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A Long-Acting Cholinesterase Inhibitor Reverses Spatial Memory Deficits in Mice

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SWEENEY, J. E., C. F. HÖHMANN, T. H. MORAN AND J. T. COYLE. *A long-lasting cholinesterase inhibitor reverses spatial memory deficits in mice.* PHARMACOL BIOCHEM BEHAV 31(1) 141–147, 1988.—The effects of the long-acting acetylcholinesterase (AChE) inhibitor, galanthamine, on spatial memory were investigated in mice. Mice received ibotenic acid or sham lesions to the nucleus basalis magnocellularis (nBM). Groups of nBM-lesioned and control mice were then trained on a modified Morris swim maze task. Each mouse was first placed on a platform and then into quadrants of the swim tank in a random order. Time required to find the hidden platform was measured. In different phases of testing, the animal had to find a platform that either remained in the same quadrant (reference memory component) or was moved daily (working memory component). The nBM-lesioned mice took significantly longer to find the platform as compared to controls on the working, but not on the reference, memory component of the task. Galanthamine (5.0 mg/kg, IP), given 3.5 hours before testing, improved performance on the working memory task in nBM-lesioned mice by 70% and strikingly impaired performance in controls. Galanthamine's ability to reverse cognitive deficits induced by nBM lesions and its comparatively long half-life suggest that it may be effective in treating the central cholinergic deficits in Alzheimer's disease patients.

Nucleus basalis lesions Acetylcholinesterase Spatial memory Mice Galanthamine Animal models for Alzheimer's disease

THE important role of central cholinergic neuronal systems in learning and memory has been recognized for a number of years (17). Pharmacological data demonstrate that central muscarinic receptor antagonists impair performance on memory tasks and cause an amnesia-like syndrome in both rodents and primates, including humans (7,18). Conversely, drugs that moderately increase central cholinergic activity enhance performance on memory tasks (5, 20, 38). More recently, lesion studies have assisted in identifying specific cholinergic systems involved in cognitive functions. In rodents and primates, the fronto-parietal cortex and hippocampus receive major cholinergic inputs from basal forebrain projections, the nucleus basalis magnocellularis (nBM) and medial septal area (MSA), respectively (27). Lesions of the nBM and MSA produce behavioral deficits in experimental animals tested on a variety of tasks including passive avoidance, T maze, radial arm maze, stone maze and water maze tasks (21,36).

Reduction of cholinergic markers in neocortex and hippocampus is the neurochemical deficit most commonly associated with Alzheimer's type dementia (AD) (10,13). Furthermore, the severity of cholinergic deficits appears to correlate with the degree of dementia and the density of senile plaques and neurofibrillary tangles in AD patients (14). Accordingly, one pharmacologic strategy for enhancing memory in AD patients has been to increase central cholinergic function by the use of inhibitors of acetylcholinesterase (AChE) to prevent the breakdown of acetylcholine (ACh). Several AChE inhibitors have been used to treat AD including physostigmine, tetrahydroamino acridine (THA), and heptylpyrolol heptylcarbamate (8, 30, 39). Physostigmine, the most widely studied of the AChE inhibitors, reverses scopolamine- and basal forebrain lesion-induced memory deficits in rodents and primates (1, 24, 25). Clinically, physostigmine has been shown to enhance short-term memory in some, but not all, AD patients (30). However, physostigmine suffers from a number of disadvantages which hamper its clinical utility and may account for some of the variable clinical results. The drug exhibits erratic absorption, low bioavailability, an unfavorable toxic to

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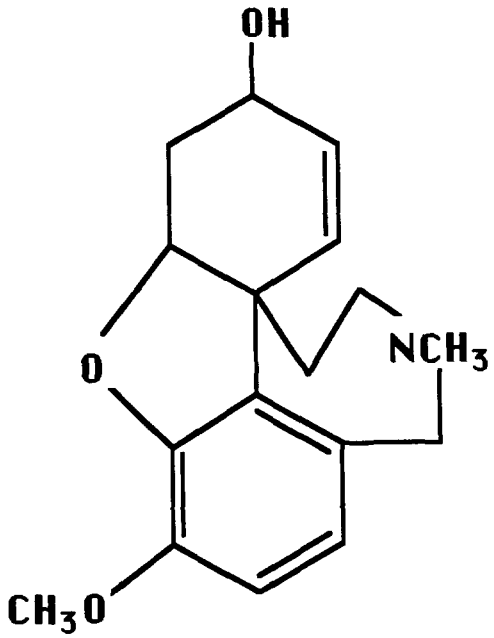

FIG. 1. The structure of galanthamine.

therapeutic ratio, and has a relatively short half-life of 20–30 minutes (16,42).

