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RAPID COMMUNICATION

Quisqualate Lesions of Rat NBM: Selective Effects on Working Memory in a Double Y-Maze

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BIGGAN, S. L., R. J. BENINGER, J. COCKHILL, K. JHAMANDAS AND R. J. BOEGMAN. *Quisqualate lesions of rat NBM: Selective effects on working memory in a double Y-maze.* BRAIN RES BULL **26**(4) 613–616, 1991.—Some authors have reported that quisqualic acid lesions of the nucleus basalis magnocellularis (NBM), although producing large cortical cholinergic losses, have little effect on memory. The purpose of the present study was to investigate the effects of quisqualic acid lesions of the NBM on working and reference memory in a double Y-maze. Each trial started with placement into one of the two end arms of the first Y-maze, and the correct response was to go down the stem (reference memory). Access was then given to the second Y-maze, the correct response being conditional upon the side of the first Y-maze from which that trial had begun (working memory). Rats were trained to an 88% correct criterion and were then given either bilateral quisqualic acid (60 nM, 0.5 μl) or sham lesions (0.9% saline, 0.5 μl) of the NBM. One week postsurgery, rats were tested on the double Y-maze task with delays of 0, 5 or 30 seconds being introduced prior to both the working and reference memory choice. NBM lesions produced a 63.2 ± 6.2% decrease of cortical choline acetyltransferase (ChAT) compared to unoperated controls. Delays affected only the working memory of the sham group. Rats with lesions showed a significant impairment of working memory at all delays, but no change in reference memory. Results indicate that quisqualic acid lesions of the NBM that produce significant reductions in cortical ChAT selectively impair working memory.

Quisqualic acid Nucleus basalis magnocellularis Working memory Reference memory Double Y-maze Acetylcholine

ANIMALS can be trained in tasks that allow a dissociation of memory for transient recent events (working memory) versus memory for components of the task that remain constant from trial to trial (reference memory) (2). It has been reported that excitotoxic lesions of the nucleus basalis magnocellularis (NBM), that produce large decreases in cortical cholinergic markers, differentially affect working and reference memory. This effect was shown with ibotenic (1, 7, 13), quinolinic (15), and kainic acid (3,8) and suggests that the cholinergic neurons of the NBM may be differentially involved in working and reference memory.

Some recent studies with quisqualic acid appear to contradict this conclusion. Thus, quisqualic acid lesions of the NBM, which resulted in cortical choline acetyltransferase (ChAT) decreases similar to those seen with other neurotoxins, failed to impair the acquisition of a conditional visual discrimination (12) or swim-maze escape task (11) or the retention of matching and nonmatching tasks (4). On the other hand, quisqualate lesions did impair reversal to the more difficult nonmatching task (4). Furthermore, it was found that quisqualate lesions impaired acquisition of a spatial navigation and passive avoidance task (5,11). Finally, it has been reported that quisqualic acid lesions of the NBM differentially affected acquisition of the working memory component of T-maze alternation and matching tasks (10,12).

Several of these studies (5, 10, 12, 14) compared the effects of NBM quisqualic acid to ibotenate and/or N-methyl-D-aspartate and clearly showed the latter two to produce greater impairments in memory in spite of similar depletions of cortical ChAT by all three excitotoxins. However, the mnemonic effects of quisqualic acid are still poorly understood. The purpose of the present study was to evaluate the effects of bilateral quisqualic acid lesions of the NBM on working and reference memory in the double Y-maze, a task that allows a clear separation of performance based on working and reference memory.

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METHOD

Subjects

Twenty male Wistar rats (Charles River, Canada), approximately 8 months old, weighed between 300–400 grams at the start of the study. All rats were maintained individually in a temperature-controlled environment (approximately 21°C) with a 12-hour light/dark cycle (lights on at 0700 h). Water was freely available in the home cage at all times. During training and testing, weights were reduced to 85% of their free-feeding level by daily feeding with measured rations.

