* PHARMAC. BIOCHEM. BEHAV. 3(1) 1–5, 1975. – Water deprived rats were trained to drink from a water spout within an apparatus on each of 2 days. On the third day, passive avoidance of the spout was induced by giving the animal a 0.3 sec, 5 mA footshock after 1 lick at the spout. A test for retention of the avoidance learning was given 48 hr following training. ECS administered through cortical electrodes at 15 sec following the footshock impaired retention. The amnesia was attenuated when amphetamine (1 mg/kg) was injected immediately but not at 6 hr following the ECS. In a second experiment, attentuation of amnesia by amphetamine was not found when the ECS occurred at 4 sec instead of 15 sec following the footshock. The results are interpreted in terms of reactivation by amphetamine of a consolidation process that was interrupted by ECS.

Electroconvulsive shock Amnesia Amphetamine Memory consolidation

ELECTROCONVULSIVE shock (ECS) is known to produce a distrubance of retention when administered following learning [10, 13, 18, 23, 27]. A widely held view is that this is due to an interference with the process of forming a permanent storage mechanism [13, 18, 23]. This is known as the consolidation hypothesis and has been notably effective in explaining the pattern of retention deficits observed following ECS. However, a long-standing difficulty with this hypothesis is that the nature of the process thought to be involved has remained obscure in spite of substantial experimentation.

One of the important questions which has arisen in the attempt to elucidate the nature of the consolidation process is whether the disturbance of consolidation is reversible. There is evidence for an affirmative answer to this question. When ECS is given following learning, the disturbance of retention is not all or none, nor is it simply graded according to the delay between training and ECS. Rather, the amount of retention depends on the time at which the ECS is given and on the strength of the ECS [16,28]. Further, if a second ECS is given following the first, the disturbance of retention can be increased [24]. This evidence suggests that the interference caused by ECS is usually a partial interruption rather than a destruction of an ongoing process. Because the substrate of the process may still be present, it might be susceptible to reactivation, if the appropriate agent could be found.

Several experimenters have now reported this kind of reactivation effect. Albert [1] obtained a reversal of the amnesic effect of cortical spreading depression by administering surface-positive polarization to the cortex. McGaugh and Hart [19] and Duncan and Hunt [8] attenuated the amnesic effect of ECS by strychnine injections. Barondes and Cohen [4] have reversed the amnesic effect of cycloheximide by administering cortisone, amphetamine, or an arousing stimulus following the amnesic treatment. The existence of this phenomenon does not stand unchallenged, however; Miller and Springer [26] have reported a failure to replicate using strychnine and ECS.

The object of the present experiments was to explore the reversal of ECS-induced amnesia further. The agent we have used is amphetamine. The interest in using this agent in conjunction with ECS is two-fold. It has proven very effective in reversing the amnesic effect of cycloheximide [4]. There is also substantial evidence that posttrial injections improve retention, and this has been attributed to a facilitation of consolidation [17]. Both strands of evidence suggest that amphetamine can in some way facilitate consolidation, and accordingly, it might effectively reverse the disturbance of consolidation by ECS.

[1]This research was supported by grants from the National Research Council of Canada (APA 192) and the University Research Committee.

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EXPERIMENT 1
REVERSAL OF AMNESIA WITH AMPHETAMINE

The effectiveness of amphetamine in reversing ECS-induced amnesia was examined in a passive avoidance training situation. ECS was given at 15 sec following training since McGaugh and Hart [19] have found that ECS-induced amnesia can be reduced by strychnine with a training-ECS delay in this range. The dose of amphetamine was 1 mg/kg, which is within the range found effective in facilitating learning by posttrial injections [7,15] and in reversing cycloheximide induced amnesia [4].

METHOD

Animals

The animals were experimentally naive, male black-hooded rats (250–300 g) from the Canadian Breeding Farms and Laboratories. They were housed in individual cages throughout the experiment.

Surgery

All animals had skull electrodes for administering ECS implanted bilaterally over the dorsolateral cortex. Electrode coordinates were 4 mm lateral to the midline and 1 mm posterior to Bregma. The electrode consisted of a pin soldered onto the end of a stainless steel jeweller's screw (1 mm dia.).

