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Scopolamine Does Not Disrupt Spatial Working Memory in Rats

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GODDING, P. R., J. R. RUSH AND W. W. BEATTY. *Scopolamine does not disrupt spatial working memory in rats.* PHARMAC. BIOCHEM. BEHAV. 16(6) 919–923, 1982.—The importance of cholinergic systems for spatial working memory was examined by injecting scopolamine at varying times during a 5 hr-long retention interval imposed between the rat's fourth and fifth choices in an 8 arm maze. Regardless of whether or not the testing procedure required the rats to adopt a spatial solution for the task, scopolamine (1.0–5.0 mg/kg) did not impair retention in a manner that was suggestive of an effect on working memory. Modest deficits observed in some conditions appeared to result from drug effects on performance. Previous findings of impaired acquisition of accurate spatial behavior by scopolamine-treated rats evidently reflect an influence of the drug on physiological systems other than those necessary to maintain working memory for spatial information.

Scopolamine Spatial memory Spatial behavior Working memory Anticholinergic

WHEN tested in radial mazes rats display working (or short-term) memory for spatial information that is unusually long lasting [7] and highly resistant to disruption by events that occur during the retention interval. For example, if a delay is imposed between choices 4 and 5, choice accuracies in excess of 85% correct on choices 5–8 are maintained in spite of sleep deprivation [10], barbiturate anesthesia [7], or training in other radial mazes [8,17] during the retention interval. Only electroconvulsive shock (ECS) [21] or electrical stimulation of the hippocampus [19] are known to degrade spatial working memory in a way that mimics natural forgetting.

The neurochemical mechanisms responsible for the robust spatial memory of rats are poorly understood at present, but a number of observations suggest that cholinergic systems, especially cholinergic inputs to the hippocampus may be important. A vast literature on the behavioral effects of hippocampal lesions attests to the importance of this structure for spatial memory and possibly other forms of memory as well [18,20]. Elderly rats exhibit selective deficits in spatial working memory [2,24] and several recent studies have linked memory defects associated with aging with the deterioration of cholinergic neural systems [3, 5, 16] including muscarinic inputs to the hippocampus [16]. Further, destruction of the medial septal nucleus, the origin of a major cholinergic input to the hippocampus, produces deficits in spatial tasks [6,26] that are comparable to those associated with lesions of the hippocampus of the fimbria-fornix. Pharmacological studies also implicate cholinergic systems in the maintenance of normal working memory. Treatment with the muscarinic antagonist, scopolamine has been reported to interfere with performance in several species and situations including delayed matching to sample (DMTS) problems in rhesus monkeys [4], non-matching to sample in rats [22], and spatial delayed alternation in mice [1] and rats [12]. Scopolamine also interferes with the acquisition and performance of accurate spatial behavior in the 8 arm maze in rats [11, 23, 25]. While the performance deficits that accompany scopolamine treatment are clear enough, it has not been established that the drug impairs retention by interfering with processes essential to memory. Bartus and Johnson [4] observed that scopolamine affected DMTS performance at long but not short delays, suggestive of an impairment in memory. However, other workers [11, 12, 22] have not found that the degree of impairment increases systematically with the length of the delay. Hence, other explanations of the performance deficit such as a drug-induced loss of stimulus control [11,12] or failure to sustain attention [9] are equally viable.

Because the memory span in most paradigms in rather short (rarely longer than a few minutes) drugs must be administered before the presentation of the to-be-remembered event. A more selective test of drug effects on mechanisms necessary for the maintenance of working memory could be achieved if drug treatments were applied after the to-be-remembered experience. If the present experiments we sought to provide such an evaluation of the effects of scopolamine on spatial working memory by capitalizing on the long span of accurate working memory displayed by rats in the 8 arm maze. This permitted us to administer scopolamine after the animals completed the first four choices.

