Effects of altered cholinergic function on working and reference memory in the rat[1]

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Many data suggest that the brain's cholinergic neurons participate in the control of memory and it has been suggested that cholinergic systems are involved differentially in working and reference memory. To test this hypothesis the effects on memory of unilateral injections of the neurotoxins, quinolinic acid or kainic acid into the cortically projecting cholinergic cells of the nucleus basalis magnocellularis (nbm) were evaluated. In experiment 1, quinolinate-injected ($n = 7$) and sham-operated ($n = 7$) rats were tested in a T-maze alternation task that requires working memory. Lesion rats performed significantly more poorly than shams and subsequent biochemical assays of cortical choline acetyltransferase (CAT) activity revealed significant reductions in the lesion rats. In experiment 2, kainate-injected ($n = 9$) and sham-operated ($n = 8$) rats were trained in an eight-arm radial maze with only four arms baited. Lesion rats made significantly more working memory errors (entries into baited arms from which the food had already been collected) than reference memory errors (entries into never baited arms). CAT assays showed that the lesion led to a decrease in cortical CAT with no significant change in hippocampal CAT. The results of these studies support the hypothesis that cholinergic neurons of the basocortical system may be differentially involved in working and reference memory.

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Plusieurs études suggèrent que les neurones cholinergiques du cerveau participent au contrôle de la mémoire et on a suggéré que les systèmes cholinergiques étaient impliqués différemment dans la mémoire de travail et dans la mémoire de référence. Afin de vérifier cette hypothèse, on a évalué, dans les cellules cholinergiques du nucleus basalis magnocellularis (nbm) se projetant corticalement, les effets sur la mémoire d'injections unilatérales des neurotoxines, acide quinolinique ou acide kaïnique. Dans l'expérience 1, on a testé des rats ayant reçu une injection de quinolinate ($n = 7$) et des rats ayant subi une opération factice ($n = 7$) dans une tâche d'alternance dans un labyrinthe en T exigeant une mémoire de travail. Les rats lésés eurent une performance significativement plus faible que les rats témoins et des analyses biochimiques subséquentes de l'activité de l'acétyltransférase de choline (ACT) corticale montrèrent des réductions significatives chez les rats lésés. Dans l'expérience 2, on a entraîné des rats ayant reçu une injection de kaïnate ($n = 9$) et des rats ayant subi un opération factice ($n = 8$) dans un labyrinthe radial à huit voies dont seulement quatre voires avaient été appâtées. Les rats lésés firent significativement plus d'erreurs de mémoire de travail (entrées dans les voies appâtées d'où la nourriture avait déjà été enlevée) que d'erreurs de mémoire de référence (entrées dans des voies n'ayant jamais été appâtées). Des analyses de l'ACT montrèrent que la lésion provoqua une diminution d'ACT corticale sans variation significative de l'ACT hippocampique. Les résultats de ces études supportent l'hypothèse voulant que les neurones cholinergiques du système basocortical puissent être impliqués différemment dans la mémoire de travail et dans la mémoire de référence.

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Introduction

In recent years, the anatomy of the brain's cholinergic systems has been described in considerable detail (Armstrong et al. 1983; Fibiger 1982; Mesulam et al. 1983). Of particular interest to the present paper are the cholinergic neurons of the basal forebrain, especially the cortically projecting cells of the nucleus basalis magnocellularis (nbm) that may be involved in the control of memorial processes.

The possible involvement of cholinergic neurotransmission in memory has been the topic of extensive research (see review by Overstreet, 1984). Deutsch and his co-workers, for example, found that the relearning of a discrimination task by rats was differentially affected by cholinergic manipulations depending on the time since original learning (Deutsch and Rogers 1979). They concluded that learning results in a systematic change in cholinergic synaptic excitability that represents a component of mnemonic processing and that lasts for at least weeks. Thus, cholinergic systems were implicated in long-term memory.

