

BRIEF COMMUNICATION

Effects of Pre-Training Administration of Scopolamine on Learning and Retention in the Cockroach, *P. americana*

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Received 6 January 1982

BARRACO, D. A. AND E. M. EISENSTEIN. *Effects of pre-training administration of scopolamine on learning and retention in the cockroach, P. americana*. PHARMACOL BIOCHEM BEHAV 20(3) 479-481, 1984.—The muscarinic cholinergic blocker, scopolamine, has no effect on acquisition or retention of the choice behavior of the cockroach. *P. americana* trained to turn right or left to avoid shock in a T-maze. Scopolamine does, however, prevent runway habituation during training, suggesting that such habituation may be dependent on central cholinergic synapses as there are no known peripheral cholinergic receptors in cockroaches. Previous work demonstrating that puromycin interferes with correct choice retention five hours after training taken together with our results demonstrating no effect of scopolamine on such retention, suggests that puromycin does not produce its amnesic effect on choice behavior by interfering with central cholinergic synaptic processes.

Cockroaches	Scopolamine	Learning	Retention	Habituation
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PREVIOUS work has indicated that cockroaches injected with puromycin (PURO) before T-maze training exhibited retention deficits of the correct turn response when tested five hours after training. However, they did not show any acquisition defect [2]. PURO also had no effect on either acquisition or retention of habituation behavior as evidenced by progressive increases in runway time with succeeding trials in the maze during both training and testing [2]. Thus, PURO may show specificity for the different types of behavioral memories that occur in any training situation and that may be mediated at different sites within the central nervous system. Similar reports of selective inhibition of memories by PURO has been observed in different vertebrates [4,7].

The mechanism by which PURO induces any retention deficits remains unclear. Cycloheximide (CXM) which causes substantially more protein synthesis inhibition than the dosages utilized for the PURO studies showed no CXM induced retention deficits of either choice or habituation learning [2]. Thus, the overall amount of protein synthesis inhibition does not appear to be the major causal factor in the retention deficits produced by PURO. Similar comparisons have been frequently noted in vertebrate preparations [1].

Some authors have theorized that PURO may be causing retention deficits by interfering with cholinergic transmission [1]. The present study represents an initial effort to

explore the possibility that the amnesic actions of PURO in the cockroach are due to interference with cholinergic transmission. The experiments used the same training and testing procedures and response measures as before [2,6]. Animals now were injected with scopolamine, a predominantly central muscarinic cholinergic antagonist one hour before training.

METHOD

Adult male cockroaches of the species *Periplaneta americana* and weighing approximately one gram were used. Scopolamine hydrobromide was obtained from Sigma Chemical Company. Initial toxicity studies indicated that a maximum dose of 500 μ g in 20 μ l insect Ringers did not alter activity level, degree of startle response, presence of the righting reflex, or produce any other unusual behavioral changes that might indicate debilitation. These observations were made over a 4-7 day period after injection of each animal.

Following this initial study, thirty-two animals were injected with either 500 μ g of scopolamine in 20 μ l of Ringers or 20 μ l of insect Ringers one hour before training in a T-maze to turn right or left to avoid an electrical shock. Animals were tested for retention five hours after the end of

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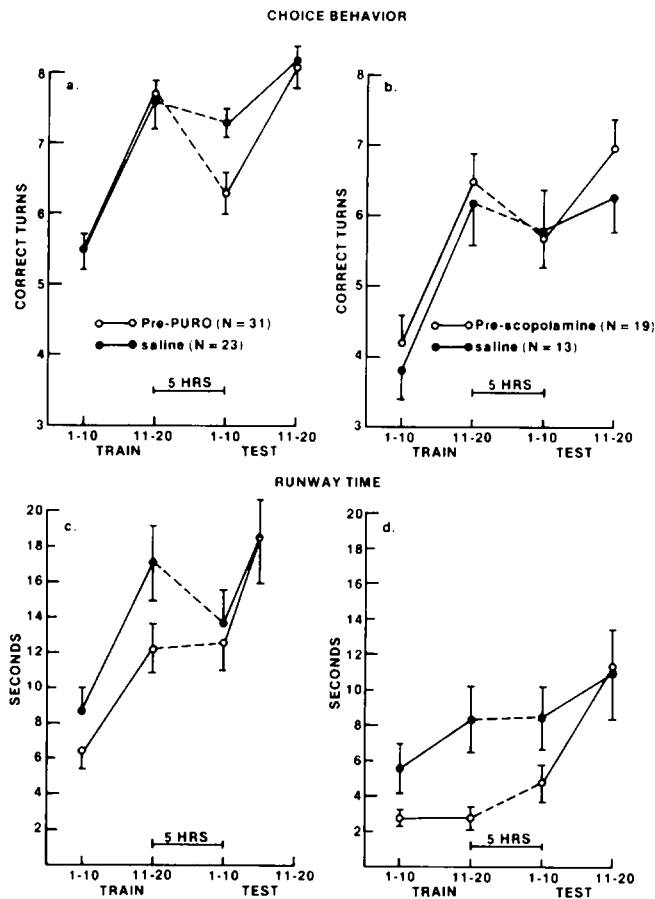


