1996

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APRIL 24, 1996

Thesis Submitted in Partial Fulfillment of a Research Honors

Acknowledgments

I would like to extend thanks first of all to my fellow "WAR" team members who helped with this project. Katherine Trickle, my partner, who put up with my mood swings and all the changes in mid course that our project has taken. Greg Tinkler and Jon Litwiller, whose help with radial arm testing was much needed and appreciated. Ed Schweitzer and William Cooper, Mary Kern and Sara Russel (the S&M girls), and Mathew Easterday and Trey Folkers, all of whom had their own projects but still managed to lend a hand.

I would also like to thank all the other people who contributed to this project. My thanks go to Lynn Worthington, the caretaker of the animal colony, and to Durwood "Dur" Cawley and Warren Johnson of the Physical Plant, who built the radial arm maze and the terrific "rat hotels."

But most of all, my thanks go to my advisor, Dr. Wayne Dornan, whose enthusiasm and commitment helped shape my own interest in neuroscience. I'm grateful for every single experience I've had in his lab, for I feel I am now well prepared for graduate school. He had the uncanny ability to see my full potential, and helped my realize it by never settling for anything but the best. And even though it made life busy, stressed, and sometimes downright insane, his support and encouragement helped me through it.

I shall dedicate this project to Greg Tinkler and Alex McCampbell. With them I spent endless days over summer break, dissecting and staining brains and discussing everything from religion to Pulp Fiction, and endless nights at Denny's, studying for genetics tests (because we'd been too busy testing animals to study earlier that week). They were my stress relief and my close friends. I couldn't have survived without them.

ABSTRACT

In this study I investigated the effects of 192-IgG saporin injections into the medial septal area (MSA) and nucleus basalis magnocellularis (NBM) on radial arm maze performance in the male rat. The results of the present study reveal that combined injections of 192-IgG saporin into the basal forebrain failed to disrupt RAM performance when compared to vehicle-injected controls. In addition, intraperitoneal injections using a muscarinic receptor blocker, scopolamine, failed to reveal a compensatory response of the cholinergic basal forebrain that may have explained the lack of behavioral effects of 192-IgG saporin. Consequently, the results of this study suggest that a selective reduction in cholinergic transmission in the basal forebrain is, by itself, insufficient to account for the functional impairments observed in spatial learning in the rat. These data do not support the use of 192-IgG saporin as a viable approach to the elucidation of the neuropathological mechanisms that are associated with the cognitive deficits seen in Alzheimer's Disease.

Alzheimer's disease (AD) is a neurodegenerative disorder that currently afflicts approximately 4 million people in the United States, with roughly 100,000 new cases diagnosed each year [19]. AD is characterized by progressive deterioration of memory, cognition, and personality [19]. Pathologically, the hallmarks of AD are the appearance of neuritic plaques and neurofibrillary tangles in various regions of the brain, and extensive neuronal damage and loss [40]. While progress has been made toward an understanding of the etiology of AD [12,18,39], currently there is no animal model that mimics the profound pathological and behavioral deficits that characterize the disease. For this reason, our laboratory has focused on the behavioral changes that occur in animals who receive bilateral injections of different fragments of ß-amyloid (the major constituent of the neuritic plaque, a pathological hallmark of AD) into the hippocampus. We have previously reported that bilateral injections of ß-amyloid in the hippocampus along with a subthreshold dose of the neurotoxin ibotenic acid induce a dramatic impairment in the acquisition of spatial learning in the rat [9]. Another approach that our laboratory has taken recently is to mimic the loss of the cholinergic projections to the hippocampus and cortex using a variety of lesion techniques and to determine how the loss of these fibers affects learning and memory in the rat. The latter approach is the focus of my project.

In AD patients, there is a decrease in the amount of cholinergic input from the basal forebrain to the hippocampus and cortex [15]. A correlation between this gradual depletion and the learning and memory deficits characteristic of AD patients has been reported in several studies [13,31,33]. The resulting theory, called the cholinergic denervation hypothesis of AD, infers a causal relationship between the loss of acetylcholine (ACh) in

the cortex and hippocampus, and the cognitive deficits. In support of the cholinergic hypothesis, these affected areas are intimately involved in learning and memory.

The most prominent projection in the mammalian basal forebrain is a projection from the medial septal area (including the diagonal band of Broca) to the hippocampus, in addition to a cortical projection that originates from the nucleus basalis magnocellularis [25]. Collectively, these two areas account for approximately 80-90% of the cholinergic input to the hippocampus and cortex respectively. Accumulating evidence from a large number of studies done in the rat reveal that disruption of the functional integrity of the cholinergic basal forebrain (CBF) projection to the hippocampus and cortex using cholinergic antagonists [22,30], or specific lesions of the medial septal area (MSA) and nucleus basalis magnocellularis (NBM), induce marked impairments of a variety of behavioral tasks, particularly those that involve spatial learning [2,6-8,14,26]. More specifically, considerable evidence suggests that, on the average, lesions of the MSA or NBM induce substantial spatial learning impairments on both the Morris water maze (MWM) and the radial arm maze (RAM). These impairments are associated with marked reductions in choline acetyltransferase (ChAT), which is an index of cholinergic activity.

In the past, many of the lesions of the MSA or NBM have been done using relatively non-selective techniques. For example, in addition to the inherent problem of damage to "fibers of passage," electrolytic lesions of the NBM often induce damage to surrounding area (e.g. globus pallidus), and not only reduce cholinergic transmitters, but other neurotransmitters as well. This is particularly problematic with MSA and NBM lesions as cholinergic neurons in these areas are scattered among a plethora of non-

cholinergic neurons. While the use of excitotoxins circumvent many of the problems inherent with electrolytic lesions, most induce considerable damage to adjacent structures via significant diffusion from the injections site. Not surprisingly, when studies have been done using the aforementioned techniques to assess the effects of lesions of the NBM and MSA on spatial learning in the rat, conflicting results have been reported. For instance, although marked impairments of spatial learning have been observed on performance of either the radial arm maze or morris water maze [23,26,27], others have found either no appreciable effects [24,32] or differential effects when assessing spatial learning with either the radial arm maze or the morris water maze [6] depending on the excitotoxin used. These studies have seriously questioned previous interpretations of spatial learning impairments induced by ibotenic acid in terms of cholinergic loss. Several investigators have suggested that the spatial impairments observed following excitotoxic lesions of the basal forebrain may be resulting from loss of non-cholinergic neurons [11].

