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## Muscimol injections into the nucleus basalis magnocellularis of rats: selective impairment of working memory in the double Y-maze

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Anatomical and neurochemical results suggest that the cortico- and amygdalopetal cholinergic neurons of the nucleus basalis magnocellularis (NBM) may receive GABAergic inputs. The present experiments were undertaken to evaluate the possible influence of intra-NBM injections of the GABA<sub>A</sub> agonist, muscimol, on memory. In two experiments, rats were chronically implanted with guide cannulae placed bilaterally into the NBM. Rats were trained to a criterion of at least 83% correct on each component in a double Y-maze task that allowed a dissociation of working and reference memory. The task began with placement into one of the two end arms of the first Y-maze and the reference memory task was to go to the stem for food. Access to the second Y was then given and the working memory task was to go to the goal arm opposite the arm in the first maze from which that trial began. In experiment 1, pre-trained rats ( $n = 7$ ) received muscimol ( $0.5 \mu\text{l}$ ) in doses of 0, 0.01, 0.1 and  $1.0 \mu\text{g}$  in a counterbalanced order with re-training to criterion between injections. In experiment 2, pre-trained rats ( $n = 8$ ) received saline, muscimol ( $0.1 \mu\text{g}$ ), the GABA<sub>A</sub> antagonist, bicuculline ( $0.01 \mu\text{g}$ ), and muscimol + bicuculline. Results of experiment 1 revealed that intra-NBM muscimol produced a dose-dependent and differential impairment of working and reference memory. A dose of  $0.1 \mu\text{g}$  impaired working memory without significantly affecting reference memory; doses of  $0.01 \mu\text{g}$  and  $1.0 \mu\text{g}$  affected neither and both types of memory, respectively. In experiment 2, the differential mnemonic effect of  $0.1 \mu\text{g}$  muscimol was replicated and co-injection with bicuculline significantly reduced this effect. Results suggest that GABA in the NBM may modulate memory, possibly by influencing cholinergic cells there.

### INTRODUCTION

The cholinergic neurons of the basal forebrain and their target structures have been described in great detail in recent years<sup>35</sup>. There are now many data from laboratory experiments implicating these neurons in memory (for reviews see refs. 4,21,26). Thus, excitotoxic lesions of the medial septum, origin of a large cholinergic projection to the hippocampus, or the nucleus basalis magnocellularis (NBM), origin of a cholinergic projection to the cortex and amygdala, produce impairments in memory in a number of tasks<sup>2,5,18,33</sup>. As excitotoxins are not specific for cholinergic neurons, however, these results do not provide unequivocal evidence for the involvement of cholinergic neurons in memory.

Clinical data also implicate basal forebrain cholinergic neurons in memory; it was found that people who

died with Alzheimer's disease, besides showing the classical neurohistopathological signs, also had lost many basal forebrain cholinergic neurons<sup>13</sup>. The severe impairment in mnemonic function in these patients may be related to the loss of these cholinergic neurons<sup>3</sup>. This suggested a treatment strategy. Based on the success of dopamine replacement therapy for the treatment of Parkinson's disease, characterized by a loss of midbrain dopaminergic neurons<sup>6</sup>, it was thought that pharmacotherapies aimed at replacing acetylcholine in the brain of dementia patients might be similarly successful. However, in spite of many clinical trials, results have been disappointing<sup>1,12,20,31</sup>.

Sarter et al.<sup>31</sup> have suggested that the contribution of cholinergic neurons to mnemonic function may be related to their phasic activation rather than to their tonic stimulation of cholinergic receptors. They argued that the failure of cholinergic agents to augment mem-

ory in dementia patients may be related to their mechanism of action. Thus, anticholinesterases lead to a reduction in synthesis and release of acetylcholine, possibly uncoupling presynaptic activity from signal transmission; the direct stimulation of cholinergic receptors with muscarinic agonists may similarly interfere with the post-synaptic effects of physiologically released acetylcholine. Better therapeutic results may be seen with the use of agents that amplify the signal in the remaining cholinergic neurons<sup>31</sup>.