Galanthamine (Fig. 1) is a centrally-acting competitive AChE inhibitor with a half-life of 4–5 hours in man (12,41). It is a hydrolysis-resistant phenantridine derivative that appears to be more readily absorbed than physostigmine and it possess only moderate toxicity (2). In normal human subjects, galanthamine has been shown to reverse scopolamine-induced impairments including drowsiness, disorientation, delusions and hallucinations (4). In rats, galanthamine antagonized scopolamine-induced amnesia on a conditioned avoidance response (9). To further assess its efficacy in reversing cholinergic deficits, we have examined the ability of this long-acting AChE inhibitor to reverse behavioral deficits in nBM-lesioned mice using a modification of the Morris swim maze task (34).

METHOD

Subjects

Male Balb/cByJ mice (Jackson Laboratories) were 6–8 weeks old at the time of surgery and weighed 26–32 grams at the start of behavioral testing. Mice were housed in groups of 4–5 in standard rodent cages with free access to water and food. Animals were maintained on a 13 hour light/11hour dark cycle with light starting at 7:00 a.m.

Surgery

Bilateral nBM or sham lesions were produced in a two-staged surgical procedure to increase the survival rate of the mice. The first lesion made to the right nucleus, and following a two-week recovery period, a second lesion was made to the contralateral nucleus.

Each mouse was anesthetized with 3% halothane (Ayerst Laboratory Inc.) at a flow of 5–8 liters per minute. The stereotaxic surgical procedure is described elsewhere (28) and summarized here. After the scalp was incised, one hole was drilled anterior to the fronto-nasal suture. The nBM in each mouse was approached by lowering an angled injection needle through the olfactory bulb and moving it in an anterior to posterior and medial to lateral direction. Thus, neither hippocampus nor cortex were directly damaged by the injection. The lesion coordinates were 2.0 mm anterior to the fronto-nasal suture and 1.5 mm lateral to the midline. The needle was lowered to 8.0 mm below the skull surface and then retracted to 7.0 mm where the first of three injections of 0.2 μl ibotenic acid (or saline in sham-treated animals) were made. Two subsequent injections at 6.5 and 6.0 mm were made.

Destruction of the nBM area was produced with the excitotoxin, ibotenic acid, which ablates neuronal perikarya at the site of injection without damaging axons of passage (11). Ibotenic acid was dissolved in a small volume of 1 N NaOH and brought up to a concentration of 10 μg/μl in 0.1 M sodium phosphate buffer, pH 7.4 (if necessary, pH of total solution adjusted to 7.4 with 1 N HCl).

Behavioral testing began following a two-week postoperative recovery period.

Behavioral Testing

Pretraining. Groups of nBM-lesioned, sham-operated, and unlesioned mice were first trained to escape to a platform submerged 1 cm below the surface of 24–26°C milk-opacified water in a 16.5-cm diameter tank. On the first day, the platform was placed against the wall of the tank. Each mouse was placed onto the platform for 30 seconds. The mouse was then placed into a random area of the tank and allowed to swim back to the hidden platform. After climbing onto the platform, the mouse was allowed to rest for 20 seconds. This procedure was repeated five times.

On the second day, training was conducted in a 30-cm diameter tank. On the wall of the tank, above the water line, different black and white patterns were displayed in each quandrant. The platform was placed approximately 5 cm from the wall into the middle of one of the quadrants. The original position of the platform varied (either north, south, east or west) for each animal. First, the animal was placed on the platform for 20 seconds. Then, the mouse was placed sequentially into the middle of each quadrant and allowed to swim back to the platform with a 20 second rest in between each trial.

