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Effects of Amphetamine on Short-Term, Protein-Independent, Memory in Day-Old Chickens

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GIBBS, M. E. *Effect of amphetamine on short-term, protein-independent, memory in day-old chickens.* PHARMAC. BIOCHEM. BEHAV. 4(3) 305–309, 1976. – When the protein synthesis inhibitor cycloheximide (CXM) is administered just before or soon after a single learning trial, the formation of permanent memory is prevented in day-old chickens. In spite of the blockage of long-term memory, which occurs by 3 hr, Mark and Watts [14] have demonstrated a short-term memory which is independent of protein synthesis and which decays over the 3 hr period. D-amphetamine sulphate, administered subcutaneously (up to 2 hr) after learning to CXM pretreated chickens, held the memory at the level exhibited by the labile memory trace at the time of injection. This close relationship between the amount of labile memory and the time of injection was still apparent 24 hr after learning. These data suggest that, provided there is sufficient labile memory in existence at the time of administration, amphetamine maintains the trace which would otherwise decay and allows its subsequent consolidation into permanent memory at a time later than normal.

Amphetamine One-trial passive avoidance learning Cycloheximide
Labile, protein-independent, short-term memory Permanent memory Memory

PHARMACOLOGICAL experiments, using day-old chickens and a one-trial passive avoidance learning task, have separated a protein-independent, short-term, labile phase of memory from long-term, protein-dependent, permanent storage [4, 7, 14]. The labile phase of memory storage is necessary for the formation of permanent memory. If protein synthesis is inhibited at the time of learning, there is memory present for a short time, but no memory at 24 hr. Drugs that disrupt the labile trace also prevent permanent memory formation.

There are reports of amphetamine, administered shortly after learning to mice and rats, preventing the amnesia produced by inhibition of protein synthesis [1,12]. Amphetamine administered after learning, in otherwise untreated mice, has been reported to facilitate memory storage of a discrimination task [6].

In the one-trial passive avoidance situation, the time courses of the 2 memory traces have been established for day-old chickens. This experimental design seemed appropriate to investigate the action of amphetamine and its interactions with the short- and long-term memory stores.

METHOD

Animals

Day-old white leghorn-black australorp cockerels were obtained from a local poultry farm on the morning of each experiment. They were housed, in pairs, in wooden boxes 20 x 25 cm which were open at the top. Food was always available, the room kept at a temperature of 27–30°C and constant humidity.

Procedure

The learning situation was essentially similar to that described by Mark and Watts [7]. Chickens have a tendency to peck at small objects. When the chickens were first placed in the wooden boxes, their attention was obtained by gently tapping the front of the compartment, and a small chromed bead (2.5 mm dia.) attached to a straight wire was presented. The chicks almost always pecked the bead within a few sec. Two of these presentations were given prior to training to encourage them to peck at strange objects entering their cage.

For the training trial a different lure, which was a 4 mm dia. chromed bead on the end of a wire with a right angle bend 1 cm from the end, was used. This was presented for 10 sec.

By making the object pecked distasteful, subsequent pecks can be inhibited. This is the basis of the one-trial learning paradigm. The large, bent wire bead was dipped into a chemical aversant, methyl anthranilate ($NH_2C_6H_4COOCH_3$). This bead was presented for only 10 sec. Chickens that did not peck on the learning trial (normally about 5%), were excluded from the data analysis. Those that did peck, immediately evidenced distaste by shaking their heads, and wiping their beaks on the ground. They rarely pecked the bead again during the learning trial.

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At various learning-retention intervals, chickens were again presented (for 10 sec), with the bent handled lure used in training but without the aversant. Whether or not the chickens pecked at this bead was recorded. The number of chickens in each group of 20 that refused to peck on the retention trial was used as the index of retention. In any one experiment, 2, 3, or 4 separate groups of 20 chickens were used; all received identical treatment and the percentage data from all groups was recorded. The mean and the range of values for percent retention in separate groups is shown in subsequent figures.