Another approach has been to evaluate the effects of altered cholinergic function on new learning. Usually, a single training session is employed with cholinergic function altered either during or immediately following training. Memory is then assessed some time later. Using this approach, Flood et al. (1981, 1983, 1984) found that intracerebroventricular (icv) injection of cholinergic antagonists impaired the retention of a discriminated active avoidance task, whereas cholinergic agonists at moderate doses improved recall. Others have used passive avoidance to study the role of cholinergic systems in new learning. Results revealed that recall was impaired if learning was carried out following injection of scopolamine (Meyers 1965) or atropine (Blozovski and Hennocq 1982). Similar results were seen following chronic treatments with diisopropylfluorophosphate that were shown to reduce the number of cortical muscarinic receptors (Gardner et al. 1984). Icv injections of the cholinergic neurotoxin, ethylcholine aziridinium ion solution (AF64A), reduced hippocampal and frontocortical acety-

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lcholine levels and impaired the memory of a passive avoidance task (Walsh et al. 1984). Aged mice and rats that have been shown to have significant reductions of cholinergic function are impaired in the retention of passive avoidance (Bartus et al. 1980; Lippa et al. 1980; Sherman et al. 1981; Strong et al. 1980). Furthermore, this impairment can be reversed with dietary choline supplements (Bartus et al. 1980) that enhance cortical cholinergic function (Beninger et al. 1984). Bilateral intrastriatal microinjections of AF64A, which reduced striatal choline acetyltransferase (CAT) but not cortical CAT or striatal or cortical glutamic acid decarboxylase, impaired retention of passive avoidance learning (Sandberg et al. 1984). The basocortical cholinergic system also has been implicated; unilateral electrolytic nbm lesions (Lo Conte et al. 1982) and bilateral kainic acid (Friedman et al. 1983) or ibotenic acid nbm lesions (Berman et al. 1983; Flicker et al. 1983; Hepler et al. 1983) reduced cortical cholinergic function and impaired passive avoidance retention. These studies implicate cholinergic systems in the control of memory.

Recently, a number of investigators have studied the possibility that cholinergic neurotransmission might be differentially involved in recent, short-term, or working memory and long-term or 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 (Honig 1978; Olton et al. 1980). 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. Tasks have been developed that allow a differentiation of these types of memory. Two such tasks are spatial alternation versus spatial discrimination in a T maze and spatial discrimination in a partially baited radial maze. Studies that have utilized these tasks to evaluate the possible differential role of cholinergic neurons in working and reference memory will be reviewed in the following sections.

T-maze tasks

In the T maze, an alternation task requires the rats to remember their choice on the previous trial to select the correct arm on the next trial. This task requires learning of the alternation "rule" (reference memory) and recall of the most recently visited arm (working memory). A spatial discrimination task in which the correct choice is always the same arm requires reference memory but makes minimal demands on working memory. Beninger et al. (1986) tested the effects of delays between trials and effects of systemic scopolamine or unilateral kainic acid lesions of the basal forebrain in these two tasks. Rats received 20 trials per day with a random 10 trials preceded by a 30-s delay during which they were confined to the start box. Results revealed significantly poorer performance following delay trials in the alternation task but no delay effect in the spatial discrimination task. This finding provided an empirical basis for the memorial distinction between the two tasks suggesting that interference during the delay impaired working memory in the alternation task but not reference memory in the spatial discrimination task. Scopolamine (0, 0.3, 0.6 mg/kg, i.p.) or unilateral kainic acid lesions of the basal forebrain resulted in an increase in errors in the alternation task but failed to significantly affect spatial discrimination performance. The lesions were shown to produce a 40% decrease in cortical CAT on the lesion side without significantly affecting hippocampal CAT. It was concluded that cholinergic neurotransmission in general, and basocortical cholinergic neurons in particular, may be differentially involved in the control of working and reference memory.

These findings are in good agreement with those of Warburton and Heise (1972) who found that scopolamine impaired alternation in a Skinner box analogue of the T maze and with Salamone et al. (1984) and Hepler et al. (1985) who reported that bilateral ibotenic acid or radiofrequency current nbm or medial septal lesions impaired T-maze alternation. Brito et al. (1983) found that bilateral microinjection of scopolamine into the dorsal hippocampus impaired T-maze alternation but not a visual discrimination, findings in excellent agreement with those of Beninger et al. (1986) and Hepler et al. (1985). These data suggest that damage to cortical cholinergic neuron terminals from nbm and cholinergic synapses in the hippocampus leads to greater deficits in working than reference memory.

