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Scopolamine Impairs Both Working and Reference Memory in Rats: A Replication and Extension

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OKAICHI, H., Y. OSHIMA AND L. E. JARRARD. *Scopolamine impairs both working and reference memory in rats: A replication and extension.* PHARMACOL BIOCHEM BEHAV **34**(3) 599–602, 1989.—Rats were trained to run in a spatial, radial maze for sucrose reward using a procedure that permitted determination of two memory functions [working memory (WM) and reference memory (RM)]. Injections of saline, 0.1, 0.4, and 0.8 mg/kg of scopolamine hydrobromide were administered using a Latin-square design; a single dose (0.4 mg/kg) of scopolamine methylbromide served as a control for peripheral drug effects. The smallest dose of scopolamine (0.1 mg/kg) had no measurable effect on performance, but as the dose was increased to 0.4 and 0.8 mg/kg there were increases in both WM and RM errors, in errors of omission, and increases in running time. These results support the view that the effects of scopolamine on performance in the radial maze is not specific for working memory, but rather the effects are more general in nature.

Scopolamine Working memory Reference memory Radial maze Rats

THE question of central concern in the present research is whether the effects of anticholinergic drugs on behavior are of a general nature or whether specific memorial processes are affected. Evidence supporting both positions can be found in the literature [for a review of the literature see (6)]. Using spatial tasks, several investigators have reported that cholinergic blockade results in specific impairments in working memory in rats (2, 5, 14, 16) and mice (8). In contrast, others have found that the effects of anticholinergic drugs are not limited to a particular type of memory, but rather have more general effects (10, 13, 15).

Especially relevant to the present research are those studies in which the effects of anticholinergic drugs on performance of the radial maze have been used. It has been reported consistently that atropine and scopolamine disrupt performance in the radial maze when all arms are baited (4,5). A problem inherent in interpreting the results of this type of testing procedure is that it is not known whether impaired performance is a result of difficulties in remembering which arms have already been visited within a trial (working memory), whether the animals do not remember those aspects of the task that are constant across trials (reference memory), or whether the problem is one of impaired attention or changes in motivation. A testing procedure that helps in interpreting the nature of drug effects on radial maze performance involves baiting a subset of arms (7,11). In the limited baiting procedure the animal must not only avoid choosing consistently nonbaited arms, but must also avoid reentering correct, baited arms already chosen during that trial; resulting errors are operationally defined as reference memory (RM) and working memory (WM), respectively.

In an earlier study by the authors (10), rats were trained to choose 4 out of 8 arms in one version of the task where all arms were similar and remained in the same spatial location over trials (place task), and to choose 4 out of 8 cues where textured floor inserts in the 8 arms were moved in location from trial to trial (cue task). A within-subjects design was employed so that each rat provided information on both tasks. Injections of scopolamine (0.5, 1.0, and 1.5 mg/kg) had a general effect on performance resulting in slowed running times, an increase in number of trials that were not completed (errors of omission), and similar impairments on both place and cue tasks and on reference and working memory. Contradictory results have been reported by Beatty and Bierley (2) and Wirsching *et al.* (16) using a place task with limited baiting and smaller doses of scopolamine (ranging from 0.25 to 0.8 mg/kg); only working memory was reported to be affected by the drug. To account for the conflicting results, Beatty and Bierley (2) suggested that the complexity of the statistical tests used by Okaichi and Jarrard may have masked a selective effect on working memory. Further, since the drug doses used in our study were generally larger than those employed in the other two studies, they also maintained that central muscarinic blockade selectively disrupts working memory at low doses, and that it may disrupt both working and reference memory at higher doses.

The present study was undertaken in an attempt to clarify the above discrepant findings by using smaller doses of scopolamine and limiting testing to performance on the spatial radial maze task with limited baiting (4 out of 8 arms). Since injections of anticholinergics result in mydriasis and a dry mouth, we had used a sucrose solution as reinforcement in our earlier study. In addition, in the present study the peripheral cholinergic blocker, scopolamine methylbromide, was used as a control injection.

