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Effects of Scopolamine and Unilateral Lesions of the Basal Forebrain on T-Maze Spatial Discrimination and Alternation in Rats

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BENINGER, R. J., K. JHAMANDAS, R. J. BOEGMAN AND S. R. EL-DEFRAWY. *Effects of scopolamine and unilateral lesions of the basal forebrain on T-maze spatial discrimination and alternation in rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1353–1360, 1986.—Cholinergic systems are thought to play a role in memory. It has been suggested that cholinergic neurons, possibly the cortically projecting cells of the nucleus basalis magnocellularis, are differentially involved in working and reference memory. To evaluate this hypothesis the effects on memory of scopolamine (0, 0.3, 0.6 mg/kg) or unilateral kainic acid (4.7 nmoles in 1 μl) lesions of the basal forebrain of rats were tested. Working memory, the recall of recent events of transient importance that is vulnerable to interference, was tested using a T-maze alternation task; reference memory, information stored over the long term that is relatively resistant to interference, was evaluated using a spatial discrimination task in the T-maze. The differential sensitivity of the two tasks to interference effects was confirmed by the finding that the insertion of a 30-sec delay between trials significantly reduced performance in the alternation but not the spatial discrimination task. Furthermore, scopolamine or the lesions significantly impaired alternation but not spatial discrimination performance. Biochemical assays of the kainate-injected brains confirmed that the cortical cholinergic marker, choline acetyltransferase, was significantly reduced. These results support the hypothesis that working and reference memory may be differentially controlled by cholinergic systems.

Scopolamine Basal forebrain Nucleus basalis magnocellularis Kainic acid Acetylcholine
Working memory Reference memory Alternation Spatial discrimination

THE anatomical locations of the brain's cholinergic systems have recently been described in some detail [1, 9, 25]. There has long been an interest in the possible role of these systems in memory. The work of Deutsch and his colleagues, for example, implicated cholinergic neurons in long term memory. They found that cholinomimetics and anticholinergics differentially affected the relearning of a discrimination task depending on the time (days) elapsed between original learning and testing [7].

Others have focused on the possibility that cholinergic systems are involved in the memory processes underlying new learning. The general approach has been to train an animal on a task, either while cholinergic function is altered or to alter cholinergic function immediately after training. The effects of these treatments are assessed in retention tests some time later. Using this approach, Flood and his coworkers [11–13] and Ridley *et al.* [33] showed that anticholinergics impaired memory retention whereas cholinergic agonists at moderate doses improved recall in active avoidance and appetitive discrimination tasks. Perhaps the most commonly used new learning procedure is passive avoidance (PA). Scopolamine or atropine given systemically [5,26] or directly into the posteroventral hippocampo-entorhinal area [5] or treatments that reduced the number of cortical muscarinic receptors [17] produced impaired recall. Aged mice and rats, known to have reduced cholinergic function, were impaired in PA retention [2, 22, 37, 38] and the deficit was reversed with dietary choline [2]. Similarly, intracerebroventricular (ICV) injections of ethylcholine aziridinium ion solution (AF64A), that reduced hippocampal and frontocortical acetylcholine levels, impaired PA retention [39]. Striatal cholinergic function was implicated in memory by the observation that intrastriatal AF64A impaired memory in a PA task [36]. Similarly, basocortical cholinergic systems have been implicated; unilateral electrolytic [23], bilateral kainic acid [15] or ibotenic acid lesions [10] of the cholinergic nucleus basalis magnocellularis (nbm) produced PA retention deficits.

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Another approach involves the use of tasks that allow a differentiation of working and reference memory. Working memory refers to the recall of recent events of transient importance and is highly vulnerable to interference effects whereas reference memory refers to information stored over the long term and is relatively resistant to interference [18,31]. The working memory components of a task are those in which information on any single trial is useful only for that trial. Reference memory components include information that is useful for all trials. In a delayed matching to sample task, for example, the matching rule applies to all trials (reference memory). The stimulus on any given trial governs the choice only on that trial and the longer the sample-test interval, the poorer the performance (working memory). Atropine [32] and scopolamine [3] produced a deficit in this task, the drug effect being greater as the sample-test interval increased.