Radial maze tasks

By training rats on, for example, an eight-arm radial maze with only four arms baited, it is possible to differentiate two types of errors; reference memory errors are entries into never baited arms and working memory errors are re-entries into arms of the baited set from which food has been eaten (Olton 1983). It has been found that animals treated with low doses of the anticholinergics, scopolamine (Wirsching et al. 1984), or atropine (Levy et al. 1983) made more working memory errors but not reference memory errors. Similarly, icv AF64A injections led to decreased striatal and hippocampal acetylcholine levels and increased working memory errors (Jarrard et al. 1984); these authors caution, however, that the neurotoxin also produced large fimbria–fornix lesions that may be responsible for the observed working memory deficit. In contrast, Murray and Fibiger (1985) observed that bilateral ibotenic acid nbm lesions, which significantly reduced cortical CAT, led to increases in working and reference memory errors that were reversed by physostigmine. Okaichi and Jarrard (1982) found that scopolamine led to an increase in both types of errors.

Experiment 1

Recently it was shown that the endogenous tryptophan metabolite, quinolinic acid, like kainic and ibotenic acid, had neurotoxic properties when injected into the nbm (El-Defrawy et al. 1985; Schwarz et al. 1983). Thus, it was shown that unilateral nbm quinolinic acid injections produced significant decreases in cortical cholinergic markers including acetylcholinesterase, Na^+-dependent high affinity choline uptake and K^+-evoked acetylcholine release on the lesion side without affecting the hippocampus. Therefore, it was of interest to test the effects of quinolinic acid lesions of nbm on T-maze alternation. It was hypothesized that quinolinic acid, like kainic acid lesions of nbm would lead to deficits in working memory in a T-maze alternation task.

Method

Fourteen male Sprague–Dawley rats, obtained from Charles River, Canada, weighing 275–350 g, were anaesthetized with halothane (Halocarbon, Malton, Ont., 2% halothane, 98% oxygen) inhalation. Seven rats received quinolinic acid (120 nmol in 1 μL) and seven rats received saline (1 μL) unilaterally at the following coordinates: 0.8 mm posterior to bregma, 2.0 mm lateral to the midline, and 8.0 mm ventral to the surface of the skull with the incisor bar set at 3.3 mm below the interaural line. Infusions were made through a Hamilton cannula (0.35 mm outer diameter) over a period of 2 min, 25 s, and the cannula was left in place for an additional 3 min. All rats received 25 mg/kg (i.p.)

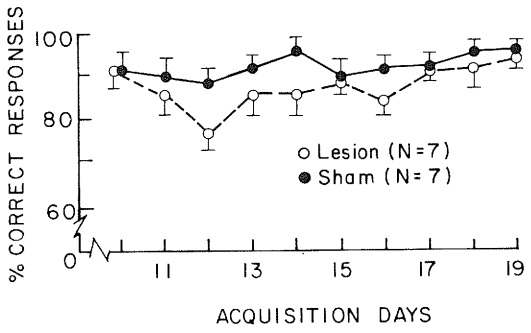

FIG. 1. Mean percent (±SEM) correct responses on days 10–19 in the T-maze alternation task for quinolinate-lesion and sham-operated rats in experiment 1. Groups differed significantly, $p < 0.04$.

TABLE 1. Mean (±SEM) choline acetyltransferase (nanomoles per milligram of protein per hour) activity in frontoparietal cortex of rats in experiment 1

	Sham ($n = 7$)	Lesion ($n = 7$)
Lesion (right) side	67.36±5.82	34.19±3.01
Intact (left) side	67.11±2.64	57.14±3.56
% decrease on lesion (right) side	−0.03±7.28	40.48±4.69

pentobarbital immediately following recovery from anaesthesia to minimize seizures. Rats were housed individually in wire-mesh cages in a colony room kept at 21 ± 1°C on a 12-h light (0700–1900 h) and dark cycle. They were maintained at 85% of their free feeding weights by daily feeding with measured rations. Water was always available in the home cage.

The polyurethane-sealed wooden T maze, with start box and goal boxes measuring 25.8 by 19.7 cm wide, consisted of a main alley 50.9 cm long by 14.2 cm wide and each arm 26.6 cm long by 14.5 cm wide. A 2.5-cm plastic food cup was located at the distal end of each goal box. All rats received 19 sessions of training in the alternation task. Each session consisted of 10 pairs of trials. The first was a forced choice, one of the goal boxes being blocked with a door and reinforcement (one 45 mg Bioserv food pellet) being available in the other. The second trial of each pair was a free choice (both goal boxes open) with the correct (reinforced) response being entry into the previously blocked goal box. For a random five pairs of trials the forced choice was to the right arm and five to the left.