Drugs and Injections

Drugs were made up in sterile NaCl 0.15M (0.9%). Cycloheximide (ACTIDIONE, Upjohn Co.) 20 μgm/chicken, or saline was administered intracranially by free-hand injection into each side of the forebrain, in volumes of 10 μl per hemisphere, using a Hamilton repeating dispenser syringe. A stop on the syringe needle regulated the injection depth to 3 mm. The location has been checked histologically [7]. These drugs were administered 5 min before learning. D-amphetamine sulphate (0.1–2.0 mg/kg) was administered subcutaneously between 10 and 120 min after learning, in 0.1 ml volumes on the ventral side of the rib cage.

RESULTS

Saline or CXM Injected before Learning

When saline was injected intracranially 5 min before learning and separate groups of chickens tested for memory 10 to 180 min after, the retention remained approximately constant (Fig. 1). Chickens injected with saline showed about the same retention as chickens not given any treatment [7]. However, when CXM was administered before learning, the percentage of chickens that pecked on tests at increasing times after learning rose; that is, retention declined over 2 to 3 hr. Tested 10 min after learning, the retention was the same as that of saline controls (72.5% cf 79.6%) but 180 min later the retention had dropped to 22% (cf 69% in controls).

Dose Response Curve for Amphetamine

Four doses of amphetaine, 0.1, 0.5, 1.0 or 2.0 mg/kg were given 10 min after learning to chickens pretreated with saline or CXM and the retention tested at 180 min (Fig. 2). The 0.1 mg/kg dose of amphetamine had an effect of reducing the retention of saline pretreated chickens. Higher doses did not affect those treated with saline. With CXM pretreated chickens, low doses (0.1, 0.5 mg/kg) of amphetamine had very little effect on the CXM amnesia but doses of 1.0 and 2.0 mg/kg resulted in retention scores that were essentially similar to those of the saline plus amphetamine group. These data indicate that the higher doses of amphetamine could reverse the CXM-induced amnesia.

Amphetamine Administration at Various Times after Learning

Amphetamine was administered to both saline and CXM groups at times corresponding to the retention intervals shown in Fig. 1. Amphetamine (1.0 mg/kg) administered subcutaneously to chickens pretreated with saline also held memory at the same level and therefore had no effect on

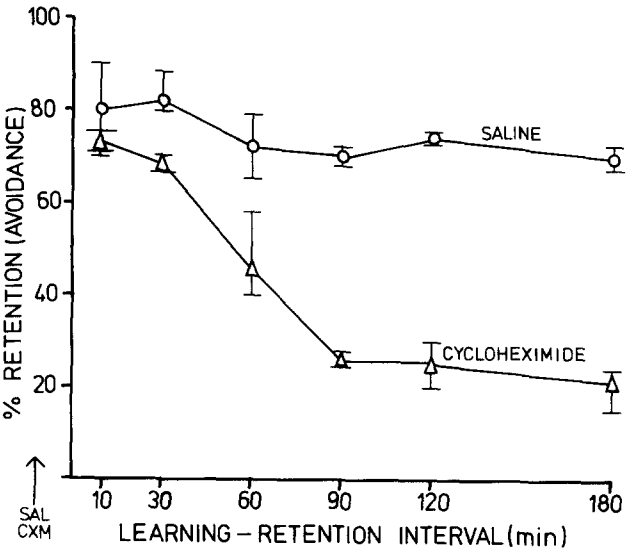

FIG. 1. Percentage retention over 180 min of separate groups of chickens given intracranial CXM (20 μgm) or saline. Each point represents the mean retention of 2 to 4 groups of 20 chickens and the bars represent the minimum and maximum percentage of the total number of groups tested at that time.

