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Running Head: SAPORIN INJECTIONS INTO THE NUCLEUS BASALIS AND THE MEDIAL SEPTAL AREA

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Acknowledgement

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and Greg Tinkler. These two have help strike that critical balance between seriousness and fun that make this work enjoyable and possible. They both will go far, if they want to. Lastly, I want to acknowledge the rats. They have had their lives disrupted and taken away from them. I just hope that it will be for some greater good.

Abstract

Alzheimer's disease currently afflicts approximately 4 million people in the United States, with 100,000 new cases being reported each year. As post mortem examination of AD patients' brains has revealed a significant decrease in the number of cholinergic neurons, one approach we have taken is to look at the correlation between the depletion of certain cholinergic markers in animals and the resulting behavioral deficits. Two regions of specific interest are the medial septal area (MSA) and the nucleus basalis magnocellularis (NBM). These regions are important because they are the major source of cholinergic neurons in the brain, they are selectively targeted during aging and AD, and there have been many reports of their importance in learning and memory tasks. Therefore in this study we examined the effects on spatial learning, as assessed by the Morris water maze (MWM), in the male rat following intracerebral injections of the selective cholinergic neurotoxin, saporin-IgG. The results of this study indicate that saporin injections into the NBM impaired the performance in the MWM when compared to controls and injections of saporin into the MSA. This was revealed by significantly longer latencies to find a submerged platform and longer latencies during the spatial discrimination test.

Introduction

Alzheimer disease currently afflicts approximately 4 million people in the United States, with 100,000 new cases being reported each year. This disease, which is characterized by specific cognitive deficits and neurohistological changes, is both financially and emotionally devastating to those effected by it. The cognitive deficits of AD include visual spatial skills, executive abilities and language (Cummings, Gorman, Shapiro, 1993), with the most prominent effect being memory impairment. While our knowledge of AD has increase dramatically since its first description in 1907, no reliable animal model exists today.

There are several different approaches that can be taken to the generation of an animal model. One approach is to mimic the specific neuropathological changes that are observed in the AD patient's brain. While the normal aging brain does undergo both morphological and histological changes, there are several changes that are amplified in AD. These include increase loss of neurons in several regions of the cortex (Coleman and Flood, 1987), changes in synaptic densities (Scheff, DeKosy, Price, 1990), and changes in acetylcholine and other neurotransmitter levels (Bartus, Reginald, Beer, Leippa, 1982). Figure 1 is a coronal section taken using magnetic resonance imaging comparing a normal aged person and a person with AD. As can be seen, both deep degeneration and marked cortical atrophy is present. In addition to the gross changes, there are two histological hallmark's of AD: neuritic plaques and neurofibrillary tangles.

Though the cause, or causes, of these morphological changes are not fully understood, much research has been focused on the protein beta amyloid (Aβ) which comprises 75% of the proteinaceous core of the neuritic plaque (Robakis, 1994). In 1989, Selkoe reported that Aβ was neurotoxic to cells in culture. Since then, our understanding of the biochemical properties and the synthetic pathways of Aβ and its precursor protein has improved dramatically (for a review see Robakis, 1994). While the evidence for the *in vitro* neurotoxicity of Aß is very strong (Yankner, Dawes, Fisher, Villa-Komaroff, Oster-Granite, Neve, 1989; Cotman and Pike, 1994), there is some controversy as to the effects *in vivo*. The controversy centers around whether or not Aβ is directly toxic to cells or only endangers them to other insults present during the aging process.

One attempt to resolve this issue was reported in 1993 by Dornan, Kang, McCampbell, Kang. In that project, a dose of Aβ was injected into the dorsal hippocampus of male rats, which were then tested on a radial arm maze for behavioral deficits. We reported no disruption in the acquisition of a spatial task in animals injected with the 25-35 fragment of Aß (there is growing evidence that it is the amino acid sequence 25-35 of Aβ that mediate its neurotoxicity (Yankner et al 1989; Cotman and Pike, 1994)).. When Aβ (25–35) was injected along with a subthreshold dose of ibotenic acid, however, a dramatic impairment in the acquisition of spatial learning was observed. These results strongly suggest that Aß kills neurons in the CNS by increasing their vulnerability to other toxins.