Following testing the rats were killed by decapitation and their brains were removed. A section of frontoparietal cortex was dissected from each hemisphere and assayed for CAT activity by the method of Fonnum (1975). Protein was measured according to Lowry et al. (1951).

Results

Mean percent correct responses for each of the last 10 alternation sessions are shown in Fig. 1. Both groups appeared to show a general improvement over time and the sham-operated group was consistently better than the lesion group. Analysis of variance (ANOVA) with groups and days as the factors analysed revealed a significant effect of days, $F(9,108) = 1.99, p < 0.05$, and of group, $F(1,12) = 5.86$, $p < 0.04$, but no significant interaction, $F(9,108) < 1, p > 0.05$, supporting this description of the data.

Results of CAT assays are shown in Table 1. While CAT activity on the sham lesion side was similar to the unoperated side, the lesion side compared with its unoperated control side showed a decrease of approximately 40%.

Discussion

Histological results are not presented here, but in a previous series of similar animals of the same strain receiving quinolinic acid nbm lesions, identical to those performed here, morphology and several biochemical markers for cortical cholinergic function were assessed (El-Defrawy et al. 1985). The quinolinic acid lesion produced significant unilateral decreases in cortical cholinergic markers comparable to the decrease in CAT reported here. Histological results showed that quinolinic acid produced a sphere of degeneration of approximately 1.0–1.5 mm diameter. Gliosis was apparent in the ventral pallidal area with some damage to perikarya in the globus pallidus. The dorsolateral boundary of the lesion did not extend past the border between the globus pallidus and striatum.

The observation that unilateral quinolinic acid lesions of nbm, which significantly reduced cortical CAT resulting in increased errors in a T-maze alternation task, is in good agreement with the effects of cortical CAT depleting unilateral kainic acid lesions of the basal forebrain (Beninger et al. 1986) and bilateral ibotenic acid or radiofrequency current lesions of nbm (Hepler et al. 1985; Salamone et al. 1984). It is noteworthy that the level of performance seen here was higher than that seen in the studies using kainic acid lesions. However, the alternation task employed here utilized paired trials rather than continuous alternation like that used in the kainic acid study. It is likely that the difference in overall peformance, seen in the sham as well as the lesion animals, reflected differences in the difficulty of the two variations of the alternation task rather than differences in the effects of the lesions. In both cases the performance of lesion rats was impaired.

Experiment 2

The only previous full report of the effects of cortical acetylcholine-decreasing nbm lesions on memory in a partially baited T maze showed a working and reference memory deficit that was reversed by physostigmine (Murray and Fibiger 1985). This finding is not consistent with the observed effects of other cholinergic manipulations on radial maze performance or on T-maze alternation versus spatial discrimination. As other studies have shown that nbm lesions lead to working memory deficits in the T maze, the present experiment was undertaken to evaluate the effects of nbm lesions on performance in the radial maze. It was hypothesized that unilateral kainic acid nbm lesions, like systemic scopolamine, would lead to a specific increase in working memory errors in a partially baited eight-arm radial maze.

Method

Twenty-one male Wistar rats received sham operations ($n = 10$) or lesions ($n = 11$) and were housed as described in experiment 1. Lesion rats received 1 μL kainic acid (Sigma) at a concentration of 4.7 nmol/μL (1.0 μg). Following testing, frontoparietal cortex and hippocampus were assayed for CAT activity as described in experiment 1.

The radial maze, elevated 50 cm above the floor, consisted of an octagonal central platform (30 cm wide) surrounded by eight 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 apparatus.

Two to three weeks postoperatively and 1 week prior to testing animals were food deprived to 80% of free-feeding weights. During this period each rat was handled daily for approximately one min. On day 5 of deprivation animals were fed Froot Loops® cereal in their home cage as small pieces subsequently were utilized as reinforcers.

Pretraining trials

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 maze 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 four arms, referred to as the baited arms. The baiting pattern remained the same throughout the experiment but varied from rat to rat. During days 10–13 the four arms were rebaited until the rat had learned to run to the end of the arm 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% cider vinegar solution.