Not surprisingly, there has been pressure to develop neurotoxins specific to the cholinergic system. One such neurotoxin, called ethylcholine aziridinium mustard ion (AF64A) was introduced by Dr. Israel Hanin about ten years ago. AF64A is selectively taken up by cells with choline uptake sites. AF64A produces reliable impairments on a variety of spatial tasks, and has been proposed as a model of the cholinergic denervation of AD [29,34]. More recently, saporin, a specific neurotoxin, has been developed. Saporin is a ribosome-inactivating protein derived from the fern *Saporin offininalis*, and when coupled to a monoclonal antibody against the p75 low affinity nerve growth factor receptor (NGFr) is a selective cholinergic toxin (192-IgG-saporin) [45]. Within the basal forebrain, the

p75 NGFr is located exclusively on cholinergic cell bodies in the MSA and NBM that project to the hippocampus and cortex, but not to the amygdala [16]. Intraventricular injections of 192 IgG saporin cause, by seven days, a permanent 80-90% depletion of acetylcholine levels in the cortex and hippocampus, while having minimal effects on other transmitter systems [5].

192-IgG-Saporin seems an idea tool for studying the components of the basal forebrain cholinergic system as they relate to memory, since it can be injected both intraventricularly and directly into the areas where a lesion is desired. However, although the neurotoxic effects of 192 IgG-saporin are specific, the effects of saporin injections into the basal forebrain on learning and memory have produced inconsistent results. In the first published study on the effects of 192-IgG saporin on spatial learning in the rat, Nilsson et al [30] reported that intraventricular injections of 192-IgG saporin which produced a 85-90% reduction of ChAT activity in the hippocampus, induced a long-lasting deficit on water maze performance in female rats when compared to controls. However, a subsequent study by Torres et al [41] found that, following intraventricular injections, there was a 70-90% depletion of AChE in the hippocampus and cortex but no deficits in water maze performance. A study by Baxter et al [3] showed that neither NBM lesions nor MSA lesions had any effect on water maze performance, although they too found a marked depletion of cholinergic markers. In contrast, Berger-Sweeney et al [4], after finding the same cholinergic depletions, found that the intraventricular and NBM lesions caused deficits in water maze performance, while the MSA lesion had hardly any effect. Clearly, the above studies indicate that despite consistent reductions of ChAT activity in the basal forebrain

following injections of 192-IgG saporin, a wide disparity of effects on spatial learning in the rat exists following injections of 192-IgG saporin. For example, thus far at least 4 studies have been published [3,4,37,38] that have reported a lack of effect on spatial learning in the rat despite a 70-90% reduction of basal forebrain acetylcholine. In the studies that have reported an impairment of spatial learning following intraventricular injections of saporin, all have noted that the effects observed may be due to loss of cerebellar NGFr-positive Purkinje cells following intraventricular injections of 192-IgG saporin. In a recent article published by Walsh et al [43], the authors conclude that although 192-IgG saporin is a highly selective cholinergic toxin, the secondary effects induced by intraventricular injections of 192-IgG saporin "makes the i.c.v. model of 192 IgG saporin problematic for studying the role of the CBF in normative behavior and in disease states." The authors further suggest that site-specific injections of 192-IgG saporin would provide a viable approach to model Alzheimer's Disease. In order to circumvent the problem of cerebellar Purkinje cell damage following i.c.v injection of 192-IgG saporin, while at the same time producing a cholinergic lesion that essentially destroys the cholinergic input to the hippocampus and cortex in the rat, we have employed a "combined lesion" technique where the animals receive stereotaxic injections into the medial septal area, and (bilateral) injections into the nucleus basalis magnocellularis. These injections produce very selective lesions of the CBF while at the same time avoiding the inherent problems associated with intraventricular injections of 192-IgG saporin.

In a previous study done in this lab using the "combined lesion" approach [10], we investigated the effects of 192-IgG saporin injections into the MSA, NBM, or combined

injections into the MSA and NBM on water maze and radial arm maze performance in the male rat. We reported a dissociation between the effects of 192-IgG saporin injections into the basal forebrain on the performance of the two tasks of spatial learning in the rat. Bilateral injections of 192-IgG saporin into the NBM, MSA, or combined MSA/NBM failed to disrupt water maze performance when compared to controls. In contrast, injections of 192-IgG saporin into the MSA, NBM, or MSA/NBM induced impairments on the radial arm maze task. Overall, the disruption of spatial learning observed in this study was relatively mild compared to deficits in spatial learning reported using less selective lesions of the cholinergic basal forebrain.

The current project attempts to expand upon the previous study. In addition to the single MSA and bilateral NBM injections, we added a second group that received two additional injections of 192-IgG saporin into the MSA (bilateral), to determine whether a more complete lesion of the MSA (and thus a greater depletion of acetylcholine) will disrupt performance of a radial arm maze spatial memory task.

All of the studies that have been published using saporin have assessed acquisition of spatial tasks. Perhaps more profound effects of selectively disrupting the cholinergic system would be observed in animals that have already learned the task. Therefore, in this study, in addition to more extensive lesions of the cholinergic basal forebrain, the animals were pretrained on the radial arm maze. After they established criteria, they were given the 192-IgG saporin injections and retested on the radial arm maze. The partially baited paradigm allows measurement of both reference memory (which arms are baited throughout all trials) and recent memory (the running list of which

arms have and have not been visited during a trial). A modified version of the RAM task was used in the second experiment, in which the trial was interrupted by a five-minute delay after the rat had visited three baited arms. This allows for measurement of two kinds of recent memory: retroactive (the rat's memory of which three baited arms it has visited before the delay) and proactive (its memory of arms visited during the postdelay session).

METHODS:

Animals

Thirty-four male Long-Evans rats, obtained from Harlan Sprague-Dawley, ranging in age between 4 and 6 months and weighing approximately 250-350 g were used in this study. The rats were individually housed in hanging stainless steel cages in a temperature-controlled environment (20°C) on a 10:14 light:dark cycle (lights on a 0600 and off at 2000). During radial arm maze testing before and after surgery, all animals were food deprived to 85% their ad libitum weight.

