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BRIEF COMMUNICATION

Alleviation of Scopolamine Amnesia by Different Retrieval Enhancing Treatments

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QUARTERMAIN, D. AND P. LEO. *Alleviation of scopolamine amnesia by different retrieval enhancing treatments.* PHARMACOL BIOCHEM BEHAV **30**(4) 1093–1096, 1988.—Mice were trained in a one-way active avoidance task to a criterion of 9/10 avoidances. Immediately following training they were injected with scopolamine hydrochloride (1 mg/kg SC) or with saline. Retention was assessed 3 days after training by 5 test trials on which the UCS was not present. Thirty min prior to the test, groups were injected with different doses of arecoline, d-amphetamine sulphate or with saline. Other scopolamine-treated mice were exposed to the CS or the UCS 24 hr prior to the test. The scopolamine-induced amnesia was attenuated by both 0.5 and 1.0 mg/kg arecoline and by 2.0 mg/kg d-amphetamine. Retention was also improved by exposure to the CS and the UCS. These data show that scopolamine amnesia can be alleviated by treatments which activate retrieval processes.

Scopolamine Amnesia Cholinergic systems and memory Retrieval Amphetamine and memory Reminder treatments

THE muscarinic cholinergic antagonist scopolamine has long been known to cause a memory loss in animals and man (see [3,20] for reviews). Because some of the characteristics of the amnesia share similarities with the memory loss associated with aging and dementia, it has been considered as a possible model for these neurological disorders ([1, 4, 5,] but see [2]). One feature of scopolamine-induced amnesia which has clinical relevance is illustrated by the recent demonstration that the memory loss can be prevented if noncholinergic as well as cholinergic agents are injected shortly following learning [6]. A related issue which has received less attention is whether pharmacological agents or other treatments can alleviate a scopolamine-induced amnesia once it is fully developed many days after learning. Studies have shown that memory loss caused by several amnestic agents can be alleviated by pharmacological and reminder treatments administered before testing [12–16]. These results have been interpreted as indicating that memory can be restored following some types of forgetting by activating retrieval processes [8–10, 12]. The purpose of the present experiment was to determine whether a 3-day-old amnesia induced by posttraining scopolamine injection could be attenuated by a cholinergic agonist (arecoline) a noncholinergic agent (d-amphetamine) and reminder treatments (CS and UCS presentation).

METHOD

Subjects

The subjects for this experiment were male Swiss Webster mice (Taconic, New York) 10 weeks of age and between 30 and 40 grams body weight. Animals were housed 4 per cage with food and water available ad lib.

Behavioral Task and Apparatus

Scopolamine-induced amnesia and its recovery by retrieval enhancing treatments were assessed by measuring retention of active avoidance learning. Mice were trained in a two compartment avoidance apparatus consisting of an aluminum V trough separated from a black Plexiglas goal box by a guillotine door. The dimensions of the trough and the goal box were 13×13×110 cm. The goal box was covered by a black lid and the trough by a clear acrylic sheet painted white except for a 5×5 cm area above the door which was left clear for observation. A 5-watt miniature lamp was mounted in the center of the lid above the trough. The walls of the trough were constructed from 2 mm sheet aluminum the bottom 10 cm of which was bent inward at 150 degree angle so that the floor consisted of a V shaped trough made up of the two wall plates suspended at 60 degrees from each

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other and separated from each other 0.5 cm at the bottom. Each of the walls was connected to one pole of a Grason Stadler constant current shocker (Model No. 700) which was activated by control circuitry to deliver a current of 0.6 mA AC.

Procedure

Training. Training was initiated by placing the mouse in the V trough facing away from the door and 5 sec later the CS (flashing light 0.5 sec on) and the latency timer were activated. After 10 sec the UCS was automatically initiated and the CS, UCS and timer were simultaneously stopped when the mouse crossed into the goal box. Mice were transferred to a holding cage for a 30 sec intertrial interval (ITI). Training trials were continued until each animal attained a criterion of 9/10 avoidances. Mice who achieved criterion in fewer than 12 or more than 35 trials were discarded (<5%).

Sham training. Some animals received sham training to assess possible nonspecific drug effects and to provide a baseline against which to compare the magnitude of the amnesia induced by scopolamine. Sham trained mice were placed in a shock chamber which had the same dimensions as the V trough but which was otherwise different and situated in an adjacent room. The walls were made of plywood and painted with black and white stripes and the floor was constructed of stainless steel rods. The chamber was covered with a clear acrylic lid. The floor of the apparatus was connected to the shocker in such a way that sham trained animals received the same intensity and duration of foot shock as their regularly trained counterparts with whom they were experimentally yoked.

