Arch. Gerontol. Geriatr., (1989) Suppl. 1, 71-90
Elsevier

(62)

Animal studies of brain acetylcholine and memory

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Summary

Memory loss is a common feature of aging in the human but not all memories are equally lost. Usually the loss is more severe for the memory of recent events and information rather than the memory of remote events and information. Associated with memory losses of this type is a reduction in levels of cortical acetylcholine and a loss of cholinergic cells of the nucleus basalis magnocellularis. In the rat it is possible to assess two types of memory analogous to recent and remote memories in humans. Reference memory would refer to information concerning the task that is invariant from trial to trial. Working memory would refer to information that changes from trial to trial. In support of the hypothesis that decreases in cholinergic funtion lead to a differential impairment of working memory, rats trained in this task and given scopolamine showed a specific increase in working memory errors. In more recent studies rats have been trained in a task and then given unilateral neurotoxic lesions of the NBM. These results raise the intriguing possibility that degeneration of the NBM and associated memory impairment seen in aging and in Alzheimer's disease may be related to a change in the ratio of these or related endogenous tryptophan metabolites.

Memory; Animals models; Acetylcholine.

Introduction

In recent years there has been a rapid proliferation of animal research concerning the possible role of the neurotransmitter, acetylcholine (ACh), in memory. Two lines of work have strongly influenced this trend. The first is the

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N.B.: The published version of this paper contained a number of errors including several omissions of text and the figures did not correspond with the captions. These errors have been corrected in this version. R.J. Beninger

improved mapping of cholinergic systems with the use of modern anatomical techniques; of particular importance has been the mapping of the basal forebrain cholinergic system including, among others, cells in the nucleus basalis magnocellularis (NBM) in rats or the homologous basal nucleus of Meynert in monkeys that project widely to the cortex (Fibiger, 1982; Mesulam et al., 1984; Woolf and Butcher, 1985, 1986; Woolf et al., 1984). The second finding that has contributed to interest in the possible role of ACh in memory is the post-mortem observation that the cholinergic cells of the basal forebrain degenerate in the brains of persons with Alzheimer's disease (Coyle et al., 1983).

A number of approaches have been used in animal studies of the possible contribution of ACh to memory. Cholinergic neurotransmission has been manipulated either by placing lesions in forebrain cholinergic nuclei or by injecting cholinergic drugs systemically or intracranially. The effects of these manipulations have been evaluated in animals tested for new learning or in animals trained in tasks requiring working and reference memory prior to treatment and then retested afterwards (Olton and Wenk, 1987). Tests of working memory require the use of recently-presented information that is not useful beyond that particular trial. Reference memory, on the other hand, refers to information that is invariant from trial to trial and therefore can be used on all trials (Honig, 1987); specific examples of tasks making demands on these two types of memory will be presented below.

Testing memory in animals is difficult. A major problem is the possible effects of manipulations on performance variables. In a recent paper, for example, Dunnett (1985) stated that "...the Morris water maze, T-maze alternation, the 8-arm radial maze and retention of passive avoidance learning ... [fail to provide] a pure test of memory performance, uncontaminated by deficits in learning capacity, spatial abilities, or more general motivational and arousal factors" (p. 357). Although we agree with this statement with regards to acquisition of these tasks and retention of passive avoidance, the other tasks can be altered in a manner that does overcome the confounding effects of changes in performance variables. Few studies, however, have used these alterations. The present chapter will review the effects of manipulations of cholinergic neurotransmission on memory in delayed matching and nonmatching, delayed alternation and radial maze tasks that were learned prior to testing. In each case, the task involved a clear separation of working and reference memory.

Delayed Matching or Nonmatching

Perhaps one of the now classical papers in the area of ACh and memory is the study of Bartus and Johnson (1976) which investigated the effects of the anticholinergic, scopolamine, in a delayed matching task. The task involved the presentation of a visual stimulus followed by a variable delay. The stimulus was the 3-sec illumination of one from nine panels in a 3×3 display. Following a

delay of 0-10 sec, during which the display was out of sight, the monkey was given access to the display and allowed to make a choice, the correct one being rewarded. Systemic scopolamine produced a delay-dependent impairment in performance, with choice accuracy being worst at the longest delay. This interaction of the effects of scopolamine with delay allowed the conclusion that cholinergic neurotransmission is involved in memory for recent events, i.e., working memory. A similar conclusion was drawn by Penatar and McDonough (1983) who observed an interaction of the effects of the anticholinergic, atropine with delay (0-16 s) in monkeys working on a delayed matching to sample task. The fact that choice accuracy was unimpaired at the shortest intervals in both studies ruled out the possibility that the drug altered perceptual, motor or motivational variables.

In a subsequent report, Bartus (1978) found that the dopamine antagonist, haloperidol, produced an impairment in choice accuracy of monkeys at all delays in the delayed matching paradigm. This finding suggested that the effects of dopamine receptor blockade were not specifically on memory and reinforced the specificity of the scopolamine or atropine effects.

Dunnett (1985) trained rats on a delayed matching task analogous to the one used by Bartus and Johnson (1976). A lever was presented and pressing it led to reward and retraction of the lever. Following a variable delay (1-16 s), two levers were presented and the correct response was to press the one originally rewarded. As expected, choice accuracy decreased with increased delay. Scopolamine or bilateral ibotenic acid lesions of the NBM produced a decrease in accuracy at all delays, however, suggesting that the effects were not specific to working memory. The NBM lesions led to a large decrease of acetylcholinesterase staining in the cortex, suggesting a loss of cortical cholinergic innervation. [For a discussion of the comparability of these types of lesions in rats to Alzheimer's disease see Arendash et al. (1987) and Pepeu et al. (1986)].

Others have similarly found that the effects of scopolamine were not specific to longer delays. Spencer et al. (1985) trained rats on a nonmatching task involving repeated 5-s presentations of either a bright or a dim light with variable interstimulus intervals (2.5-10 s). Rewarded responses were those made when there was a change in the brightness of the stimulus from one presentation to the next. Thus, the only source of information regarding the appropriateness of responding was the recall of the previous stimulus. Scopolamine produced a uniform decrease in responding after all delay intervals suggesting that its effects were not specific to working memory.