EXPERIMENT 1

METHOD

Animals

The subjects were 17 albino male rats, obtained from the Holtzman Co., Madison, WI, at about 3 months of age. They

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were caged singly with free access to water in an air-conditioned animal room (22±3°C) that was illuminated from 0700–2100 by overhead fluorescent lights. The rats were maintained on a restricted feeding schedule of Purina Lab Chow pellets designed to maintain body weight at 85% of the free-feeding level adjusted for growth. Behavioral tests occurred during the daylight portion of the L:D cycle.

Apparatus

Behavioral testing was conducted in an elevated 8 arm maze made of wood painted white which was shaped like a rimless wagon wheel. Each arm (74×9 cm) extended from an octagonally shaped central hub (36 cm across). Black plastic sidewalls (3.5 cm high) extended the length of each arm. Small metal cups, mounted at the end of each arm, served as receptacles for reinforcers. Guillotine doors surrounded the hub and controlled access to each arm. The room housing the maze (3 m^2) was cluttered with running wheels, a steam line with valves and hoses and other surplus equipment which provided numerous extra-maze cues.

Preliminary Training

The rats were initially adapted to the maze by allowing them to explore and eat from the food cups at the end of each arm. When the rats entered and ate from all 8 arms in 10 min or less, pretraining began. At the start of each session a single 190 mg Noyes pellet was placed in the food cup in each alley. The rat was placed in the center hub and the guillotine doors were raised, permitting access to any of the arms. As food was never replenished during a session the rat was required to learn a win-shift food-searching strategy. Reentries into an arm previously visited on a single test day were scored as errors. Since the session continued until the rat visited all 8 arms, the total number of errors per day was potentially unlimited and included all errors made during the session. Training at the rate of one session per day continued until the rat achieved 5 criterial days in succession. A criterial day occurred when the rat entered 7 different arms in the first 8 choices and all 8 arms in the first 10 choices. Next a delay of 1 hr was imposed between the rat's fourth and fifth choices. As performance stabilized the delay was increased to 4 hr and then to 5 hr. After 74 days of preliminary training, when all rats consistently maintained performance at least 80% correct on choices 5–8 at the 5 hr delay, formal testing began.

Phase 1. To assess the effects of cholinergic receptor blockade on spatial memory the rats received ip injections of scopolamine HBr (0, 1.0 or 2.0 mg/kg expressed as the weight of the salt) dissolved in physiological saline 0 or 2 hr after completion of the first 4 choices. The retention interval was 5 hr. At the higher scopolamine dose several of the rats had obvious difficulty consuming the food pellets during the retention tests and on two occasions animals failed to complete the retention tests. For this reason the results were inconclusive.

Phase 2. To circumvent the problem of ingestion described above we substituted 0.25 cc of an 8% sucrose solution for the Noyes pellet rewards. The rats were retrained with a delay interval of 5 hr between choices 4 and 5 using 8% sucrose as the reward. When performance was stable, which required about 2 weeks, testing began again. Each rat received ip injections of scopolamine HBr (0, 1.0, 2.0, or 5.0 mg/kg expressed as the weight of the salt) 0 or 2 hr after choice 4 or 3 hr before the first 4 choices. The latter condition served as a control for possible proactive influences of the drug on performance during the retention tests. The time of injection was varied because Shavalia *et al.* [21] found that the effects of ECS on spatial memory were time dependent; treatment with ECS 2 hr after choice 4 caused profound amnesia, but treatment immediately after choice 4 was ineffective. Each rat was tested once at each of the drug treatment conditions. Drug tests were conducted every third day; on the intervening days spatial memory was assessed in the usual way but no treatment was administered. The order of drug treatments was counterbalanced among subjects.

The rats consumed the liquid sucrose solution without apparent difficulty. Nevertheless, 3 rats failed to complete the retention tests when the 5 mg/kg dose of scopolamine was administered 2 hr after choice 4, 1 rat failed to complete the test at the 2 mg/kg dose given 2 hr after choice 4 and 1 rat failed to complete the test with the 2 mg/kg dose given immediately after choice 4.

