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Differential Effects of Scopolamine on Working and Reference Memory of Rats in the Radial Maze

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WIRSCHING, B. A., R. J. BENINGER, K. JHAMANDAS, R. J. BOEGMAN AND S. R. EL-DEFRAWY. *Differential effects of scopolamine on working and reference memory of rats in the radial maze.* PHARMACOL BIOCHEM BEHAV **20**(5) 659–662, 1984.—Anticholinergics have often been found to impair choice accuracy in the radial maze. Some researchers have suggested that this indicates involvement of cholinergically innervated structures in cognitive mapping while others argue that these structures mediate working memory. However, most results are open to either interpretation since the baiting method did not allow a distinction between reference and working memory errors. To further test these hypotheses this study examined the effects of systemic scopolamine on radial maze performance, using a 4-out-of-8 baiting procedure. Food-deprived Wistar rats were pretrained until working memory choice accuracy stabilized to a criterion of 87% or better. Scopolamine (0.1, 0.4 and 0.8 mg/kg, IP, 30 min before a session) significantly increased the number of working memory errors (re-entries into baited arms) whereas reference memory errors (entries into never baited arms) did not change significantly. Observed deficits appeared not to be attributable to a drug-induced disruption of motivational systems. Results confirm the behavioural similarities between the memorial effects of hippocampectomy and anticholinergics, and implicate cholinergically innervated structures in working memory.

Working memory Reference memory Radial maze Scopolamine Acetylcholine Cholinergic neurons

IT has generally been accepted that the hippocampus plays a critical role in human memory. With the repeated observation that hippocampal lesions severely disrupt accuracy in the radial-arm maze this view has been extended to include animal memory [8,15]. However, in the animal literature, there is little consensus regarding the type of memory systems with which the hippocampus is involved [11, 12, 13, 14, 15].

O'Keefe and associates [11,12] proposed a cognitive mapping theory suggesting that the hippocampus processes and stores spatial information. This system provides the animal with a mapping strategy which enables it to locate itself in a geographic environment as well as generate place hypotheses about that environment. In support of this notion it has been found that hippocampal damage leads to a marked deficit in ability to perform maze tasks, particularly those requiring place learning [11].

In contrast to this spatial interpretation is the working memory hypothesis [13, 14, 15] suggesting that behavioural impairments observed in maze performance following hippocampal ablations stem from an inability to solve the working memory component. For instance, Olton and Papas [15] showed a differential involvement of the hippocampal system in reference versus working memory by only baiting 8 of 17 arms in a radial maze. The unbaited set formed a reference memory component of the task since the information that no food was to be found on these arms was useful for all trials. The baited arms, on the other hand, formed a working memory component since the information concerning which of the baited arms had already been visited was only useful for that particular trial. It was reasoned that hippocampal rats would err on both the baited and unbaited arms if the impairment reflected a cognitive deficit. On the other hand, if the impairment was of working memory, animals would re-enter the baited arms from which food had already been eaten. Results indicated that rats with fimbria-fornix lesions performed at chance levels on the working memory component of the task while reference memory was unimpaired. These results support the hypothesis that the hippocampal system may be selectively involved in working memory.

Since the hippocampal region is strongly innervated with cholinergic fibres [3,9] studies utilizing anticholinergics such as scopolamine have also attempted to evaluate the cognitive mapping versus working memory hypotheses. Although anticholinergics produce behavioural effects on radial maze performance that closely parallel those arising from hippocampal ablations [2, 5, 17, 18], it has not been possible to conclusively argue in favour of either hypothesis, since the baiting method employed did not allow a distinction between working and reference memory. The present study was undertaken to further test the two hypotheses by examining the effects of scopolamine on performance in a partially baited radial maze, with only 4 of the 8 arms baited. According to the cognitive mapping theory drugged rats should err

TABLE 1
DRUG DESIGN

Order									
1	Baseline	Saline 1	BL	0.1*	BL	0.4*	BL	0.8*	BL	Saline 2
2	BL	Saline 1	BL	0.1	BL	0.8	BL	0.4	BL	Saline 2
3	BL	Saline 1	BL	0.4	BL	0.8	BL	0.1	BL	Saline 2
4	BL	Saline 1	BL	0.4	BL	0.1	BL	0.8	BL	Saline 2
5	BL	Saline 1	BL	0.8	BL	0.4	BL	0.1	BL	Saline 2
6	BL	Saline 1	BL	0.8	BL	0.1	BL	0.4	BL	Saline 2

*Dose of scopolamine (mg/kg).