Besides the species difference between the studies of Bartus and Johnson (1976) and Penetar and McDonough (1983), on the one hand, and those of Dunnett (1985) and Spencer et al. (1985), on the other, there was another important difference between these two sets of studies that may account for the apparent discrepancy in the observed effects of anticholinergic or NBM lesions on working memory. Both of the former studies included a condition where there was no delay (0-s delay) between presentation of the to-be-remembered stimulus and the choice stimuli whereas, in the latter studies, there was not a no-delay

condition. Possibly, the latter studies failed to observe a greater effect of reduced cholinergic function with increasing delay because the delays were all long enough to produce an impairment. In support of this analysis, it can be seen that the interaction between dose of anticholinergic and delay in the former studies was primarily the result of minimal effects at the 0-s delay.

Further support for this point of view is provided by the results of Viscardi and Heise (1986). They trained rats on a delayed discrimination task involving the presentation of one of two stimuli followed by a variable delay (0-2.5 s); a signal then indicated that reward could be attained if the correct response occurred. The correct response was either to respond on a lever or not respond, depending on the previous signal. Results showed that performance was decreased with increasing delay and, furthermore, that the effects of scopolamine were greatest at the longest delay. Thus, the anticholinergic seemed to produce an effect specific to working memory in excellent agreement with the results of Bartus and Johnson (1976) and Penetar and McDonough (1983). These results provide good evidence that the discrepant results reviewed above were not simply attributable to species difference; working memory appears to be specifically affected by treatments that decreased cholinergic function in monkeys or rats tested in procedures involving a no-delay condition.

In a recent study, Santi et al. (1987) trained pigeons to perform two different tasks in each testing session in a Skinner box. One task was a simple discrimination between two different line orientations presented on the key; the correct choice was always the same. The other task involved the presentation of one of a pair of stimuli followed by a variable delay (1-8 s) and then a choice between the two stimuli presented on the two side keys; this was a standard delayed matching to sample task. As expected, performance decreased with increasing delay in the matching task. In addition, they found that scopolamine impaired the performance of the matching task but not the simple discrimination. As the matching task involved working memory whereas there was no working memory component in the discrimination task, the results suggest that cholinergic neurotransmission is involved in working memory. Santi et al. (1987) failed to find an increasing effect of scopolamine with increasing delay. However, as discussed above, this may have been the consequence of not employing a no-delay condition.

Similar conclusions can be made with reference to a recent paper by Weisman et al. (1987). They trained rats on a delayed discrimination (DD) or a delayed conditional discrimination task (DCD). The DD task involved the presentation of a tone or a clicking stimulus followed by a delay (5-40 s) and then the presentation of a lever that could be pressed to produce food if the preceding stimulus was the S+ (the tone for some rats and the click for others). The DCD involved the same two stimuli followed by a delay (1-20 s) and then the presentation of one of two additional stimuli, a bright light or the house light, and the lever. Which of these two latter stimuli was followed by a rewarded lever

press was conditional on the initial stimulus. For example, if the initial stimulus was the tone then responses during the house light produced reward or if the initial stimulus was the click, responses during the bright light produced reward but not vice- versa.

Results (Fig.1) showed that for both tasks, errors increased with delay. Furthermore, scopolamine produced a dose-dependent impairment in both tasks; however, the effects of scopolamine were different in the two tasks. Whereas

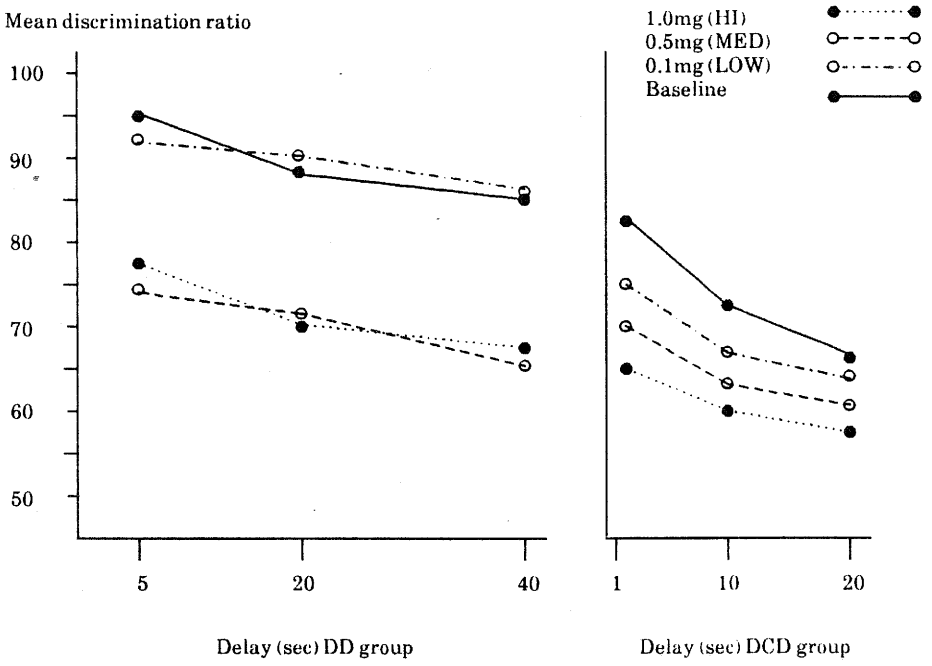

Fig. 1. Mean discrimination ratios as a function of delays and scopolamine dose (0.1, 0.5, and 1.0 mg/kg) for delayed simple (DD) and conditional (DCD) discriminations. Twenty-four food-deprived rats were trained on a DD or DCD task. In the DD task the presentation of a stimulus (tone or click) was followed by a delay (5-40s) and then the presentation of a lever that could be pressed to produce food if the preceding stimulus was the S+. In the DCD task the same two stimuli were followed by a delay (1-20s) and then the presentation of one of two additional stimuli, and the lever. Which of these two stimuli was followed by a rewarded lever press was conditional on the initial stimulus. Discrimination ratios were calculated by dividing responses during the S+ trials by the sum of responding during S+ and S-trials and multiplying by 100. Values of 50 indicate chance performance and 100 indicates perfect performance. In both tasks, errors increased with delay and scopolamine produced a differential dose-dependent impairment. Baseline indicates performance without drugs.

the lowest dose (0.1 mg/kg) produced no significant effect in the DD task, it reduced performance in the DCD task. Performance in drug-free trials and the differential effect of delay in the two groups suggest that the DCD task was more

difficult than the DD task. As both tasks involved working memory, the demands on this type of memory may have been greater in the DCD task. The differential effects of scopolamine in the two tasks may therefore be taken as further evidence that cholinergic systems are involved in working memory. As was the case for the papers reviewed above, the lack of a greater effect of scopolamine with increasing delay may have been related to the failure to include a no-delay condition.