Data Analysis

Statistical analyses of two dependent variables, percent correct on choices 5–8 and total number of errors per day, were conducted. The data from no treatment sessions were initially examined for evidence of systematic changes in performance over time and testing. No such trends were evident so the data were pooled to yield a single value for the No Trt condition on each measure. One way repeated measures analyses of variance were performed including all conditions. Subsequent pairwise comparisons with F tests were conducted if the omnibus analysis revealed reliable overall treatment effects. Missing data from Phase 2 were accounted for by entering the treatment mean for the missing value and correcting the df and error variance appropriately.

In addition the number of errors involving repetitions of choices made during the retention tests (retention errors) was examined. Normal rats make most of their errors by reentering arms visited during the first four choices [7]. ECS produces selective amnesia for alleys visited during the first 4 choices without increasing the rate of retention errors [21]. Hence, a significant increase in retention errors following scopolamine treatment would suggest that the drug was not selectively degrading spatial working memory.

RESULTS

Figure 1 depicts the mean percent correct on choices 5–8 for the various treatment conditions examined during Phase 2. Statistical analysis revealed a significant effect of drug treatment, $F(11,171)=2.06$, $p<0.03$. Subsequent analyses demonstrated that administering 5 mg/kg scopolamine 3 hr before the first 4 choices reduced the accuracy of performance relative to the No Trt condition ($p<0.05$). The same dose given 2 hr after choice 4 produced a qualitatively similar effect which fell short of statistical significance ($p<0.08$). No other comparisons yielded reliable differences.

A similar pattern of treatment effects was observed in the analysis of the average number of errors per day, but the effect was not significant ($F=1.65$). The number of retention errors was too small for a detailed statistical analysis. Overall 10.2% of the total errors were retention errors for the control conditions (No Trt and Saline combined) versus 14.5% on days when scopolamine was injected.

Using the testing procedure of Phase 2 in which the rats were allowed to complete the first 4 choices in any order

