# THE EFFECT OF SCOPOLAMINE HYDROBROMIDE ON MAZE ACQUISITION IN THE WHITE RAT

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## PREFACE

Many studies have been conducted to investigate the effects of atropine upon various discrimination tasks. These effects generally take the form of performance decrements, presumably due to a blockage of central nervous system cue-processing centers. The decrements are more pronounced when greater demands are made upon the cue-processing centers, as when multiple cues are used in the discrimination task. The question remains as to whether these effects are limited to atropine, or are characteristic of other related belladonna alkaloids as well. The purpose of this study is to investigate the effects of atropine's closest chemical relative, scopolamine, in a multiple-cue discrimination task.

Indebtedness is acknowledged to Drs. Larry Brown, Roy Gladstone, and Kenneth Sandvold for their valuable guidance. I especially wish to acknowledge my indebtedness to Dr. James M. Whitehouse of the University of Montana, under whose guidance this study was conceived and carried out.

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# CHAPTER I

## REVIEW OF THE LITERATURE AND STATEMENT OF THE PROBLEM

Atropine is the best known member of a group of drugs blocking the action of acetylcholine (ACH) on postganglionic parasympathetic nerves. Besides atropine, this group of drugs includes another natural beladonna alkaloid, scopolamine, and several semi-synthetic quaternary ammonia derivatives of the beladonna alkaloids. Also belonging to this group are a large number of synthetic anti-muscarinic compounds, mostly quite unrelated structurally to the natural alkaloids. While the actions of this group are chiefly anti-muscarinic, high concentrations of atropine and scopolamine are capable of blocking transmission at autonomic ganglia. The quaternary ammonia derivatives exhibit the lowest amount of ganglionic blocking activity. (For further pharamacological information, see Goodman and Gilman, 1965, Chapter 25.)

Whitehouse (1964) demonstrated that atropine sulfate is capable of producing a detrimental effect on the learning of a successive discrimination task involving multiple cues. This deleterious effect of atropine was noted on both acquisition of new responses and on the performance of responses previously learned. This study also produced indirect evidence that atropine's action in this case was due primarily to Central Nervous System (CNS) blockage and not because of any peripheral blocking effects. To rule out the possibility that the impairment of discriminatory learning could possibly be due to

peripheral actions rather than CNS activity, as indirectly indicated earlier, another experiment (Whitehouse, 1964) was designed employing essentially the same procedures. The effects on a group of rats injected with methylatropine were compared to an atropine group. (Methylatropine was employed because it is a potent peripheral blocking agent whose CNS actions are quite limited when compared to those of atropine (Goodman and Gilman, 1965, Chapter 25).) By comparing the results of atropine and methylatropine on maze acquisition, more direct evidence as to the actual nature of atropine's effect on learning was obtained. With both trials to criterion and mean errors being measured, the atropine sulfate group learned significantly slower than either the control or methylatropine groups, and also had significantly more errors. The methylatropine and control group did not differ on either number of errors or trials to criterion, indicating no interference on this type of task by methylatropine. Peripheral effects were matched for both atropine groups by means of food intake. The atropine groups did not differ on food intake either on injection days or on the rest of the test days, although both atropine groups consumed much less than the control group. This food-intake comparison indicates that the peripheral action of the two drugs was comparable (presumably through salivary inhibition), and, therefore, that the process of atropine's impairment of maze acquisition is due to the CNS action of the drug.

These earlier studies on the nature of atropine's action suggested further that atropine may have differential effects in the maze situation depending on the method (simultaneous or successive) of cue presentation or the number of cues employed. Another study by Whitehouse (unpublished, 1965) resulted in the observation that when a single cue

was used (regardless of type: visual, auditory, or tactual), atropine produced no impairment of discrimination learning; only on the multiple-cue situation did atropine produce its characteristic retarding effects. The impairment occurred using both simultaneous and successive methods of cue presentation, with the successive method producing a more pronounced impairment. An experiment (Whitehouse, unpublished, 1965) designed to check the influence of cue presentation on atropine's effect indicated that atropine's inhibition is related to the use of the cues and not to the method of cue presentation. It appeared that experimental subjects were unable to utilize multiple cues to increase their learning rate as normal subjects were able to do. To pursue further this line of reasoning, another experiment (Whitehouse, unpublished, 1966) was performed whereby irrelevant cues were added to a single relevant cue for both simultaneous and successive discrimination situations. Since the characteristic impairment of discrimination appeared when irrelevant cues were added to the atropinized group but not when added to the control group, the hypothesis that CNS cholinergic blockage by atropine interferes with cue processing in some way was further strengthened.