While the results of this project were promising, a more satisfactory model would be one in which this Aβ-induced vulnerability could be observed with an endogenous substance rather than an artificial one, e.g. ibotenic acid. One specific candidate that emerged was the stress hormone cortisol. The rational for choosing cortisol is outlined below.

In a study by Davis, Davis, Greenwald, Mohs, Mathe, Johns, and Horvath in 1986, they reported that hypercotisolemia and a reduced negative feedback inhibition of cortisol secretion was found in AD patients when compared to age-matched controls. In a more recent study, de Leon, McRae, and Tsai, in 1988 reported that the magnitude of the human cortisol response to an IV glucose load correlated positively with dementia severity and hippocampal atrophy in 9 AD cases. In addition, a series of animals studies have demonstrated that chronic corticosterone treatment amplifies various neurological insults, including hypoxia-ischemia, oxidative stress and kainic and ibotenic acid induced lesions (Sapolsky, 1991). Therefore, one neuropathological approach to the Aß-induced vulnerability hypothesis would be to examine the interaction between stress hormones and Aß. In a study presented last year, we reported a potentiation of the behavioral as well as the neuropathological effects of Aß when combined with chronic injections of the stress hormone, corticosterone (Dornan, Giordano, McCampbell, Wijeweera, Pequette, Champman, Bond, Peterson, and Hickman, 1994; Giordano, Dornan, Bannon, Kowall, McCampbell, Peterson, and Tinkler, 1994).

That is one approach to the generation of an animal model for AD. Another approach that we have taken is to mimic the neurotransmitter changes that occur. The cholinergic hypothesis of aging was formalized by Bartus and colleagues (Bartus et al., 1982), who drew upon work completed during the 1970s. This hypothesis draws on evidence from human and animal studies that cognitive and behavioral deficits displayed in aging animals can be in part attributed to the degeneration of the main cholinergic systems of the basal forebrain and its projections to the neocortex and hippocampal formations. The changes in neuronal numbers in AD patients occurs largely in the same regions as in ageing people, however, the degree to which it occurs is much greater (Coleman and Flood, 1987 1987).

Among the areas most severely effected are the nucleus basalis of Meynert (NBM)[1], the hippocampal complex (including the CA1-CA4 regions of Ammon's horn and the dentate gyrus), the amygdala, the medial septal area (MSA) and the anterior olfactory nucleus (Coleman and Flood, 1987). The largest depletion is found in the NBM, with a 90% depletion of choline acetyltransferase activity compared to age matched controls (Coleman and Flood, 1987). Figure 2 is a schematic representation of the main cholinergic projections in the human and rat brain. Two areas illustrated in this figure, the MSA and NBM have received considerable attention because they supply 90% of the cholinergic innervations

[1]The analogous structure in the rat is the nucleus basalis magnocellularis.

within the brain.

The nucleus basalis magnocellularis is a collection of large cells, diffusely located in the forebrain of the rodent (Dekker, Conner, and Thal, 1991). While cholinergic neurons do predominate (approximately 80% of the neurons in this region are cholinergic (Mesulam, Murson, Wainer, and Levey, 1983), there are other neurotransmitters present (Johnston, McKinney, and Coyle, 1979). The major projection of the NBM is to the cortex, where it supplies as much as 75-80% of the cholinergic innervation.

The medial septal area includes the medial septal nucleus and the vertical limb of the diagonal band of Broca. These neurons primarily project to the hippocampus, terminating primarily in the granular layer of the dentate gyrus. Like the NBM, the MSA undergoes extensive neuronal loss during AD.