By training rats on, for example, an 8-arm radial maze with only 4 arms baited it is possible to differentiate reference memory errors, entries into never-baited arms, from working memory errors, re-entries into arms of the baited set from which food has been eaten [30]. Animals treated with low doses of scopolamine [42] or atropine [21] made more working but not reference memory errors. Similarly, ICV AF64A injections led to decreased striatal and hippocampal acetylcholine levels and increased working memory errors [19]. In contrast, bilateral nbm ibotenic acid lesions [27] or high doses of scopolamine [29] led to increases in working and reference memory errors in rats. Two T-maze tasks also can be employed to differentiate between working and reference memory. Working memory is tested using an alternation task that requires the rats to remember their choice on the previous trial to select the correct arm on the next trial. Reference memory is evaluated using a spatial discrimination task in which the correct choice is always the same arm. Brito *et al.* [6] found that scopolamine into the dorsal hippocampus impaired alternation but not a visual discrimination in the same T-maze. Similarly, systemic scopolamine [40] and bilateral ibotenic acid nbm lesions [35] impaired T-maze alternation. However, neither of these latter studies employed an alternate task with minimal working memory requirements, making interpretation of the results with respect to memory difficult.

The present study was carried out to test the hypothesis that working and reference memory in the T-maze may be differentially controlled by cholinergic systems. Performance on the alternation task should be more subject to interference than the spatial discrimination task. To test this, a 30-sec delay was imposed between half the trials on each task and it was expected to lead to a selective impairment on the alternation task. To test the hypothesis that anticholinergics or lesions of the nbm lead to working memory impairments, the effects of scopolamine or unilateral kainic acid lesions of the basal forebrain were tested. Unilateral lesions were used as previous studies have shown that these are sufficient to produce memory deficits [23]. If the lesions and scopolamine produce task-specific memory impairments and if the lesions reduce cortical cholinergic function, then the hypothesis that basocortical cholinergic systems are involved in working memory will be supported.

METHOD

Subjects

Eighty-four experimentally naive male albino rats of the Sprague-Dawley strain were obtained from Charles River Canada, weighed 250–400 g and were maintained at 80–85% of these free feeding weights by daily feeding with measured rations. Rats were housed individually in wire mesh cages in a climatically controlled (21±1°C) colony room kept on a 12 hr light (0700–1900 hr)/dark cycle; water was continuously available.

Surgery

Forty-three rats were anaesthetized with halothane (Halocarbon, Malton, Ont.; 2% halothane, 98% oxygen) inhalation and positioned in a stereotaxic surgery apparatus. With the incisor bar set at 3.3 mm below the interaural line, unilateral microinjections were aimed at the nbm on the right side with coordinates from bregma being 0.8 mm posterior, 2.6 mm lateral to the midline and 8.0 mm ventral to the surface of the skull. Nineteen rats were infused over a 2.5 min period with 1.0 μl containing 4.7 nmoles (1.0 μg) kainic acid (Sigma, Lot 32F-0867), dissolved in 0.9% saline titrated to pH 7.4 with 1.0 N NaOH, using a Hamilton cannula (0.35 mm o.d.). The remaining 24 rats received 1.0 μl injections of 0.9% saline. Following infusions, the cannula was left in place for 3.0 min to allow for diffusion and then withdrawn. The scalp was apposed with sutures. Immediately following surgery, kainic acid treated rats received an IP injection of 25.0 mg/kg sodium pentobarbital (M.T.C. Pharmaceuticals) to prevent seizures and remote damage associated with these seizures [4]. At least one week elapsed prior to the beginning of testing.

Apparatus

The T-maze was constructed of wood, sealed with polyurethane and covered with Plexiglas. The start box and two goal boxes measured 25.8 by 19.7 cm wide, the main alley was 50.9 cm in length and 14.2 cm wide and each arm was 26.6 cm long and 14.5 cm wide. A 2.5 cm dia. plastic food cup was located at the distal end of each goal box.