Surgery

Once all animals had established criteria (i.e., group means of < 2 recent memory errors and ≤ 1 reference memory errors), they were brought back to their ad libitum weights in preparation for surgery. The animals were assigned to four groups such that the mean performance of all measures (reference and recent memory errors, and choice latency) in every group was statistically identical. Since it has been demonstrated that injections of 192-IgG alone do not produce neurotoxic damage, or effects on spatial

learning [4,45], all control injections were done using the 0.05% sodium azide solution (0.6 µl/per injection). At this dose, sodium azide does not affect spatial learning [10]. Each rat was anesthetized with sodium pentobarbitol (50 mg/kg) and received stereotaxic injections via a 1 µL Hamilton syringe of 0.84µg/µL of 192-IgG saporin (Lot # 31795031, Chemicon) dissolved in a 0.05% sodium azide solution. One half of the animals received two bilateral injections into the nucleus basalis magnocellularis (AP: -0.8mm; ML: +3.6mm; DV: -5.9mm) and one injection into the medial septal area (AP: +1.8mm; ML: 0mm; DV: -6.0mm). The other half received these injections plus two additional injections into the medial septal area (AP: +1.8mm; ML: +1.0, -1.0mm; DV: -7.0mm). All coordinates are from dura.

In order to prevent backflow and minimize tissue damage, all injections took place over a three minute interval, and the needle was left in place after the injection for an additional five minutes. The needle was also raised and lowered over an interval of four minutes. The coordinates for all injections were empirically determined using the Atlas of Paxinos and Watson as a guide [35].

Apparatus and Behavioral Testing

Experiment 1: Standard RAM Task

The testing apparatus consisted of a partially baited 8-arm (5 arms baited) radial arm maze (RAM). Prior to surgery, all animals were trained to perform the RAM task to criteria, which was designated as making no more than two errors. The animals were exposed to a habituation period of four days, during which reinforcers (cheerios) were liberally scattered on the RAM. Three animals were placed on the center platform and allowed to explore for 5 minutes. Following the adaptation phase, each animal was tested

alone over a period of three weeks until it met criteria. The RAM consisted of 8 arms, of which 5 were baited with reinforcers. The baited and unbaited arms remained constant throughout the experiment. At the beginning of the RAM task, each animal was placed in the center of the maze and permitted to choose among the arms until it had successfully completed the task (obtained all five reinforcers), or until five minutes had elapsed. The following behavioral parameters were recorded: 1) recent memory errors: total number of reentries into any arms, 2) reference memory errors: first entry into arms that were never baited, and 3) choice latency, calculated by dividing total trial latency by the total number of choices.

Six days after the animals had reached criteria, stereotaxic surgery was performed. Following a two week recovery period, all animals were given at least three trials to reestablish a baseline performance on the RAM. If the animal did not complete the task within five minutes, it was removed from the maze and the data from that trial was not counted in the final analyses. Testing continued for a total of sixteen days, with a two-week break between the twelfth and thirteenth day of testing.

Experiment 2: Delay Condition

Following completion of the standard testing, there was a 5 minute delay imposed in the middle of the trial. The rat was allowed to find three of the reinforcers, then removed from the maze and made to wait five minutes in the carrier. The rat was then returned to the maze, and only the two of the original five arms remained baited (the two it had not previously visited). The rat was then allowed to run until it had found the remaining reinforcers. The following parameters from the postdelay session of the trial were

recorded: 1) Reference memory errors : entry into an arm that was never baited; 2) Retroactive memory errors: entry into an arm that had been baited and visited during the predelay session; 3) Proactive memory errors: reentries into any arm. Animals that did not finish either the predelay or postdelay task within five minutes were excluded from the analysis for that day. Testing continued for a total of nine days. There was an added distraction as well; in the break between experiment one and two there was a central "doorway" which included guillotine doors leading to each arm installed onto the maze. These were not used during the course of the remaining experiments and were kept open at all times.

Scopolamine Treatment and Behavioral Testing

Following the completion of the delay testing, all animals were randomly assigned to receive one of three drug treatments. They received an IP injection of either sterile saline, scopolamine at a low dose (0.03mg/kg), or scopolamine at a high dose (0.3 mg/kg). Fifteen minutes after the injections, the animals were again tested on the RAM, with a five-minute delay between the third and forth choices. Animals were tested again two days later, after the drug had worn off, to ensure that there were no carryover effects from the injections. They then received another different dose of the drug, followed by the RAM test immediately after and two days later. Animals in this way received two doses of the drug and one control injection.

Neurochemical Analysis and Histological Verification and ChAT Analysis:

The brains of the animals will be processed following completion of the testing.

RESULTS:

Of the 34 animals that began the experiment, one died of an infection unrelated to the saporin lesions during Experiment 2. That animal's data was included up to the point that it could no longer run (in block 2). That left 33 animals for data analysis: MSA (1)/ NBM (saporin), n=12; MSA (3)/ NBM (saporin), n=11; MSA (1)/ NBM (vehicle), n=5; and MSA(3)/ NBM (vehicle), n=5.

Experiment 1:

Animals were tested for sixteen days. All scores were summed and averaged over blocks comprised of two days per block. It was clear that the two control groups did not differ in their performance during the first few blocks, and separate ANOVA's by injection site in the control groups did reveal a non significant difference ($p > 0.05$). All subsequent data for the two groups were combined and the group collectively referred to as "controls."

The mean number of reference memory errors and recent memory errors during the eight blocks of standard RAM testing are illustrated in Figures 1a and 1b, respectively. The average latency per choice is shown in Figure 1c.

A 3 x 8 mixed ANOVA with blocks as the repeated measure and injection condition as the between measure revealed a significant main effect of injection site for reference memory errors, $F(2,64) = 6.12$, $p < 0.003$. However, the analysis revealed a non-significant block by injection condition interaction $F(14, 448) = 1.19$, $p > 0.05$. Pos hoc analysis revealed that on blocks 5 and 7, the MSA 1/NBM group was significantly different from the MSA 3/ NBM group, and on block 6, the control group was significantly different

than the MSA 3/ NBM group.

Analysis of recent memory errors revealed no main effect, $F(2,64) = 0.14$, $p > 0.05$, and no interaction, $F(14, 448=1.49)$, $p > 0.05$. This demonstrated that saporin injections into the MSA and NBM combined had no effect on recent memory as measured by the RAM task.

Analysis of choice latency showed both a significant main effect of injection site, $F(2,64) = 7.06$, $p < 0.002$. There was also significant block by injection condition interaction $F(14,448) = 2.28$, $p < 0.005$. Post hoc analysis revealed that, overall, the animals with 3 injections to the MSA and 2 to the NBM had significantly faster choice latencies than the other two groups on all blocks except 5 and 8. This is illustrated in Figure 1c.

Experiment 2:

Animals were tested for a total of nine days, and the data was combined into blocks of 3 days per block. Mean reference memory errors, retroactive errors, and proactive errors, are displayed in Figure 2a, 2b, and 2c, respectively.