TABLE 2
COMPARISON OF SALINE WITH PRECEDING BASELINE

	BL	S_1	BL	S_2
Working Memory Errors Mean (±SEM)	0.08 (±0.08)	0.33 (±0.19)	0.67 (±0.22)	0.58 (±0.23)
Reference Memory Errors Mean (±SEM)	1.8 (±0.11)	2.4 (±0.36)	0.33 (±0.14)	0.58 (±0.23)

n=12.

on both the unbaited and baited arms. The working memory hypothesis, on the other hand, would predict disrupted performance on only the baited arms.

METHOD

Subjects

Eighteen experimentally naive male albino rats of the Wistar strain were individually housed in a climatically controlled room on a 12 hr light/dark cycle. Initial free feeding weights of 200 to 270 g were decreased to 80% (adjusted for growth) by daily feeding with measured rations.

Apparatus

The radial maze, elevated 50 cm above the floor, consisted of an octagonal central platform (30 cm wide) surrounded by 8 equally spaced radial arms (65 cm long × 10 cm wide). Food wells, located 1 cm from the end of each arm were 1.0 cm deep and 1.5 cm in diameter.

Testing was carried out in a white painted room lit by 70 W fluorescent tubes. Several visually distinct cues (e.g., door, shelf) were present in the room and remained in the same position with respect to the maze.

Procedure

Deprivation was begun one week prior to testing. During this period each rat was handled daily for approximately one min. On day 5 of deprivation animals were fed a small quantity of Froot Loops cereal in their home cage as small pieces subsequently were utilized as reinforcers.

Pretraining. On day 8 of deprivation animals were placed for 10 min in pairs on the central hub of the maze with Froot Loop pieces scattered on the platform and arms. On day 10 rats were placed singly on the maze. Again food was scattered on the platform and along a randomly predetermined subset of only 4 arms, referred to as the baited arms. The baiting pattern remained the same throughout the experiment but varied from rat to rat. During days 10 to 13 the 4 arms were rebaited until the rat learned to run to the end and collect the food in 10 min or less. Type of arm entry (baited or unbaited) was recorded. An arm entry was defined as crossing a line 10 cm into each arm. After each trial the maze was cleaned with a 2.5% cidar vinegar solution.

Formal training. Each rat received one session per day, 7 days a week. At the start of each session, the 4 predetermined arms were baited at their distal end (note that arms were not rebaited within a session). Each rat was placed on the platform and left until all 4 baits were collected, 14 choices were made or 10 min had elapsed, whichever came first. Training continued until choice accuracy stabilized over 4 days to an average criterion of 87% or better; thus a score of 100% (4 out of 4 unrepeated baited arm entries within the first 4 choices) on at least 2 of the 4 days was required.

Drug testing. This phase consisted of 5 4-day blocks of drug treatment with one session per day. Each treatment block was followed by non-drug baseline sessions that continued until the 4-day criterion of 87% or better was reestablished. Each session of the first and fifth treatment block was preceded 30 min by an IP injection of saline (1 ml/kg). Sessions of the second, third and fourth treatment blocks were preceded 30 min by an IP injection of scopolamine hydrobromide (Sigma Chemical Co.) dissolved in distilled water, at doses of 0.1, 0.4 or 0.8 mg/kg. Rats were randomly assigned to one of the six possible dosage orders (see Table 1).