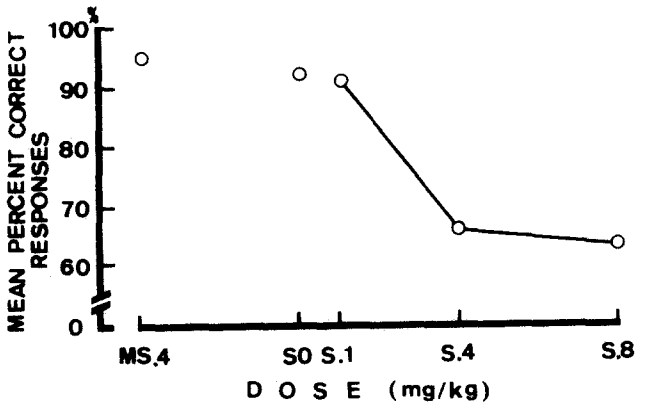

FIG. 1. Mean percent correct responses in the first 4 choices as a function of dose of scopolamine hydrobromide and methylscopolamine. (MS.4 = 0.4 mg/kg of methylscopolamine; S0 = saline; S.1, S.4, S.8 = 0.1, 0.4, 0.8 mg/kg of scopolamine hydrobromide, respectively.)

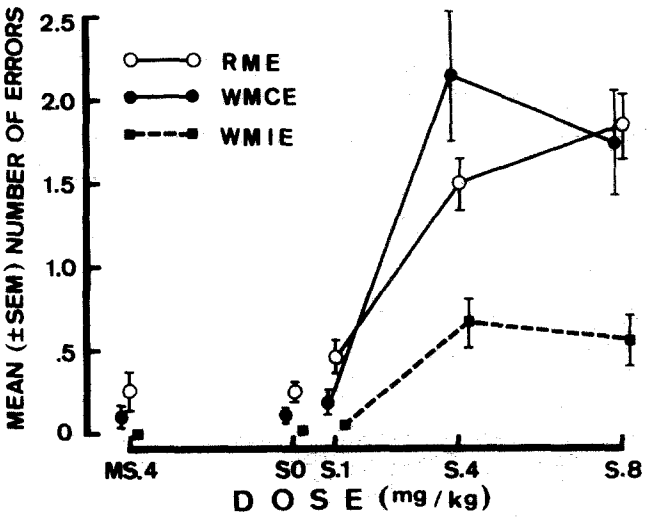

FIG. 2. Mean (±SEM) number of three memory errors (RME, WMCE, and WMIE) for all choices as a function of dose of scopolamine hydrobromide and methylscopolamine. (MS.4 = 0.4 mg/kg of methylscopolamine; S0 = saline; S.1, S.4, S.8 = 0.1, 0.4, 0.8 mg/kg of scopolamine hydrobromide, respectively.)

METHOD

Subjects

Seventeen experimentally naive male albino rats of the Wistar strain were used as subjects. The animals were 68–70 days old, weighed 276–302 g at the beginning of the experiment, and were housed individually in standard rat cages with water available at all times. Illumination of the room was maintained on a 12:12-hr light/dark cycle.

Apparatus

The wooden radial arm maze was elevated 54.6 cm above the floor and had a center platform with eight arms radiating from the center. The platform was 35.0 cm in diameter, and each arm was 71.0 cm long and 9.0 cm wide. Plexiglas walls 5.5 cm high extended along the length of each arm. Holes 1.3 cm in diameter and 0.4 cm deep drilled at the distal end of each arm served as wells for the sucrose solution.

The room in which the maze was located was well lighted and contained several obvious extramaze cues including fluorescent ceiling lights, tables, chairs, rack of cages, shelves, a sink, doors with curtain, and the experimenter.

Procedure

Subjects were handled for 5 min each day for 5 days with the deprivation schedule starting on the first day of handling. The amount of food was adjusted daily so that body weight was maintained at 85% of the expected ad lib weight, with an approximately 5 g gain being permitted during each week of the experiment.