All of these experiments on the nature of atropine's cholinergic blockage contribute evidence to support Whitehouse's hypothesis that the cholinergic system of the brain is involved in cue processing, and that subsequent blockage, as by atropine, interferes in some manner with this processing. These experiments, especially with irrelevant cues, would further lend support to Carleton's view (Carleton, 1963) that the cholinergic system of the brain is involved in the extinction of non-reinforced responses.

Applying this view to the extinction of irrelevant responses involved in extinction, Carleton arrived at an expectation that variations in cholinergic activity will lead to corresponding changes in acquisition. (This follows, since it can be assumed that learning to make a correct choice involves the extinction of tendencies to make the wrong ones.) This would especially seem to be the case when multiple cues make greater demands on the cholinergic system, since multiple cues increase the number of specific response tendencies that must be extinguished. The studies by Whitehouse investigating atropine's effect on maze acquisition would support this view. An experiment by Carleton (1961) is also relevant. This study involved a two-stage response chain in which rats were required to press the correct one of five keys, then cross the response chamber and depress a lever to receive the reward. Rats were required to learn the correct key, which was changed every 20 trials, by trial and error. Both scopolamine and atropine increased the number of errors made in the course of obtaining 20 reinforcements. Carleton relates this effect to the increased probability of intrusion of incorrect responses due to an attenuation of the normal effects of non-reinforcement. Unfortunately, the results of this experiment, depended entirely on the speed with which the animal switched from a key response when it was no longer reinforced.

The question still remains as to whether the impairment of discrimination in T-maze acquisition is unique to atropine alone, or is also characteristic of other closely related beladonna alkaloids. The purpose of this study was to attempt to apply the actions and characteristics of atropine to those of atropine's nearest chemical relative, scopolamine, using the procedure established by Whitehouse with

atropine. Scopolamine, by a similar CNS blockage, should produce the same impairment of maze acquisition with multiple cues as atropine does, while not impairing performance involving single cues. Multiple cues presented successively was the condition chosen because this seems to produce the most severe blockage when atropine is employed (Whitehouse, unpublished, 1965). It was considered unnecessary to include a scopolamine methylbromide group to rule out possible peripheral effects, as Whitehouse has conclusively shown the action of atropine in this type of task to be due to CNS action. Any inhibiting action of scopolamine in the same situation should be due also to CNS blockage.

## CHAPTER II

## APPARATUS

The pretraining apparatus was a straight runway of wood painted gray, with black or white inserts alternated in the goal box. The training apparatus was a modified T maze (Figure 1). For the multiple cue conditions the subject was presented on each trial of a successive discrimination problem with a complex of cues. On those trials in which a right turn was rewarded, the center runway was black with a rough floor and a buzzer was sounded. On those trials in which a left-turning response was rewarded, the center runway was white with a smooth floor and no buzzer was sounded. In the single cue conditions, only the visual cues were used. On these a left turn was rewarded when a white center runway was present and a right turn was rewarded when a black center runway was present. In the multiple-cue conditions the rough-floor cue was provided by $\frac{1}{4}$" hardware cloth covering plexiglass. Plexiglass covering $\frac{1}{4}$-inch hardware cloth was the smooth floor cue. The auditory stimulus for multiple-cue conditions was provided by an Edwards No. 15-0 6 volt door buzzer of about 60 db. On the multiple-cue trials where the buzzer was employed, it was begun upon opening the start box and was continued until subject entered one of the goal boxes.