Dependent measures. Type of error and reinforcement receipts were recorded. The first entry into a baited arm regardless of whether or not the bait was collected was scored as a correct choice, while a re-entry into that arm was scored as a working memory error. Entries into never baited

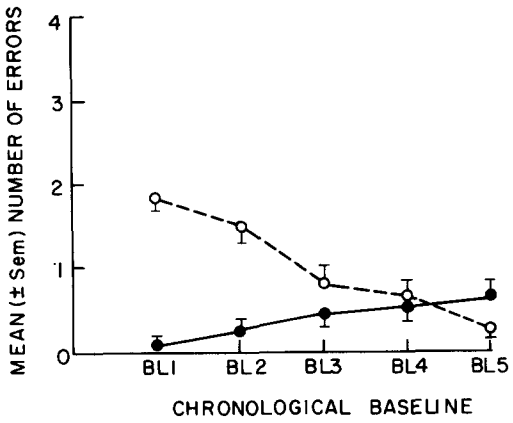

FIG. 1. Mean (±sem) type of error within the first 4 choices as a function of chronological baseline. Reference memory errors (○— —), working memory errors (●—), n=12.

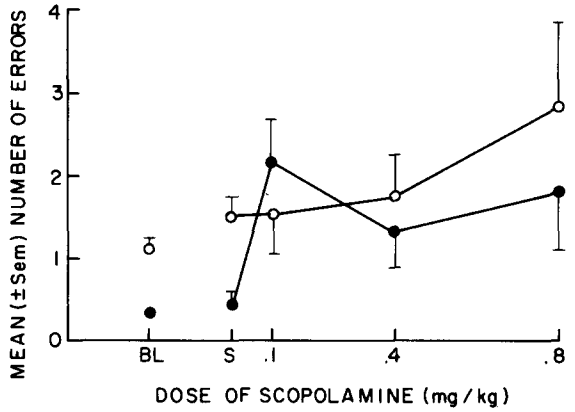

FIG. 2. Mean (±sem) type of error within the first 4 choices at various doses of scopolamine. Baseline (BL), saline (S), reference memory errors (○—), working memory errors (●—), n=12.

arms were scored as reference memory errors. The scoring system for working memory may have a problem. Namely, if a rat entered one of the baited arms and did not collect the food a re-entry could be considered correct. Therefore another scoring system for working memory errors was employed: re-entries into an arm that still contained the bait were scored as correct choices.

RESULTS

Data from animals that died for reasons unrelated to the experimental design (n=2), failed to learn the task (n=2) or did not run on at least 2 of the 4 drug sessions (n=2) were excluded from statistical analyses.

One way repeated measures analyses of variance were performed on two dependent variables: total number of working memory errors (both scoring systems) and total number of reference memory errors. Scores were summed over each 4-day testing block and were based on choices within the first 4 arm entries. Data from the first and fifth 4-day non-drug baseline blocks were compared to saline 1 (S1) and saline 2 (S2), respectively. There was no significant effect of saline in either case for the number of working memory errors or reference memory errors (see Table 2).

Non-drug baseline blocks were examined for chronological changes in performance. Figure 1 shows the mean (±SEM) type of error within the first four choices for the chronological baseline blocks 1 to 5. Reference memory errors significantly decreased during the course of baseline testing, $F(4,44)=15.07$, $p<0.0001$. Although working memory errors showed a marginal increase the change was not statistically reliable, $F(4,44)=2.4$, $0.05<p<0.10$. Hence despite the non-drug baseline criterion of 87% or better, performance continued to improve throughout baseline testing.

Analyses of the type of error revealed a selective impairment of working memory (see Fig. 2). The mean number of working memory errors increased under drug treatment, $F(4,44)=6.3$, $p<0.001$. The mean number of reference memory errors, on the other hand, did not change significantly, $F(4,44)=1.6$, $p>0.10$. These data suggest that low doses of scopolamine (0.1 and 0.4 mg/kg) selectively disrupted the working memory component of the radial maze task. Subsequent post-hoc comparisons with Scheffe's F test conducted