From this point of view, it is important to identify possible neurotransmitters or neuromodulators that may influence the activity of basal forebrain cholinergic neurons themselves as a first step to developing agents that may be more successful in treating mnemonic deficits in dementia patients. It is also necessary to show that these neurotransmitters or neuromodulators can influence mnemonic abilities. One candidate is the inhibitory neurotransmitter,  $\gamma$ -aminobutyric acid (GABA), which has been shown to influence the activity of cholinergic neurons of the NBM. Local infusion of GABA or the GABA<sub>A</sub> agonist, muscimol, was shown to lead to significant reductions in the level of acetylcholine release in target cortical areas<sup>9,34</sup>. Anatomical studies showed that GABAergic neurons also make synaptic contact with amygdalopetal cholinergic neurons in the NBM<sup>36</sup>. However, the mnemonic effects of basal forebrain infusions of GABAergic agents have not been widely tested.

In two studies, the effects of local injections of the GABA<sub>A</sub> agonist, muscimol, into the medial septum have been evaluated: Brioni et al.<sup>8</sup> found that learning of the Morris water maze task was impaired by intraseptal infusions of muscimol that also decreased hippocampal high-affinity choline uptake; Chrobak et al.<sup>10</sup> similarly found a dose-dependent impairment of working memory in a radial maze task. Although not ruling out a possible influence of muscimol on non-cholinergic neurons of the medial septum, these results might suggest that GABA influences the mnemonic function of cholinergic neurons projecting from the medial septum to the hippocampus.

Chronic unilateral infusion of GABA into the NBM of rats was found to lead to impairments in radial maze and passive avoidance learning<sup>22</sup>. Similarly, bilateral intra-NBM injections of muscimol impaired passive avoidance<sup>24</sup>. Another study reported that infusions of muscimol into the NBM led to impairments in a visual discrimination task<sup>14</sup>. The tasks used by these investigators did not allow an unequivocal conclusion that mnemonic abilities were affected. Impairments could have arisen as a result of non-mnemonic effects of the treatments; for example, infusions of GABA or musci-

mol could have affected motivation, perception or motor abilities of the rats<sup>25</sup>.

One task that allows for a more specific assessment of mnemonic effects of various treatments is the double Y-maze<sup>5</sup>. In this task, rats begin each trial from one of the end arms of the first Y-maze and must always enter the stem, common to both Y-mazes, to get food. They are then released into the second Y-maze where the choice of the correct (baited) arm is based on the starting position in the first Y-maze. The only way to perform the second portion of the task at a level better than chance is to remember the starting position on that particular trial; this has been defined as a working memory problem. The first portion of the task does not require the recall of recent information. The correct choice is invariant from trial to trial and from day to day, i.e. go to the stem regardless of starting position. This has been defined as a reference memory problem<sup>19</sup>. It is noteworthy that the motivational, perceptual and motor demands of each half of the double Y-maze task are identical; the components differ only in their mnemonic demands.

If a treatment is shown to selectively impair the working memory component of the double Y-maze task, it can be concluded that such a treatment affected the animals' memory. Previous studies have shown that the imposition of a delay between the two components of the task or lesions placed in the NBM using the excitotoxin, quisqualic acid, selectively impaired working memory<sup>5</sup>. In unpublished studies, we have found that systemic injections with the anticholinergic, scopolamine also selectively impaired working memory. Thus, the double Y-maze task provides a powerful tool for assessing the mnemonic effects of various treatments.

The purpose of the present studies was to assess the mnemonic effects of intra-NBM injections of the GABA<sub>A</sub> agonist, muscimol. In the first study, it was found that muscimol produced a dose-dependent impairment of working memory. The second study evaluated the ability of the GABA<sub>A</sub> antagonist, bicuculline, to reverse the working memory impairments produced by muscimol.

## MATERIALS AND METHODS

Treatment of the rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy, and was approved by the Queen's University Animal Care Committee.

### Subjects

Male Sprague-Dawley rats, 16 for each of experiments 1 and 2, were purchased from Charles River, Canada. Rats weighed between 200 and 225 g at the time of arrival and were individually housed in

hanging wire cages in a temperature controlled ( $21 \pm 1^\circ\text{C}$ ) colony room maintained on a 12-h light/dark cycle (lights on at 07.00 h). Water was available ad libitum in the home cage. Food was rationed daily to maintain the rats at 85% or 80% of their free-feeding weights, adjusted for growth, for experiments 1 and 2, respectively.

### Surgery

Surgery followed training in experiment 1 but preceded training in experiment 2 (see below). Rats were anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg, i.p.) and implanted bilaterally with chronic indwelling guide cannulae (0.64 mm diam) aimed at a site 1 mm dorsal to the NBM. With the incisor bar set at 3.3 mm below the horizontal plane passing through the interaural line<sup>28</sup>, co-ordinates for experiment 1 were 0.8 mm posterior to bregma, 2.6 mm lateral to the midline and 7.0 mm ventral to the surface of the skull. Corresponding co-ordinates for experiment 2 were 1.3, 2.6 and 6.8 mm, respectively. The cannulae were anchored to the skull with stainless steel screws and dental acrylic cement. Between injections the guide cannulae were occluded with stainless steel wire pins.