Procedure

In 2 experiments, rats were trained in a spatial discrimination task and in 2 experiments rats were trained in an alternation task. Each rat received one session per day at approximately the same time each day, 5 days a week. For either task, a session began with placement in the start box and opening the start box door. For half the rats in the spatial discrimination task, reinforcement consisting of one 45 mg food pellet (Bio Serv) always was available in the food cup in the right goal box; for half food always was in the left. Once a goal box was entered (and the food eaten if the choice was correct), the rat was picked up and placed back into the start box (end of first trial) and the door opened to begin the next trial. There were 20 trials per session. For rats in the alternation task, food was available in either goal box on the first trial each day. Subsequently, food always was found only in the goal box opposite the one entered on the preceding trial, regardless of whether or not the preceding goal box entry was correct. There were 20 alternation trials per session following the first free choice trial. Sessions continued until two consecutive days at 70% or better correct responding occurred. After this, a 30-sec delay during which rats were confined to the start box was inserted prior to 10 trials randomly selected from the 20 trials each session. The dependent variables were: (1) percent correct responses on 0-sec

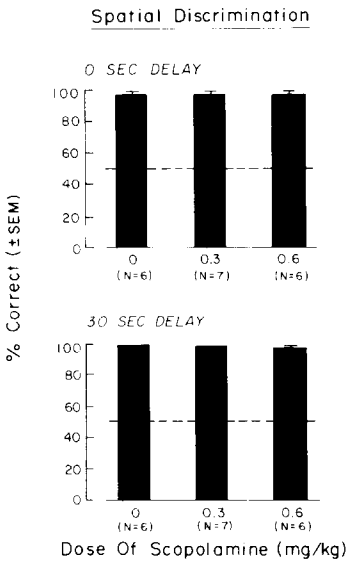

FIG. 1. Mean (±SEM) percent correct responses for no-delay and delay trials in the spatial discrimination task for groups receiving saline (n=6), 0.3 mg/kg (n=7) or 0.6 mg/kg (n=6) scopolamine, averaged over the 4 treatment sessions in Experiment 1.

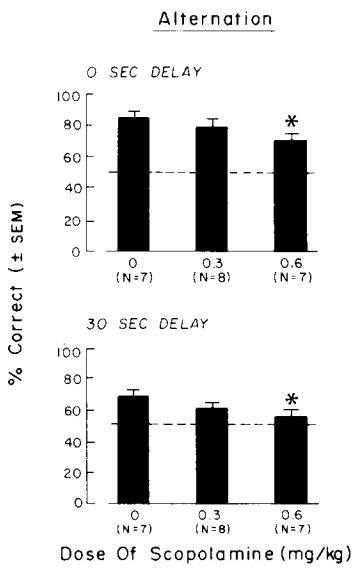

FIG. 2. Mean (±SEM) percent correct responses for delay and no-delay trials in the T-maze alternation task for groups receiving saline (n=7), 0.3 mg/kg (n=8) or 0.6 mg/kg (n=7) scopolamine averaged over the 2–4 treatment sessions of Experiment 2. Overall there was a significant effect of delay ($p<0.01$) and dose ($p<0.01$). *Different from 0 mg/kg dose ($p<0.02$).

and 30-sec delay trials each session; (2) time per trial, calculated by determining total session time, subtracting delay time and dividing by the total number of trials.

In Experiment 1, 19 rats received 5 spatial discrimination sessions without delay followed by 3 delay sessions. On the next 2 days, 30 min prior to each delay session rats were injected IP with saline (n=6) or scopolamine hydrobromide (Sigma) dissolved in distilled water at doses of 0.3 mg/kg (n=7) or 0.6 mg/kg (n=6). Rats were randomly assigned to dose groups. Three recovery, no-drug delay sessions were followed by 2 more drug sessions. In Experiment 2, 15 rats received 10–16 alternation sessions without delay followed by 9 delay sessions. (Note that this task was more difficult than the spatial discrimination and therefore required more training sessions.) Rats were randomly assigned to dose groups and on the next 2 days, 30 min prior to each delay session scopolamine was injected in doses of 0.3 mg/kg (n=8) or 0.6 mg/kg (n=7). Three recovery, no-drug delay sessions were followed by 2 more drug sessions. The high dose group then was trained for another 3 no-drug recovery sessions followed by 2 saline sessions.