After handling, the animals were placed individually on the center platform of the maze for a 5-min period on each of 5 days. A 23% sucrose solution was placed in a small glass jar at the center of the platform. Following the habituation period, each rat was given 2 training trials a day for a minimum of 15 days, until it reached a criterion of at most 1 error, i.e., 1 entry to unbaited arms or 1 re-entry to baited arms, over 5 successive trials. At the start of each trial, the 4 arms that were correct were baited with 0.4 ml of a 23% sucrose solution, and each rat was placed at the center of the platform. The animals remained on the maze until all 4 reinforcements had been consumed, until 16 choices were made, or until 5 min had elapsed, whichever occurred first. Both the choices of arms and total running times were recorded. The intertrial interval was approximately 15 min.

On drug days, 20 or 27 min prior to the test trial, rats received intraperitoneal injections of 0 (saline), 0.1, 0.4, or 0.8 mg/kg scopolamine hydrobromide, or 0.4 mg/kg scopolamine methylbromide (Nakarai Chemical, Co., Ltd., Kyoto). Drugs were diluted in physiological saline, and every dose was administered in 1 ml/kg body weight. The order of treatments was counter-balanced according to a Latin-square design. The Latin-square design was repeated so that each subject was tested on 2 trials a day on 2 separate days at each dose level, a total of 4 test trials at each dose level in 10 test trial days. The next dose level of a drug was not given until choice accuracy stabilized at a criterion of at most 1 error over 5 successive trials.

Data Analysis

The data for each dose level were averaged over the 4 trials of 2 test days. Memory errors were broken down into: 1) reference memory errors (RME, entry into arms that were never baited, thus reflecting the failure of reference memory), 2) working memory correct errors (WMCE, repeated entry into arms that were baited, thus reflecting the failure of working memory), and 3) working memory incorrect errors (WMIE, repeated entry into arms that were never baited, thus reflecting the combined effects of failures of both working and reference memory).

Repeated subject analyses of variance (ANOVA) were employed to assess overall effects of scopolamine hydrobromide on percent of the responses that were correct in the first 4 choices, the 3 types of memory errors, and running time. Further analysis of the data was carried out using the Newman-Keuls' multiple-range test.

RESULTS AND DISCUSSION

Initial comparison of performance following the saline and methylscopolamine injections indicated that neither choice accuracy, type of memory error, nor running time were affected by these control injections.

TABLE 1

NUMBER OF SUBJECTS THAT ENTERED 4 BAITED ARMS AND NUMBER OF TRIALS WHICH WERE COMPLETED IN THE 4 TEST TRIALS

Drug	Methyl-scopolamine	Scopolamine H Br (mg/kg) 0	0.1	0.4	0.8
Number of Rats	17	17	16	5	7
Number of Trials	68	68	66	42	49

Maximum number of rats is 17. Maximum number of trials is 68.

The overall accuracy of performance is shown in Fig. 1, where percent correct responses in the first 4 choices are plotted for the different dose levels of scopolamine hydrobromide. A repeated subject ANOVA showed that there was a significant decrease in choice accuracy with increasing dose levels, $F(3,48) = 40.39$, $p<0.001$. Further analysis with the Newman-Keuls' test indicated that the saline and 0.1 mg/kg doses did not differ, doses of 0.4 and 0.8 mg/kg did not differ, but the smaller doses did differ from the two larger doses ($p<0.05$).

In Fig. 2 is plotted the mean number of each of the three types of errors that were made for each drug dose for all choices. These data were evaluated with ANOVA in which Dose and Error Type served as within-subjects factors. The analysis revealed significant main effects for Dose, $F(3,48) = 36.88$, $p<0.001$, and Error Type, $F(2,32) = 20.32$, $p<0.001$. Additional analysis with the Newman-Keuls' test indicated that the two largest doses (0.4 and 0.8 mg/kg) differed from the smallest dose for all types of errors ($p<0.05$). Of special interest is the significant interaction of Dose × Error Type, $F(6,96) = 9.87$, $p<0.001$, indicating that the types of errors made were differentially affected by the different dose levels. This significant effect was due primarily to a greater effect of the higher doses on RME and WMCE compared to WMIE ($p<0.05$), WMCE being more affected than RME by 0.4 mg doses ($p<0.05$).

The number of subjects that completed each of the 4 test trials and the number of trials on which rats entered all 4 baited arms on test trials are shown in Table 1. After receiving 0.4 and 0.8 mg/kg of scopolamine hydrobromide, one-third of the subjects did not complete all of the trials. In contrast, almost all subjects completed the trials after administration of saline and 0.1 mg/kg.