Apparatus

The passive avoidance apparatus used was a box 40 X 40 X 40 cm. Inside was painted flat black and there was a grid floor. Midway along one wall and 2 cm above the floor was a 6 X 6 cm opening to a nook in which a drinking spout was housed (a more thorough description of the apparatus is contained in Albert and Mah [2,3]).

The latency to drink at the spout was recorded by means of an automatic timer connected to a drinkometer. The length of the training session was controlled by a preset clock which also started the latency timer.

Footshock administered during passive avoidance training was delivered automatically following the first lick at the spout and the shock was generated by a 60 Hz 460 V transformer in series with 85,000 ohm resistance.

ECS (55 mA, 0.4 sec) was generated through a 60 Hz, 820 V transformer connected in series to a 15,000 ohm resistor. Full tonic–clonic convulsions were reliably elicited.

General Training Procedure

Twenty-four hr before surgery, the animals were put on a 23 hr water deprivation schedule which continued for the remainder of the experiment. On the fifth day following surgery (Day 1), the animals were given the first of two daily sessions of approach training in the passive avoidance apparatus. Each animal was placed into the training box and left for the duration of the session which lasted 5 min. The latency to make 20 licks at the spout was automatically recorded. (This measure rather than the first lick latency was used because we found it to reflect more validly the animals' behavior in this task both before and after passive avoidance training.)

On Day 3, the animals were given passive avoidance training. Wire leads for administering ECS were first attached to the animal's skull electrodes before the animals were placed into the apparatus. For passive avoidance training, the animals were automatically given a footshock immediately after making one lick at the spout.

Following passive avoidance training each animal was given additional treatments as required by the experimental design and returned to its cage. About 1.5 hr after the last animal was trained, water was made available to all animals for 1 hr.

Experimental Design

The groups used in this experiment were all given two days of approach training. On the 3rd day the groups were treated as follows. One group (FS-delECS-Saline; N = 7) received a footshock (FS) and ECS a sufficient length of time later (6 hr) so as to be ineffective in disturbing retention. Immediately after the ECS they were given a 1 cc injection of 0.9% NaCl. A second group (FS-ECS-Saline; N = 13) was given a footshock and then 15 sec later an ECS followed immediately by a 1 cc injection of 0.9% NaCl. These two groups should show that there is a time-dependent amnesic effect of ECS with this task. A third group (FS-ECS-Amph; N = 15) received a footshock followed at 15 sec by ECS and an injection of amphetamine (1.0 mg/kg) immediately after the ECS. This group was intended to demonstrate the attenuation of amnesia by post-ECS amphetamine injections. A fourth group was intended to show that the effect of the amphetamine injection was time dependent. This group (FS-ECS-delAmph; N = 8) was given a footshock followed 15 sec later by an ECS and then 6 hr later by an injection of amphetamine (1.0 mg/kg). All groups given ECS at 15 sec remained in the box in the interval following the footshock. The delayed ECS and delayed amphetamine groups were returned to their cages until the time for the treatment arrived and then were brought back into the training room for the treatment.

No treatment was given on the day following avoidance training but the animals were all allowed one hour access to water in their home cage at the usual time.

Two days after passive avoidance training (Day 5), the animals were tested for retention of the avoidance training given on Day 3. Each animal was placed into the apparatus and the latency to make 20 licks at the spout was automatically recorded. If the animal did not lick at the spout at least 20 times by the end of the test session which lasted 5 min, it was assigned a score of 300 sec.

RESULTS AND DISCUSSION

Figure 1 shows the median latency to make 20 licks by each group on the test day. Animals given footshock followed 6 hr later by ECS showed virtually complete avoidance of the spout on the test day (Group FS-delECS-Saline; median latency = 300 sec). In contrast, when the ECS was given 15 sec following the footshock, the avoidance was significantly less (Group FS-ECS-Saline; median latency = 230 sec; Mann Whitney U Test, two-tailed; U = 14, $p<0.02$). These results confirm the decrement in retention observed when ECS is given shortly but not long after training [13, 20, 23].