Apparatus

The double Y-maze (see Fig. 1) was elevated 76 cm above the floor. The center stem of the maze was 55 cm long and 15 cm wide and each arm extended 35 cm from the stem at an angle of 120°. Removable wooden barriers could be inserted at the end of each arm and in the middle of the stem to provide 15-cm compartments. The floor consisted of steel grids spaced approximately one cm apart except at the junctions of the three arms where the floor consisted of a triangular piece of Plexiglas. The maze walls (15 cm high) and barriers were painted light gray. Plastic food containers were placed in the center of the end wall of the goal box of each arm and in the center of the end wall of the stem. Froot Loops cereal was used as a reward and pieces of the cereal were scattered under the grid floor to mask possible odour cues. Testing was carried out in a small room in which several visual cues (lights, door frame) were within sight of the maze.

Surgeries

All rats were trained in the double Y-maze task before surgeries and met criterion (see below). Eighteen rats were anaesthetized with an oxygen flow containing 4% halothane and then maintained during surgery on a 2% flow (Halocarbon, Malton, Ontario). Rats were placed in a stereotaxic apparatus with the incisor bar set at 3.3 mm below the horizontal plane passing through the interaural line. Using a 10-μl Hamilton syringe attached to an electric pump (Sage Instruments), bilateral microinjections (0.5 μl) of quisqualic acid (60 nM; 10 rats) or saline (0.9%; 8 rats) were infused at these coordinates: 2.6 mm lateral and 0.8 mm posterior to bregma and 8.0 mm ventral to the surface of the skull. Injections lasted 73 seconds and the cannula was left in place for 3 min to allow for diffusion. Once the cannula was removed, the hole in the skull was filled with bone wax and the scalp apposed with sutures. Rats were on free food for 5 days postsurgery and then were again food-deprived.

Procedure

Training. Food deprivation began five days before training. On day 4 of deprivation, rats were fed Froot Loops cereal in their home cages. Pieces of the cereal were subsequently used as food reward in the double Y-maze task.

Rats were given five days of habituation trials during which they were free to move throughout the maze for a 5-min period. During habituation, Froot Loops were scattered throughout the maze. Following this period, the rats were given one session of twelve trials per day, seven days per week, at approximately the same time each day. Each trial began by placing the rat in one of the end arms of the first "Y." The barrier was then removed and the rat was rewarded for going down the stem, the distal end of which was blocked by a removable barrier (see Fig. 1). Upon entering this region located in the middle of the stem, a barrier

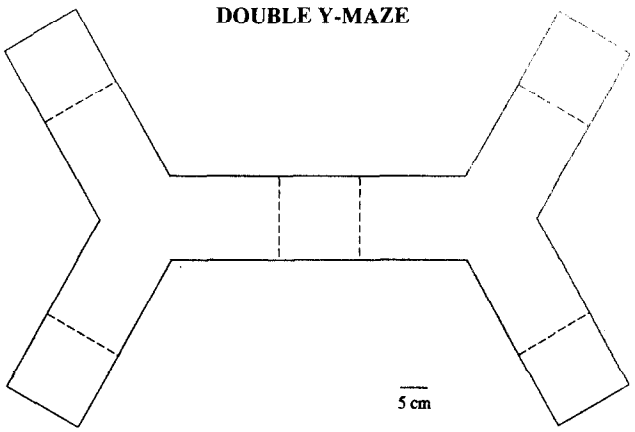

FIG. 1. Double Y-maze. Broken lines indicate manually operated barriers which could be used to restrict access to each part of the maze. The Y-maze to the left was where rats made the reference memory choice, and the Y-maze to the right was where the working memory choice was made.

was dropped into place behind the rat preventing reentry into the first "Y." The barrier in front was then removed to allow access into the second "Y." The rat continued up the stem, and was rewarded again for entry into the appropriate goal box of the second "Y."

The correct choices required the use of both working and reference memory. The reference memory component was to always go down the stem in the first "Y" and enter the start box in the middle of the stem regardless of which end arm of the first "Y" was the starting position. The working memory choice was counterbalanced among rats. For half, the working memory choice was to enter the arm of the second "Y" on the side of the maze opposite the side of the first "Y" from which that particular trial had begun. For the other half of the rats, the correct working memory choice was to enter the arm of the second "Y" on the same side as the side of the first "Y" from which that trial had begun. If an incorrect working memory choice was made, the rat was removed from the maze and that trial ended. Entry into the appropriate arm was considered correct when the rat's hind legs crossed completely onto the grid floor of the arm. Choice of the start location in the first "Y" varied randomly with the condition that no more than 6 trials per day be given from the same side. Training continued until the rats reached a criterion of at least 88% (32/36) choice accuracy on both memory components over a 3-day block.