In summary, tasks involving delayed matching or nonmatching or conditional discriminations require the use of working memory. Performance was impaired with increasing delay between sample and test stimuli. Performance also was impaired if cholinergic transmission was reduced. Although increases in delay were not always accompanied by increases in the effects of anticholinergics, this profile was seen in monkeys or rats if a 0-s delay condition was included. Results suggest a role for cholinergic neurotransmission in working memory.

Alternation

Alternation tasks can be viewed as a type of nonmatching to sample task. Animals are usually tested in a T-maze or a Skinner box outfitted with two levers using either continuous or discrete trials. In the discrete trial version of this task, animals receive pairs of trials. In the initial trial in the T-maze only one of the goal arms is open and a reward is found there; after a variable delay, a second trial is given with both arms open and the correct choice is to go to the arm not visited on the first trial. In the continuous version in the T-maze, the correct choice on each trial is to go to the arm not previously visited. This task has a clear working memory component. The only way the animal can consistently make a correct choice is to remember where it was on the preceding trial. With increasing delay between trials, choice accuracy should be seen to deteriorate. If anticholinergics impair working memory, their effects should be greatest at the longer intervals.

Using a two-lever Skinner box analogue of the T-maze, Heise et al. (1976) trained rats in a simple discrimination or delayed alternation task. In the simple discrimination a light above one of the two levers signalled the appropriate lever to press for reward in discrete trials. In the delayed alternation, a light located midway between the two levers indicated that reward was available but choosing the appropriate lever to press required remembering the one pressed on the previous trial and selecting the alternate one. A delay of between 2.5 and 40 s intervened between trials. Results showed that choice accuracy in the alternation task decreased with increasing delay, as expected. Whereas accuracy of the discrimination was not altered by scopolamine treatments, accuracy in the delayed alternation task was significantly reduced. However, the effects of scopolamine were consistent across delays, there being no significant interaction between treatment and delay.

Others have used a similar procedure in the T-maze. Wenk et al. (1987) trained rats in a discrete trial delayed alternation task with delays of 5 to 60 s between the sample trial and the choice trial. They found that ibotenic acid lesions of the basal forebrain cholinergic system that resulted in significant depletions of hippocampal and cortical choline acetyltransferase (ChAT) led to a reduction in choice accuracy. Moreover, there was a greater effect with increasing delay. An interesting addition to the study of Wenk et al. (1987) was groups that underwent neurotoxic lesions of the noradrenergic or serotoninergic systems. Although the former treatment was without significant effect, they found that rats receiving 5,7-dihydroxytryptamine lesions of the medial and dorsal raphe were significantly impaired at the longest delay only.

The study by Heise et al. (1986) found that the effects of reduced cholinergic function on a delayed alternation task were to produce an impairment in choice accuracy but not an effect specific to longer delays. Results were in good agreement with those of Dunnett (1985) and Spencer et al. (1985), reviewed above, which showed a similar effect of decreased cholinergic function in delayed matching or nonmatching tasks. The delayed alternation procedures of Heise et al. (1976) also shared with the latter two studies the lack of a no-delay condition. As already discussed, the failure to observe an interaction between treatment and delay in these studies may have been related to this variable. It is noteworthy, however, that Wenk et al. (1987) also did not have a no-delay condition but did observe an interaction of treatment with delay. Perhaps the extensive cholinergic deficits produced by the combined NBM and medial septal lesions in their study enhanced the susceptibility of the rats to delays leading to the observed interaction.

A number of studies have investigated the effects of treatments that reduced cholinergic function in alternation tasks that did not include a delay. However, like the study of Heise et al. (1976), they included a related task that did not have a working memory component.

One of the most ingenious approaches to this problem was taken by Chrobak et al. (1987) who trained rats in a T-maze using a discrete trial procedure. Each trial began by placing the rat in one of the goal boxes. The correct response was to go to the start box where reward was found. Then, to obtain another reward the rat had to go to the other goal box. In this task the first choice was always the same from trial to trial, i.e., go to the start box and, therefore, the first choice required only reference memory. The correct second choice, however, was always determined by the origin for the first trial and, therefore, required remembering where the first trial began. Thus, the second choice required working memory. A particularly attractive feature of this task is that the two types of memory were tested in exactly the same apparatus and the response demands of the two were the same. In spite of these task similarities, Chrobak et al. (1987) found that well-trained rats receiving intracerebroventricular (ICV) injections of the cholinergic neurotoxin, ethylcholine aziridinium ion (AF64A) showed an increase in working but not reference memory errors. As the treatment resulted

in a significant decrease in hippocampal ChAT, the cholinergic projections to this structure were implicated in working memory.