The critical experimental group is the one receiving amphetamine immediately following the ECS. The animals of this group, almost without exception, avoided the spout

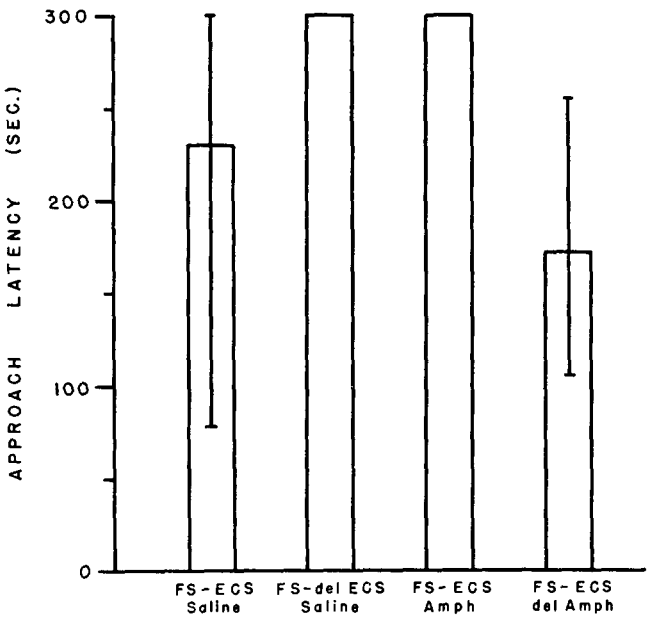

FIG. 1. Median latency to make 20 licks at the spout on the test day. ECS was given at 15 sec or 6 hr (delECS) following footshock. Amphetamine or saline was injected immediately following the ECS or after 6 hr (delAmph). The vertical line within a bar represents the interquartile range.

on the test day (Group FS-ECS-Amph; median latency = 300 sec). This avoidance level is significantly greater than the comparably treated group injected with saline (Group FS-ECS-Saline, median latency = 230 sec, U = 43, $p<0.02$), but is not reliably different from the group which received a delayed ECS (Group FS-delECS-Saline, U = 42, $p>0.10$).

The results also indicate that the effect of the amphetamine is time dependent. The group injected with amphetamine 6 hr following the ECS treatment (Group FS-ECS-delAmph) showed the typical decrement in retention found with ECS (median latency = 173 sec). This group was not different from the group given ECS at 15 sec and injected with saline immediately afterwards (U = 45, $p>0.10$) but did avoid significantly less than the animals injected with amphetamine immediately following the ECS (U = 17, $p<0.02$).

The amphetamine injections given immediately following ECS appear to prevent the disturbance of retention caused by ECS. This is shown by comparison of the animals injected with amphetamine and those injected with saline immediately following learning. The effect of the amphetamine is time dependent indicating that the improved avoidance level on the test day cannot be attributed to a general pharmacological action at the time of testing. Two respects in which the present findings are ambiguous is that there was no assessment of the effect of the closely paired ECS-amphetamine treatment on latency to drink in comparison to ECS-Saline. It is possible that the pairing of ECS and amphetamine causes an increased latency to drink independently of the footshock. A second possibility is that the effect of the amphetamine is to attenuate the physiological effects of ECS and thereby reduce its amnesic effectiveness. Both of these will be controlled for in Experiment 2, which was conducted simultaneously with this experiment.

The present results resemble very closely those obtained by McGaugh and Hart [19] and Duncan and Hunt [8] using post-ECS injections of strychnine instead of amphetamine. Our results are also consistent with the findings of Barondes and Cohen [4], who found that amphetamine could reverse the amnesia induced by cycloheximide.

EXPERIMENT 2
THE EFFECT OF TIME OF ECS ON THE REVERSAL OF AMNESIA BY AMPHETAMINE

The results of McGaugh and Hart [19] showed that reversal of the ECS-induced amnesia depended on the time the amnesia disrupting agent (ECS) was given as well as on the time the consolidation facilitating agent was given. In the previous experiment we have confirmed part of this conclusion by showing that amphetamine produces an attenuation of amnesia only if it is given shortly following ECS. However, we did not determine whether the attenuation of amnesia was also dependent on the time the ECS was given. According to McGaugh and Hart [19] if the ECS is given shortly after training (within 8 sec) the amnesia should not be reversible.

The intent of the present experiment was to determine whether the effect of amphetamine is similar to that of strychnine in this respect. ECS was given at 4 sec following footshock (instead of 15 sec as in Experiment 1) and amphetamine was injected immediately afterward.

METHOD

The animals and apparatus were the same as in the previous experiment. As before, the animals also had ECS cortical electrodes implanted bilaterally and were placed on 23 hr water deprivation 6 days before training began.