The hippocampal formation which is selectively damaged during both normal aging and AD (Coleman and Flood, 1987) is considered by most neuroanatomists to be comprised of the entorhinal cortex, dentate gyrus, hippocampus proper, and subiculum. The term "hippocampus" is used to refer to the dentate gyrus and CA1-CA4 pyramidal cell region. The hippocampus has been the subject of many investigations, and there is not enough space to do an extensive review of the findings here. Briefly, numerous reports have demonstrated that the hippocampus is important for spatial learning in the rat and human. Furthermore, Sapolsky and colleagues have demonstrated that long term stress has a deleterious

effect on the cholinergic neurons of the hippocampus (Sapolsky, 1992).

Neurotoxins of different neuronal selectivity have been employed, e.g.., ibotenic acid (Hepler, Olton, Wenk, and Coyle, 1985), quisqualic acid (Hagan, Salamone, Simpson, Iversen, and Morris, 1988), and AF64A (McGurk, Hartgraves, Kelly, Gordon, and Butcher, 1987). All have induced deficits in spatial learning in the rat when injected into the MSA and NBM. Recently a controversy has emerged with respect to changes in cholinergic pathways, as assessed by change in either choline acetyltransferase (ChAT) or acetylcholinesterase (AChE)[2] and behavior spatial learning.

Indeed, a series of reports in the mid-1980s brought the whole hypothesis of the role cholinergic neurons in the NBM and MSA in spatial tasks into question. The articles examined the effects of different lesions by different agents and how their resulting behavioral deficits correlated with the cholinergic depletion. The agents were the neurotoxins, quisqualic acid and ibotenic acid. While the quisqualic acid produced a greater depletion in ChAT and AChE levels, the ibotenic acid caused a greater behavioral deficit (Dunnett, Wishaw, Jones, and Bunch, 1987; Robbins, Everitt, Ryan, Martson, Jones, and Page, 1989; Markowska, Wenk, and Olton, 1990; Conner, Langlais, and Thal, 1991; Fibiger,

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[2]ChAT and AChE are enzymes responsible for the synthesis and breakdown of acetyl choline. There are a variety of methods to quantitatively measure the amount of these enzymes present. They are generally regarded as reliable measures of degree of cholinergic neuron depletion.

1991). These results suggest that many of the previously used toxins were non-selective.

The best means to resolve this debate would be to use a neurotoxin that is highly specific for cholinergic neurons. In 1981, Manitone, Fisher, and Hanin proposed that the ethylcholine mustard aziridinium ion (AF64A) was such a toxin. They reported that the AF64A was taken up by the high affinity acetylcholine receptors, and subsequently prevented the neurons from firing. Though the exact mechanism has not been elucidated, this prevention of neuronal firing, leads to necrosis. As promising as the initial results were, more recent reports have cast doubt as to the selectivity of AF64A.

Recently, Wiley and colleagues have introduced a new lesioning technique which early evidence indicates is very selective to cholinergic neurons (Roßner, Hartig, Schliebs, Bruckner, Brauer, Perez-Polo, Wiley, and Bigl, 1994). The technique relies on the concept of immunolesioning. A toxin is coupled to an antibody that is directed against a receptor that is expressed by only those neurons that one wishes to destroy. In this case, the toxin used is saporin. Saporin is derived from the fern *Saporin officinalis*, and cleaves the mRNA sequence that codes for the 28S small ribosomal subunit in eukaryotes (Lappi, Maher, Martineau, Baird, 1991). The saporin is conjugated to a monoclonal antibody that is directed against the p75 low affinity nerve growth factor receptor (NGFr) (Heckers, Ohtake, Wilery, Lappi, Geula, and Mesulam 1994). Cholinergic neurons in the