Training reference memory component. During this phase of training, the platform remained in the same position on each day for a given animal. Since the position in space to which the mouse had to swim did not change, this was considered the reference memory component of this task.

For five days, performance was assessed in a 72-cm diameter tank which contained the same patterns as the smaller tank in each of its quadrants (Fig. 2). The platform was placed approximately 10 cm from the wall in the middle of one of the quadrants. The position of the platform was the same as during pretraining for each animal.

First the animal was placed onto the platform for 20 seconds. Then the animal was placed into the middle of each quadrant, except the one that contained the platform, in a random order. Time to find the hidden platform was measured. If the animals did not find the platform in 120 seconds, it was placed onto the platform for 20 seconds.

Each animal received one training session per day which contained three trials, one from each of the three quadrants (not containing the platform). Training was conducted be-

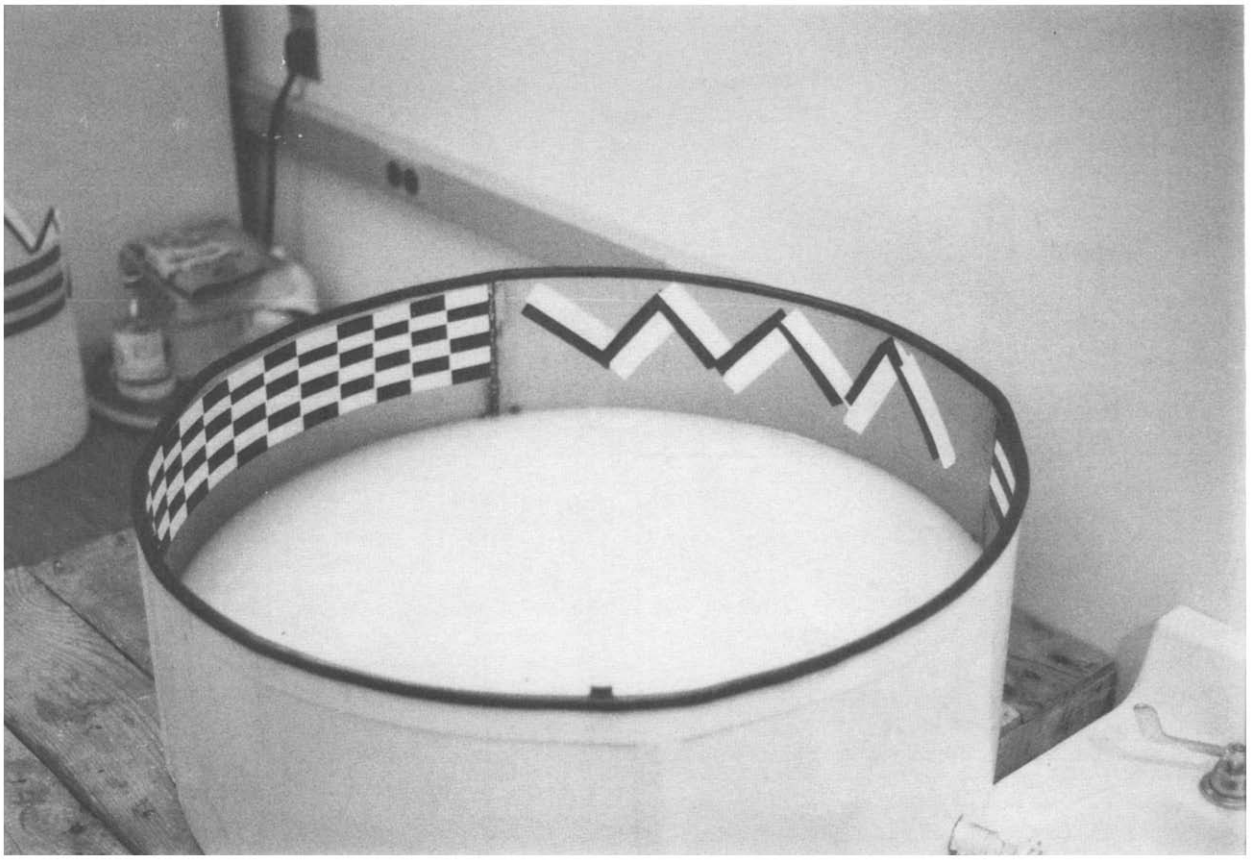

FIG. 2. Photograph of 72-cm diameter swim tank with intramaze cues.

tween 13:00 and 16:00 hours each day. A criterion level was chosen of ≤50 seconds/session for two consecutive days.