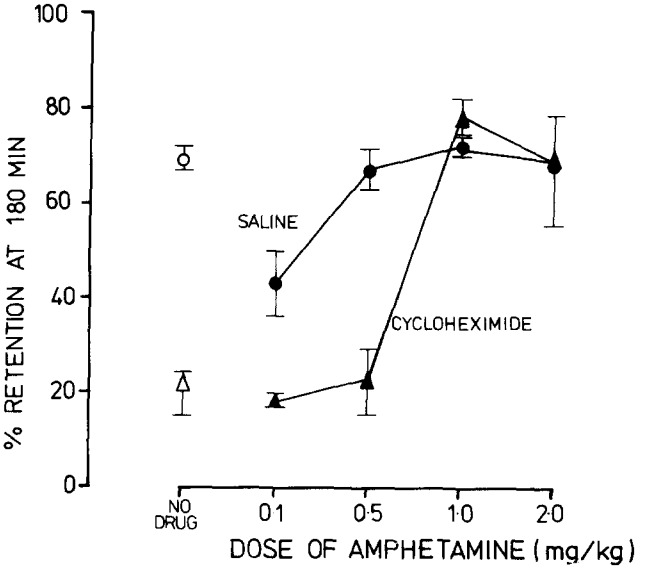

FIG. 2. Percentage retention on 180 min test of chickens injected with a range of concentrations of amphetamine and pretreated with either CXM or saline.

retention levels at 3 hr (Fig. 3B). If chickens were pretreated with CXM and memory was therefore declining; amphetamine, when administered, seemed to hold the memory at precisely the level existing at the time of injection (Fig. 3A). When amphetamine was given to CXM chickens at 10 min, the predicted retention from Fig. 1 would have been 72.5%. The retention measured at 180 min was 77.8%. Thirty min after learning, retention in CXM pretreated chicks, would normally have been 68.4%. Retention measured at 180 min when amphetamine was administered at 30 min, was 62.9%. When amphetamine was given at a time when retention after CXM administra-

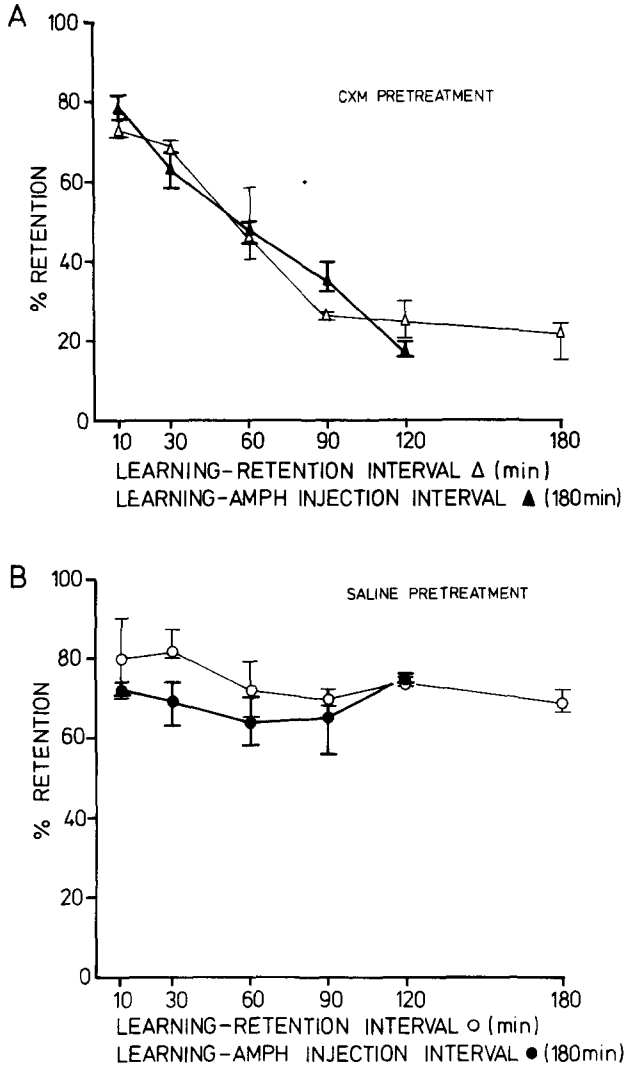

FIG. 3. Retention tested between 10 and 180 min following intracranial CXM (A) or saline (B) compared with the retention seen at 180 min when amphetamine (1.0 mg/kg) was given 10 to 120 min after learning. The learning-retention interval data is the same as in Fig. 1. Note that the two graphs have been superimposed, the amphetamine ordinate is the time between learning and injection of amphetamine and all testing for these groups was carried out at 180 min.

tion was low (33.3% at 90 min) the measured retention at 180 min was also low (35.1%).