In Experiment 3, lesion (n=7) and sham-operated (n=11) rats received 16–24 no-delay spatial discrimination training sessions. (The greater number of sessions than in Experiment 1 was required as the lesion slowed acquisition.) After a minimum of 16 sessions and attainment of a criterion of 2 consecutive sessions at 70% or better, 2 delay sessions followed. (To allow for within-animal comparisons of the two tasks, the 11 sham-operated rats and 5 rats from the lesion group received 10 no-delay alternation trials following the spatial discrimination experiment.) In Experiment 4, unoperated (n=6), sham operated (n=7) and lesion (n=6) rats were trained for 17–20 no-delay alternation sessions. (The slower acquisition than in Experiment 2 again appeared to be due to the lesion.) After a minimum of 17 sessions and attainment of a criterion of 2 consecutive sessions at 70% or better, 10 delay sessions followed.

Histology

Six kainic acid injected animals and 6 sham operated animals were killed 7 days post lesion and their brains fixed in formalin for 10–20 days. Brains were then frozen, sectioned at 40 μ, mounted and stained with cresyl violet for light microscopic examination.

Biochemistry

Following behavioral testing, the rats from Experiments 3 and 4 were killed by decapitation and their brains removed. A section of fronto-parietal cortex was dissected from each hemisphere and assayed for choline acetyltransferase (CAT) activity by the method of Fonnum [14]. Additionally, for animals from Experiment 3, similar procedures were followed to determine dorsal hippocampal CAT activity in both hemispheres. Protein was measured according to Lowry *et al.* [24].

RESULTS

Experiment 1

Mean (±SEM) percent correct responses for delay and no-delay trials in the spatial discrimination task for groups receiving saline (n=6), 0.3 mg/kg (n=7) or 0.6 mg/kg scopolamine (n=6), averaged over the 4 treatment sessions are shown in Fig. 1. Kruskal-Wallis analysis of variance by ranks revealed no significant effect of groups at either delay, $H(2)=2.50$, $p>0.05$ and $H(2)=5.24$, $p>0.05$. Wilcoxon matched-pairs signed-ranks tests for delay effects in each group also revealed no significant differences. The mean (±SEM) time (sec) per trial for the 3 groups was 5.3 (±0.2),

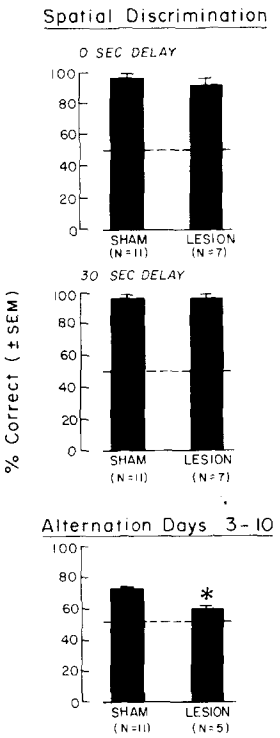

FIG. 3. Upper two panels: Mean (±SEM) percent correct responses for delay and no-delay trials in the spatial discrimination task for sham-operated (n=11) and lesion (n=7) groups in Experiment 3. Lower panel: Performance of the same groups over 8 no-delay alternation sessions that followed Experiment 3. *The group difference was significant ($p<0.01$).