A 3 x 3 mixed ANOVA with blocks as the repeated measure and injection condition as the between variable on reference memory errors revealed a non-significant main effect of injection condition, $F(2,99) = 0.15$, $p > 0.05$. There was also a non-significant block by injection condition interaction, $F(4,198)=0.25$, $p > 0.05$. The results show that 192-Saporin lesions of the MSA and NBM had no significant effect on performance of the RAM task when compared to controls, even after a delay of five minutes was imposed during the middle of the trial.

Likewise, analyses of retroactive errors and proactive errors also revealed non-significant main effects of injection conditions, $F(2,99) = 0.401$, $p>0.05$; and $F(2,99) = 0.08$, $p > 0.05$, respectively. It also revealed non-significant interactions of block and injection condition $F(4,198) = 0.34, P>0.05$; and $F(4,198)= 0.34$, $p>0.05$.

Analysis of choice latency revealed a non-significant main effect of injection condition, $F(2,99)=0.91$, $p>0.05$, and a non-significant interaction, $F(4,198)=0.90$, $p > 0.05$. (graph not shown).

Overall, these data indicate that 192-IgG saporin, injected into the basal forebrain, had no effect on any of the measures of this task in rats when compared to vehicle-injected controls.

Experiment 3:

Figure 3a, 3b, and 3c illustrate reference memory errors, retroactive memory errors, and proactive errors in animals which have received either saline or scopolamine injections fifteen minutes prior to the trial.

A 3 x 3 mixed ANOVA with injection condition as the first between variable and scopolamine dose as the within variable on reference memory errors revealed a non-significant main effect of injection condition, $F(2,30)=2.95$, $p > 0.05$, and a non-significant injection condition by scopolamine dose interaction $F(4,60)=0.25$, $p > 0.05$.

A similar analysis of retroactive memory errors also revealed a non-significant main effect of injection condition, $F(2,30)=0.17$, $p > 0.05$, as well as a non-significant injection condition by scopolamine dose interaction, $F(4,60)=1.15$, $p > 0.05$.

An analysis of proactive errors did reveal a significant main effect of injection site,

$F(2,30)=4.54$, $p < 0.02$, as well as an injection site by scopolamine dose interaction, $F(4,60)=4.54$, $p < 0.003$. Post hoc analysis of proactive errors, however, revealed that the interaction was only seen using the highest dose of scopolamine. The MSA 1/ NBM group made significantly more errors than did the MSA 3/ NBM or control groups. There were no differences between the MSA 3/ NBM and control group at the highest dose, nor were there any differences between groups at either the saline or the low scopolamine dose.

Analysis of choice latencies (graph not shown) did not reveal a significant main effect, $F(2,30)=1.86$, $p > 0.05$. It did not reveal any injection condition by scopolamine dose interaction either, $F(4,60)=1.09$, $p > 0.05$.

Overall, there were no significant differences between the 192-IgG saporin-lesioned animals and the controls at either the saline or the low dose of scopolamine. The only parameter significantly affected at the high dose was the proactive errors.

DISCUSSION:

The results of this study did not show any appreciable deficit of either recent or reference memory as measured by the RAM task following lesions of the basal forebrain using 192-IgG-saporin. The first experiment showed that the MSA 3/ NBM 2 group made significantly faster choices when compared to the two other groups, but otherwise there were no differences between groups on any other measures.

In the light of other studies using saporin, these results are neither surprising nor unexpected. Another study conducted in our lab at the same time as this one, in which saporin from the same batch was used to lesion the MSA only, revealed that the saporin caused a marked depletion of AChE levels in the hippocampus, showing the saporin was working. We are thus reasonably certain that there was a marked cholinergic depletion in our animals, although we have not yet confirmed this with either neurochemical or histological analyses.

There are a number of factors that may explain our not seeing an impairment. A study by Waite et al [42] concluded that at least a 85-95% depletion of ChAT was necessary before a behavioral impairment would be seen. They compared it to the >90% loss of dopaminergic neurons needed before the behavioral symptoms of contralateral turning become apparent (in the rat model of Parkinson's Disease). They suggest that perhaps this is the reason that the quisqualic and AMPA lesions of the NBM also did not produce a behavioral deficit, since they only depleted ChAT by 70-80%. It is possible that, even in our animals with five lesions, we did not induce a large enough depletion of ACh to see a deficit and that the brain was able to compensate for the loss.

If this was the case, however, we expected that we would see an impairment following injections of a subthreshold dose of scopolamine, just as Steckler et al had expected [20]. We believed that scopolamine would cause a greater deficit on the RAM in the 192-IgG saporin lesioned animals than in controls. In our other study, rats with MSA lesions only did show greater memory deficits when given subthreshold doses of scopolamine. Since the subthreshold dose had no measurable effects on our rats, we cannot conclude anything from that experiment.

However, the "compensation" explanation does not explain why such profound deficits are seen with general antagonists like scopolamine or other excitotoxins such as ibotenic acid, which produce the same amount of cell loss and cholinergic depletion as quisqualic acid and AMPA. One plausible explanation is that scopolamine affects peripheral ACh receptors as well. These include cholinergic systems controlling salivation, heart rate, gastrointestinal motility, temperature, blood pressure, and efficient functioning of the lungs and bronchial tract [1]. Animals on high doses of scopolamine not only exhibit motor and breathing problems, but their dry mouths prevent them from eating the reinforcers. In this study, only 50% of the rats on the high dose of scopolamine were able to complete the task. Thus, the observed deficits may be less attributable to memory deficits, and more due to the side effects.

Another explanation is that excitotoxic damage is nonspecific. In fact, nearly every study using saporin that has found behavioral deficits has used either intraventricular injections (known to affect cerebellar Purkinje cells) or has been at a high enough dose to affect other neurotransmitters as well [43]. These results, along with numerous studies

involving manipulations of other transmitters in combination with acetylcholine, clearly show the involvement of other neurotransmitter systems in learning and memory. There is evidence for interactions between the cholinergic system and other systems such as serotonergic, GABAergic, and noradrenergic systems [6,17,28,36]. Numerous studies have also shown that lesions to the NBM produce deficits on attentional tasks, leading Wellman et al [44] to conclude that the deficits induced by NBM lesions may be mediated by attention and motivation.