Working memory component. In this phase of testing, the position of the platform was changed daily. Since the position in space to which the animal had to swim changed daily, this was considered the working memory component of the task.

First, the animal was placed on the platform for 20 seconds for orientation. Then, as in the reference phase, the animal was placed into the three quadrants of the tank, except the one that contained the platform, in a random sequence. Time to find the hidden platform was measured.

Drug Testing

For the three groups of animals (nBM-lesioned, sham-operated and unlesioned), the effects of galanthamine on performance of the working memory task were assessed. Once an animal had reached a criterion level of performance on the reference component (day 6 after pretraining), each animal received a saline injection (0.1 cc, IP) one hour before the start of the working memory component of the task. The following day (day 7), the three groups received galanthamine (5.0 mg/kg, IP dissolved in saline to 2.0 μg/ml) 3.5 hours before behavioral testing. Each animal was retested 24 hours after administration of the drug.

Biochemistry

Within one week of the completion of behavioral testing, animals were sacrificed for biochemical and histological analyses to examine the efficacy of the lesions and the amounts of cholinergic depletion. Each mouse was decapitated, and the brain rapidly removed onto an ice-cooled metal plate. Tissue samples (approximately 17 mg/hemisphere) were taken from fronto-parietal cortex, not including the cingulate area, and stored at −70°C until the time of assay. The activity of choline acetyltransferase (ChAT) was measured by a modified method of Fonnum (23) using [^{14}C]-Acetyl Coenzyme A (New England Nuclear, 57.2 mCi/mmol; total Acetyl CoA concentration of 500 μM) as substrate. Subsequent separation of the reactants from the product was carried out via an organic ([^{14}C]-acetylcholine extracted into tetraphenyl boron): inorganic (Acetyl CoA into the aqueous phase) separation. Protein was measured according to the method of Lowry (32). All assays were performed in triplicate.

Histology

After removal of samples for biochemical analysis, the remaining brain tissue was fixed by submersion in 4% phosphate buffered formalin, pH 7.4 and 20% sucrose solution (w/v). Frozen brains were sectioned on a sliding microtome into 50 μm coronal sections. Sections through the lesion site were mounted and stained for Nissl substance.

Statistics

Behavioral data were analyzed by a repeated measures analysis of variance (ANOVA): the repeated measure was

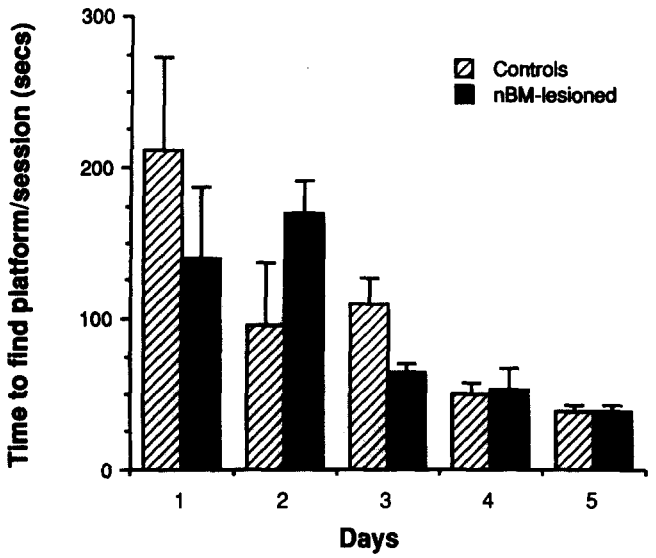

FIG. 3. Acquisition for the reference component of the swim maze task in nBM-lesioned (n=7) and control mice (n=11). The mean time to find the platform/session ±S.E.M. are presented.

the animal's performance at three different times: before treatment with galanthamine, 3.5 hours and 24 hours after its administration. Differences between specific means were assessed using planned *t*-comparisons.