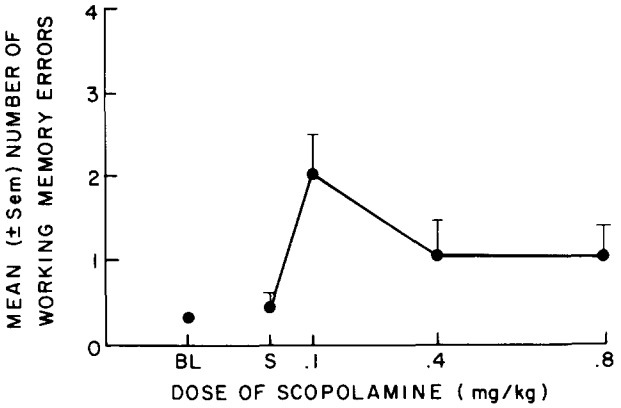

FIG. 3. Mean (±sem) total number of working memory errors from the alternate scoring system (see Procedure section in text). Points show results for baseline (BL), saline (S) and various doses of scopolamine. (n=12).

on working memory errors showed that the combined means of baseline and saline differed significantly from the combined means of the 3 drug doses ($p<0.05$). Scopolamine also increased the number of working memory errors relative to the saline condition alone ($p<0.05$). Although administration of 0.1 mg/kg of scopolamine produced the largest increase in working memory errors, this dose did not differ significantly from 0.4 or 0.8 mg/kg. Similarly there was no significant difference between 0.4 and 0.8 mg/kg.

Results from the alternate scoring system of working memory errors are depicted in Fig. 3. In contrast to the scores shown in Fig. 2, re-entries into arms of the baited set where the food was not eaten were scored as correct rather than incorrect choices (see Procedure section). Comparison of Figs. 2 and 3 suggests that failures to eat occurred most often under the higher doses (0.4 and 0.8 mg/kg) of scopolamine. Nevertheless, even when re-entries into still baited arms were scored as correct working memory choices, scopolamine administration significantly increased the mean number of working memory errors, $F(4,44)=6.1$, $p<0.001$. Hence, the results from both scoring systems were similar.

DISCUSSION

Results of the present experiment are consistent with previous reports showing that anticholinergic drug treatments, like hippocampectomy, severely impair choice accuracy in the radial-arm maze [2, 17, 18]. Eckerman *et al.* [2], for example, showed that 0.1 mg/kg of scopolamine, like hippocampal lesions reduced continuous choice accuracy in the 8-arm maze. More importantly the present findings demonstrated that low doses of scopolamine (0.1 and 0.4) selectively disrupted the working memory component of this task, a finding consistent with the effects of hippocampectomy [8,15], and cholinergic blockade [10]. Within a session scopolamine-treated animals had difficulty remembering which of the arms had already been visited. This result is in agreement with Olton and associates' [13, 14, 15] working memory proposal. It does not, however, support O'Keefe and colleagues' [11,12] cognitive mapping theory since scopolamine administration did not significantly increase the number of responses to the unbaited radial arms; spatial abilities apparently were not deficient. Scopolamine thus appears to have deleterious and selective effects on working memory.

One possible explanation for this selective action lies in the degree of interference to which each type of memory system is susceptible. Reference memory is composed of permanent representations and is relatively immune to disruption, while working memory is composed of newly formed representations and is vulnerable to disruption [6,14]. If animals with disrupted cholinergic function are more distractable or reactive to environmental stimuli, interference effects might be enhanced resulting in the selective impairment of working memory. This possibility gains some support from Carlton's [1] suggestion that cholinergic systems have an inhibitory effect on behaviour and the observation that anticholinergics lead to prolonged reactivity to sensory stimuli [7].

It could be argued that the scopolamine-induced deficit reported here may be attributed to a disruption of motivational systems rather than a selective interference with the processes essential for working memory since anticholinergics produce dry mouth effects possibly making food unpalatable [4]. Although refusal of the food was observed during the course of drug testing especially at the higher doses, analysis of the alternate scoring system revealed that when these instances were excluded the mean number of working memory errors still increased significantly. These results are consistent with previous reports that neither scopolamine nor peripherally acting methylscopolamine altered reinforcer effectiveness [2,16] and support the notion that scopolamine may have selective effects on memorial processes.

In conclusion, scopolamine like hippocampectomy, produced a selective impairment of working memory. This might suggest that cholinergic neurons projecting to the hippocampus play a major role in this memory process. Work is presently under way to test this hypothesis by selectively depleting the hippocampus of cholinergic afferents with localized injections of neurotoxins.

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