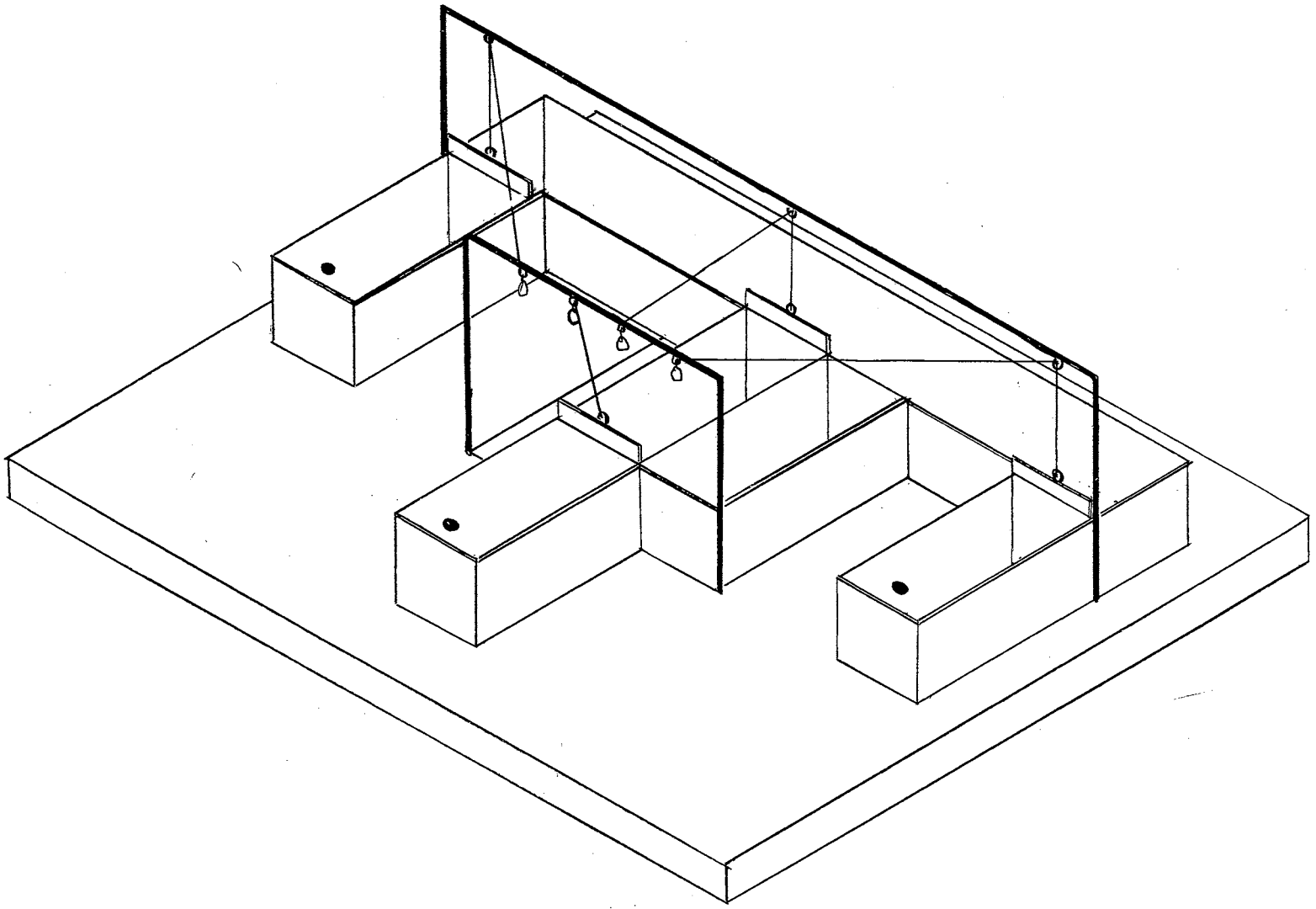

Figure 1. Modified T Maze

## CHAPTER III

## SUBJECTS AND EXPERIMENTAL CONDITIONS

Subjects were forty male Sprague-Dawley albino rats obtained from Simonsen Laboratories (Gilroy, California). They were approximately 60 days old at the beginning of the experiment, and were divided into four groups of 10 rats each.

For both single-cue and multiple-cue conditions there were two groups, a saline control and a scopolamine group. There were, therefore, four groups: Control, Single Cue (CSC); Scopolamine, Single Cue (SSC); Control, Multiple Cue (CMC); and Scopolamine, Multiple Cue (SMC). The two scopolamine groups received injections of .3 mg./kg. body weight of Scopolamine Hydrobromide from the first training day until criterion was reached. The Scopolamine Hydrobromide was prepared in saline solution such that each cubic centimeter of solution contained a kilogram of body weight dosage. The two control groups received injections of a saline placebo from the first training day until they reached criterion. All injections were administered intraperitoneally approximately 60 minutes before the start of each daily training.