On the fifth day following surgery (Day 1), the rats were given the first of two daily sessions of approach training in which the animal was simply allowed 5 min in which to drink from the water spout within the apparatus. On Day 3, the animals were all given a passive avoidance trial. With wire leads attached to the ECS electrodes, the animals were given a shock to the feet after making one lick at the spout.

Following the footshock the animals were given one of the following three treatments. One group of rats received an ECS at 4 sec following the footshock followed immediately by a 1 cc injection of 0.9% NaCl (Group FS-ECS-Saline; N = 10). The second group was treated in the same way except that it was injected with 1 mg/kg amphetamine following the ECS (Group FS-ECS-Amph; N = 11). The third group had a 6 hr interval between footshock and ECS and was then immediately injected with 1 cc of saline (FS-delECS-Saline; N = 7). One and one-half hr following the training of the last animal, all animals had water available in their home cage for 1 hr.

Two days following these treatments (Day 5), a retention test was given to all animals and the latency to make 20 licks at the spout was recorded.

RESULTS AND DISCUSSION

The median latency for each group to make 20 licks at the spout on the test day are summarized in Fig. 2. There was no difference in the avoidance behavior of the animals

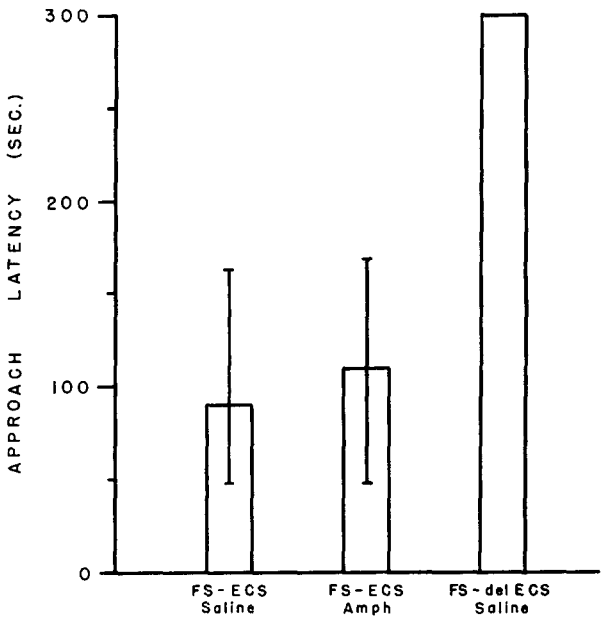

FIG. 2. Median latency to make 20 licks at the spout on the test day. ECS was given either at 4 sec or 6 hr (delECS) following footshock. Amphetamine or saline was injected immediately following ECS. The vertical line within a bar represents the interquartile range.

given ECS at 4 sec following footshock and injected with saline and those given ECS and injected with amphetamine. The median response latency for animals injected with amphetamine (Group FS-ECS-Amph) was 110 sec, while that of the animals injected with saline (Group FS-ECS-Saline) was 90 sec ($U = 47.5$; $p>0.10$). In contrast, when rats were given footshock, ECS 6 hr later and then a saline injection (Group FS-ECS-delECS), they avoided for a median of 300 sec. This is a significantly longer latency than that of either the group given ECS at 4 sec and then injected with either amphetamine ($U = 14$; $p<0.05$) or saline ($U = 9$; $p<0.02$).

The conclusion suggested by these results is that amphetamine does not attenuate amnesia when the ECS is given at 4 sec following training. This supports McGaugh and Hart's similar finding with strychnine [19]. It also obviates the criticism of the previous experiment that the pairing of ECS and amphetamine itself produces a general increase in response latency, or that amphetamine following ECS produces an attenuation of amnesia by reducing the physiological disturbance caused by ECS.

GENERAL DISCUSSION

The principle finding is a reduction in ECS-induced amnesia when amphetamine is administered immediately following ECS. As we have already pointed out, the dependence of the amphetamine-induced attenuation of amnesia on both the time of administering the amphetamine and the time of ECS argues against the results being due to a direct effect of the drug on avoidance behavior or to a reduction in the physiological disturbance caused by ECS. On the other hand, the time dependence of the attenuation of amnesia is consistent with the suggestion that the amphetamine is affecting time-dependent processes in the storage of memory.