Testing. Retention was tested by employing the standard training procedure except that the UCS was not scheduled. Animals were given 5 trials with a 30 sec ITI and animals failing to cross within 60 sec on any trial were given the maximum latency as a test score.

Amnestic agent. Amnesia for active avoidance training was induced by injecting 1.0 mg/kg scopolamine hydrochloride SC immediately after training. The drug was dissolved in saline and adjusted for individual animals by injecting 0.01 ml per gram body weight.

Pretest treatments. The following treatments were administered prior to testing:

(1) The catecholamine stimulating agent d-amphetamine sulphate 0.5, 1.0, 2.0 mg/kg SC 30 min before testing.
(2) The cholinergic agonist arecoline 0.5, 1.0 mg/kg SC 30 min pretest.
(3) A CS reminder. Twenty-four hours prior to testing animals were placed in the V trough with the door closed. After 5 sec the CS was introduced for a 10-sec period. After an interval of 30 sec the procedure was repeated. Following the second exposured the mice were removed and returned to the home cage. The UCS never followed the CS in this reminder treatment.
(4) A UCS reminder. Twenty-four hours prior to testing mice were placed in a novel shock chamber situated in a different room from the one in which avoidance training was conducted. The floor of the chamber was 10 cm square and constructed from steel rods. The walls were 20 cm high and painted with gray and white horizontal stripes. Mice were removed from the chamber via a hinged door. Mice were place in the chamber and given two 1.0 sec duration 0.6 mA shocks separated by an interval of 30 sec.

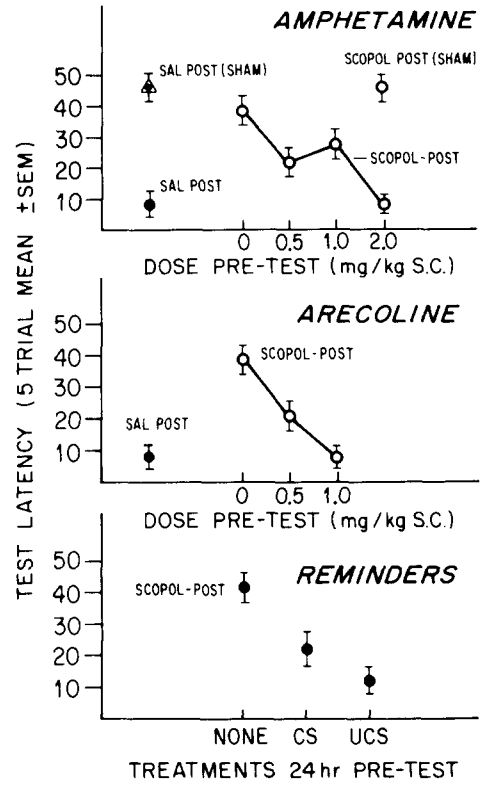

FIG. 1. Mean 5 trial test latency for the 3 retrieval enhancing treatments. Undisturbed memory is represented by the group injected with saline posttraining. As amphetamine and arecoline were tested in a single experiment, the same control groups are shown in both panels of the figure.

Procedure

Pharmacological agents pretest. Mice were trained as described above and immediately following attainment of the learning criterion injected with either saline (N=12) or scopolamine (N=70). Retention was tested 3 days after training. Thirty min prior to the test, saline controls were injected with a second saline injection and the scopolamine-treated mice were injected with either saline (N=12) or one of the following doses of amphetamine, 0.5 (N=12), 1.0 (N=12), 2.0 (N=11) mg/kg SC or arecoline 0.5 (N=12), 1.0 (N=11). Seven mice were trained in each session and assigned in a quasirandom fashion to the treatment groups in a manner that resulted in approximately equal mean trials to criterion across the 7 groups.

An additional 24 mice were given sham training. Each of these mice was yoked to a trained animal and at the completion of sham training injected with either saline (N=12) or scopolamine (N=12). Thirty min prior to being tested in the regular apparatus the saline-treated mice were injected with saline while those which had received scopolamine posttraining were given an injection of 2.0 mg/kg d-amphetamine.

Reminder treatments pretest. The reminder treatments were run as a separate study following the completion of the pharmacological experiment. Thirty-four mice were trained and all were injected with scopolamine immediately following training. Twenty-four hours prior to testing they were randomly assigned to 1 of the three groups and given expo-

sure to the UCS (N=12), exposure to the CS (N=11) or placed in an empty cage for 60 sec in the vivarium (no exposure).