Permanency of Amphetamine Reversal of CXM Amnesia

The effect of amphetamine on memory in CXM pretreated chicks lasted at least 24 hr. When the retention test was delayed and given 24 hr after learning, it was evident that the amphetamine effect was still dependent on the time of amphetamine administration (Fig. 4). The results were close to those seen in the experiments using a 3 hr retention test. Once again, with saline pretreatment, amphetamine had no effect when administered 10 min or later after learning.

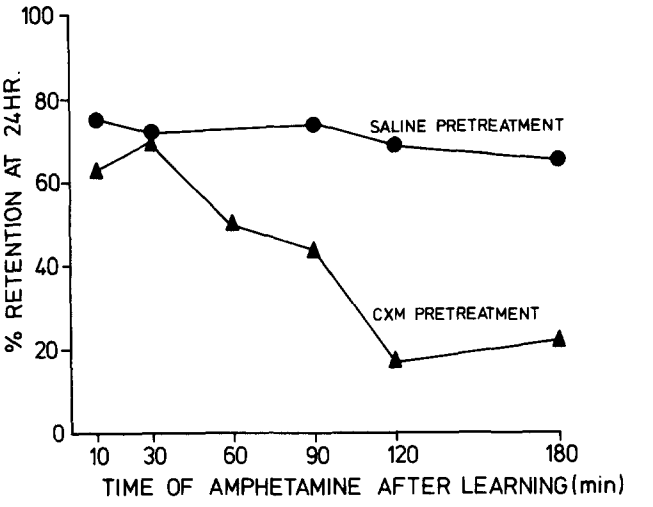

FIG. 4. Retention of memory at 24 hr after CXM or saline pretreatment as a function of time of amphetamine administration after the learning trial.

Time Course of the Amphetamine Effect on CXM Amnesia

The question arises as to whether amphetamine's effect in overcoming CXM-induced amnesia occurs immediately, or after a time lapse.

Chickens were pretreated with CXM or saline and given amphetamine 10 min after the learning trial. Retention was measured in different groups at times up to 180 min (Fig. 5). Both the saline and CXM pretreated chickens given amphetamine showed an apparent loss of memory; i.e., an increase in pecking with a maximum effect at 90 min. However, at later times both retentions return to the levels seen at 10 and 30 min. As this increase in pecking at 90 min occurred in both saline and CXM pretreated chickens it must have been a transient effect of amphetamine on performance.

DISCUSSION

Amphetamine overcame the amnesia from CXM only if it was administered while short-term, protein-independent, memory was still present. Subsequent testing showed that amphetamine held the decaying protein-indpendent memory, for at least 24 hr, to the level existing at the time the amphetamine was administered. It is as if amphetamine arrested the decline of memory at the time it was injected and allowed the consolidation of just that amount of memory. Amphetamine did not reverse the action of CXM, in that retention was not returned to the original levels (i.e. saline control values) when injected at later times after learning. Likewise, amphetamine did not improve the performance of saline treated birds in this experiment, even though control retention was usually less than 80%.

The experiments to determine when the action of amphetamine occurred indicated that amphetamine had 2 effects; it increased pecking at 90 min in both saline and CXM pretreated chicks possibly as a result of arousal changes, but more importantly, it had an immediate protective effect on memory in CXM pretreated birds. Several workers have shown that high doses of amphetamine in