Biochemical data were analyzed using a one-way ANOVA. The data were subjected, post hoc, to Dunnett's test to identify specific differences between the groups.

RESULTS

Behavior

Animals in the nBM-lesioned group that did not have at least a 15% decrease in ChAT activity as compared to controls were excluded from behavioral analyses because it was assumed that the lesion was not successfully located in the nBM region.

Reference memory component. Mean values for the time to find the platform/session were similar for the sham-operated and unlesioned groups. A two-factor ANOVA showed that the two groups did not differ significantly, $F(2,20)=0.79$, $p=0.39$. Therefore, data from these two groups of controls were combined for subsequent behavioral analyses.

Eighty-five percent of the animals in both the nBM-lesioned and control groups achieved criterion levels of performance within five days of the beginning of training (Fig. 3). The two groups performed similarly on the reference memory component of the task. The mean values for the time to find the platform/session for control and lesioned groups did not differ significantly, even though the controls were slightly better than nBM-lesioned animals on days 2 and 4 of the test. On day 5 of the reference component of the test, the mean time to find the platform/session was 38.0±4.0 seconds (±S.E.M.) for nBM-lesioned animals, and was 39.0±4.9 seconds for controls.

Working memory drug treatment. The performance of control and nBM-lesioned mice in the working memory component of behavioral testing depended upon the drug treatment (Fig. 4). There was a highly significant interaction

