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Time Dependent Changes in Anterograde Scopolamine-Induced Amnesia in Rats

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MOSS, D. E., J. B. ROGERS, J. A. DEUTSCH AND R. R. SALOME. *Time dependent changes in anterograde scopolamine-induced amnesia in rats.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 321–323, 1981.—These experiments studied the effect of scopolamine on memory formation and subsequent memory recall. Different groups of rats were trained on a Y-maze brightness discrimination task 20 min after IP injection of 2 mg/kg scopolamine HBr, an anticholinergic. Retention tests were then conducted 1 day or 2, 4, or 6 weeks after training. Deficits in retention performance were observed at 1 day and 2 weeks after training but not at the longer intervals. In addition, other rats were trained in the same manner and after the same dose of scopolamine but were then retention tested 20 min after 0.5 mg/kg physostigmine salicylate, a cholinesterase inhibitor. These subjects also showed deficits at 1 day and 2 weeks but were not different from controls at the longer intervals. Amnesia was not, however, produced after treatment with scopolamine methyl nitrate or by injections of scopolamine HBr administered immediately after training. These results suggest that scopolamine, present in the central nervous system during training or within the first few moments thereafter, modifies the formation of the memory trace in such a way that memory is not available for recall for a period of weeks.

Amnesia Memory Cholinergic mechanisms Scopolamine Physostigmine Rats

RECENT evidence suggests that memory processes involve the action of certain neurotransmitters in the central nervous system. The most thorough investigation of the role of a neurotransmitter in learning and memory processes appears to be the study of cholinergic mechanisms in rodents, monkeys, and humans. Specifically, cholinergic blocking agents and anticholinesterase compounds have been shown to alter retention in a wide variety of tasks and experimental procedures [1, 2, 5, 6, 7, 9]. One of the clearest demonstrations that memory is affected by cholinergic drugs is presented by Deutsch and his associates who have shown that memory can be systematically manipulated over days or weeks with anticholinergic compounds and anticholinesterases given only before the retention tests [5]. These several experiments have led to the hypothesis that a set of cholinergic synapses required for the recall of memory change in excitability over a period of time after learning [5].

One problem, however, which has received insufficient attention is the effect of cholinergic drugs given before training on memory recall days or weeks later. The anticholinergic drugs, scopolamine and atropine, are particularly interesting in this regard because they reliably produce amnesia when given before training [3, 4, 8, 10]. In addition, Dennis [4] has found that the amnestic effects of scopolamine appear to be time dependent in that mice trained under the effects of scopolamine showed poor retention one day after training but improved retention at one week. On the basis of these data, Dennis [4] suggested that the effects of scopolamine should be considered in terms of temporal aspects of the memory process itself rather than as a case of state dependence. The purpose of the present study was, therefore, to examine the effects of scopolamine administered before training on the ability of animals to recall the task at various times after training under various drug conditions. Specifically, animals were retention tested either without drugs or with physostigmine, a cholinesterase inhibitor which produces a pharmacological effect opposite to that of scopolamine.

METHOD

Holtzman strain albino rats of both sexes reared in this laboratory were trained to escape a 0.75 mA foot-shock by entering the lighted arm of a Y-maze essentially identical to that employed by Deutsch and his colleagues [5]. In the main experiments, training was completed to a criterion of 10 correct responses out of any 10 trials or to a maximum of 80 trials. A criterion of 8 correct responses out of any 10 trials was used in one supplementary experiment. Each arm of the maze was 48×13 cm with metal walls 15 cm high. The end wall of each arm was made of translucent plastic behind which was a 60 W bulb which, when lighted, was supplied

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with 65 V. Once the animal was placed in the maze it was not removed until the end of training insofar as the correct goal alley on one trial was the start alley on the next trial. Training was conducted 20 min after an IP injection of either 2.0 mg/kg scopolamine HBr, 2.0 mg/kg scopolamine methyl nitrate, or after a control injection of water.

The retention tests were conducted at various times after original learning with each animal only being retention tested once. The retention test was a relearning retention test using a procedure exactly like the training procedure except that an intertrial interval of 10 sec was used. Retention tests were initiated 20 min after an IP injection of 0.5 mg/kg physostigmine salicylate or water. All injections were in a volume of 1.0 ml/kg. The subjects were retention tested at one day, two, four, or six weeks after training.