Formal training

Each rat received one session per day, 7 days a week. At the start of each session, the four 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 four baits were collected, 14 choices were made or 10 min had elapsed, whichever came first. Animals were trained for a minimum period of 28 days, or a maximum period of 40 days if working memory choice accuracy did not stabilize over 4 days to an average criterion of 87% or better by day 28.

Dependent measures

Type of error and number of 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 arms were scored as reference memory errors.

Results

Data from animals that did not run for at least 28 consecutive days (n=4) were excluded from statistical analyses. To assess treatment effects a one-way ANOVA was performed on the number of days to criterion. (Animals which did not reach criterion were assigned the maximum score of 40 days.) Two-way ANOVAs with one repeated measure were performed on two dependent variables: total number of working memory errors and total number of reference memory errors. Error scores, based on choices within the first four arm entries, were summed over 4 days yielding seven 4-day training blocks. An examination of the working memory data revealed that there were no instances of re-entering arms that still contained the bait. Hence, the analysis of working memory errors represents cases wherein animals re-entered arms from which the bait had already been collected.

The mean (±SEM) number of days to criterion for nbm lesion animals and sham controls was 36.9 (±3.1) and 24.3 (±1.9), respectively. Lesion animals took significantly longer to acquire the task as compared with the shams, $F(1,15) = 11.25$, $p < 0.005$. In fact, by day 40, eight of the nine lesion animals had failed to reach the 87% criterion. By contrast, all of the sham animals reached criterion by the minimum period of 28 days.

The mean (±SEM) total number of working and reference memory errors as a function of training blocks is depicted in Figs. 2 and 3, respectively. The nbm group made significantly more working memory errors, $F(1,15) = 6.69$, $p < 0.03$. The effect of training blocks was not statistically reliable, $F(6,90) = 1.72$, $p > 0.05$, nor was the interaction, $F(6,90) < 1$, $p > 0.05$. By contrast, reference memory errors did not differ significantly between groups, $F(1,15) < 1$, $p > 0.10$. The effect of training blocks was significant, $F(6,90) = 7.02$, $p < 0.0001$, as was the interaction, $F(6,90) = 2.79$, $p < 0.02$. Tests of simple main effects revealed that reference memory errors decreased with training for both sham, $F(6,90) = 6.7$, $p < 0.001$, and lesion animals, $F(6,90) = 2.9$, $p < 0.05$, but did not differ reliably at any of the seven training blocks. Hence, the group × block interaction reflects differential within group changes in the total number of reference memory errors over days: with training both groups were learning to inhibit responses to the unbaited arms. Overall, the analyses suggest that lesions of the nbm more reliably impair the acquisition of the working memory component of the radial maze task.

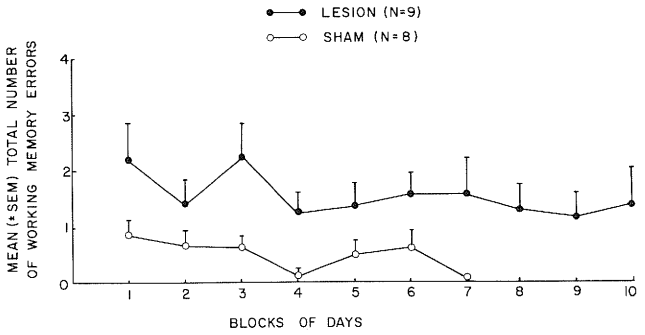

FIG. 2. Mean (±SEM) number of working memory errors on the first four choices summed into 4-day blocks for kainic acid nbm lesion and sham-operated rats in experiment 2. Groups differed significantly, $p < 0.03$.