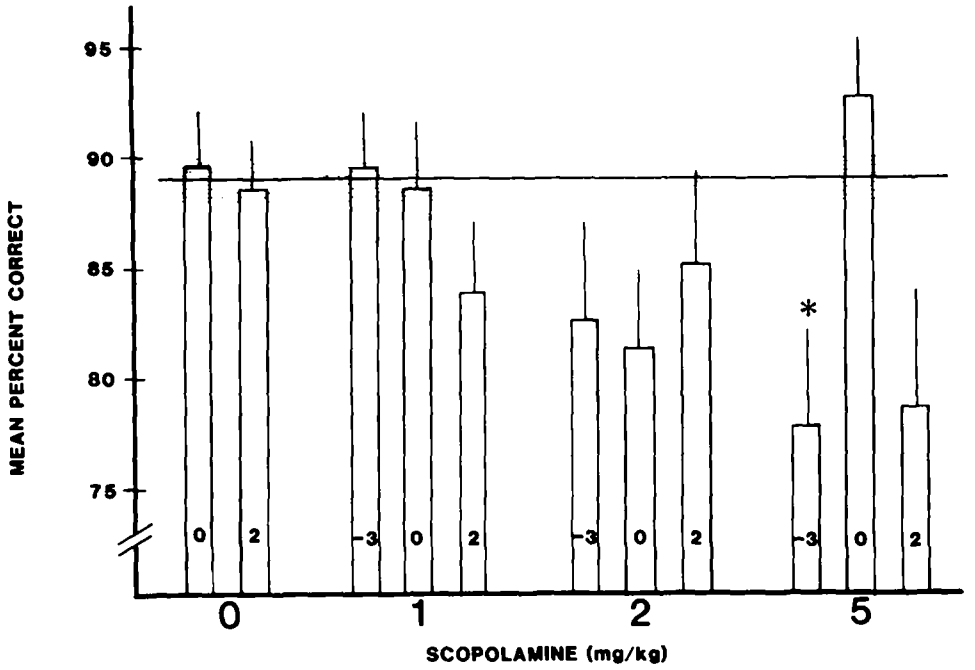

FIG. 1. Mean percent correct on choices 5–8 at varying doses of scopolamine in Experiment 1. Numbers on the histograms indicate the time in hours of drug treatment in relation to the first 4 choices. Solid horizontal line and shaded area indicate the mean ± SEM for the No Trt condition. Vertical lines above each histogram indicate SEM for that condition. *Significantly different from No Trt.

they wished, administering scopolamine during the retention interval did not degrade spatial memory. While this finding might suggest that muscarinic cholinergic circuits are not crucial to the maintenance of spatial working memory, another explanation is plausible. Stevens and colleagues [23,25] have reported that scopolamine interferes with the acquisition of accurate choice behavior in the radial maze only if the rats actually employ a spatial strategy for solution. When allowed to select the first 4 choices in whatever order they wish, highly experienced rats often adopt response patterns, usually involving selection of 4 adjacent arms [7,8]. Such a strategy, which is not essential to accurate memory over long retention intervals by normal rats [7], might simplify the problem for animals treated with scopolamine. For example, by adopting a response strategy, the rat might convert a problem in spatial memory to a problem requiring only memory for landmarks or even orientation.

To test this idea the proportion of tests on which the rats entered 4 adjacent arms (in any order) on the first 4 choices was determined for each animal. Four rats entered 4 adjacent arms on more than 35% of the tests (Range: 36.8–77.2). These animals were arbitrarily considered nonspatial responders and their data excluded from further analysis. The remaining 13 rats entered 4 adjacent arms on fewer than 25% of the tests (Mdn=5.3, Range: 1.8–22.8). Reanalysis of the data on these "spatial" subjects revealed a reliable treatment effect on the percent correct measure, $F(11,128)=2.21$, $p<0.02$, but not on the total errors per day measure ($F=1.54$). Mean percent correct on choices 5–8 was 87.8 for the No Trt condition, 72.7 when 5 mg/kg scopolamine was injected 2 hr after choice 4 and 75.0 when the same dose was injected 3 hr before the start of the daily session. Both of these high dose treatments reduced the percentage of correct choices relative to the untreated condition ($p<0.05$), but the degree of disruption was comparable and did not vary reliably as a function of the time of injection in relation to testing. Thus, even when only animals that appeared to pursue a spatial strategy are considered, the data provided no evidence that scopolamine disrupted retention performance by selectively affecting memory.

EXPERIMENT 2

Although selecting 4 adjacent arms during the first 4 choices is the most obvious way the rat might simplify the problem of remembering which arms it had already visited, there might be other response patterns that could aid solution and attenuate the effects of scopolamine treatment. To test this possibility we repeated the experiment using a testing procedure in which the rats were forced to make their first four choices in a predetermined randomly selected order which was changed each day. Because some rats had failed to run in the maze after injection of 2 or 5 mg/kg scopolamine, only the 1 mg/kg dose was employed.

METHOD

The animals, apparatus, and general maintenance procedures were the same as in Phase 2 of Experiment 1. The testing procedure was identical as well except that during the first 4 choices the rats were forced to enter preselected arms in a randomly determined sequence that varied from rat to rat and was changed every day. This was accomplished by raising the guillotine doors one at a time during the first 4 choices. Again correct choices were rewarded with 0.25 cc

of an 8% sucrose solution. During retention tests the rats could enter any of the 8 arms at any time.