Given the evidence against the exclusive role of ACh in learning and memory, it is somewhat surprising that most of the therapeutic drugs currently being tested for AD are specific to acetylcholine. In fact, drugs such as tacrine (Cognex), physostigmine, velmacrine, and others are currently being used to treat patients with the disease [21]. These drugs work by increasing the amount of acetylcholine available in the affected areas of the brain, and they are meeting with limited success. Part of the reason for the failure of these drugs is that their mode of action is presynaptic. As AChE inhibitors, they prevent the breakdown of ACh and allow more of it to remain in the cleft. Unfortunately, in Alzheimer's Disease, the presynaptic fibers are degenerating. Thus there is less ACh present to begin with. AChE inhibitors may be useful in early stages of the disease, but for later stages there is not enough ACh for them to act on.

One final possibility for our not finding behavioral deficits was that we may not have been using the right tests. As mentioned earlier, most of the saporin studies tested acquisition. We decided to test retention, our rationale being that any deficits we found would be due to the lesion, not due to a slower-learning group. By pretraining the rats,

we may have made the task too familiar. If the MSA and NBM are involved in acquisition and consolidation of events, then we of course would not have seen deficits. The second experiment was designed to control for that effect by introducing a new trial-specific element by interrupting the task and forcing the rats to remember where they had been before on that day. It may be that five minutes was not a long enough delay for effects to be seen.

In conclusion, further studies on the interaction of neurotransmitter systems and the cholinergic system are warranted. While selective depletion of acetylcholine by saporin is not in itself a good model for AD, it will be incredibly useful in conjunction with toxins specific to other systems for determining these possible interactions. We plan to study the GABA system next, by giving these animals a GABA enhancing drug and testing them on the water maze.

FIGURE LEGENDS:

Figure 1:

A: Effects of combined immunolesions using 192-IgG Saporin on mean number of reference memory errors (entries into unbaited arms) during a standard partially-baited RAM task by male rats.

B. Effects of combined immunolesions using 192-IgG saporin on mean number of recent memory errors (reentries into arms) during a standard partially-baited RAM task by male rats. Mean number of recent memory errors (reentries into arms). **C**. Effects of combined immunolesions using 192-IgG Saporin on the average latency per choice (total trial latency divided by number of choices) during a standard partially-baited RAM task by male rats. Each rat received one trial per day for sixteen days. Vertical lines represent standard errors. Groups are as follows: MSA 1/ NBM 2, n=12; MSA 3/ NBM 2, n=12; Controls, n=10. (*) indicates significantly different from the MSA 3/ NBM 192-IgG saporin lesioned group. (**) indicates significantly different from both other groups. (***) indicates significantly different from the MSA 1/ NBM 192- IgG saporin lesioned group.

Figure 2

A: Effects of combined immunolesions of the MSA and NBM on the mean number of reference memory errors (entries into arms that were never baited) during performance of a standard RAM task with a five-minute delay between the third and forth choices. **B:** Effects of combined immunolesions of the MSA and NBM on the mean number of retroactive memory errors (entries into unbaited arms that were baited and visited during

the pre-delay session of the trial) during performance of a standard RAM task with a five-minute delay between the third and forth choices. **C**: Effects of combined immunolesions of the MSA and NBM on the mean number of proactive errors (reentries into any arm during the post delay session) during performance of a standard RAM task with a five-minute delay between the third and forth choices. Vertical lines represent standard errors. Groups are as follows: MSA 1/ NBM 2, n=12; MSA 3/ NBM 2, n=11; Controls, n=10.

Figure 3:

A: Effects of two doses of scopolamine on the mean number of reference memory errors (entries into arms that were never baited) during performance of a standard RAM task with a five minute delay by rats with combined immunolesions of the MSA and NBM. **B:** Effects of two doses of scopolamine on the mean number of retroactive memory errors (entries into unbaited arms that were baited and visited during the pre-delay session of the trial) during performance of a standard RAM task with a five minute delay by rats with combined immunolesions of the MSA and NBM. **C**: Effects of two doses of scopolamine on the mean number of proactive errors (reentries into any arm during the post delay session) during performance of a standard RAM task with a five minute delay by rats with combined immunolesions of the MSA and NBM. Each rat received one trial per day for nine days. Vertical lines represent standard errors. Groups are as follows: MSA 1/ NBM 2, n=12; MSA 3/ NBM 2, n=11; Controls, n=10. (*) indicates that this group was significantly different from both of the other two groups.

REFERENCES:

1. Andrews JS, Jansen JHM, Linders S, Princen A. Effects of disrupting the cholinergic system on short term spatial memory in rats. Psychopharmacology 1994; 115:485-494.

2. Arendt T, Allen Y, Marchbanks RM, Schugens MM, Sinden J, Lantos PL, et al. Cholinergic system and memory in the rat: effects of chronic ethanol, embryonic basal forebrain brain transplants and excitotoxic lesions of cholinergic basal forebrain projection system. Neuroscience 1989; 33:435-462.

3. Baxter MG, Bucci DJ, Gallagher M, Gorman LK, Wiley RG. Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. Behavioral Neuroscience 1995; 109:714-722.

4. Berger-Sweeney J, Heckers S, Lappi DA, Marsel Mesulam M, Sharma M, Wiley RG. Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. The Journal of Neuroscience 1994; 14:4507-4519.

5. Chen AC, Lappi DA, Thal LJ, Waite JJ, Wardlow ML, Wiley RG. Time course of cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. Neuroscience Letters 1994; 169:154-158.

6. Decker MW, McGaugh JL. The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. SYNAPSE 1991; 7:151-168.

7. Decker MW, Radek RJ, Majchrzak MJ, Anderson DJ. Differential effects of medial septal lesions on spatial memory tasks. Psychobiology 1992; 20:9-17.

8. Dekker AJAM, Connor DJ, Thal LJ. The role of cholinergic projections from the nucleus basalis in memory. Neuroscience & Biobehavioral Reviews 1991; 15:299-317.

9. Dornan WA, Kang DE, McCampbell A, Kang EE. Bilateral injections of Beta Amyloid (25-35)+IBO into the hippocampus disrupts acquisition of spatial learning in the rat. Neuroreport 1993; 5:165-168.

10. Dornan WA, McCampbell ARV, Tinkler GP, Hickman LJ, Bannon AW, Decker MW, et al. Comparison of site-specific injections into the basal forebrain on water maze and radial arm maze performance in the male rat after immunolesioning with 192-IgG saporin. Behavioral Brain Research 1996; In press.

11. Dunnett SB, Whishaw IQ, Jones GH, Bunch ST. Behavioural, biochemical and histochemical effects of different neurotoxic amino acids injected into nucleus basalis magnocellularis of rats. Neuroscience 1987; 20:653-669.

12. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F B-amyloid precursor protein. Nature 1995; 373:523-527.

13. Gaykema RA, Nyakas C, Horvath E, Hersh LB, Majtenyi C, Luiten PM. Cholinergic fiber aberrations in nucleus basalis lesioned rat and Alzheimer's disease. Neurobiology of Aging 1992; 13:441-448.

14. Gaykema RPA, Nyakas C, Horvath E, Hersh LB, Majtenyi C, Luiten PGM. Cholinergic fiber aberrations in nucleus basalis lesioned rat and Alzheimer's disease. Neurobiology of Aging 1992; 13:441-448.

15. Guela C, Mesulam M-M. Cholinergic systems and related neuropathological predeliction patterns in alzheimer disease. In: Terry RD, Katzman R, Bick KL, editors. Alzheimer Disease. New York: Raven Press, Ltd. 1994:263-304.

16. Heckers S, Ohtake T, Wiley RG, Lappi DA, Geula C, Mesulam M-M. Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. J Neurosci 1994; 14:1271-1289.

17. Hortnagl H, Potter PE, Kindel G., Hanin I. Noradrenaline depletion protects cholinergic neurons in rat hippocampus against AF64A-induced damage. Journal of Neuroscience Methods 1989; 27:103-108.

18. Hyman BT, Tanzi R. Molecular epidemiology of Alzheimer's disease. The New England Journal of Medicine 1996; 332:1283-1284.

19. Katzman R, Kawas CH. The Epidemiology of Dementia and Alzheimer's Disease. In: Terry RD, Katzman R, Bick KL, editors. Alzheimer's Disease. New York: Raven Press, 1994:105-122.

20. Keith AB, Sahgal A, Steckler T, Wiley RG. Cholinergic lesions by 192 IgG-saporin and short-term recognition memory:role of the septohippocampal projection. Neuroscience 1995; 66 No. 1:101-114.

21. Lamy PP. The role of cholinesterase inhibitors in Alzheimer's disease. CNS Drugs 1994; 1:146-165.

22. Leventer SM, Wulfert E, Hanin I. Time course of ethylcholine aziridinium ion

(AF64A)-induced cholinotoxicity in vivo. Neuropharmacology 1987; 26:361-365.

23. Levin ED, Rose JE. Nicotinic and muscarinic interactions and choice accuracy in the radial-arm maze. Brain Research Bulletin 1991; 27:125-128.

24. Maier DL, Isaacson RL. Behavioral and neuroanatomical consequences of a unilateral intraventricular infusion of AF64A and limitations on the neuroprotective effects of nimodipine. Brain Res 1994; 648:281-290.

25. Mantione CR, Zigmond MJ, Fisher A, Hanin I. Selective presynaptic cholinergic neurotoxicity following intrahippocampal AF64A injection in rats. Journal of Neurochemistry 1983; 41:251-255.

26. McAlonan GM, Wilkinson LS, Robbins TW, Everitt BJ. The effects of AMPA-induced lesions of the Septo-hippocampal cholinergic projection on aversive conditioning to explicit and contextual cues and spatial learning in the water maze. European Journal of Neuroscience 1995; 7:281-292.

27. Mesulam MM, Mufson EF, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on a alternative nomenclature. Neuroscience, 1983.

28. Myhrer T. Animal models of Alzheimer's disease: Glutamatergic denervation as an alternative approach to cholinergic denervation. Neurosci Biobehav Rev 1993; 17:195-202.

29. Nakamura S, Nakagawa Y, Kawai M, Tohyama M, Ishihara T. AF64A (ethylcholine aziridinium ion)-induced basal forebrain lesion impairs maze performance. Behavioural Brain Research 1988; 29:119-126.

30. Nilsson A, Leanza G, Rosenblad C, Lappi DA, Wiley RG, Bjorklund A. Spatial learning impairments in rats with selective immunolesion of the forebrain cholinergic system. Neuroreport 1992; 3:1005-1008.

31. Nilsson OG, Gage FH. Anticholinergic sensitivity in the aging rat septohippocampal system as assessed in a spatial memory task. Neurobiol Aging 1993; 14:487-497.

32. Olton DS. Dementia: Animal models of the cognitive impairments following damage to the basal forebrain cholinergic system. Brain Research Bulletin 1990; 259:499-502.

33. Opello KD, Stackman RW, Ackerman S, Walsh TJ. AF64A (ethylcholine mustard aziridinium) impairs acquisition and performance of a spatial, but not a cued water maze task: Relation to cholinergic hypofunction. Physiol Behav 1993; 54:1227-1233.

34. Opello KD, Stackman RW, Ackerman S, Walsh TJ. AF64A (ethylcholine mustard

aziridinium) impairs acquisition and performance of a spatial, but not a cued water maze task:relation to cholinergic hypofunction. Physiology and Behavior 1993; 54:1227-1233.

35. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Sydney: Academic Press, 1986.

36. Riekkinen PJ, Sirvio J, Riekkinen P. Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. Brain Research 1990; 527:342-345.

37. Smith G. Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Research Review 1988; 13:103-118.

38. Tamer AE, Corey J, Wulfert E, Hanin I. Reversible cholinergic changes induced by AF64A in rat hippocampus and possible septal compensatory effect. Neuropharmacology 1992; 31:397-402.

39. Tanzi R. Clinical implications of basic research: a promising animal model of Alzheimer's disease. The New England Journal of Medicine 1996; 332:1512-1513.

40. Terry RD, Masliah E, Hansen LA. Structural Basis of the Cognitive Alterations in Alzheimer Disease. In: Terry RD, Katzman R, Bick KL, editors. Alzheimer Disease. New York: Raven Press, Ltd. 1994:179-196.

41. Torres EM, Perry TA, Blokland A, Wilkinson LS, Wiley RG, Lappi DA, et al. Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. Neuroscience 1994; 63:95-122.

42. Waite JJ, Wardlow ML, Chen AC, Lappi DA, Wiley RG, Thal LJ. Time course of cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. Neurosci Lett 1994; 169:154-158.

43. Walsh TJ, Kelly RM, Dougherty KD, Stackman RW, Wiley RG, Kutscher CL. Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: cholinergic and non-cholinergic effects following i.c.v. injection. Brain Research 1995; 702:233-245.

44. Wellman CL, Logue SF, Sengelaub DR. Maze learning and morphology of frontal cortex in adult and aged basal forbrain-lesioned rats. Behavioral Neuroscience 1995; 109:837-850.