Other studies employing two tasks with differential demands on working memory include those of Brito et al. (1983) and Hepler et al. (1985). In the former study, rats were trained on a visual discrimination in a T-maze and on an alternation task in the same T-maze. Brito et al. (1983) found that intrahippocampal microinjections of scopolamine impaired performance of the alternation but not the discrimination task. In the latter experiment rats were trained in a T-maze with a double stem requiring a choice of the right or left side. Only one side led to the choice point for the two goal arms; the other side was a blind alley. A curtain blocked the rats' view of the distal end of the stem. The correct goal box was determined by a discrete trial procedure consisting of a first run with one of the goal arms blocked followed by a choice of the two goal arms, the one not entered on the first run being correct. Thus, working memory was not required to complete the stem choice, one side always being correct, but the consistent correct choice of goal arm required remembering where reward was found on the previous trial, a working memory demand. Hepler et al. (1985) pretrained rats on this task and then made ibotenic acid lesions of either the NBM, medial septum or both. The former lesion resulted in significant decreases in cortical ChAT, the latter in decreases of ChAT in both terminal areas. Behavioral results revealed that all three lesions produced a selective decrease in choice accuracy in the alternation task, an effect specific for working memory.

In summary, alternation experiments have revealed that cholinergic systems in general and the septal-hippocampal and basocortical cholinergic systems in particular may be involved in working memory. Data from discrete trial alternation experiments with variable delays, although showing an impairment following disrupted cholinergic function, failed to show an increasing effect with increasing delay. Perhaps this failure related to the lack of a no-delay condition as suggested by the results of the matching and nonmatching experiments. On the other hand, experiments employing tasks which allowed the independent assessment of working and reference memory consistently showed that impaired cholinergic function selectively produced working memory errors.

Radial Maze

A radial maze consists of a central platform with a number of alleyways (arms), frequently eight, radiating from it (Olton, 1983; Olton and Samuelson, 1976). Rats are trained to find food rewards at the ends of the arms. This task has a clear working memory component because the animal must remember which arms have been visited (or not visited) during any one trial. Many investigators have trained rats with all eight arms baited on each trial and then observed that scopolamine, for example, produced an impairment in performance. Unfortunately, with this procedure it is not possible to conclude unequivocally that working memory was impaired as the impairment could have reflected, for example, a loss of orientation in space, perceptual difficulties or some other

nonspecific effect of the drug. For the same reasons, the observation of an impairment in the acquisition of the radial maze task after a treatment does not necessarily mean that working memory was impaired.

For these reasons, only radial maze experiments that involved training the animals prior to treatments and only those experiments that employed procedures that allowed an independant assessment of working and reference memory will be reviewed here.

Two approaches have been taken to independently assess working and reference memory in the radial maze. One involves the imposition of a delay following some of the choices, for example after the first four in an eight arm maze. Errors involving re-entries into arms from which the food had already been taken, i.e., working memory errors should increase with increasing delay. If the cholinergic systems are involved in working memory, the effects of treatments that reduce cholinergic function should be greatest at the longest delays. A second approach is to consistently bait only a subset of arms, for example, four of eight. This is an excellent procedure for differentiating types of errors. Once the animal has learned this task to a high level of proficiency, two possible types of errors can be identified. Reference memory errors would be said to occur when entries into arms of the never-baited set are made; this type of error might suggest that the animal had forgotten the basic task which is to find food in a particular subset of four places. Working memory errors would be said to occur when the animal re-enters an arm of the baited set from which food had been eaten. In this case the animal apparently remembers the task (select the particular subset of four arms) but forgets which of the four arms it has visited.

Employing a delay (0-40 min) following the first four trials in a fully baited eight arm radial maze, Buresova et al. (1986) observed a significant increase in errors with increasing delay. Furthermore, scopolamine produced an increase in errors and the magnitude of the scopolamine effect increased with delay. Similar results for a related experiment using a twelve arm maze were reported (Buresova and Bures, 1982) supporting the hypothesis that cholinergic neurotransmission was involved in working memory. Complementary results were reported by Peele and Baron (1988) and Decker and Gallagher (1987). The latter study also showed that 6-OHDA lesions of the dorsal noradrenergic bundle were without effect on working memory, a finding in agreement with that of Wenk et al. (1987) in their studies of delayed alternation; however, they did find that the effects of scopolamine were enhanced in the noradrenaline-depleted rats. Thus, there may be an interaction of the cholinergic and noradrenergic systems in the control of working memory.

Bartus et al. (1985) trained rats in an eight arm radial maze with a delay (0-24 h) imposed after four trials. They found that bilateral ibotenic acid lesions of the NBM resulted in decreased cortical ChAT and increased errors with the largest effect being at the longest delay intervals. In summary, studies of working memory in the radial maze with a delay intervening after the first half of the choices were made are in general agreement that the effects of anticholinergic treatments are to impair working memory. A similar

impairment can be produced by NBM lesions implicating the basocortical cholinergic system in working memory.

Studies employing the partially baited radial maze to simultaneously investigate the effects of treatments affecting cholinergic function on working and reference memory have similarly found good evidence for a differential sensitivity of the two types of memory to disruptions of cholinergic function. We trained rats to a high level of proficiency in a four-out-of-eight radial maze task and then investigated the effects of several doses of scopolamine given in a counterbalanced order (Wirsching et al., 1983). Results (Fig. 2) showed that low doses specifically increased working but not reference memory errors. Similar results have been reported by others using scopolamine (Beatty and Bierley, 1985) or atropine (Levy et al., 1983). Okaichi and Jarrard (1982) reported that scopolamine increased both working and reference memory errors in mice but the doses were higher than those used by Wirsching et al. (1983), who also found a disruption of reference memory at their highest dose (Fig. 2).