The rats were first accustomed to the modified testing procedure which required about 10 days. Then they were given scopolamine HBr IP (0 or 1.0 mg/kg expressed as the weight of the salt) 0 or 2 hr after choice 4 or 3 hr before the first 4 choices. Two tests at each drug condition were conducted and drug tests occurred every third day. The order of treatment was counterbalanced and performance on days without treatment (No Trt) served as a baseline. During the experiment 5 rats refused to run in the maze. Such refusals seemed unrelated to the administration of drugs and the animals appeared to be healthy. Their partial data were excluded from analysis.

RESULTS

Mean performance on the retention tests is shown in Fig. 2. Although scopolamine tended to reduce the mean percent correct on choices 5–8, the trend was not reliable ($F=1.79$). However, the drug treatment effect was significant on the total errors per day measure, $F(5,55)=2.44$, $p<0.05$. Subsequent analyses showed that scopolamine increased the number of errors (relative to the No Trt condition) when it was given 2 hr after choice 4 or 3 hr before the first 4 choices ($p<0.05$), but there was no reliable difference between the latter two conditions. No other drug treatment affected performance on the errors measure. Again retention errors were too infrequent for a detailed statistical analysis, but overall they accounted for 11.3% of errors on control sessions (No Trt and saline combined) and 17.1% of errors on scopolamine treatment days.

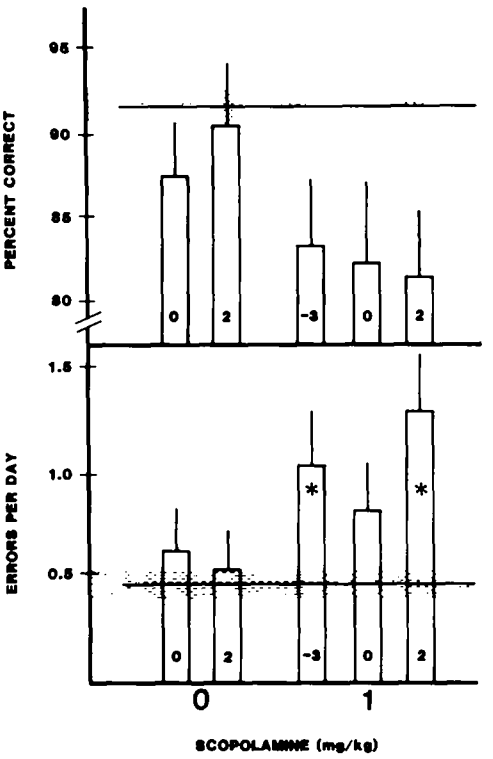

FIG. 2. Mean percent correct on choices 5–8 (upper panel) and total errors per day (lower panel) at varying doses of scopolamine in Experiment 2. Numbers on the histograms indicate time in hours of drug treatment in relation to the first 4 choices. Solid horizontal line and shaded area are mean±SEM for the No Trt condition. Vertical lines above each histogram indicate SEM for that condition. *Significantly different from No Trt.

GENERAL DISCUSSION

Honig [13] has suggested that memory includes two partially distinguishable components, working memory and reference memory. Working memory, which is similar to short-term memory, is the memory for specific cues or events (e.g., the sample in a DMTS task) and is typically transient. Reference memory is usually longer lasting and often includes rules for the general solution of a problem which are applicable to all instances (e.g., "Select the comparison stimulus that matches the sample you saw earlier," again, in a DMTS task). In the radial maze, as in DMTS problem, accurate retention requires that both working and reference memory be functional. To display accurate spatial memory the rat must remember the general win-shift rule ("Don't go back to an arm that you have already visited"), which is presumably stored in reference memory as well as the specific arms that it has entered on a particular test session which is assumed to be recorded in working memory, at least temporarily.

To support the conclusion that a particular amnesic treatment has produced a retention deficit by interfering specifically with mechanisms that maintain working memory for spatial information two additional facts must hold: (1) the proportion of retention errors must be similar after exposure to the amnesic agent and the control conditions and (2) the magnitude of the memory defect caused by exposure to the treatment at some time within the retention interval must be significantly greater than that observed when the same amnesic treatment is administered at a comparable time before the to-be-remembered event. Our previous work [21] demonstrated that the retention deficits observed after ECS met both of the above criteria; thus ECS can degrade mechanisms necessary to sustain accurate spatial working memory.