45. Wiley RG, Oeltmann TN, Lappi DA. Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. Brain Research 1991; 562:149-153.

FIGURE 3

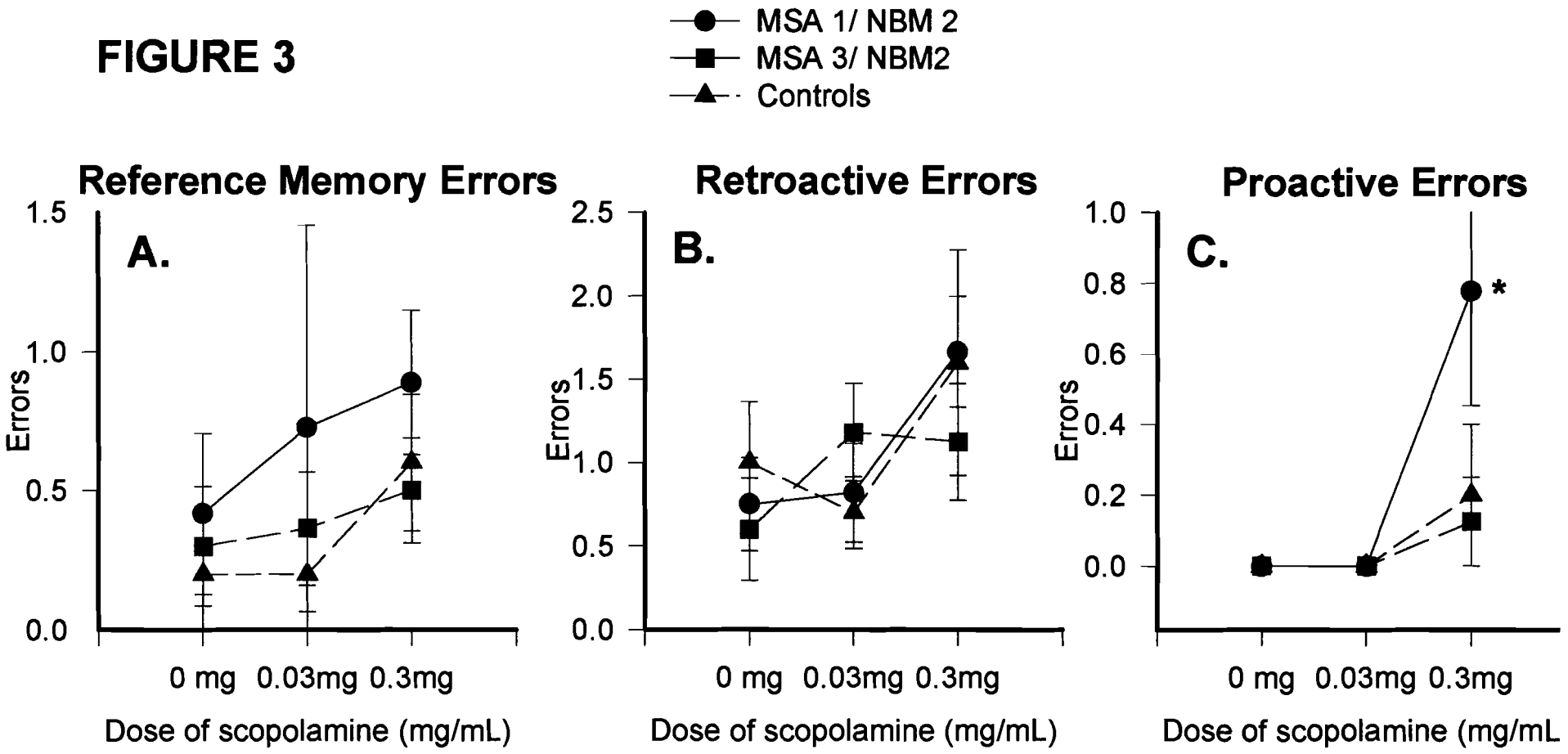

MSA 1/ NBM 2
MSA 3/ NBM2
Controls
Reference Memory Errors
A.
Errors
1.5
1.0
0.5
0.0
0 mg 0.03mg 0.3mg
Dose of scopolamine (mg/mL)
Retroactive Errors
B.
Errors
2.5
2.0
1.5
1.0
0.5
0.0
0 mg 0.03mg 0.3mg
Dose of scopolamine (mg/mL)
Proactive Errors
C.
*
Errors
1.0
0.8
0.6
0.4
0.2
0.0
0 mg 0.03mg 0.3mg
Dose of scopolamine (mg/mL