We would be reluctant to conclude from these results that the effect of amphetamine is to reactivate the consolidation process following ECS without the supporting evidence in the literature. This evidence consists primarily of experiments reporting a facilitation of retention when amphetamine is injected following learning. In three of these experiments, positive results have been found using a Lashley III maze [5], a three-choice discrimination [11], and a two-way active avoidance [9]. Unfortunately, these studies have not controlled for the possibility that the effect of the drug is on behavior at the test session (24 hr later) rather than on a process that persists following training. However, Kosman ([14] two-choice water maze) and Johnson and Waite [12] step-down avoidance) have allowed 7 days between training and testing so that a persisting drug effect is unlikely and they still find a facilitation of retention. The most effectively controlled experiments are those of Krivanek and McGaugh ([15] discriminated approach learning) and Doty and Doty ([7] simple avoidance, discriminated avoidance). Each of these shows that the effect of posttrial amphetamine injections is dependent on the drug being injected shortly following the acquisition period. Taken together, these findings provide reasonable support for the conclusion that amphetamine can improve retention by facilitating consolidation.

More directly related to our experiment is the question of whether there is evidence for the conclusion that amphetamine might be able to reactivate consolidation following ECS. As we have outlined in the Introduction, the evidence on consolidation suggests to us that this question is not fundamentally different from the question of whether amphetamine can facilitate retention by posttrial injections since consolidation appears to continue in some degree following ECS. However, there is no direct evidence other than our own that amphetamine can reverse ECS-induced amnesia when given shortly following ECS. Consistent with our finding, nevertheless, is that of Barondes and Cohen [4] who have found that amphetamine reversed the amnesic effect of cycloheximide and this effect was time dependent. A more complex result is that of Serota, Roberts, and Flexner [30]. They found that amphetamine attenuated the amnesic effect of acetoxycycloheximide, but the amnesia they were working with was temporary (6 days) so that the interpretation of this result is not straightforward.

The evidence that amphetamine is able to reverse the amnesia induced by ECS is supported by a larger body of findings derived from studies of facilitation of retention with posttrial administration of a number of other stimulants including strychnine, picrotoxin, and pentylenetetrazol (see [17,22] for reviews of this literature). In addition, with every amnesic agent studied to date the disturbance of retention is reversible if an appropriate agent is applied shortly after the disrupting agent [1, 4, 8, 19]. The mechanism by which either the facilitation of retention or the attenuation of amnesia occurs has not been subjected to substantial investigation. On the basis of their findings, Barondes and Cohen [4] suggest that the reactivation effect depends on the generation of a neurophysiological effect that is normally correlated with arousal.

Our experiments were substantially guided by McGaugh and Dawson's [18] theoretical elaboration of consolidation theory which attempts to account for the relation of the

reactivation effect to the time at which the ECS is given. In both their experiments and our own, the ECS-induced amnesia is reversible only if there is a delay of a number of seconds between the training and ECS. In explaining the failure to find a reversal of amnesia when ECS comes within a few seconds following learning, McGaugh and Dawson [18] have relied on a parallel finding of McGaugh and Landfield [21] which shows that when ECS is given within 8 sec following training, a retention test at 1 hr reveals no short-term retention. When the ECS is delayed until about 20 sec following training, there is short-term retention at 1 hr, but no long-term retention at 24 hr. McGaugh and Dawson have suggested that the failure of strychnine to attenuate amnesia when the ECS comes at 8 sec following training may be due to the absence of short-term memory. If short-term memory is destroyed, it may be that consolidation cannot continue even if all other conditions are properly present.

Our results are consistent with McGaugh and Dawson's interpretation, but there is one obvious alternative. With the training and ECS parameters we have used, the short latency ECS administered in Experiment 2 may simply produce a disruption of consolidation which is sufficiently great that the amphetamine is unable to reactivate the consolidation process. In Experiment 1 where we found an attenuation of amnesia, the FS-ECS-Saline group showed a median avoidance latency of 230 sec. In Experiment 2 with ECS at 4 sec following footshock, the median avoidance latency of the FS-ECS-Saline group was much shorter (90 sec) and there was no attenuation of amnesia by amphetamine. From this point of view, the failure of amphetamine to reverse amnesia with the 4 sec training-ECS interval may be related to the severity of the disturbance of consolidation rather than to the presence or absence of short-term memory.

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