There were basically three experimental conditions studied in these experiments. There was a control group trained after a placebo injection of water and retention tested after another placebo injection. In addition, there were two experimental groups; both trained after 2.0 mg/kg scopolamine HBr but one group was retention tested after a placebo injection of water and the other was injected with physostigmine prior to retention testing. In addition, another control group was trained and retention tested only at the one day interval to insure that the effects of scopolamine were, in fact, due to effects on the central nervous system. This control group was trained after receiving 2.0 mg/kg scopolamine methyl nitrate which produces all of the peripheral effects of scopolamine HBr but does not cross the blood-brain barrier to produce central effects. This control group was retention tested after a placebo injection of water. There were twelve animals in each of the three experimental conditions at each time interval studied. Therefore, there were 156 rats trained and retention tested in these main experimental conditions.

In supplementary experiments, other rats were trained without prior injection of drug but were injected with either 2 mg/kg scopolamine HBr immediately *after* training to 10 correct responses out of 10 trials or with 2, 4, or 8 mg/kg of scopolamine HBr immediately after training to a criterion of 8 correct responses out of any 10 trials. The purpose of these post-training injection groups was to determine if scopolamine had to be present during training in order to have its effects on memory.

RESULTS

Training

There were no differences between training scores obtained after injections of water or scopolamine. Animals injected with water learned the maze to a criterion of 10 correct responses out of 10 trials in an average of 49 trials while animals trained after an injection of scopolamine required an average of 53 trials. Animals trained to a criterion of only 8 correct responses out of any 10 trials required an average of only 22 trials to reach this less rigorous level of training.

Retention Tests

The results of the main experimental groups are shown in Fig. 1. Single classification analysis of variance of the data from the three experimental conditions indicated that there was a significant difference in the retention performance of the groups at 1 day, $F(2,33)=9.57$, $p<0.01$, and at two weeks, $F(2,33)=4.66$, $p<0.05$, but not at other intervals. A factorial analysis of variance (3 treatment conditions by 4 time intervals) indicated that there was a significant main effect of drug condition, $F(2,132)=6.355$, $p<0.01$, but no significant main effect of time over the six weeks when all groups were combined. The interaction between drug condition and time was, however, also significant, $F(6,132)=3.224$, $p<0.01$.

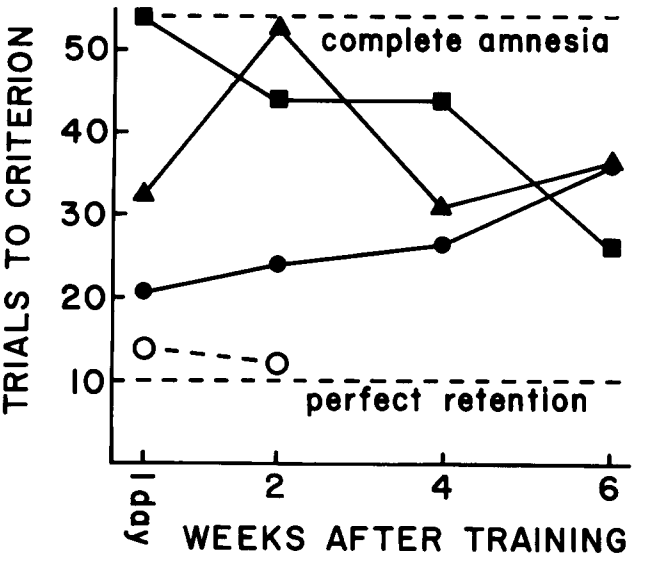

FIG. 1. Retention as a function of time after training. The criterion was the number of trials required to obtain 10 correct responses out of 10 trials. The dashed line showing complete amnesia is the mean number of trials required to train naive animals to the same criterion. The solid circles represent the retention performance of the control groups (trained without drug and retention tested without drug). The triangles represent the performance of groups trained after scopolamine and retention tested without drug. The squares represent the performance of groups trained after scopolamine and retention tested after physostigmine. There were 12 animals in each of the previous groups. The open circles with the dashed line represent the retention of two groups of 6 animals injected with scopolamine immediately after training and retention tested without drug.