By contrast, the present experiments suggest that while blockade of central and peripheral muscarinic receptors with scopolamine can produce modest deficits in retention in the radial maze, there is no reason to believe that these deficits arose from a direct effect of the drug on working memory. In the present study scopolamine treatment immediately after choice 4 never affected retention. When given 2 hr after choice 4 (3 hr before the retention test) scopolamine, in certain doses, produced deficits on some measures, but these deficits were never of greater magnitude than when the same scopolamine dose was given 3 hr before the to-be-remembered event. Thus, the modest deficits we observed might reflect either an influence of the drug on reference memory or some aspect of performance. Although the data are quite limited, examination of the proportion of total errors that involved reentries of arms visited on the retention test (retention errors) suggested that scopolamine had little effect on this measure. This implies that the rats probably remembered the general win-shift strategy and argues against a major drug influence on reference memory. Most likely the modest changes we observed on spatial memory reflect an influence of the drug on some performance factor such as attention. Similar conclusions regarding the effects of scopolamine on behavior in the radial maze and other tests of working memory have been reached by others [9, 11, 12, 22].

On the other hand Stevens and his associates have found that scopolamine retards the acquisition of accurate choice behavior in the radial maze provided that the animals adopt a spatial solution to the problem [23,25]. However, even when we employed procedures that forced the rats to utilize a spatial solution, there was no evidence that scopolamine disrupted spatial working memory, so it is unlikely that Stevens' results reflect a drug effect on working memory either. In light of the current findings two explanations of Stevens' data seem tenable. First, the drug-induced impairment could reflect a change in performance unrelated to memory. This possibility is rendered somewhat less likely by the fact that in Stevens' studies scopolamine did not affect acquisition by rats that did not employ a spatial strategy, but since all drugs were injected prior to training and no controls for the peripheral effects of scopolamine were provided, effects on performance cannot be ruled out. Alternatively, scopolamine might have affected some component of spatial memory that is especially vulnerable during the acquisition phase such as the reference memory for the win-shift habit required by the task. Studies with selective lesions of the hippocampus or its afferents and efferents [14,15] reveal differential effects on acquisition and retention of accurate choice behavior in the 8 arm maze. Thus, lesions restricted to cell field CA1 or to the alveus impair postoperative acquisition but are without effect on retention of preoperatively established performance. Apparently acquisition and retention may be differentially affected by scopolamine as well.

Since the present tests were conducted when the rats were between 3 and 10 months of age, caution should be exercised in generalizing the findings to older animals, especially considering the body of data linking various memorial defects in aged animals with deterioriation of cholinergic neural systems [3, 5, 16].