### Central injections

Two Hamilton microsyringes (10.0  $\mu\text{l}$ ) mounted in an infusion pump (Sage Instruments, Model 355) were used to infuse the drugs at a constant rate of 1.0  $\mu\text{l}$  per min. The two sides were injected in sequence. The volume of all injections, including vehicle, was 0.5  $\mu\text{l}$ . Injection cannulae, made of stainless steel tubing (0.31 mm diam) were cut to extend 1.0 mm beyond the tips of the guide cannulae and were attached to the microsyringes by polyethylene tubing. To ensure diffusion of the drug, the injection cannulae were kept in position for an additional 30–60 s following infusion.

### Drugs

Muscimol hydrobromide (Research Biochemicals Inc.) and bicuculline methyl bromide (Research Biochemicals Inc.) were dissolved in normal saline. Muscimol was freshly prepared daily and bicuculline was prepared immediately prior to each injection.

### 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, also 15 cm wide, extended 35 cm from the stem at an angle of  $120^\circ$ . Removable wooden barriers could be inserted at the end of each arm and in the middle of the stem to provide 15 cm<sup>2</sup> 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

(26 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 (removable) end wall of the stem. Froot Loops cereal was used as the reward and pieces were scattered under the grid floor to mask possible odour cues. Testing was carried out in a small room in which several visual cues (e.g. lights, door frame) were within sight of the maze.

### Procedure

**General training.** Food deprivation began eight days before the beginning of training. During the first five days, rats were handled daily and fed Froot Loops in their home cages. Pieces of the cereal were used subsequently as rewards in the double Y-maze. On the next three days rats received 10-min habituation sessions during which they were free to move about the maze.

Training sessions were conducted at approximately the same time each day, seven days a week. For the first 10 days, rats received approximately 40 (experiment 1) or 30 (experiment 2) trials a day. On the following days, as the animals began to acquire the task, the number of trials was reduced to 12 in experiment 1 and 24 in experiment 2.

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 the region located in the middle of the stem, a barrier was dropped into place behind the rat preventing re-entry into the first 'Y'. The barrier in front of the rat was then removed to allow access to the second 'Y'. The rat continued along 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 correct working memory choice was to enter the arm of the second 'Y' on the side of the maze diagonally opposite the side of the first 'Y' from which that particular trial had begun. (Previous studies from this laboratory have shown similar results when the working memory component required entry into the arm directly opposite the start location<sup>5</sup>. For simplicity, only the diagonally opposite condition was employed here.) During the first 10 training days, rats were allowed to make reference or working memory errors and then to enter the correct arm to obtain the Froot Loops reward. During the remaining trials and days, an incorrect choice was followed by removal from the maze. A choice was defined to have taken place when the hind legs crossed onto the grid floor of the arm. Choice of the start location in the first 'Y' varied randomly with the condition that 50% of the trials began from each side.

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.

**Experiment 1.** Training continued at 12 trials per day until choice accuracy reached a criterion of at least 88% correct on both working and reference memory components over a 3-day block. This required at least 32 out of 36 correct choices for each component over three days. Animals then underwent surgery for bilateral implantation of guide cannulae into the NBM (see Surgery section). Following recovery from surgery, approximately eight days of training were required to re-establish criterion.

A within-subjects design was used to examine the mnemonic effects of muscimol injections into the NBM. Each rat received four treatments, the order being different from animal to animal. The treatments were saline (0.5  $\mu\text{l}$ ) and three doses of muscimol (0.01, 0.1 and 1.0  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ). Originally, it was intended to give each treatment twice, once on each of two consecutive days, to provide a better assessment of the effects of muscimol. However, the animals did not tolerate the two consecutive injections very well (see below). After a total of 28 pairs of injections, it was found that the correlation between the number of correct working memory choices on the