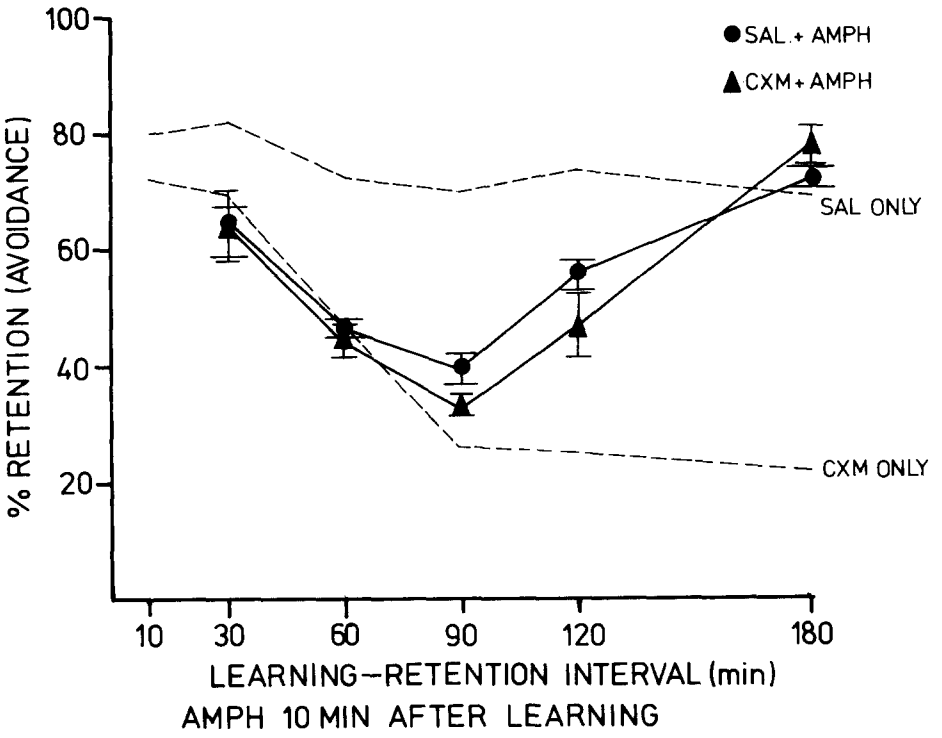

FIG. 5. Percentage retention measured following pretreatment with intracranial CXM or saline and subcutaneous amphetamine (1.0 mg/kg) 10 min after learning. This was compared with data obtained from CXM or saline pretreatment only.

chickens produce many physiological and behavioural effects, such as increased vocal activity, postural changes, motor excitement [13], and increased locomotor activity [11]. Although these effects are seen at higher dosage levels not comparable to those used in the present experiments, they may explain the transient decrease in avoidance at 90 min as merely a behavioural effect. At 3 hr when the saline retention had returned to normal levels, the amphetamine effect on CXM pretreated birds is more likely to be an effect on memory rather than due to performance changes.

At present there is no satisfactory explanation of amphetamine's central nervous system effects and accordingly interactions between protein synthesis inhibitors and amphetamine are difficult to interpret. Barondes and Cohen [1] found that amphetamine, corticosteroids, and even noncontingent foot-shock, could cause memory enhancement in CXM pretreated mice. They suggested that an appropriate state of arousal, induced when short-term memory was still present, could specifically direct the establishment of long-term memory (p. 928). Amphetamine, and perhaps other stimulating drugs, effect nonspecific states such as arousal and some authors [5,8] believe that these effects may have an influence on the consolidation of short-term to long-term permanent memory.

An alternative explanation [2, 3, 9, 10, 12] comes from the suggestion that CXM is producing amnesia by the inhibition of tyrosine hydroxylase synthesis, resulting in reduced norepinephrine levels in the brain. Amphetamine and other centrally acting drugs may overcome the CXM-induced amnesia by releasing norepinephrine, which would reinstate the memory formation.

Leaving aside the possible mode of action, certain conclusions can be drawn from the data presented in the paper. Amphetamine clearly arrests the decline of memory in a brain under the influence of CXM, and hence it must be interacting with the process that holds the labile memory. It could do this by direct antagonism to the molecular action of CXM but preliminary biochemical experiments indicate that this is not the case (Jeffrey and Gibbs in preparation). Alternatively, it could enhance the duration or intensity of the labile store, so that the labile memory trace has a much longer than normal duration and, therefore, outlasts the inhibition of protein synthesis. Since amphetamine cannot restore memory that has already declined; that is, it cannot remind birds that have already forgotten, it seems likely that it affects the labile, protein-independent, phase of memory storage.

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