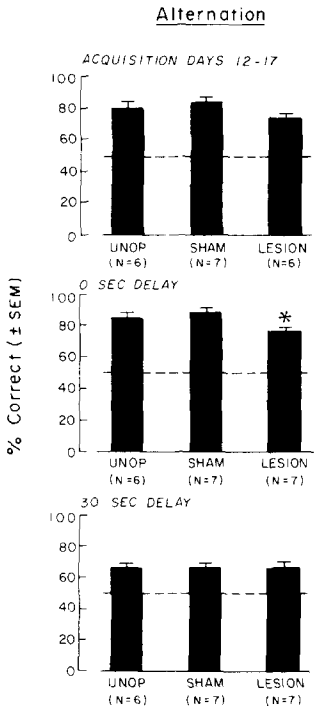

FIG. 4. Upper panel: Mean (±SEM) percent correct responses for acquisition sessions 12–17 in the no-delay alternation task for unoperated (n=6), sham-operated (n=7) and lesion rats (n=6) in Experiment 4. Group differences approached significance ($p<0.06$). Lower panels: Performance on the no-delay and delay sessions of Experiment 4. The delay effect was significant ($p<0.01$) and groups differed on no-delay trials ($p<0.01$). *Different from sham ($p<0.01$) and unoperated groups ($p<0.05$).

5.4 (±0.2) and 6.3 (±0.8), respectively. Analysis of variance (ANOVA) revealed that these means did not differ significantly, $F(2,16)=1.29$, $p>0.05$.

Experiment 2

Mean (±SEM) percent correct responses for no-delay and delay trials in the T-maze alternation task for groups receiving saline (n=7), 0.3 mg/kg (n=8) or 0.6 mg/kg scopolamine (n=7) averaged over the 2–4 treatment sessions are shown in Fig. 2. The groups appeared to perform more poorly under delay conditions and with increasing dose of scopolamine. Statistical analyses confirmed this impression. A 2-way repeated measures ANOVA of the saline and 0.6 mg/kg doses yielded effects of dose, $F(1,6)=44.34$, $p<0.001$, and delay, $F(1,6)=17.49$, $p<0.01$, but no significant interaction, $F(1,6)<1$, $p>0.05$. Post hoc ANOVAs showed that the dose effect was significant on no-delay, $F(1,6)=10.23$, $p<0.02$, and delay trials, $F(1,6)=23.74$, $p<0.01$. In separate mixed 2-way ANOVAs comparing saline and 0.3 mg/kg or 0.3 mg/kg and 0.6 m/kg doses, groups did not differ significantly, $F(1,13)=1.85$, $p>0.05$ and $F(1,13)=2.05$, $p>0.05$, respectively, the interactions were insignificant, $F(1,13)<1$, $p>0.05$ in each case, but the delay effect was significant in both cases, $F(1,13)=28.99$, $p<0.001$ and $F(1,13)=47.86$, $p<0.001$, respectively. The mean (±SEM) time (sec) per trial for saline, 0.3 mg/kg and 0.6 mg/kg scopolamine groups was 7.8 (±1.9), 7.8 (±0.6) and 8.3 (±1.0), respectively. These means did not differ significantly.

Experiment 3

Mean (±SEM) percent correct responses for no-delay and delay trials averaged over 2 sessions in the spatial discrimination task for sham-operated (n=11) and lesion (n=7) groups are shown in the upper two panels of Fig. 3. Mann-Whitney rank tests for independent samples revealed no significant group differences at either delay, $U(7,11)=23.5$, $p>0.05$ and $U(7,11)=31.0$, $p>0.05$. Wilcoxon matched-pairs signed-ranks tests for delay effects in each group also revealed no significant differences. The mean (±SEM) times (sec) per trial were 9.1 (±1.3) for the sham group and 12.9 (±1.9) for the lesion group and did not differ significantly, $F(1,16)=2.94$, $p>0.05$.