In the reference memory component, entries into the arm of the first "Y" that was never baited were scored as reference memory errors. In the working memory component, entries into the arm of the second "Y" opposite the appropriate arm for that trial were scored as working memory errors. The number of working and reference memory errors was recorded daily for all trials.

Testing. Rats began testing one week postsurgery. The task remained the same as in training trials, the only difference being the use of variable delays (0, 5 and 30 s). The reference memory component of each trial began by placing the rat into one of the end arms of the first "Y" with the barrier in place for the appropriate delay. Once the rat entered the center region of the stem, the barrier behind the rat was dropped into place but the barrier in front was not removed until after the appropriate delay. The rat was then released into the second "Y" to make the working memory choice. The delay prior to each choice was the same for

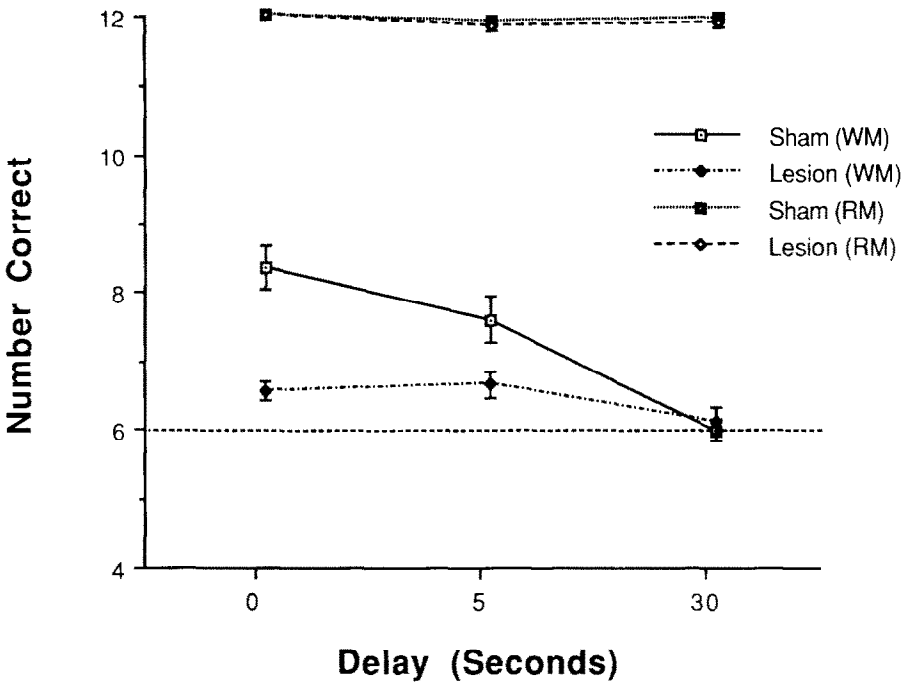

FIG. 2. Mean (±SEM) number of correct working (WM) and reference memory (RM) choices at 0, 5 and 30 s delays following either quisqualic acid or sham (saline) lesions of the NBM. Data have been collapsed across blocks. The horizontal broken line at 6 correct choices indicates chance performance. Analysis of working memory choices revealed a significant delay × group interaction ($p<0.01$); the lesion group performed poorly at all delays whereas the performance of the sham group decreased with increasing delay. There appeared to be little effect of the lesion or delay on reference memory.

any individual trial. Rats were given 12 trials per day, with 4 trials at each delay. Again, the choice of the starting goal box in the first "Y" was randomly determined, with the provision that no more than 6 trials per day began from either side. Rats were tested with delays for 15 days.