# CHAPTER III

## PROCEDURE

All of the subjects were handled for approximately five minutes daily for one week prior to pretraining. Three days prior to the beginning of pretraining all subjects were put on a 22-hour deprivation schedule and, once testing began, were fed for two hours after each daily test. All subjects were given free access to water between daily testings. This schedule was maintained throughout the experiment.

Pretraining consisted of 20 trials in the straight runway, and 20 trials in the testing apparatus. On the first four days each subject was given five trials each day in the straight runway. For the next two days each subject received 10 trials per day in the testing apparatus. For pretraining trials in the testing apparatus an approach runway was constructed which did not provide any differential cues, and ten trials were given to each side. This was done by allowing the subject to choose on odd-numbered trials; on even-numbered trials, the subject was forced to the side opposite that just previously chosen. All subjects received the same pretraining. The subjects were randomly assigned to groups following pretraining. A single 45-mg Noyes food pellet was used as a reward for each trial during both pretraining and testing.

Following pretraining all subjects were given 10 trials per day in the maze for at least 200 trials, even if the criterion was reached

earlier. The learning criterion was 18 errorless trials out of 20 trials administered on 2 consecutive days. The order of correct choice alternated daily between LRRLLRLRRL and RLLRRLRLLR. The measures recorded were trials to criterion and number of errors. An error was defined as entry of the whole body exclusive of tail into the incorrect goal box, and only one error was possible on each trial.

The subjects were brought into the experimental room in groups of ten, and trials were alternated among subjects. Therefore, each subject received Trial 1 before any subject in the same group received Trial 2. Between trials each subject remained in an individual compartment of a transport cage, which was sound-deadened to reduce chances of subjects hearing the auditory stimulus between trials. For each subject there was a 2-3 minute period between trials.

## CHAPTER V

## RESULTS

The learning curves for all groups are presented in Figure 2. The mean trials to criterion for the CSC group was 293.33, while that for the SSC group was 300.00. Using the Mann-Whitney U test, this difference was found to be statistically non-significant. For the multiple-cue condition the mean trials to criterion for the CMC group was 145.55, compared to 241.11 for the SMC group. This difference is significant ($U = 16$, $P < .01$).

Mean number of total errors was 131.11 for the CSC group, and 137.77 for the SSC group. This difference was not significant. A significant difference ($U = 22$, $P < .05$) was found between the mean errors of the CMC group (67.1) and the mean errors (108.33) of the SMC group.

Since all of the animals in the single-cue condition were not run to criterion, the possibility does exist that a drug effect might have eventually emerged had testing of these two groups been continued.

There seemed to be no discernable difference between scopolamine and control groups in the animal's readiness to consume the reward, indicating that the peripheral effects of scopolamine were minimal.