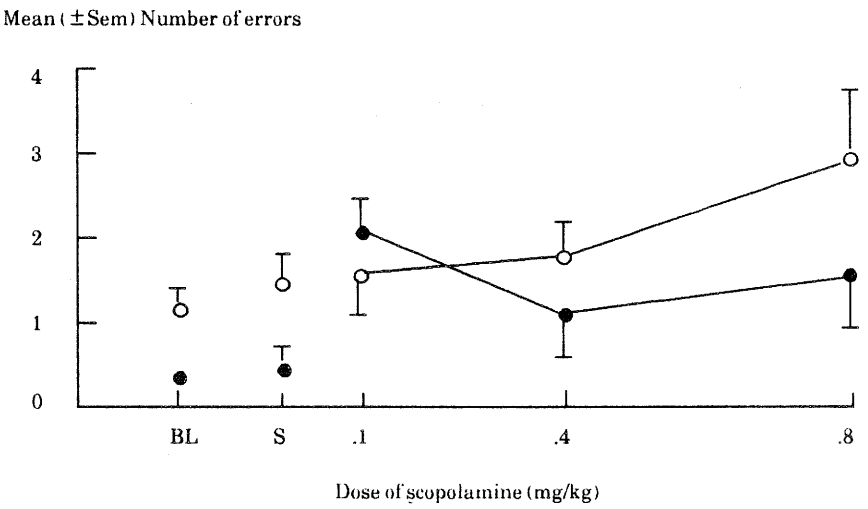

Fig. 2. Mean (± SEM) total number of working (•) and reference (o) memory errors at the various doses of scopolamine. Error scores were based on the first four choices and summed over 4 days; Twelve food-deprived total rats were pretrained on a four-out-of-eight radial maze task until choice accuracy stabilized over 4 days to a criterion of 87% correct. Scopolamine (0.0, 0.1, 0.4 or 0.8 mg/kg, ip) was given for 4 consecutive days, a treatment block, with one session per day. Each treatment block was followed by non-drug baseline sessions that continued until the 4-day 87% criterion was re-established. Every rat received every dose and order of dose was counterbalanced. Low doses of scopolamine significantly increased working but not reference memory errors. BL: Baseline; S:Saline.

We have recently utilized the partially baited radial maze to investigate the mnemonic effects of unilateral quinolinic acid lesions of the NBM (Wirsching et al., 1988) [for a discussion of some of the similarities and differences in the

behavioral and neurochemical effects of unilateral and bilateral NBM lesions see Casementi et al. (1988)]. Quinolinic acid is an endogenous tryptophan metabolite that has excitotoxic effects probably on cells bearing N-methyl-D-aspartate receptors (El-Defrawy et al., 1986). Table I shows the percent decreases in cortical ChAT on the injected side compared to the uninjected side produced by various concentrations of quinolinic acid unilaterally injected into the NBM. The data indicate increasing loss of ChAT with increasing concentration and suggest that quinolinic acid was destroying the cholinergic cells of the NBM, a conclusion that was supported by histological studies (Boegman et al., 1984; El-Defrawy et al., 1985). Regional specificity was suggested by the finding of no significant effect of intra-NBM quinolinic acid on hippocampal or striatal ChAT (El-Defrawy et al., 1986).

TABLE I

Percent decrease in cortical choline acetytransferase of rats on the injected side compared to the uninjected side following unilateral microinjections (0.5 ul) of various endogenous tryptophan metabolites into the nuclues basalis magnocellularis either alone or in combination.

Compound	Concentration (n mol)	Percent Decrease (±SEM)
Saline		13.0±4.6
Quinolinic acid	45	15.0±9.9
	60	18.8±9.4
	75	33.5±4.1
	90	46.6±4.1
	120	53.5±6.9
	150	56.6±4.7
Kynurenic acid	360	11.0±4.7
Quinolinic acid plus	120+45	20.0±5.0
Kynurenic acid	120+120	3.6±3.0
	120+240	8.0±3.0
Picolinic acid	120	11.3±3.9
	240	22.7±2.4
	360	13.5±3.2
	480	19.8±4.0
Quinolinic acid plus	120+60	41.3±6.5
Picolinic acid	120+120	32.3±7.0
	120+240	12.6±5.5
	120+360	9.4±4.5
	120+480	6.0±3.5

One week following injection a section of fronto-parietal cortex was dissected from each hemisphere and assayed for ChAT according to the method of Fonnum (1975). ChAT values on the uninjected side varied from rat to rat probably due to slight differences in the origin of the tissue and ranged from 27 to 45 nmoles per mg of protein per hr.

Wirsching et al. (1988) trained rats in an eight-arm radial maze with four arms baited until they attained a high level of proficiency, viz., no more than a total of two errors in the combined first four choices from four consecutive days. Rats then underwent unilateral injections of quinolinic acid (120 nmol in 1 µl) into the NBM. After recovery from surgery, testing resumed. Results revealed a significant increase in working memory errors in the quinolinic acid-injected animals compared to sham operated controls (Fig. 3).