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REFERENCES

1. Alpern, H. P. and S. J. Jackson. Short-term memory: A neuropharmacologically distinct process. *Behav. Biol.* **22:** 133–146, 1978.
2. Barnes, C. A., L. Nadel and W. K. Honig. Spatial memory deficit in senescent rats. *Can. J. Psychol.* **34:** 29–39, 1980.
3. Bartus, R. T. Physostigmine and recent memory: Effects in young and aged nonhuman primates. *Science* **206:** 1087–1089, 1979.
4. Bartus, R. T. and H. R. Johnson. Short-term memory in the rhesus monkey: Disruption from the anti-cholinergic scopolamine. *Pharmac. Biochem. Behav.* **5:** 39–46, 1976.
5. Bartus, R. T., R. L. Dean, A. J. Goas and A. S. Lippa. Age-related changes in passive avoidance retention: Modulation with dietary choline. *Science* **209:** 301–303, 1980.
6. Beatty, W. W. and C. P. Carbone. Septal lesions, intramaze cues and spatial behavior in rats. *Physiol. Behav.* **24:** 675–678, 1980.
7. Beatty, W. W. and D. A. Shavalia. Spatial memory in rats: Time course of working memory and effect of anesthetics. *Behav. Neural Biol.* **28:** 454–462, 1980.
8. Beatty, W. W. and D. A. Shavalia. Rat spatial memory: Resistance to retroactive interference at long delay intervals. *Anim. Learn. Behav.* **8:** 550–552, 1980.
9. Cheal, M. Scopolamine disrupts maintenance of attention rather than memory processes. *Behav. Neural Biol.* **33:** 163–187, 1981.
10. Dodge, A. M. and W. W. Beatty. Sleep deprivation does not affect spatial memory in rats. *Bull. Psychon. Soc.* **16:** 408–409, 1980.
11. Eckerman, D. A., W. A. Gordon, J. D. Edwards, R. C. Mac Phail and M. I. Gage. Effects of scopolamine, pentobarbital, and amphetamine on radial arm maze performance in the rat. *Pharmac. Biochem. Behav.* **12:** 595–602, 1980.
12. Heise, G. A., R. Conner and R. A. Martin. Effects of scopolamine on variable interval spatial alternation and memory in the rat. *Psychopharmacology* **49:** 131–137, 1976.
13. Honig, W. K. Studies of working memory in the pigeon. In: *Cognitive Processes in Animal Behavior*, edited by S. H. Hulse, H. Fowler and W. K. Honig. Hillsdale, NJ: Lawrence Erlbaum, 1978, pp. 211–248.
14. Jarrard, L. E. Selective hippocampal lesions: Differential effects on performance by rats of spatial tasks with preoperative versus postoperative training. *J. comp. physiol. Psychol.* **92:** 1119–1127, 1978.
15. Jarrard, L. E. Selective hippocampal lesions and behavior. *Physiol. Psychol.* **8:** 198–206, 1980.
16. Lippa, A. S., R. W. Pelham, B. Beer, D. S. Critchett, R. L. Dean and R. T. Bartus. Brain cholinergic dysfunction and memory in aged rats. *Neurobiol. Aging* **1:** 13–19, 1980.
17. Maki, W. S., S. Brokofsky and B. Berg. Rat spatial memory: Resistance to retroactive interference. *Anim. Learn. Behav.* **7:** 25–30, 1979.
18. O'Keefe, J. and L. Nadel. *The Hippocampus as a Cognitive Map*. Oxford: Clarendon Press, 1978.
19. Olton, D. S. The function of septal-hippocampal connections in spatially organized behavior. In: *Functions of the Septo-Hippocampal System*. Ciba Foundation Symposium 58. Amsterdam: Elsevier, 1978, pp. 327–342.
20. Olton, D. S., J. T. Becker and G. E. Handelman. Hippocampal function: Working memory or cognitive mapping? *Physiol. Psychol.* **8:** 239–246, 1980.
21. Shavalia, D. A., A. M. Dodge and W. W. Beatty. Time-dependent effects of ECS on spatial memory in rats. *Behav. Neural Biol.* **31:** 261–273, 1981.
22. Spencer, D. G. Central cholinergic function and short-term memory in the rat. *Soc. Neurosci. Abstr.* **7:** 524, 1981.
23. Stevens, R. Scopolamine impairs spatial maze performance in rats. *Physiol. Behav.* **27:** 385–386, 1981.
24. Wallace, J. E., E. E. Krauter and B. A. Campbell. Animal models of declining memory in the aged: Short-term and spatial memory in the aged rat. *J. Geront.* **35:** 355–363, 1980.
25. Watt, J., R. Stevens and C. Robinson. Effects on scopolamine on radial maze performance in rats. *Pharmac. Biochem. Behav.* **12:** 595–602, 1980.
26. Winson, J. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* **201:** 160–163, 1978.