The results of the no-delay alternation trials that followed the spatial discrimination experiment are shown in the lower panel of Fig. 3. Only the last 8 of the 10 alternation sessions are included in the means as most of the animals failed to complete 20 trials on the first 2 training days in this task probably as a result of negative transfer. The data suggest that rats with lesions were impaired and ANOVA results revealed a significant group difference, $F(1,14)=12.53$,

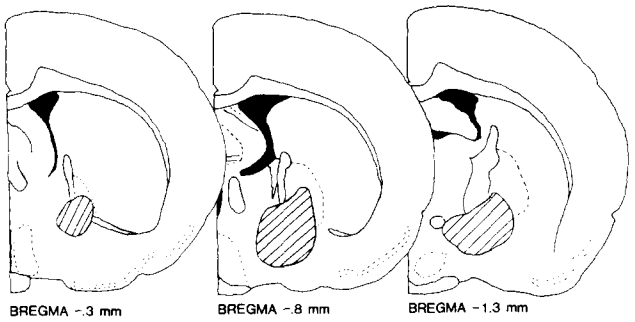

FIG. 5. Frontal sections showing the extent of damage produced by basal forebrain injections of kainic acid.

$p<0.01$. There was also an effect of days, $F(7,98)=3.94$, $p<0.01$, but no significant interaction, $F(7,98)<1$, $p>0.05$, suggesting that group differences did not change over days.

Experiment 4

Mean (±SEM) percent correct responses for acquisition, no-delay and delay trials in the T-maze alternation task for the unoperated (n=6), sham-operated (n=7) and lesion groups (n=7) are shown in Fig. 4. While learning the task during acquisition, many rats failed to complete 20 trials in the allotted time of 15 min on one or more days over the first 11 training sessions. However, all but 1 lesion rat completed sessions 12 to 17 and results are shown in the upper panel of Fig. 4. It appeared that the performance of the lesion group was poorest, with the difference between the sham and unoperated groups being smaller and a one-way group ANOVA revealed an effect that approached significance, $F(2,16)=3.41$, $p=0.059$. There was no significant effect of days, $F(5,80)<1$, $p>0.05$, or interaction, $F(10,80)<1$, $p>0.05$.

On day 18, delay sessions began except for those lesion rats (n=2) that had not reached criterion of 2 consecutive sessions at 70% or better. These rats continued to receive no-delay alternation trials for 4–5 additional sessions by which time they reached criterion. The mean (±SEM) percent correct for unoperated, sham and lesion groups on the 2 days preceding delay sessions was 84.2 (±4.3), 83.2 (±2.7) and 80.7 (±2.0), respectively, and did not differ significantly, $F(2,17)<1$, $p>0.05$.

Results for the 10 sessions with no-delay and delay trials are shown in the lower 2 panels of Fig. 4. There appeared to be an effect of delay in every group and the lesion group appeared poorer than sham and unoperated rats at the no-delay trials only. A two-way mixed design ANOVA revealed no significant group effect, $F(2,17)=1.87$, $p>0.05$, a delay effect, $F(1,17)=140.56$, $p<0.001$, and an interaction, $F(2,17)=5.30$, $p<0.02$. Post hoc ANOVAs at each delay revealed a group effect at no delay, $F(2,17)=10.57$, $p<0.01$ but not in the 30-sec delay condition, $F(2,17)<1$, $p>0.05$. Finally, Tukey post hoc individual comparisons showed that at no-delay, lesion rats differed from shams ($p<0.01$) and unoperated rats ($p<0.05$), the latter 2 not differing significantly.

Mean (±SEM) times (sec) per trial for unoperated, sham and lesion groups during acquisition days 12–17 were 7.3 (±0.5), 10.2 (±1.6) and 13.1 (±0.5), respectively. A one-way ANOVA revealed a significant group effect, $F(2,16)=6.40$, $p<0.01$, and post hoc (Tukey) tests showed only the difference between unoperated and lesion groups to be significant ($p<0.01$). For the 10 days with delays, respective means (±SEM) were 5.9 (±0.3), 7.7 (±1.3) and 9.4 (±0.5) sec. A one-way ANOVA again showed these means to differ significantly, $F(2,17)=34.35$, $p<0.05$ and again Tukey tests showed only the difference between unoperated and lesion groups to be significant ($p<0.05$).