FIG. 1. Mean number of correct turns (a and b) and mean time spent in the runway (c and d) as a function of either puromycin or scopolamine injection. Standard errors are shown. Separate saline control groups were run with the PURO and scopolamine groups.

training. Both training and testing consisted of twenty trials as in previous work [2]. The number of correct turns in each block of ten trials was noted.

In addition, the time taken by the animal to proceed down the runway of the maze was measured on each trial and the group mean was calculated for each block of ten trials. Habituation was measured as an increase in runway time over trials. Statistical comparisons were done by means of a two-tail *t*-test. Unless otherwise stated significance levels were $p \leq 0.05$.

RESULTS

The figure shows the effects of pre-training administration of scopolamine and saline on acquisition and retention. Figures and data for previous work utilizing pre-training injections of PURO also are presented for comparison.

Choice Behavior

The curves of the scopolamine group and its saline control are statistically indistinguishable (Fig. 1b). In addition, the significant increase in the mean number of correct turns from training trials 1-10 to trials 11-20 demonstrates that

both groups exhibit the shorter-term memory necessary for learning. Thus scopolamine has no effect on acquisition of the avoidance training.

A comparison of the mean number of correct turns from training trials 11-20 to testing trials 1-10 can be used as a measure of retention five hours later [2]. The decrease for both groups is not significant. Thus, both scopolamine and saline injected animals exhibit excellent retention of the avoidance training over a five hour retention interval.

Runway Time

While the acquisition and retention curves for the choice behavior are identical (Fig. 1b) the curves for the runway time are quite different. Figure 1d shows that the scopolamine animals run the maze faster during training than the saline animals. The difference between the runway times of the saline and scopolamine animals in trials 1-10 show a level of significance at $p = 0.07$, two-tail. Additionally, the scopolamine group does not show the progressive increase in runway time with successive trials during training that has previously been observed as habituation [2]. However, during testing, five hours after the cessation of training, a comparison of the scopolamine runway times for trials 1-10 with 11-20 does show a significant increase indicating that habituation is now present.

DISCUSSION

The importance of cholinergic transmission in learning and memory processes has been demonstrated often in vertebrates through the use of muscarinic blockers such as atropine and scopolamine [9]. Our investigations suggest that scopolamine and PURO affect differently the two types of behavioral plasticity studied in the cockroach in this one-session T-maze training procedure.

PURO appears specific for interference with retention of the choice behavior (Fig. 1a). Scopolamine has no effect on acquisition or retention of the choice behavior (Fig. 1b). This suggests that central cholinergic synapses are not involved in choice learning and retention in the cockroach and that PURO is probably not acting to prevent retention by interfering with cholinergic transmission.

Scopolamine does, however, have an effect on habituation behavior (Fig. 1d). It interferes with runway habituation during training. PURO does not (Fig. 1c). The effect is not permanent as can be seen during testing when the habituation behavior returns (Fig. 1d). The latter effect is probably due to the fact that the animals were injected seven hours before testing and the drug was most likely metabolized. In fact, belladonna alkaloids are known to be rapidly excreted from the blood of vertebrates [5]. Scopolamine effects on runway habituation suggest that central cholinergic synapses are involved in such behavior since there are no known peripheral cholinergic receptors in the cockroach [3,8].

CONCLUSIONS

(1) Scopolamine has no effect on acquisition or retention of the choice behavior of cockroaches trained in a T-maze to turn right or left to avoid a shock. (2) Scopolamine does prevent runway habituation during training suggesting that such habituation may be dependent upon central cholinergic synapses as there are no known peripheral cholinergic receptors in cockroaches. (3) The PURO and scopolamine data taken together suggest that PURO is not interfering with

central cholinergic synapses to produce its amnesic effect on choice behavior. Furthermore, the PURO and scopolamine results support our previous suggestion [2] that choice behavior learning and runway habituation may be mediated at different sites in the central nervous system and most likely by different mechanisms.

ACKNOWLEDGEMENT

This work was done in the laboratory of the second author while he was a faculty member at Michigan State University. We wish to thank the College of Natural Science of Michigan State University for research funds received from the NIH Biomedical Research Support Grant.

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