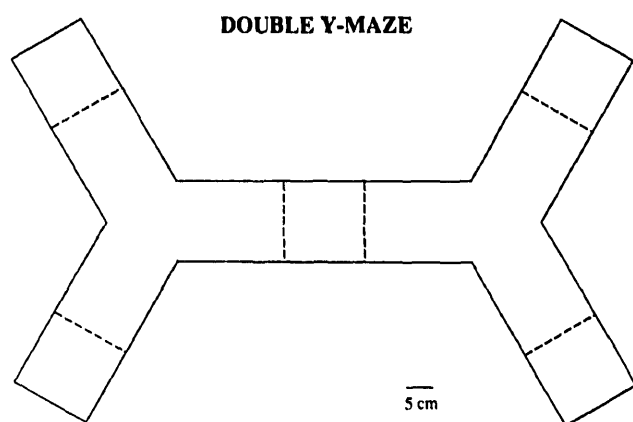


Fig. 1. Double Y-maze. Broken lines indicate manually operated barriers which could be used to restrict access to each part of the maze. Rats made the reference memory choice in the Y-maze to the right and the working memory choice was made in the Y-maze to the left.

first and second treatment days was 0.88 and the corresponding correlation for reference memory was 0.80. Therefore, it was decided to use only one day at each treatment for the remainder of the study. Animals received 3–8 sessions of training between injections to re-establish criterion level performance.

**Experiment 2.** Animals received bilateral cannulae into the NBM prior to food deprivation or any experience with the maze (see Surgery section). Training continued at 24 trials per day until choice accuracy reached a criterion of at least 83% correct on both working and reference memory components over a 3-day block. This required at least 60 out of 72 correct choices for each component over three days.

A within-subjects design was used to examine the mnemonic effects of muscimol and bicuculline injections into the NBM. Each rat received four treatments, the order being different from animal to animal. The treatments were saline (0.5  $\mu$ l), muscimol (0.1  $\mu$ g in 0.5  $\mu$ l), bicuculline (0.01  $\mu$ g in 0.5  $\mu$ l), and muscimol (0.1  $\mu$ l) + bicuculline (0.01  $\mu$ l); the combined treatment was given as a mixture in a single 0.5  $\mu$ l injection. The dose of muscimol was selected from experiment 1 where it was seen to produce a significant decrease in working memory performance without significantly affecting reference memory performance (see below); the dose of bicuculline was determined from pilot studies with three animals as the dose that produced minimal mnemonic effects when given alone. Animals received one day at each treatment. At least four sessions of training were given between injections to re-establish criterion level performance.

#### Histology

After the completion of behavioural testing, the rats were injected with a lethal dose of sodium pentobarbital and then perfused

intracardially with saline followed by 4% formalin solution. The brains were extracted and stored in 4% formalin for at least four days before being frozen and sliced into 50  $\mu$ m coronal sections. The sections were mounted on glass slides and stained with thionin to verify cannulae placements.

## RESULTS

#### Histology

**Experiment 1.** Of the original 16 rats, one died under anaesthetic. Of the remaining 15 rats, four animals receiving central injections of either saline or muscimol stopped eating and drinking and died within 24–72 h of the injections. Autopsies revealed several of these animals to have enlarged kidneys, blood clots in the lungs and enlarged cerebral vessels and ventricles. Some of these animals had received central injections on two consecutive days and that procedure was abandoned as discussed above. Data from these animals were not used in the analyses presented below. Locations of the cannulae tips of the remaining 11 animals, all having completed the training and testing protocol of experiment 1, are shown in Fig. 2A; seven were classified as hits and four as misses. All behavioural