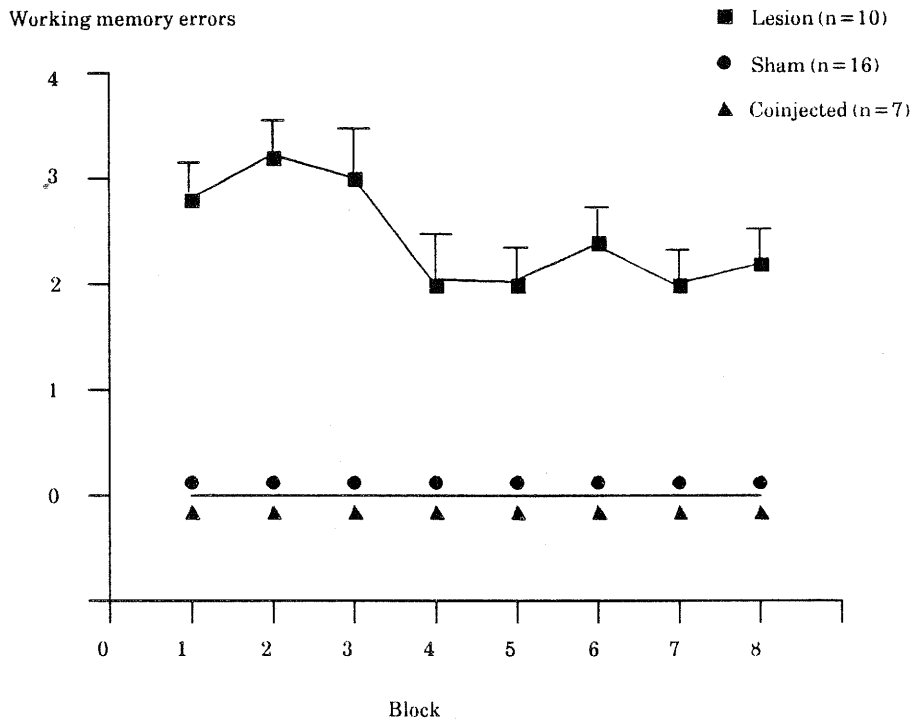

Fig. 3. Mean (± SEM) total number of working memory errors for the lesion (quinolinic acid), co-injected (quinolinic and kynurenic acid) and sham groups. Thirty-three food-deprived rats were pretrained on a four-out-of-eight radial maze task until choice accuracy stabilized over 4 days to a criterion of 87% correct. Rats were then randomly assigned to receive either injections of quinolinic acid (120 nmol/1.0 µl), co-injections of quinolinic acid and kynurenic acid (360 mol/1.0 µl) or saline (0.9%) unilaterally into the NBM. Following at least one week of recovery animals were retested on the radial maze for 32 consecutive days. Errors, based on the first four choices, were summed over 4 days yielding eight 4-day blocks. The lesion group showed a significant increase in working memory errors which persisted over blocks.

Reference memory errors increased in both groups after surgery and then decreased in the sham group but not in the quinolinic acid group (Fig. 4) post hoc tests of group differences at each test block revealed that groups did not differ significantly on the first block but did on the subsequent blocks. Thus, the NBM

lesion produced a relatively specific decrease in working memory performance during the first four-day postoperative test block. Perhaps the lesion group failed to improve in reference memory performance on subsequent blocks because they were impaired in their ability to relearn the task. It has often been reported that new learning is impaired after NBM lesions (Murray and Fibiger, 1985) providing some support for this suggestion.

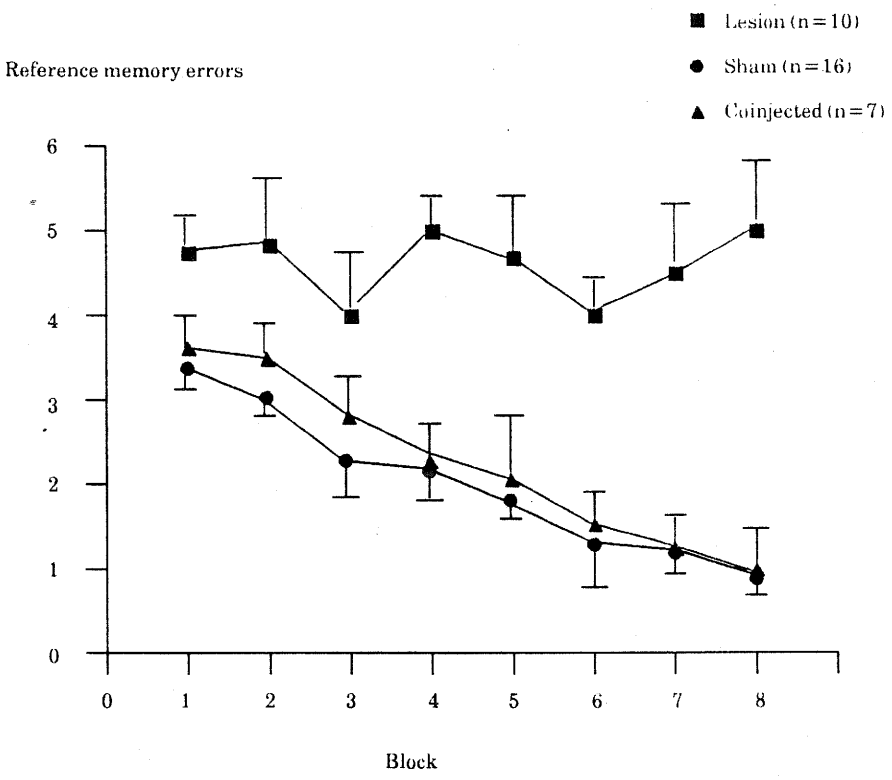

Fig. 4 Mean (± SEM) total number of reference memory errors for the lesion, co-injected and sham groups as described in the caption of Fig. 3. The main effect of group and group x block interaction were significant. Further analyses revealed that reference memory errors significantly decreased over blocks for both the co-injected and sham groups. The lesion group, by contrast, did not show a reliable decrease in reference memory errors. Post-hoc tests of group differences at each block revealed that the lesion group did not differ dignificantly from the other 2 groups on the first block but did on the subsequent blocks. the sham and co-injected groups did not differ reliably at any of the blocks.

In a similar study, Kesner et al. (1987b) recently reported that rats pretrained in a four-out-of-eight radial maze task and then undergoing bilateral ibotenic acid NBM lesions showed only reference memory errors when tested

postoperatively. However, they combined their data into ten-day blocks. Perhaps the groups were initially similar but the sham group improved over the ten days whereas the lesion group did not, possibly being impaired in relearning the task. Examination of their figure 6 supports the hypothesis that the sham group was improving. This profile of effects was observed in the Wirsching et al. (1988) study. As Wirsching et al. (1988) analyzed their data in four-day blocks they were able to detect the initial poor postoperative performance of the sham group. Possibly a similar effect in the Kesner et al. (1987b) study was not seen with the use of ten-day blocks. The failure of Kesner et al. (1987b) to observe working memory errors is more difficult to reconcile with our results. Perhaps with the animals making so many reference memory errors, the opportunity to make working memory errors was greatly reduced and consequently few were seen.

Wirsching et al. (1988) also tested a group of rats that received a co-injection of quinolinic acid (120 nmol in 1 µl) and another endogenous tryptophan metabolite, kynurenic acid (360 nmol in 1 µl) following pretraining. Kynurenic acid, like quinolinic acid, is an endogenous tryptophan metabolite; it is not neurotoxic on its own but has been found to antagonize the neurotoxic effects of quinolinic acid when co-injected with it (Boegman et al., 1984). Table I shows the percent decreases in cortical ChAT on the injected side compared to the uninjected side produced by various concentrations of kynurenic acid injected alone or co-injected with quinolinic acid (120 nmol) unilaterally into the NBM. Wirsching et al. (1988) found that kynurenic acid produced a total protection against the mnemonic effects of quinolinic acid (Fig. 3 and 4). These results were in good agreement with a preliminary report from our laboratories (Beninger et al., 1986).