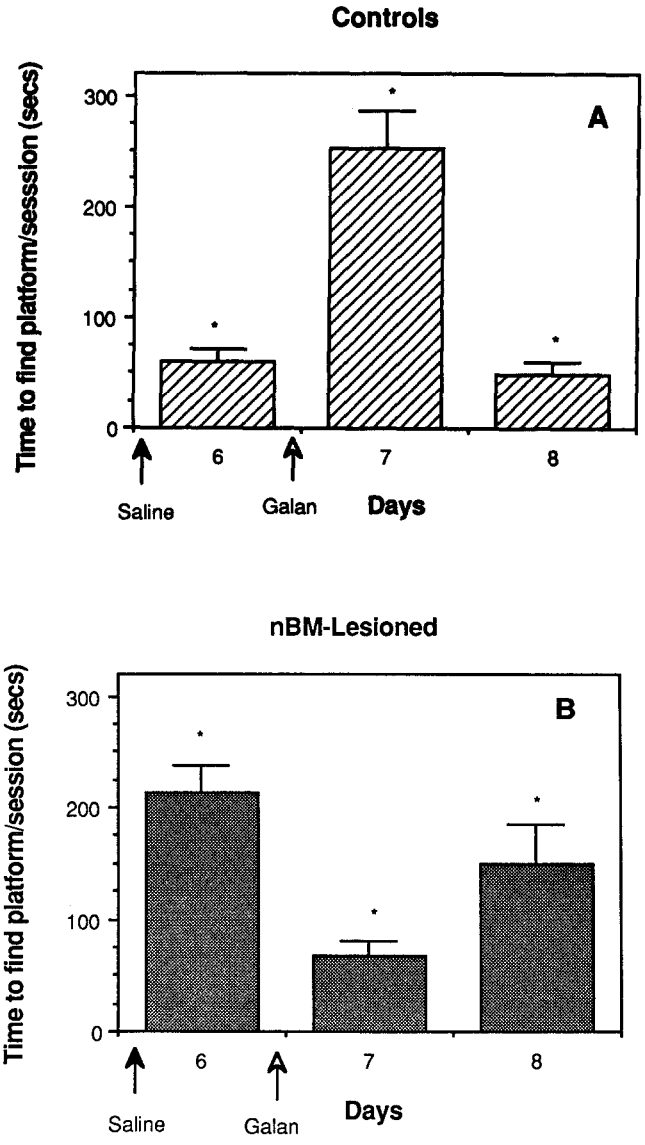

FIG. 4. Effects of saline (0.1 cc, IP, 1 hour before testing) and galanthamine (galan) (5.0 mg/kg, IP, 3.5 hours before testing) on the mean time to find the platform/session ±S.E.M. in control (A) and nBM-lesioned (B) animals on the working memory component of the task. *Repeated measure ANOVA, $F(2,26)=21.2$, $p<0.001$, for interaction effect nBM-lesioned mice vs. controls. Significant differences existed between the groups on day 6, $t(26)=2.92$, $p<0.01$; on day 7, $t(26)=5.44$, $p<0.001$; and on day 8, $t(26)=2.1$, $p<0.05$ (Planned *t*-test). Significant differences also existed before and after the galanthamine treatment in controls, $t(26)=4.65$, $p<0.001$; and nBM-lesioned animals, $t(26)=3.71$, $p=0.001$.

between lesion status and drug treatment, $F(2,26)=21.2$, $p<0.001$. On day 6 of testing, one hour after saline injections, the nBM-lesioned mice demonstrated a clear deficit relative to controls. The mean time to locate the platform/session was 62.3±11.2 seconds for controls and 212.7±25.2 seconds for nBM-lesioned animals ($t=2.92$, $p<0.01$).

Administration of galanthamine (5.0 mg/kg, 3.5 hours before testing) on day 7 significantly decreased the time to find the platform/session in nBM-lesioned animals by 70% to

TABLE 1

ChAT ACTIVITY IN FRONTO-PARIETAL CORTEX DETERMINED 6–8 WEEKS AFTER nBM LESIONS

Groups	ChAT Activity (nmol ACh/mg protein/hr)	% Change
Controls (n=8)	76.9 ± 3.2	—
Sham-Operated (n=3)	73.5 ± 2.5	↓ 0–5%
nBM-Lesioned (n=7)	57.4 ± 2.9*	↓ 15–34%

*F(5,17), $p=0.003$ (one-way ANOVA). nBMs vs. controls $q(10)=4.45$, $p<0.01$; nBMs vs. shams $q(10)=3.47$, $p<0.01$ (Dunnett's test).

67.9±13.4 seconds ($t=3.71$, $p=0.001$). Galanthamine injections produced the opposite effect in controls; the mean time to find the platform/session increased by approximately 400% to 252±35.9 seconds ($t=4.65$, $p<0.001$). The performances of the two groups under the galanthamine treatment were significantly different ($t=5.44$, $p<0.001$).

Twenty-four hours after the drug administration, the mean time to find the platform in controls decreased to 53.7±11.0 seconds, similar to predrug levels. In nBM-lesioned animals, the mean time to find the platform increased to 149.3±35.7 seconds. The two groups were significantly different on day 8 ($t=2.1$, $p<0.05$).

Biochemistry

Levels of ChAT activity in the fronto-parietal cortex were measured 6–8 weeks after surgery in the unlesioned, sham-operated and nBM-lesioned groups (see Table 1). ChAT activity was decreased significantly from 15 to 34% in nBM-lesioned animals as compared to controls. In two nBM-lesioned animals, there was no decrease in ChAT activity in the fronto-parietal cortex. It is interesting to note that in these animals, galanthamine impaired performance of the working memory task, similar to results in control animals.

Histology

In the lesioned animals, the needle tract could be followed from substantia innominata, just lateral to the anterior commissure, to the ventral medial globus pallidus. Gliosis around the needle tract and loss of magnocellular neurons indicated destruction of the nBM.

DISCUSSION

In our study, nBM-lesioned mice demonstrated severe impairment on the working memory, but not the reference memory, component of a swim maze task. Galanthamine (5.0 mg/kg given 3.5 hours before behavioral testing) significantly improved performance of the working memory task in the previously impaired nBM-lesioned animals and significantly impaired performance in controls.