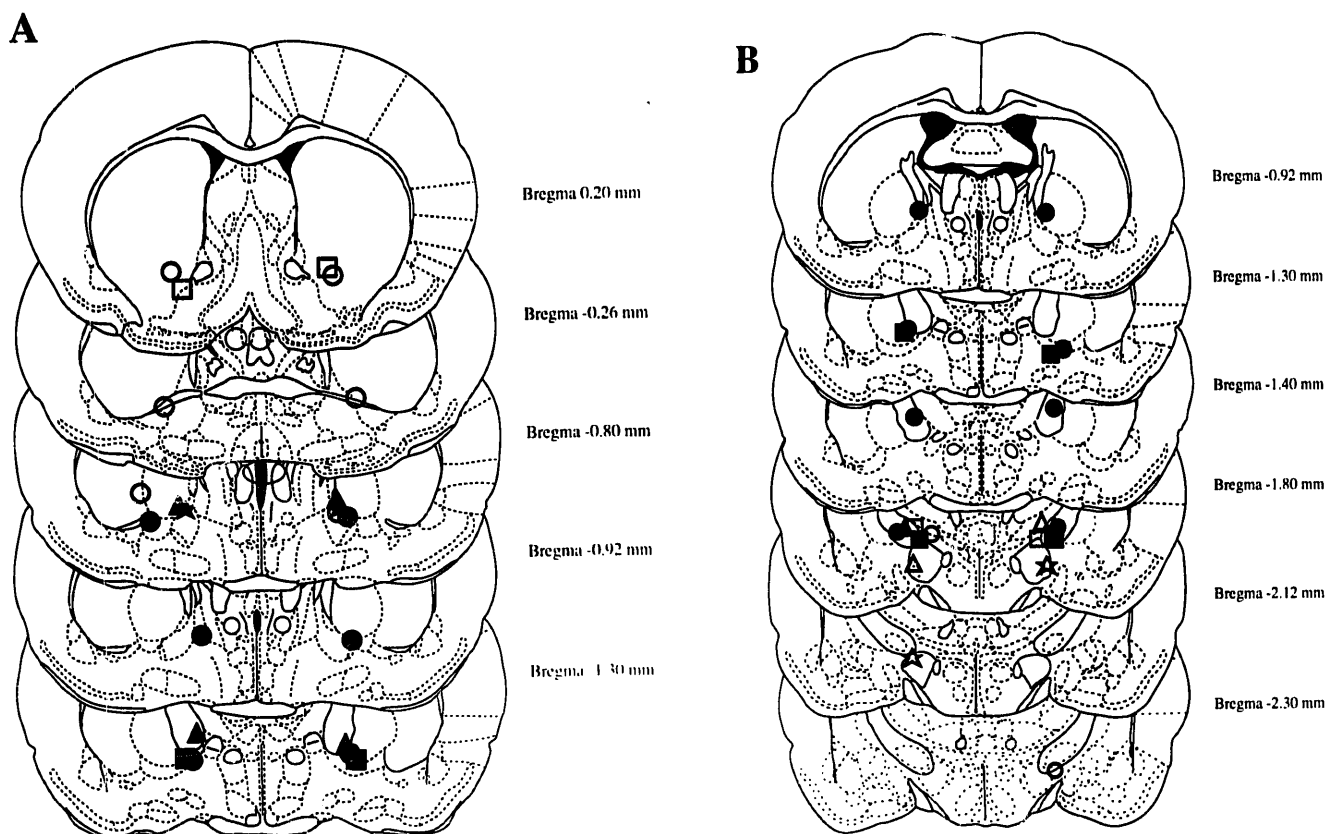


Fig. 2. Location of intracerebral injection sites. The position of the cannulae tips aimed at the nucleus basalis magnocellularis for experiments 1 and 2 are shown in A and B, respectively. Coronal sections were taken from the atlas of Paxinos and Watson<sup>28</sup>. The anterior–posterior co-ordinates, relative to bregma, are located to the right of the sections. Open symbols represent misses and filled symbols represent hits. Each rat was implanted with two cannulae and the shapes of the symbols (circles, squares, triangles, stars) indicate the pairs of placements for a particular rat.

analyses were based on the data of the seven animals classified as hits.

**Experiment 2.** Three rats failed to reach behavioural criterion and two lost their cannulae mounts prior to the completion of testing; therefore, histological examinations were carried out on 11 rats. Of these, eight had bilateral cannulae placements in the NBM and three were classified as misses (see Fig. 2B). A single injection was given for each treatment, as in the latter portion of experiment 1, and no health problems similar to the animals in experiment 1 were noted.

### Behaviour

**Experiment 1.** Some animals received some treatments on two consecutive days; in these instances, the percent correct working memory choices was averaged over the two days, as was the number of correct reference memory choices. The number of correct choices on each memory component each day was converted to a percentage of the total number of trials of that type. A baseline score was derived for non-treatment days by averaging together the percent of correct working memory choices for the three non-treatment days at criterion that preceded each of the four treatments. Thus, there were five scores for each rat for each memory type: baseline, saline, and muscimol 0.01, 0.1 and 1.0  $\mu\text{g}/0.5 \mu\text{l}$ .

Working memory was impaired in a dose-dependent manner by muscimol (see Fig. 3A). There was little effect of saline or the 0.01  $\mu\text{g}$  dose whereas the 0.1 and 1.0  $\mu\text{g}$  doses produced large decreases in the percent of correct responses. Reference memory choices were also impaired by muscimol but only at the 1.0  $\mu\text{g}$  dose (see Fig. 3B). Thus, a muscimol dose of 0.1  $\mu\text{g}$  selectively impaired working memory; a higher dose affected both working and reference memory, while a lower dose affected neither.