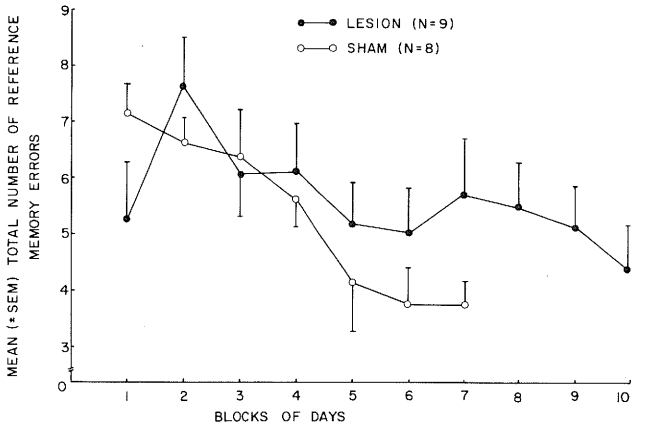

FIG. 3. Mean (±SEM) number of reference memory errors on the first four choices summed into 4-day blocks for kainic acid nbm lesion and sham-operated rats in experiment 2. Group differences were not significant, $p > 0.05$ but the block by group interaction was, $p < 0.02$.

Results from the cortical and hippocampal CAT assays are presented in Table 2. Data were analyzed by a two-way ANOVA with one repeated measure. The effect of interest was the group by side interaction which was significant for cortical CAT, $F(1,14) = 150.09$, $p < 0.0001$. Tests of simple main effects indicated that lesion animals showed a large and reliable depletion in right cortical CAT as compared with left cortical CAT, $F(1,14) = 348.0$, $p < 0.0001$. There was no significant difference between right and left cortical CAT for sham controls, $F(1,14) = 1.9$, $p > 0.05$. Rats with nbm lesions also showed a significant decrease in right cortical CAT as compared with sham controls, $F(1,28) = 219.05$, $p < 0.0001$. There was no significant difference in left cortical CAT between the lesion and sham groups, $F(1,28) = 3.19$, $p > 0.05$.

TABLE 2. Cortical and hippocampal choline acetyltransferase activity (nanomoles per milligram of protein per hour) for rats in experiment 2

	Lesion*	Sham**
Right cortex	18.57±2.03***	51.73±1.50
Left cortex	50.06±1.49	54.06±1.24
Right hippocampus	91.06±2.66	101.80±12.97
Left hippocampus	80.00±2.12	79.42±3.30

NOTE: Values are mean ± SEM.
*n = 8; one lesion animal was unavailable for the CAT assay.
**n = 8; one value for hippocampal CAT was unavailable thereby reducing that n to 7.
***Different from left cortex, $p < 0.0001$, and from right cortex of shams, $p < 0.0001$.

For hippocampal CAT the interaction between side and group was not statistically reliable, $F(1,13) < 1.0, p > 0.05$, indicating that left and right hipocampal CAT activity was not significantly affected by either nbm or sham lesions.

Discussion

Histological results were not presented here. However, as discussed in experiment 1, we have previously presented evidence for histological damage to nbm in animals receiving quinolinic and kainic acid nbm lesions identical to those performed here (El-Defrawy et al. 1985). The kainic acid lesions produced significant decreases in cortical cholinergic markers comparable to the decreases in CAT reported here. Kainic acid produced a sphere of cellular degeneration with a diameter of approximately 1.5 mm which included the ventral pallidal area and as much as two-thirds of the globus pallidus, and a portion of the lateral hypothalamus and preoptic area but not the caudate–putamen. It should be noted that others have reported damage remote to the site of injection following kainic acid lesions (Nadler et al. 1978). However, it was suggested that remote damage is secondary to seizures occurring postoperatively (Ben-Ari et al. 1980) and it was shown that remote damage was attenuated if seizures were prevented with a barbiturate (Fuller and Olney 1981; Zaczek et al. 1978). This practice was followed in the present and previous studies from our laboratory (El-Defrawy et al. 1985) and it was found that neither hippocampal morphology (El-Defrawy et al. 1985) nor CAT, as reported here, was significantly affected.

Our finding that unilateral cortical CAT-reducing nbm kainic acid lesions produced a significant impairment in working memory is in good agreement with some previous findings from radial maze experiments. Thus, systemic scopolamine (Wirsching et al. 1984), atropine (Levy et al. 1983), and icv AF64A injections that depleted hippocampal and striatal acetylcholine (Jarrard et al. 1984) produced increases in working memory errors in a partially baited radial maze. However, Okaichi and Jarrard (1982) found that scopolamine resulted in increases in working and reference memory errors, and Murray and Fibiger (1985) found that bilateral ibotenic acid nbm lesions increased working and reference memory errors.