The behavioral task that we have described has clearly distinguishable components assessing performance of both reference and working memory (35). During the acquisition/reference memory phases, the platform remained in the same position each day. Therefore, the rules of performing the task, and the position in space to which the mouse swam, did not change (trial-independent). In contrast, once the platform was moved and the animal was placed on the platform in the new position each day, it was required to remember where the platform was on that particular day and specifically where in space to swim to reach the platform (trial-dependent). The nBM-lesioned animals demonstrated severe impairment on the working, but not the reference, component of the task. In the acquisition/reference memory component, the nBM-lesioned mice demonstrated slightly more variability between days than controls; however, the difference was not statistically significant. Therefore, our study provides indirect evidence that in mice the nBM projection to the cortex is involved primarily in working and not reference memory. Other studies have shown that cholinergic projections to the hippocampus and cortex are involved in working memory, but not reference memory (26,35). However, conflicting data exist, demonstrating the involvement of the nBM projection in reference memory (31,33). These studies were performed in rats and the behavioral tasks were different; therefore, we are unable to compare our results directly to those previously reported.

One drawback to our study was that we were unable to record directly the path length that the mice were swimming. Therefore, we are unable to distinguish at this time whether the working memory deficits in the nBM animals resulted because they were unable to unlearn the first position once the platform was switched, or whether the deficit was in remembering the new position.

The groups of animals (nBM-lesioned and controls) performed similarly on the reference memory component of the task; therefore, the conditions were ideal to test the efficacy of galanthamine on the working memory deficit. Since the groups performed similarly during the first phase of the task, motivation, motor skills, visual acuity and other factors necessary to learn the task were assumed to be similar for the groups. The deficits noted between the groups became clear only on the working memory component of the task, and these specific deficits were reversed by galanthamine. Hence, the improvement seen in the nBM-lesioned animals was most likely related to improvement in memory and not factors, such as improved motor activity.

Other studies further support the hypothesis that we are looking at a memory related phenomenon and not merely alterations in motor activity. In one study, neostigmine, the pheripherally-acting AChE inhibitor, did not improve performance of nBM-lesioned rats on a passive avoidance task, while physostigmine, the centrally-acting compound, did (25). In another study, unilateral ibotenic acid nBM lesions did not affect the speed of swimming in rats (19).

Interestingly, administration of galanthamine severely impaired performance in sham-operated and unlesioned animals at the same dose that it improved performance in nBM-lesioned animals. The dose of galanthamine chosen was based on the highest dose that had produced a consistent behavioral effect in reported literature. It is possible that galanthamine, similar to other AChE inhibitors, exhibits an inverted U-shaped dose-response curve (22,25). In other words, at low doses, these drugs can enhance performance, but higher doses result in impaired performance. Similar findings have been noted in clinical studies in which large doses of physostigmine were given to normal subjects (3,15). Further studies are being carried out to determine the dose-response curve and duration of effect of galanthamine in experimental animals. One explanation for this inverted U-shaped

dose-response curve is that accurate performance of working memory tasks requires optimal levels of ACh at cortical synapses. If this is true, then either insufficient or excessive levels of ACh would impair performance (43). The former would occur if there was a loss of cholinergic input to the cortex after nBM lesions; the latter would occur if ACh breakdown was dramatically inhibited in normals.

ChAT depletion in the cortices in our animals ranged from 15 to 34% which is lower than those reported in many other studies. It is possible that there was recovery of ChAT activity in our animals by the time they were sacrificed 6–8 weeks after the first nBM lesion. Therefore, the ChAT activity reported may not accurately describe ChAT activity at the time of behavioral testing. Several studies report recovery of ChAT activity following unilateral nBM lesions (29,40) and bilateral lesions (37). However, conflicting evidence about the recovery of ChAT activity following bilateral nBM lesions exists (6).

In conclusion, this study provides compelling evidence that galanthamine (5.0 mg/kg, IP) can significantly improve performance of a spatial memory task in nBM-lesioned mice, even when given 3.5 hours before behavioral testing. Clearly, since dysfunction of cholinergic neurons is not the sole cause of the cognitive deficits seen in AD patients, an AChE inhibitor could not be expected to ameliorate all symptoms and restore functions to normal. Nevertheless, these data encourage the expectation that appropriate pharmacological manipulations of the cholinergic system may eventually be developed to alleviate some of the cognitive impairments associated with dementia, such as that seen in Alzheimer's disease.

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