This description of the data was supported by statistical analyses. Repeated measures one-way analyses of variance (ANOVA), using the Greenhouse–Geisser adjusted degrees of freedom for repeated measures, of the percent of correct working memory choices revealed a significant treatment effect,  $F_{2,16,12.95} = 68.08$ ,  $P < 0.0001$ . Dunnett's post-hoc tests, comparing each dose to saline, revealed that 0.1 and 1.0  $\mu\text{g}$  doses of muscimol produced significantly less correct working memory responses than saline,  $P < 0.01$ . Similarly, the percent of correct reference memory responses was affected by treatment condition,  $F_{1,31,7.87} = 58.67$ ,  $P < 0.0001$ , and Dunnett's tests revealed that the 1.0  $\mu\text{g}$  dose led to a reduced number of responses compared to the saline treatment,  $P < 0.01$ .

**Experiment 2.** The number of correct choices on

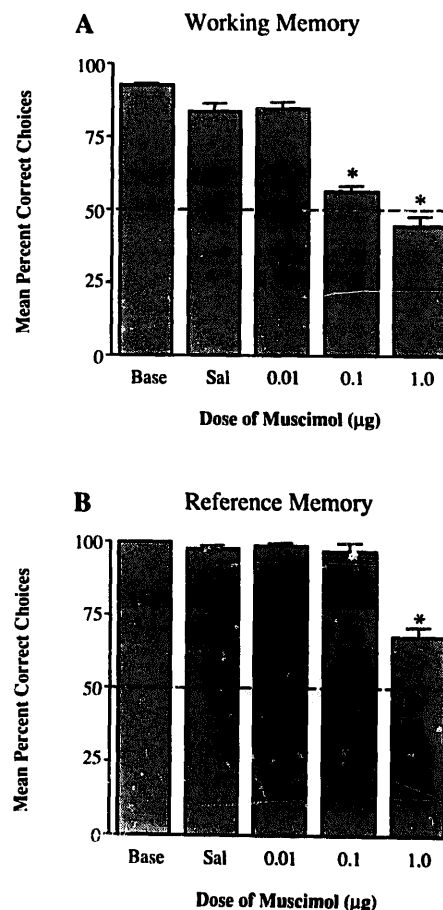


Fig. 3. Mean ( $\pm$ S.E.M.) percent correct working (A) and reference (B) memory choices in the double Y-maze in experiment 1. Data indicate baseline (Base) performance and the effects of saline (Sal) and three doses of muscimol. The broken horizontal line at 50 percent indicates chance performance. Both memory types were significantly ( $P < 0.0001$ ) affected by muscimol. However, a dose of 0.1  $\mu\text{g}$  differentially affected working and reference memory. \* Significantly ( $P < 0.01$ ) different from saline by Dunnett's tests.

each memory component each day was converted to a percent of the total number of trials of that type. There were four scores for each rat for each memory type: saline, muscimol (0.1  $\mu\text{g}/0.5 \mu\text{l}$ ), bicuculline (0.01  $\mu\text{g}/0.5 \mu\text{l}$ ) and muscimol (0.1  $\mu\text{g}$ ) + bicuculline (0.01  $\mu\text{g}$ ). Results are shown in Fig. 4A,B. None of the treatments affected percent correct reference memory choices. Percent correct working memory choices was reduced to chance level by muscimol (0.1  $\mu\text{g}$ ), replicating the effect of that dose in experiment 1. Bicuculline (0.01  $\mu\text{g}$ ) alone produced a small but significant impairment of working memory; however, this dose also produced a partial reversal of the mnemonic deficit resulting from muscimol.

This description of the data is supported by the results of statistical analyses. One-way repeated measures ANOVA, using the Greenhouse–Geisser adjusted degrees of freedom, of the effects of the four treatment conditions on percent correct working mem-