It may be possible to reconcile these apparently contradictory data by considering the dose of anticholinergic or neurotoxin injected and the memorial demands of the task. Wirsching et al. (1984), for example, found that low doses of scopolamine (0.1, 0.4 mg/kg) produced a selective increase in working memory errors while a dose of 0.8 mg/kg produced an insignificant trend towards greater working *and* reference memory errors. Okaichi and Jarrard (1982) utilized doses of scopolamine ranging from 0.5 to 1.5 mg/kg and observed increases in both working and reference memory errors. Both tasks utilized a four-out-of-eight baiting method; possibly with higher doses of scopolamine both types of memory are affected. Murray and Fibiger (1985) used *bilateral* nbm lesions and a memorially demanding 9-out-of-16 arm radial maze task. Possibly the increase in reference memory errors that they observed was a result of these factors. Finally, it is noteworthy that even in the present experiment, there was a significant interaction of groups and blocks in the reference memory data (Fig. 3). Although post hoc pairwise comparisons failed to reveal significant differences, it did appear that sham-operated rats were improving faster than the lesion group. Thus, cholinergic systems may be differentially involved in working and reference memory but not necessarily exclusively involved in either.

General discussion

Radial maze experiments utilizing the partial baiting procedure (Olton 1983) and T-maze experiments contrasting performance on spatial discrimination versus alternation tasks have made possible the independent assessment of working and reference memory. These two types of memory have also been assessed utilizing operant matching-to-sample tasks with a variable time period between sample and test stimuli (Honig 1978). The results of many studies utilizing these tasks in conjunction with manipulations of the brain's cholinergic function have provided good evidence for a differential role for cholinergic neurons in working and reference memory. Thus, working memory errors were seen to be selectively increased in the radial maze following scopolamine (Wirsching et al. 1984), atropine (Levy et al. 1983), icv AF64A (Jarrard et al. 1984), and unilateral kainic acid nbm lesions (experiment 2). Working memory in the T-maze alternation task was impaired by systemic scopolamine (Beninger et al. 1986; Warburton and Heise 1972), bilateral intrahippocampal microinjections of scopolamine (Brito et al. 1983), bilateral ibotenic acid lesions of the medial septum (Hepler et al. 1985), unilateral kainic acid nbm lesions (Beninger et al. 1986), unilateral quinolinic acid nbm lesions (experiment 1), and bilateral ibotenic acid or radiofrequency current nbm lesions (Hepler et al. 1985; Salamone et al. 1984). Working memory in a delayed matching-to-sample task was impaired by scopolamine (Bartus and Johnson 1976) and atropine (Penetar and McDonough 1983). These studies support the conclusion that cholinergic systems, including the basocortical system and hippocampally projecting cholinergic neurons play a differential role in working and reference memory. It is important to note that results do not argue for an exclusive role for cholinergic systems in working memory. Indeed, some studies utilizing memorially demanding tasks have observed that bilateral ibotenic acid nbm lesions also affect reference memory (Murray and Fibiger 1985).

Others have assessed the effects of manipulations of cholinergic systems on new learning. Possibly, impaired recall of new learning reflects a working memory deficit resulting in difficulty in transferring new information to long-term memory. Consistent with this hypothesis, many studies have shown that the recall of new learning is impaired if cholinergic function was disrupted near the time of learning. Thus, icv anticholinergics (Flood et al. 1981), icv AF64A (Walsh et al. 1984), systemic anticholinergics (Blozovski and Hennocq 1982; Meyers 1965; Ridley et al. 1984), intrastriatal AF64A (Sandberg et al. 1984), unilateral electrolytic lesions of nbm (Lo Conte et al. 1982), bilateral kainic acid nbm lesions (Friedman et al. 1983), and bilateral ibotenic acid nbm lesions (Berman et al. 1983; Flicker

et al. 1983; Hepler et al. 1983) impaired the recall of new learning. Conversely, icv cholinergic agonists enhanced recall (Flood et al. 1983, 1984).

In conclusion, there is good evidence for the involvement of cholinergic systems in memorial processes. Furthermore, it appears that cholinergic neurons may be differentially involved in working and reference memory. Many recent studies have investigated the possibility that individual cholinergic systems may play a differential role. Results suggest that both basocortical neurons and the cholinergic terminals in the hippocampus are differentially involved in working and reference memory.

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