As shown by the gentle upward trend in Fig. 1, the control retention performance appears to decline with time after training. While a gradual deterioration in learned performance over 6 weeks is not surprising, it is important to know if the significant interaction obtained in the factorial analysis is due to deterioration of control performance or improvement in the drug conditions or both. In order to determine the source of the interaction, single classification analysis of variance was computed for each drug condition across time. There was, however, no significant difference in control performance across time, $F(3,44)=1.235$, n.s., and the differences in the scopolamine/water groups performance only approached significance, $F(3,44)=2.148$, n.s. However, the differences in performance in the scopolamine/physostigmine groups across time was highly significant, $F(3,44)=4.310$, $p<0.01$. Insofar as the three drug conditions were significantly different from each other at 1 day and 2 weeks (see above) but not at 4 and 6 weeks and there was no significant change in the controls, it appears that most of the effect is due to improvement in drug group retention performance with time.

Rats trained after receiving 2.0 mg/kg of scopolamine methyl nitrate and retention tested after 1 day showed retention virtually identical to water injected controls. Water in-

jected controls reached retention criterion in an average of 20.8 trials while the scopolamine methyl nitrate group required an average of 21.1 trials.

The subjects in the supplementary experiment which received 2.0 mg/kg scopolamine HBr immediately after training to the 10/10 criterion did not show any decrement in retention and, in fact, seemed to show a slight facilitation. These animals relearned to 10/10 criterion in an average of only 12.4 trials compared with the water injected controls discussed above who required 20.8 trials. This apparent facilitation was not statistically significant and it appeared that a ceiling effect may have prevented a bigger difference. Therefore, in order to test the possibility that post-training injections of scopolamine might facilitate recall at one day, approximately 60 additional animals were trained to a criterion of only 8 correct responses out of 10 trials in order to remove the apparent ceiling effect in the retention data observed above. One group of animals received a post-training injection of water (controls), and each of 3 other groups received either 2 mg/kg, 4 mg/kg, or 8 mg/kg scopolamine HBr. Again, a slight but not statistically significant facilitation was observed in the retention scores of those animals which received scopolamine immediately after training.

DISCUSSION

The effect of scopolamine administered before training on learning performance suggests that the learning process itself and, in addition, the short-term memory required for normal trial-to-trial improvement in performance are probably not muscarinic cholinergic processes. This conclusion is supported by the observation that scopolamine treated animals were not distinguishable from controls during learning on the basis of total number of trials required nor general appearance. In addition, the retention performance of rats treated with scopolamine before training was virtually identical to control retention performance at 6 weeks after training. These observations argue that the initial learning under the effect of scopolamine was equal to the initial learning of the control animals.

The amnesia observed after training animals under the effects of scopolamine, however, is probably a very complex phenomenon. Explanations of these results based upon the argument that scopolamine interfered with learning seem to be unsatisfactory for the reasons discussed above which indicate normal learning. In addition, the post-training injection experiments demonstrated that the poor performance of the scopolamine treated groups was not due to a performance decrement produced by some nonspecific interaction between scopolamine and the physiological sequel to the stress of training. Another possible explanation for apparent amnesia after training during a drug effect is, of course, state dependence. Currently, however, the major theories of state dependent learning [11] do not provide an explanation for the time-dependent changes in memory recall observed in these experiments.

The data related to the time-dependent effects suggest that learning took place and the memory was formed and maintained intact during the entire time studied. However, it appears that memory was simply not available for recall in scopolamine HBr treated groups until about 4 weeks later. The experiments using scopolamine methyl nitrate and the experiments using post-training injections of scopolamine HBr suggest that the scopolamine must be present in the central nervous system during training or within the first moments thereafter (i.e., so early that a post-training IP injection has no effect) in order to produce amnesia. It appears that scopolamine, in the central nervous system at the time of training, interacts with the initiation of the memory trace in such a way that the memory formed cannot be recalled for a period of time after training. It may be that scopolamine, administered before training, modified the initiation of a neurophysiological substrate for memory which, in itself, may be a time-dependent process.

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