Biochemistry. Following the final test session, all rats were killed by decapitation and their brains removed. A section of the fronto-parietal cortex was dissected from each hemisphere and assayed for ChAT (6). Protein was measured according to the method of Lowrey et al. (9).

RESULTS

Biochemical analyses revealed a 63.2±6.2% cortical ChAT decrease in the quisqualic acid-injected group, while saline injections produced a ChAT reduction of 15±3.55% relative to uninjected control animals.

Working memory scores from 15 days of delay testing were subjected to 3-factor analyses of variance with variables being group (lesion, sham), delay (0, 5, 30 seconds), and 3-day block (5 blocks). The Greenhouse-Geisser adjusted degrees of freedom were used for variables involving repeated measures to reduce possible Type 1 error associated with violation of homogeneity of covariance assumptions. The analysis of working memory results (Fig. 2) revealed a significant effect of the lesion, $F(1,16)=6.67$, $p<0.03$, a significant delay effect, $F(1.51,24.10)=17.47$, $p<0.001$, and a delay by lesion interaction, $F(1.51,24.10)=7.43$, $p<0.01$. In a breakdown of the delay by group interaction, only the sham group showed a significant delay effect, $F(1.14,7.99)=17.61$, $p<0.001$.

Because of the small variances and high level of performance on the reference memory component, statistical analysis was not performed. However, considering the means (averaged over delays) for reference memory choice accuracy by both the lesion (Mean=11.95) and sham group (Mean=11.91), there did not appear to be any difference.

DISCUSSION

One of the defining characteristics of working versus reference memory is their differential susceptibility to delay effects. The observation in the sham group of a significant delay effect on the working but not reference memory component of the double Y-maze task supports the use of this paradigm for assessing these two types of memory. One advantage of the double Y-maze over other paradigms previously used to evaluate working and reference memory is that the task demands of each memory component are the same, viz., choose one of two arms.

The observation that quisqualate-produced cortical ChAT-depleting lesions of the NBM resulted in relatively selective deficits of working memory is in excellent agreement with findings in a split-stem T-maze (10,14) that allowed an independent assessment of the two memory types. These results are also in agreement with those showing intra-NBM quisqualate produced deficits in the acquisition of spatial navigation and passive avoidance learning (5,11).

Other findings are more difficult to reconcile with these results. It has been found that cortical ChAT-depleting lesions of the basal forebrain had little effect on the acquisition of a conditional discrimination in rats (12). Perhaps quisqualate-produced deficits are only seen with large cortical ChAT depletions. In the present study, frontocortical ChAT values were decreased by

more than 60% which is comparable to decreases of more than 70% (10,14) and 50% (5) in studies with similar findings. By contrast, ChAT decreases in studies which found no effect of quisqualate-produced lesions were 42.9% (12). Indeed, it was reported that when ChAT depletions were as high as 60%, learning deficits were produced (12).

The level of decrease in cortical ChAT following NBM injections of quisqualic acid may also be relevant to results which revealed no effect on pretrained matching and nonmatching tasks following ChAT decreases of about 55% (4). Although depletions in this range (5) or even lower [about 40% in (11)] were found to produce deficits in other studies, acquisition was being evaluated rather than performance of pretrained tasks. Perhaps another variable that interacts with the level of cortical ChAT depletions produced by quisqualate lesions of the NBM is the amount of pretraining or difficulty of the task. In support of this speculation, deficits in animals with NBM quisqualate lesions were seen when they were switched from the matching to the more difficult nonmatching task (4).

In four previous reports (5, 10, 12, 14) results clearly showed that quisqualic acid produced cortical ChAT depletions comparable to those seen following ibotenic acid, but significantly smaller mnemonic deficits. This provocative finding suggests that ibotenic acid may damage noncholinergic cells in the region of the NBM that also participate in memory.

The present and previous finding that cortical ChAT-depleting quisqualic acid lesions of the NBM produce mnemonic deficits are in good agreement with an extensive literature implicating ACh in memory (2). Although the differential mnemonic effects of various excitotoxins in the NBM suggest the possible involvement of noncholinergic cells, significant mnemonic deficits observed after quisqualic acid lesions strongly suggest that a rejection of a role for ACh in memory may not be justified.

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