Figure 3 shows the increases in running time that resulted from the different doses of the drug, $F(3,48) = 26.81$, $p<0.001$. Further tests showed that there were significant differences between the smallest dose (0.1 mg/kg) and each of the larger doses (0.4 and 0.8 mg/kg) ($p<0.05$).

In the present study, the smallest dose of scopolamine (0.1 mg/kg) had no measurable effect on radial maze performance, but as the dose was increased to 0.4 and 0.8 mg/kg, there were increases in both RM and WM errors, in errors of omission, and increases in running time. Working memory was more affected than reference memory only at the 0.4 mg/kg dose. This pattern of results is similar to that found in our previous study using larger doses of scopolamine (0.5, 1.0, and 1.5 mg/kg), and, thus, further supports the view that the effect of scopolamine on performance in the radial maze is general in nature (10).

A point of concern is why we find that a wide range of doses of scopolamine result in increases in both RM and WM errors in

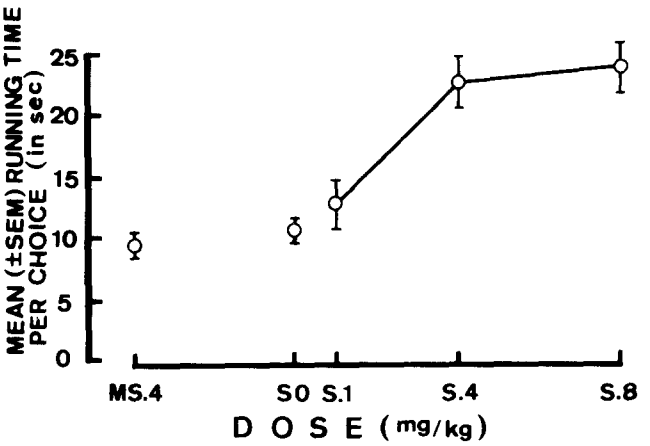

FIG. 3. Mean (±SEM) running time per choice as a function of dose of scopolamine hydrobromide and methylscopolamine. (MS.4 = 0.4 mg/kg of methylscopolamine; S0 = saline; S.1, S.4, S.8 = 0.1, 0.4, 0.8 mg/kg of scopolamine hydrobromide, respectively.)

the radial maze, while Beatty and Bierley (2) and Wirsching *et al.* (16) find that WM is selectively affected by cholinergic drugs. While there are minor procedural differences, it would appear that there are more obvious explanations for the differences. The animals in the Beatty and Bierley study had been trained originally with food reinforcement and used in other drug studies, but the reinforcement was changed to sucrose solution for the scopolamine study. It was reported that the change in reinforcement resulted in a selective deterioration in performance only on the RM component of the task (errors increased from 0.68 to 1.28 per session under food and sucrose reinforcement, respectively), while WM errors stayed essentially the same. Under these circumstances one might expect that WM errors would be more easily disrupted by the administration of a drug. While Wirsching *et al.* reported that scopolamine had an effect only on WM, inspection of their data indicated a considerable increase in RM errors with increasing doses and a marked heterogeneity of variance between saline- and scopolamine-treatment conditions [see (6)].

Further support for a more general involvement of the cholinergic system in memory is provided in a recent study (1). Using a radial maze task and procedure similar to that employed in our earlier study (10), it was reported that cholinergic-rich implants of brain tissue improved equally RM and WM, and place and cue performance in rats that suffered from alcohol-induced memory impairments. Other research (9) reported that ibotenic acid lesions of the nucleus of Basalis, which reduced cortical cholinergic activity and increased both reference and working memory errors, were equally reversed by physostigmine. While there continue to be studies published that report selective impairments in WM following disruption of the cholinergic systems (3,12), and others that fail to find support for a specific type of memory being affected (9,13), our research indicates that both high (10) and low (present results) doses of scopolamine have a general effect on performance in the radial maze. In view of these results, and the recent report (15) that the cholinergic system is more involved in the selection of movements and strategies, it would appear that caution should be observed in attributing to the cholinergic system a selective role in a particular type of memory.

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