RESULTS

The results are shown in Fig. 1. Scopolamine injected immediately after training produced a robust amnesia when latencies were compared with saline controls, $t(22)=4.87$, $p=0.001$. The extent of the amnesia was relatively complete as evidenced by the finding that there was no significant difference in test latency between untrained (sham) mice and those given scopolamine after avoidance training, $t(22)=0.79$. The amnesia induced by scopolamine was alleviated in a dose dependent fashion by d-amphetamine. A one-way ANOVA of the scopolamine-amphetamine data indicated a significant difference in test latency among the groups, $F(3,43)=4.87$, $p=0.005$. Post hoc comparisons were carried out using t-tests where the per comparison alpha was corrected according to the Dunn Method or the Bonferroni inequality [7]. Results showed that neither 0.5 nor 1.0 mg/kg d-amphetamine produced a significant attenuation of the amnesia but that a dose of 2 mg/kg resulted in an almost complete alleviation of forgetting [2 mg/kg vs. scopolamine; $t(21)=4.47$, $p=<0.001$]. That the decreased test latencies following amphetamine were not the result of increased activity or other nonmemorial factors is indicated by the different effect amphetamine had on the test latencies of trained and sham-trained animals, $t(18)=7.24$, $p=<0.001$. Amphetamine clearly failed to decrease test latencies in the sham group whose performance was virtually indistinguishable from sham trained mice given saline before testing. These results confirm previous findings (e.g., [13,14]) which have shown that amphetamine and other drugs administered before testing do not facilitate performance in animals not previously exposed to the training contingencies.

The effects of arecoline on scopolamine amnesia was analysed by a one-way ANOVA. Results indicated a significant difference among the groups, $F(2,32)=11.55$, $p=<0.001$, and post hoc t-tests revealed that both the 0.5 mg dose, $t(22)=2.59$, $p=0.016$, and the 1.0 mg dose, $t(21)=4.59$, $p=<0.001$, attenuated the amnesia when compared with the latencies of scopolamine animals treated with saline prior to testing.

A one-way ANOVA was also carried out on the reminder data shown in Fig. 1. Results showed a significant difference among the three groups, $F(2,31)=8.48$, $p=<0.001$. Subsequent t-tests indicated that mice exposed to both the CS, $t(20)=2.15$, $p=<0.04$, and the UCS, $t(21)=3.97$, $p=<0.001$, had faster test latencies than scopolamine-treated mice who received no exposure.

DISCUSSION

The results of this study indicate that forgetting induced by blockade of cholinergic receptors can be alleviated by treatments administered long after events which caused the memory loss had occurred. This finding adds to a growing body of evidence (e.g., [11, 13–15]) which indicates that forgetting resulting from diverse sources can be alleviated by activating retrieval processes.

In this study both the catecholamine stimulating agent d-amphetamine and the cholinergic agonist arecoline facilitated remembering. There is a suggestion that the cholinergic agonist may be a more effective retrieval enhancer than amphetamine following cholinergic blockade since improved retention occurred at lower doses with arecoline. However caution must be exercised in assigning relative potencies to different drugs because of unknown differences in absorbtion, distribution and pharmacokinetics. The data do however, clearly indicate that noncholinergic agents can facilitate memory loss resulting from disruption of cholinergic neurotransmission. This extends a recent finding which showed that amnesia induced by posttraining scopolamine injection could be prevented from developing by administering a variety of pharmacological agents within 45 min of training [6].

The present results suggest that amphetamine and arecoline may be influencing remembering by their effect on a nonspecific retrieval system. Thompson [19] has recently shown that lesions in a functionally interconnected system including parts of the basal ganglia, the limbic midbrain and the brain stem reticular formation impair retention in a wide variety of tasks. This general memory system may be the neural substrate of a nonspecific retrieval system through which many different classes of drugs can influence memory processing.

The results of the present study indicate that memory loss caused by disruption of cholinergic functioning can also be ameliorated by nonpharmacological means. Many studies have shown that reminder treatments can restore remembering following amnesia (see [8,18] for a review). It has been established that these treatments do not improve performance by providing additional training but instead function as retrieval cues [8]. It is not known whether these reminder cues influence memory through the same neural systems that mediate the effects of drugs on retrieval. The possibility that these two classes of retrieval cues may share a common mechanism is suggested by a preliminary study which showed that the retrieval enhancing effects of a UCS reminder were attenuated by pretreatment with the dopamine antagonist haloperidol [10].

The findings discussed above underscore the importance of retrieval processes in the amelioration of forgetting. Studies concerned with the attenuation of amnesia have typically concentrated on introducing drugs around the time of training in an attempt to block the development of amnesia. A strategy which may have more relevance for memory loss associated with aging and dementia is to introduce drugs prior to testing with the objective of facilitating the retrieval of weakly stored memories.

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