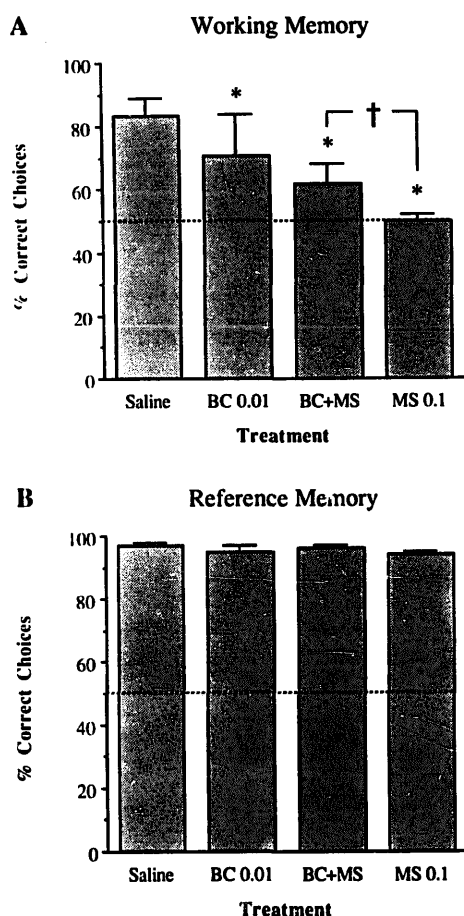


Fig. 4. Mean ( $\pm$  S.E.M.) percent correct working (A) and reference (B) memory choices in the double Y-maze in experiment 2. Treatments include saline, 0.01  $\mu$ g bicuculline (BC 0.01), bicuculline + muscimol (BC + MS), and 0.1  $\mu$ g muscimol (MS 0.1). The broken horizontal line at 50 percent indicates chance performance. Working, but not reference, memory was significantly ( $P < 0.0001$ ) affected by drug treatments. \* Significantly ( $P < 0.01$ ) different from saline by Dunnett's tests. † Significantly ( $P < 0.001$ ) different from each other by a planned  $t$ -test.

ory choices revealed a significant treatment effect,  $F_{1,67,11.69} = 32.21$ ,  $P < 0.0001$ . Dunnett's tests revealed that each treatment differed significantly from saline,  $P < 0.01$ . Planned comparisons of the muscimol alone treatment with the muscimol + bicuculline treatment revealed a significant difference,  $t_7 = 5.38$ ,  $P < 0.001$ , showing that bicuculline partially reversed the working memory deficit produced by muscimol. Finally, there was no significant treatment effect on the percent correct reference memory choices,  $F_{1,81,12.68} = 1.21$ ,  $P > 0.05$ .

## DISCUSSION

Results of experiment 1 revealed that the GABA<sub>A</sub> agonist, muscimol, when injected bilaterally into the NBM produced a dose-dependent impairment of both working and reference memory in the double Y-maze.

However, the two types of memory were differentially sensitive to muscimol. A dose of 0.1  $\mu$ g produced an impairment of working memory without significantly affecting reference memory while, the higher 1.0  $\mu$ g dose affected both memory types. The selective effect of the 0.1  $\mu$ g dose of muscimol on working memory was replicated in experiment 2. Results of experiment 2 also showed that the GABA<sub>A</sub> antagonist, bicuculline, partially reversed the working memory deficit produced by muscimol, confirming that the effects of muscimol on memory were mediated by GABA<sub>A</sub> receptors. These results show that GABA in the NBM modulates memory, but the use of agonists precludes definite conclusions concerning the function of endogenous GABA. Results might suggest that GABAergic modulation of NBM (possibly cholinergic) neurons significantly influences mnemonic function.

It is possible that a drug-induced generalized motor hypoactivity might differentially affect working and reference memory. Thus, if the animal was hypoactive and it took longer to travel from the end compartment of the first Y-maze to the working memory choice point of the second Y-maze, working memory might have been impaired because of increased interference or decay during the lengthened memory interval. Although latency data were not systematically collected in the present experiments, the effects of muscimol on working memory did not appear to result from a confounding effect of motor changes. In both experiments 1 and 2, doses of muscimol of 0.01 or 0.1  $\mu$ g did not have any apparent motor effect. Yet the 0.1  $\mu$ g dose differentially impaired working memory. The muscimol dose of 1.0  $\mu$ g did produce some slowing of completion of the reference memory component of experiment 1 but this appeared to result from an impaired ability to orient in space. This interpretation is supported by the observation that the 1.0  $\mu$ g dose of muscimol impaired both reference and working memory. The apparent mnemonic deficits seen in rats following bilateral intra-NBM injections of muscimol (0.1  $\mu$ g) were probably the result of an effect on mechanisms of memory rather than changes in motor activity.