FIGURE 2

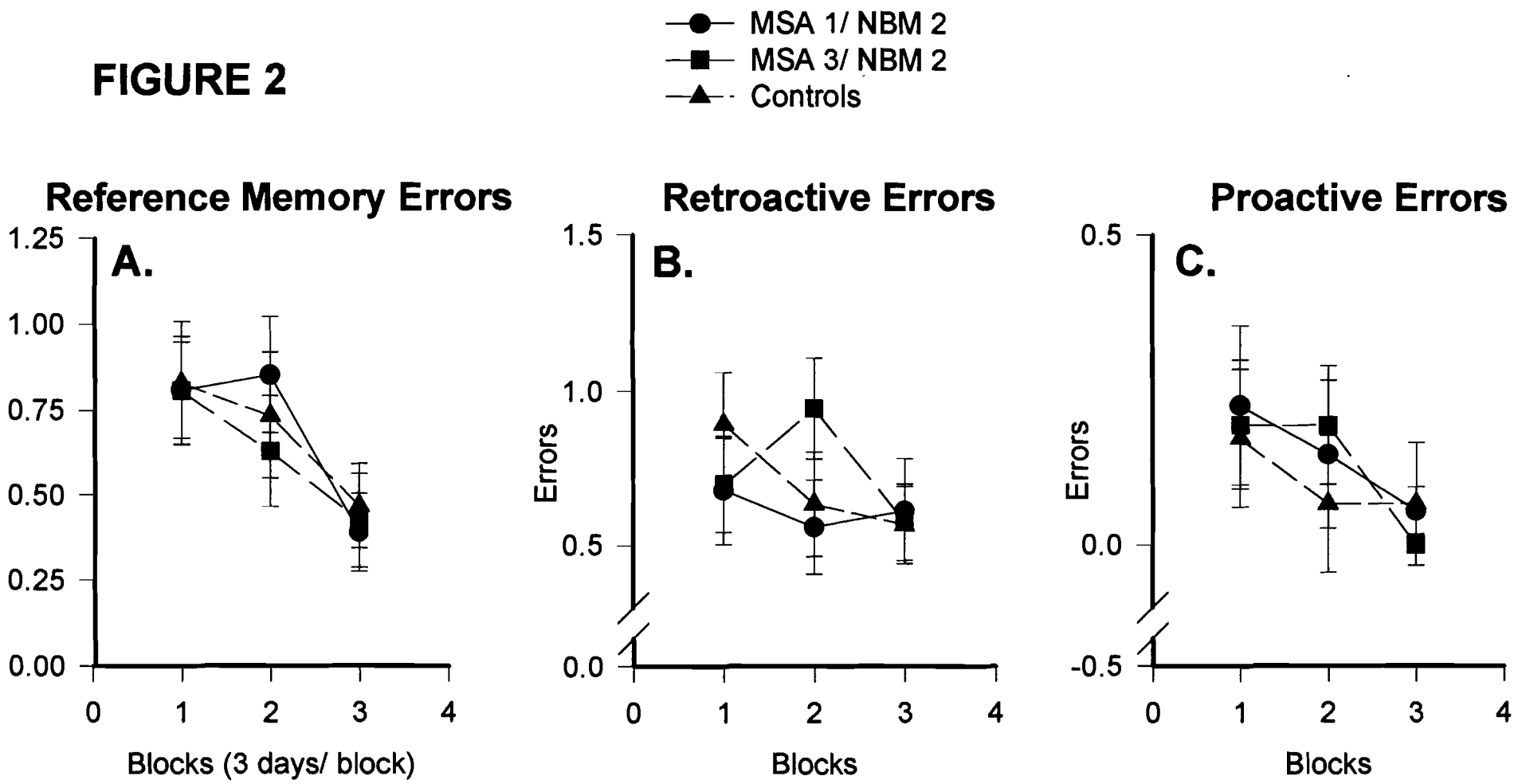
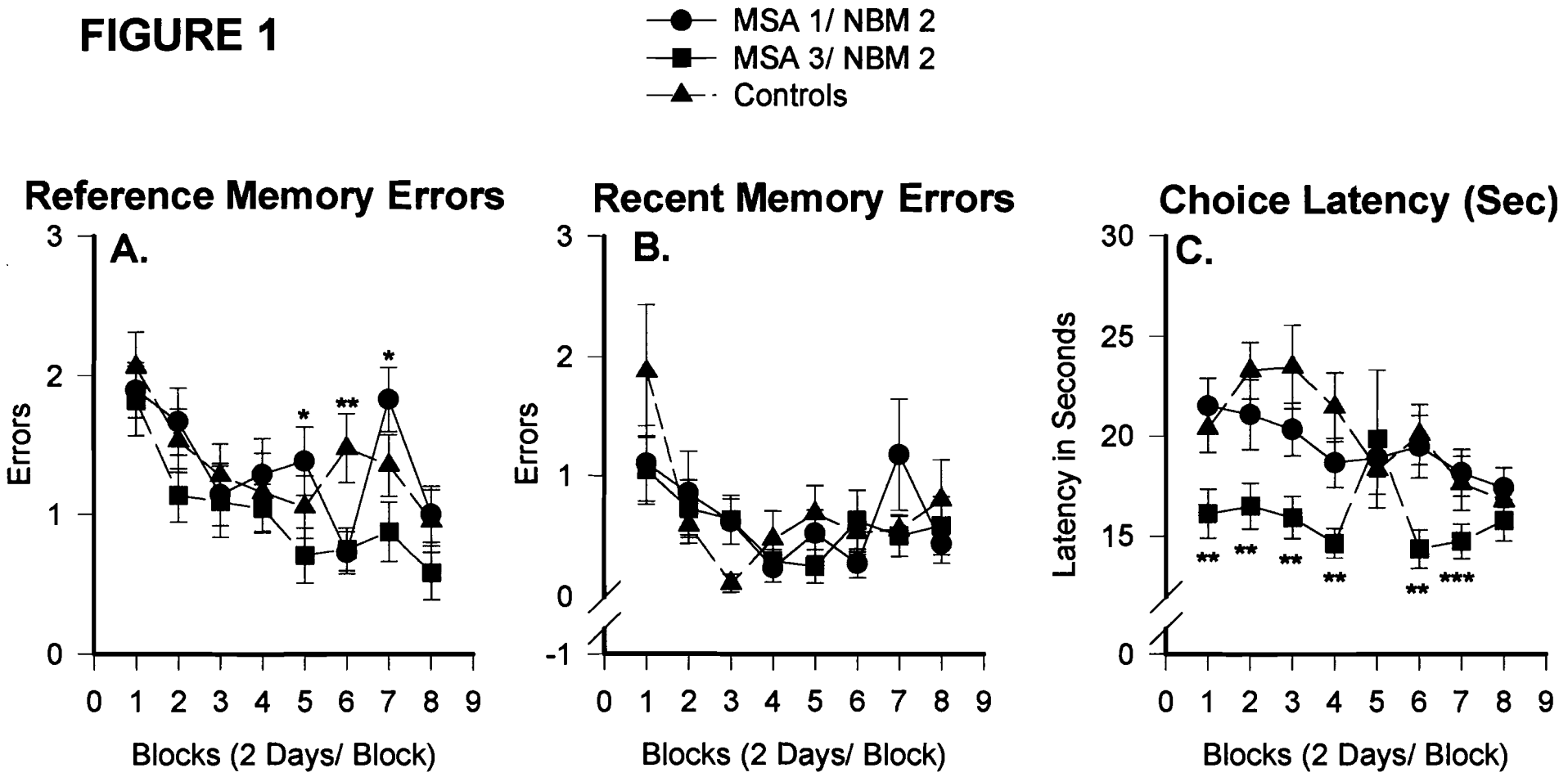

MSA 1/ NBM 2
MSA 3/ NBM 2
Controls
Reference Memory Errors
A.
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
Blocks (3 days/ block)
Retroactive Errors
B.
1.5
1.0
0.5
0.0
Errors
0
1
2
3
4
Blocks
Proactive Errors
C.
0.5
0.0
-0.5
Errors
0
1
2
3
4
Blocks

FIGURE 1


MSA 1/ NBM 2
MSA 3/ NBM 2
Controls
Reference Memory Errors
A.
Errors
Blocks (2 Days/ Block)
Recent Memory Errors
B.
Errors
Blocks (2 Days/ Block)
Choice Latency (Sec)
C.
Latency in Seconds
Blocks (2 Days/ Block)