Recent unpublished studies from our laboratories have investigated the ability of physostigmine or 3,4-diaminopyridine to improve memory in animals with quinolinic acid lesions of the NBM. The latter compound enhances the release of ACh, probably by blocking K^+ channels and prolonging depolarization (Glover, 1982), making it a candidate for treatment of disorders associated with impaired cholinergic function. Results (Fig. 5 and 6) showed that although physostigmine decreased working memory errors in a dose-related fashion, 3,4-diaminopyridine was without significant effect on working or reference memory errors. The physostigmine data further support the hypothesis that the mnemonic deficit seen after quinolinic acid lesions of the NBM is related to the loss of cortical ACh.

Yet another endogenous tryptophan metabolite, picolinic acid, is not strongly neurotoxic on its own and protects against the neurotoxicity of quinolinic acid when co-injected with it (Table I). In preliminary studies we have found that picolinic acid (360 nmol in 1 µl) produced only partial protection against the memory impairments seen following quinolinic acid (120 nmol in 1 µl). These studies of tryptophan metabolites are of particular interest because they raise the possibility that the memory-impairing loss of basal forebrain cholinergic cells in humans may be related to the action of endogenous excitotoxic substances. For example, a change in the concentrations of quinolinic acid and

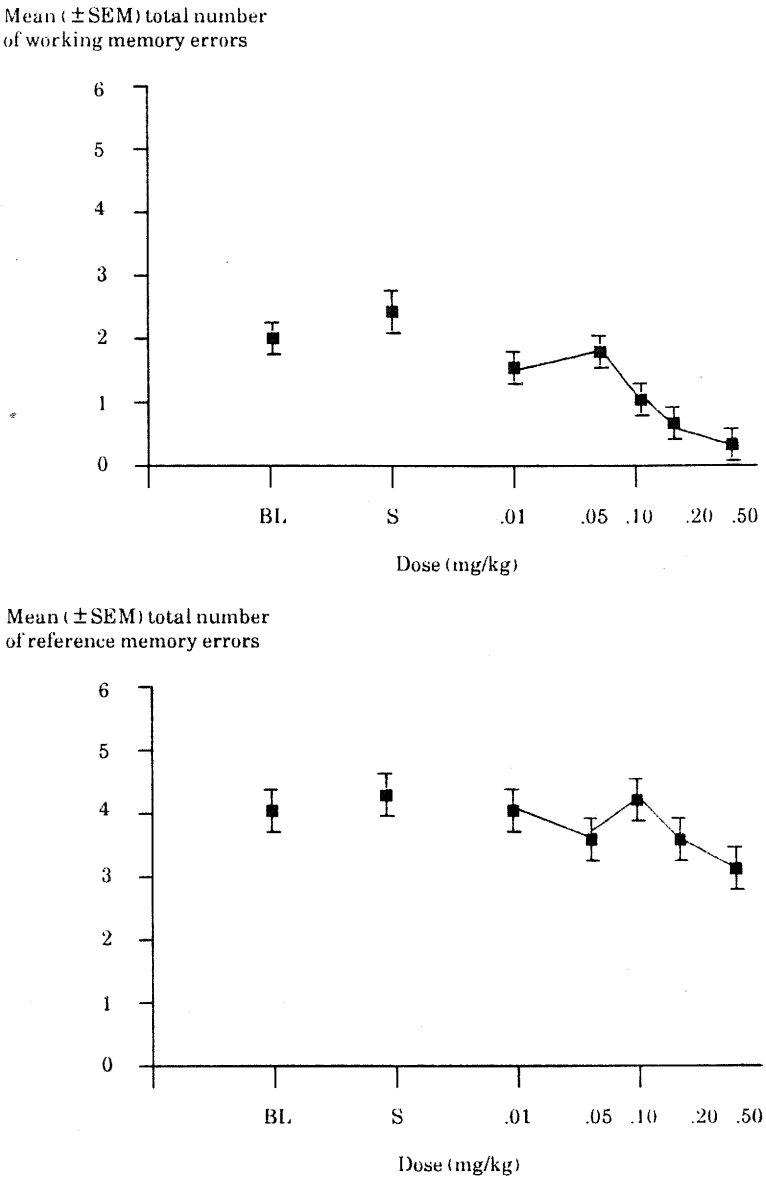

Fig. 5. Mean (± SEM) total type of error at the various doses of physostigmine. Error scores were based on the first four choices and summed over 4 days. Ten food-deprived rats were pretrained on a four-out-of eight radial maze task until choice accuracy stabilized over 4 days to a criterion of 87% correct. Rats then underwent unilateral infusions of quinolinic acid (120 nmol/1.0 ul) into the NBM. Following recovery, rats received physostigmine (0.0, 0.01, 0.05, 0.10, 0.20 or 0.50 mg/kg, ip) in a counterbalanced order. Drugs were given daily for 4 consecutive days followed by 4 non-drug baseline days. Physostigmine led to a significant dose-dependent decrease in working memory errors; reference memory errors were not significantly affected. BL: Baseline; S: Saline.