TABLE 1

CHOLINE ACETYLTRANSFERASE (nmoles/mg PROTEIN/HR) FOR SPATIAL DISCRIMINATION RATS IN EXPERIMENT 3

	Sham (N=11)	NBM Lesion (N=7)
Fronto-Parietal Cortex		
Lesion (Right) Side	45.4 ± 2.7	25.7 ± 3.4
Unlesion (Left) Side	41.3 ± 2.3	44.2 ± 2.1
RT ÷ LT × 100 (%)	107.7 ± 4.7	58.6 ± 7.3
Sham vs. lesion (%): $p<0.0001$.		
Hippocampus		
Right Side	65.5 ± 5.7	73.0 ± 5.3
Left Side	62.7 ± 4.7	62.7 ± 3.0
RT ÷ LT × 100 (%)	106.0 ± 4.6	116.6 ± 7.0
Sham vs. lesion (%): N.S.		

Histology

Kainic acid produced a sphere of cellular degeneration with a diameter of approximately 1.5–2.0 mm which included the ventral pallidal area and as much as two-thirds of the globus pallidus. There was some degeneration of cell bodies in the lateral hypothalamus and pre-optic area but not the caudate-putamen (Fig. 5). No cell loss was seen in the dorsal or ventral hippocampus. Sham operated animals showed no cell loss in the basal forebrain.

Biochemistry

Mean (±SEM) levels of CAT activity (nmoles/mg protein/hr) in the fronto-parietal cortex and dorsal hippocampus on each side of the brain of the sham-operated and lesion rats from Experiment 3 are shown in Table 1 and fronto-parietal cortical CAT for unoperated, sham and lesion rats from Experiment 4 is shown in Table 2. In Experiment 3, the lesion reduced CAT activity on the right side to 58.6% of the left side whereas the sham operation resulted in no reduction; the difference was highly significant, $F(1,16)=35.40$, $p<0.001$. The lesion had no significant effect on hippocampal CAT, $F(1,16)=1.72$, $p>0.05$. In Experiment 4, CAT activity on the side of the lesion was found to be 57.9% of the intact side. A one-way group ANOVA showed that percent scores differed for the 3 groups, $F(2,17)=5.12$, $p<0.02$ and post hoc pairwise comparisons showed that both unoperated and sham operated groups differed significantly from the lesion group ($p<0.03$) but not from each other.

TABLE 2

CHOLINE ACETYLTRANSFERASE (nmoles/mg PROTEIN/HR) FOR ALTERNATION RATS IN EXPERIMENT 4

	Fronto-Parietal Cortex		NBM
	Unoperated (N=6)	Sham (N=7)	Lesion (N=7)
Lesion (Right) Side	25.3 ± 2.5	30.1 ± 3.3	14.2 ± 1.6
Unlesion (Left) Side	23.1 ± 4.0	32.2 ± 3.3	24.3 ± 1.2
RT ÷ LT × 100 (%)	121.9 ± 19.3	101.8 ± 16.4	57.9 ± 5.0

ANOVA on groups (%): $p<0.01$.
Unoperated vs. sham: N.S.
Unoperated vs. lesion: $p<0.01$.
Sham vs. lesion: $p<0.03$.

DISCUSSION

The results can be summarized as follows. Performance in the alternation task was impaired when a 30-sec delay was inserted between trials whereas spatial discrimination was not significantly affected. Furthermore, the anticholinergic drug, scopolamine or unilateral kainic acid lesions of the basal forebrain that were shown to significantly deplete cortical CAT, impaired alternation but not spatial discrimination performance. These findings support the hypothesis that working and reference memory may be differentially controlled by cholinergic systems.

Kainic acid lesions produced decreases in cortical CAT and a loss of cell bodies in the basal forebrain similar to those seen previously [8]. Others have reported damage remote to the site of injection following kainic acid lesions [28]. However, it was suggested that remote damage is secondary to seizures occurring postoperatively [4] and seizures were prevented with a barbiturate [16,43]. This practice was followed in the present and previous studies from our laboratory [8] and neither hippocampal morphology nor CAT was significantly affected.