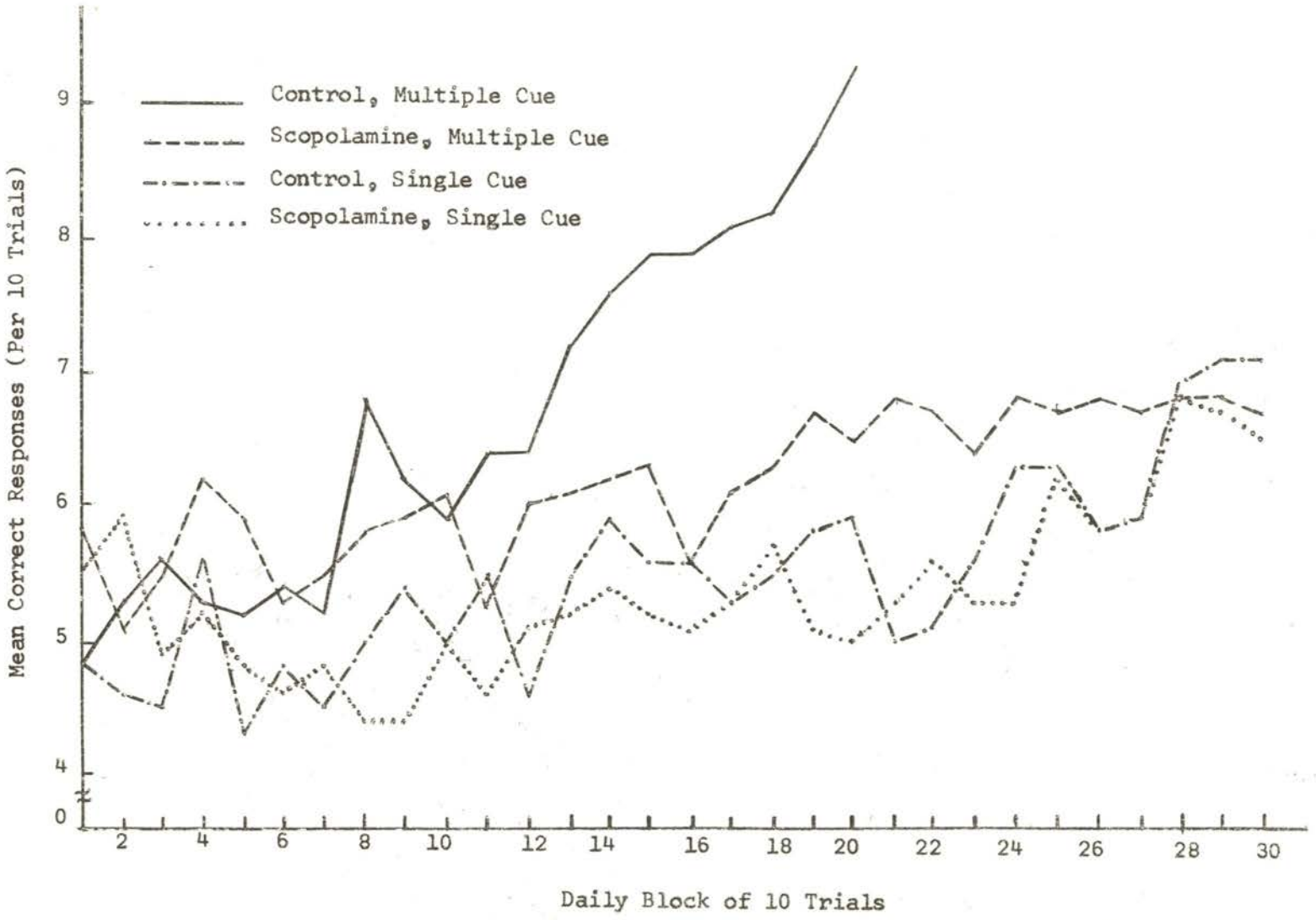


Figure 2. Learning Curves for All Groups.

## CHAPTER VI

## INTERPRETATION OF RESULTS

The results indicated that a decrement in acquisition, similar to that produced with atropine, occurred under the multiple-cue, but not single-cue, conditions. Carleton's (1963) hypothesis can handle these data, if it is assumed that multiple cues make greater demands on the cholinergic system by increasing the number of specific responses that must be extinguished. Cholinergic blockage under this condition should produce a greater acquisition decrement than with single cues. If the cholinergic system is involved in cue processing, as Whitehouse (1965) hypothesizes, multiple cues would again place greater demands on the system, resulting in a greater acquisition decrement under this condition. The results obtained here would also support this view.

The decrement produced in this study by scopolamine differs from that demonstrated by Whitehouse using atropine in that it did not begin to appear until the 12th daily trial, while the atropine decrement appeared as early as the 6th trial. This difference may obviously be due to differences in the drug employed, or perhaps to a lack of comparable dosages. It is interesting to note, however, that Whitehouse used hooded rats, while an albino strain was employed here. In comparing the multiple-cue control groups of each strain, the hooded animals learned the discrimination much faster than the albinos. It appears, therefore, that strain differences may contribute to the nature of the

decrement produced by cholinergic blockage. A difference in time of appearance of decrements between the hooded and albino strains would be expected, if it were assumed that cue processing and/or the ability to extinguish non-reinforced responses is most crucial at some particular point in the learning process. If cholinergic blockage interrupts these activities, then lengthening the learning process may result in a later period during which cue processing and/or extinction of non-reinforced responses are most important. The decrement in acquisition would then appear later, as in the albino strain here, corresponding to a lengthening of the learning process. This factor of strain differences may not be important, but, on the other hand, it may produce enough variation to warrant taking it into account. To investigate further these possibilities a particular blocking drug with a set dosage could be tested on several strains of animals. If the drug produces its effects during a particular period of the learning process, the decrements observed should appear at different times in different species, assuming that the different control groups of the different species show different acquisition rates.

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