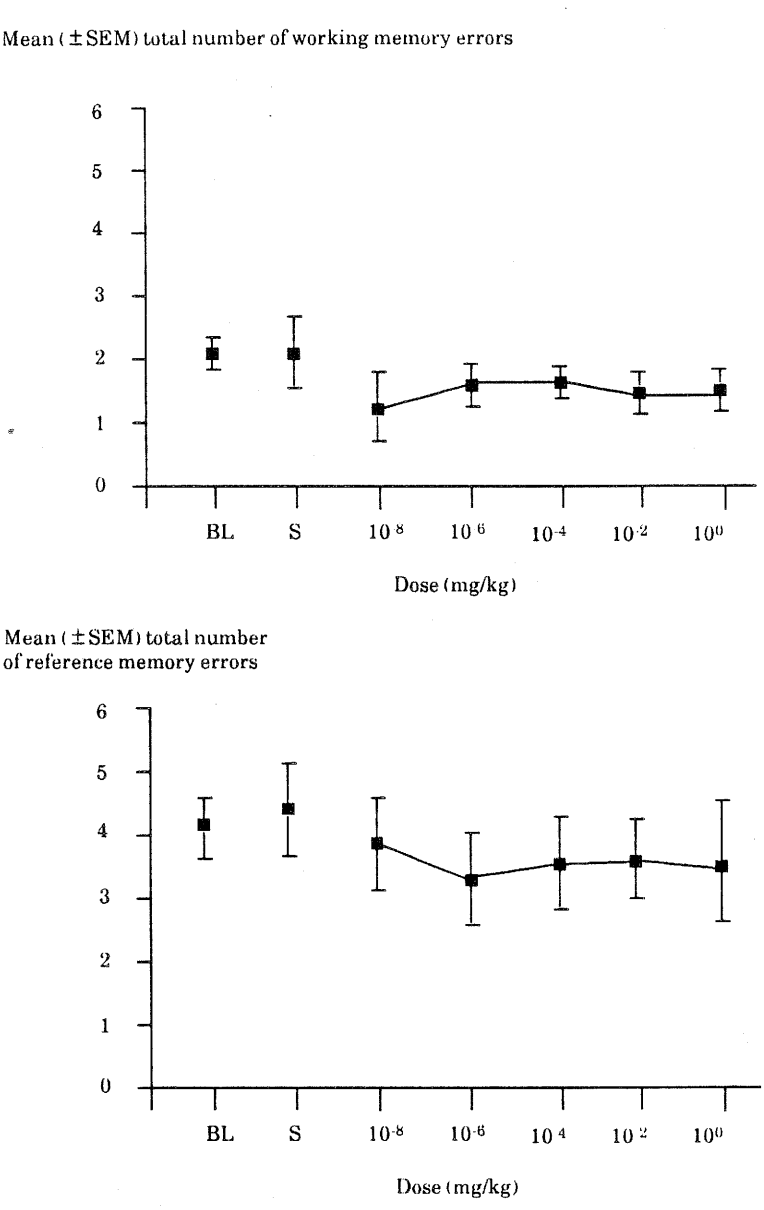

Fig. 6. Mean (± SEM) total type of error as a function of 3,4-diaminopyridine treatment. Error scores were based on the first four choices and summed over 4 days; Nine rats were pretrained and underwent unilateral quinolinic acid NBM lesions as specified in the caption of Fig. 4. Rats then received 3,4-diaminopyridine (0.0, 0.00000001, 0.000001, 0.0001, 0.01 or 1.0 mg/kg, ip) daily for 4 consecutive days followed by 4 non-drug baseline days. 3,4-diaminopyridine did not produce a reliable dose-dependent decrease in either working or reference memory errors. BL: Baseline; S: Saline.

kynurenic acid leading to an enhanced relative concentration of quinolinic acid may lead to the toxic action of this compound (Stone and Connick, 1985).

Finally, the ingenious work of Kesner et al. (1986) provides further support for the cholinergic hypothesis of working memory deficits. Rats were trained in an eight-arm radial maze with all arms baited until a high level of efficiency was reached. Animals than began "serial-order probe training". These trials consisted of first allowing the rat to collect all eight baits but in a predetermined order; this was achieved with the use of a series of doors around the central hub of the maze. Then the rat was given access to only two alleys. The task was to select the one that had been visited earlier in the sequence. Probes included a choice between arms one and two of the predetermined order, arms four and five or arms seven and eight. Control animals showed a classical serial position curve for performance on probe trials, they were more accurate at correctly selecting from the first two or last two arms of the sequence than the middle two. Correct performance of this task required remembering the sequence of arm visits during the initial part of the session, a working memory demand. Kesner et al (1986) observed that ibotenic acid lesions of the NBM that depleted cortical ACh impaired performance of this task. Recently, Kesner et al (1987a) replicated these findings on rats and reported in addition that humans with dementia of the Alzheimer's type showed the same profile of memory impairment in a task demanding recall of the order of a series of items.

Conclusion

The present review has restricted itself to animal studies of the role of brain ACh in memory that met the following two criteria. First, only studies in which the animals were trained on the task prior to any manipulation of cholinergic function were included. Thus, studies involving the acquisition of a task were not covered. Second, only reports that included methodologies allowing a clear separation of working and reference memory were reviewed. This separation was achieved either by employing two tasks that made differential demands on the types of memory or by employing single tasks that incorporated both a working and reference memory component.

Results from delayed matching or nonmatching tasks consistently showed that performance decreased with increasing delays between sample and test stimuli, a good indication that the tasks were assessing working memory. Studies of either rats or monkeys showed that scopolamine produced an impairment in this task that was delay-dependent. Those failing to find a treatment by delay interaction did not employ a no-delay condition. Failures to

find a differential effect of scopolamine at different delays in delayed alternation experiments may have been related to the same independent variable. Experiments utilizing alternation and discrimination tasks making differential demands on working and reference memory have consistently found that treatments leading to impaired hippocampal or cortical cholinergic function reduced choice accuracy in working but not reference memory tasks. In the radial maze, results generally showed that working memory was more impaired than reference memory following systemic anticholinergics or neurotoxic destruction of the NBM and physostigmine reversed these effects. Thus, there is good agreement from a number of different paradigms that the brain's cholinergic systems, in particular the basal forebrain cholinergic system, may play an important role in the control of working memory.

Finally, it has been found that the brain contains a substance, quinolinic acid, that can act as an excitotoxin when injected locally into the NBM. This substance can produce a lesion of cholinergic cells that leads to working memory impairments. Furthermore, other endogenous substances, kynurenic or picolinic acid can protect against the neurotoxic effects of quinolinic acid. Further studies of endogenous compounds may lead to the discovery of new possibilities for retarding the loss of cholinergic function that seems to be associated with working memory impairments accompanying Alzheimer's disease.

Acknowledgements

This chapter is dedicated to Tyrina and Steve; Preparation of the manuscript was supported by a grant from the Ontario Mental Health Foundation. The senior author is supported by a grant from the Ontario Ministry of Health.

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