Another possibility is that the differential mnemonic difficulty of the two components of the double Y-maze task may contribute to the observed differential effect of muscimol (0.1  $\mu$ g) on the two components. Thus, the reference memory component was learned to criterion relatively quickly; many more trials were necessary to learn the working memory component to criterion. It is not possible from the present results to rule out the possibility that the differential effects of muscimol on working and reference memory were related to the differential difficulty of the two tasks. However, one

study has addressed this question. Hepler et al.<sup>18</sup> trained rats on conditional discrimination in the reference memory component of a split-stem T-maze task and found that acquisition of this and the working memory component were similar. Subsequently excitotoxin lesions of either the medial septum or the NBM produced differential impairments of working memory. This result shows that differential difficulty of working and reference memory tasks does not provide an adequate explanation of the differential effects of manipulations of the neurons of the NBM on these two types of memory. It would appear that the selective impairment of working memory produced by muscimol (0.1  $\mu$ g) in the present experiments was not attributable to the relative difficulty of that component.

The results of the present experiments are in general agreement with those of other investigations of the possible mnemonic effects of GABAergic agents injected into the NBM. Thus, Majchrzak et al.<sup>22</sup> found that chronic unilateral infusions of GABA into the NBM of rats impaired performance in a radial maze task and acquisition of passive avoidance. Nagel and Huston<sup>24</sup> found that bilateral intra-NBM muscimol injected immediately following the training trial disrupted performance in a retention test of a passive avoidance task. Dudchenko and Sarter<sup>14</sup> trained rats on conditional visual discrimination; they found that intra-NBM muscimol dose-dependently impaired performance of this task. In the present experiment we found that muscimol, at the highest dose tested (1.0  $\mu$ g) impaired both working and reference memory in the double Y-maze. The observation of an impairment in the reference memory component is in agreement with the above findings but it is difficult to rule out possible non-mnemonic effects when reference memory apparently is being affected. For example, similar impairments might be seen if treatments influenced motivation, motor performance or perceptual abilities<sup>25</sup>. However, the present observation of differential effects of muscimol (0.1  $\mu$ g) on working and reference memory, in a task that places equal motor, sensory and motivational demands on the two components of the task, makes nonmnemonic interpretations difficult. Our previous report<sup>5</sup> that performance of the working but not the reference memory component of the double Y-maze task was impaired by the insertion of delays before the choice further shows that this task differentially assesses working and reference memory. It can be concluded that GABAergic modulation of NBM neurons significantly influences memory. As intra-NBM muscimol or GABA has been shown to depress the turnover of cortical acetylcholine<sup>9,34</sup>, the mnemonic effects of muscimol reported here may result from

depression of NBM cholinergic neurons. However, further studies are needed to evaluate possible effects of GABA agonists on non-cholinergic neurons of the NBM and the possible role of these neurons in memory.

The present results also generally agree with those of studies investigating the mnemonic effects of intraseptal muscimol. Chrobak et al.<sup>10</sup> trained rats in a working memory task on the radial maze and found that muscimol injected into the medial septum immediately following exposure to the to-be-remembered stimuli impaired recall in a subsequent test. Brioni et al.<sup>8</sup> found that intraseptal muscimol impaired acquisition of learning in a water maze. From these results, and those of the present and previous studies investigating the mnemonic effects of intra-NBM administration of GABAergic compounds, it is possible that the role in memory of both the septohippocampal and basocortical-basoamygdalar cholinergic systems is modulated by GABA.

In recent years the importance of basocortical cholinergic projections in memory has been brought into question (e.g. ref. 17). Particularly damning has been the observation that the excitotoxins, quisqualic acid or  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), although producing decreases in cortical cholinergic markers equal to or *greater* than those produced by excitotoxins such as ibotenic acid, following injection into the NBM, produced significantly *less* mnemonic impairment<sup>11,15,16,23,27,29,30,32</sup>. However, in neurochemical experiments we have found that ibotenic and quisqualic acid differentially affected basoamygdalar cholinergic projections. Whereas ibotenic acid destroyed basal forebrain cholinergic projections to the cortex and amygdala about equally, quisqualic acid more potently destroyed projections from the NBM to the cortex<sup>7</sup>. Anatomical<sup>36</sup> and neurochemical evidence<sup>9,34</sup> reveals the interaction of GABA afferents with both cholinergic projections. However, it is not clear which of the two projections plays a more significant role in the depression of working memory produced by muscimol. It will remain the challenge of future research to determine whether the NBM neurons projecting to the cortex or amygdala or possibly non-cholinergic neurons of the NBM are modulated by GABA with regard to its influence on memory.

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