Several months elapsed between the time of the lesion and CAT assays. The observation of significant reductions in CAT after this time is contrary to a report of recovery of function 3 months following a unilateral ibotenic acid lesion of nbm [41]. In a study designed to specifically test the possibility of recovery we also observed little evidence of this phenomenon [20]. Inspection of Tables 1 and 2 reveals considerable variability in the absolute value of CAT activity observed in our assay; nevertheless, in both Experiments 3 and 4, only the lesion groups showed a significant decrease and the magnitude was the same. Experiment 4 was actually conducted prior to Experiment 3 and the somewhat low CAT values observed may reflect the use of a very small amount of cortical tissue possibly receiving less cholinergic innervation than the larger section of fronto-parietal cortex subsequently used. Furthermore, 60 min incubation times for CAT assays in Experiment 4 were longer than the 15 min used in Experiment 3, possibly resulting in differences in cortical CAT activity [34].

Behavioural performance in the alternation task was impaired when a delay was inserted between trials whereas performance of the spatial discrimination task was not significantly affected. One of the defining characteristics of working memory is that it is highly susceptible to interference effects whereas reference memory is resistant to interference effects [18,31]. The delay effect, therefore, provides an empirical basis for the memorial distinction between the two tasks.

Scopolamine or unilateral kainic acid lesions of the basal forebrain were found to impair alternation but not spatial discrimination. These differential effects on the two tasks might be observed if the treatments impaired motor performance resulting, for example, in increased running time. Thus, as the two tasks are differentially sensitive to interference effects, treatment-produced enhanced running time might differentially increase working memory errors. However, any nonspecific motor effects of spopolamine or the lesions should be apparent in both tasks. Analysis of time per trial did not yield significant scopolamine effects in either task. The mean time per trial was longer for lesion than for sham or unoperated rats in both tasks; however, the difference was significant only in the alternation experiment. These data suggest that significantly lengthened running times for lesion animals may have been the consequence of memorial impairments in the alternation tasks rather than nonspecific effects of the lesion on performance.

The observation that scopolamine or cortical CAT-depleting unilateral kainic acid lesions impaired T-maze alternation but not spatial discrimination is in excellent agreement with the T-maze findings of Brito *et al.* [6] following injections of scopolamine into the dorsal hippocampus. Similar impairments have been reported following systemic scopolamine [40] or bilateral ibotenic acid lesions of nbm [35]. Others, employing the radial maze have found a selective effect on working memory; this was seen following systemic scopolamine [42] or atropine [21] or ICV AF64A [19]. In contrast, Murray and Fibiger [27] found that bilateral ibotenic acid nbm lesions impaired working and reference memory. Others found that the effects of atropine [32] or scopolamine [3] on matching to sample accuracy interacted with delay. This further supports a differential role for cholinergic systems in working and reference memory.

The present findings are in agreement with previous work showing that the retention of new learning is impaired by treatments or conditions that reduce cholinergic function at the time of learning. These include: (1) systemic anticholinergics [5, 26, 33]; (2) ICV anticholinergics [11]; (3) age [2, 22, 37, 38]; (4) ICV AF64A [39]; (5) bilateral intrahippocampal microinjections of atropine or scopolamine [5,6]; (6) bilateral intrastriatal microinjections of AF64A [36]; and (7) unilateral electrolytic or bilateral neurotoxic destruction of nbm [10, 15, 23]. Furthermore, enhanced cholinergic function has been shown to lead to an enhancement of new learning [2, 11–13].

In conclusion, there is good evidence for cholinergic involvement in memory. Evidence supports a role for acetylcholine in both reference [7] and working memory. However, experiments that utilize procedures for assessing both frequently find that cholinergic systems are differentially involved. Advances in the anatomical localization of cholinergic systems have led to the assessment of the role of individual pathways in memory. Considerable evidence now suggests that the cortically projecting nbm cholinergic system is involved in working memory (this study, [10, 15, 23, 35]). However, hippocampal neurons are also strongly implicated [5,6] and recent work might suggest a role for striatal cholinergic neurons [36].

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