NBM and MSA have very high concentration of this NGF receptor. Other aspects of saporin help make it a very attractive chemical to use. Most reports indicate that a 90-100% depletion of Ach occurs within 2-3 days following injections into the lateral ventricles, and after 3-5 days for injections directly into either the NBM or MSA (Berger-Sweeney, Heckers, Mesulam, Wiley, Lappi, and Sharma, 1994; Hekkers et al., 1994; Roßner, et al., 1994) with no significant change in other transmitter levels (Waite, Wardlow,m Chen, Lappi, Wiley, and Tahl, 1994). In addition, while many studies with ibotenic and quisqualic acid have reported a high mortality rate, so far, few deaths have been reported with the saporin-IgG complex. Ibotenic and quisqualic lesions of the NBM typically have resulted in the animals refusal of food and water, and some groups have resorted to force feeding to insure survival of the subjects (Decker, et al., 1991) So far these problems have not been reported by those using saporin. Furthermore, while compensatory effects have been reported with AF64A (Tamer, Corey, Wülfert, and Hanin, 1992), there has been no changes in other neurotransmitter levels after saporin injections (Heckers, et al., 1994). Behaviorally, when injected into the NBM, a profound deficit in place navigation (Berger-Sweeney, et al., 1994) has been reported.

At this point it is necessary to provide a detailed discussion of the different type of behavioral tasks commonly used to assess spatial ability. The two most common tests are the radial arm maze task and the Morris water maze (aka spatial

navigation) After this description, there is a discussion of the findings of Berger-Sweeney, et al.

Morris Water Maze

The Morris Water Maze was first introduced in 1981 as a new method for testing an animals ability to complete a spatial task. Since then, it has grown in popularity and several variations have been introduced. Brandeis, Brandys, and Yehuda, recently reviewed the MWM literature, and it is from this article that most of this information is taken. Pools have ranged in size from .8 m to 2.2 m in diameter. The pool is divided into quadrants typically, with the platform always being situated at one position (see figure 3). As described above, the rat is trained to that position, and then a probe trial is performed. The probe trial is the test when the platform has been removed.

Two variations of the MWM are the 1) cued navigation, and 2) spatial discrimination tasks. The cued navigation task acts as a control to insure that the animal is not impaired due to secondary effects of non-generalized brain damage. The rat is introduced into a pool in which the platform is visible and the latency to escape is measured. If the animal cannot complete this task successfully, then a secondary explanation outside of lack of spatial ability, must be sought. Other possible deficits include lack of sufficient motor control to complete the task, lack of necessary sensory input (i.e. blind, or nearly blind), or an attentional deficits.

Another variation of the MWM is the spatial discrimination task introduced

by Dekker in 1991. In the spatial discrimination task, two identical platforms are placed in the pool. One platform is a false platform, that will tip when the rat attempts to stand on it. The other platform is stable and will support the animal. The rat is introduced once from each quadrant that lacks a platform. The latency to the correct platform, the number of attempts on the false platform, and the path is generally recorded. This is a good test to distinguish between those animals that have learned to solve a task purely through a system, as opposed to using spatial cues.

Radial Arm Maze (RAM)

The testing apparatus consist of an elevated platform with a range of 8 to 16 arms spaced at regular intervals around the central platform. The original task was designed with having all the arms baited, and the animal was to retrieve the baits within a given time interval. A common variation is to only bait some of the arms, thereby preventing the animal from running a simple pattern.

Lesion effects

Hippocampus. The work of Jarrard and colleagues has focused on the hippocampus and the effects of the lesions on the acquisition of spatial tasks. Though there is still theoretical and mechanistic issues to be resolved, many believe that the hippocampus plays a large but not an exclusive role in spatial acquisition (Morris, Schenk, Tweedle, and Jarrard, 1990). It has been generally reported that the lesions of the hippocampus produces severe impairments on the above tasks.

Medial Septal Area. In the articles reviewed here, it appears that the MSA is important for performance in both the RAM and the MWM (Hepler et al., 1985; Miyamoto, Kato, Narumi, and Nagaoka, 1987; Messer, Sibbe, Bohnett, 1991; Hagan et al., 1988; Kelsey and Landry, 1988). All of the experiments were conducted with lesioning techniques that generated non-specific tissue damage, except for Berger-Sweeney et al., which reported no deficit in MWM performance. However, the spatial discrimination task which is reported to be very sensitive to MSA injections, was not tested.

Nucleus basalis magnocellularis. In a review article, Dunnett et al., conclude that NBM is probably important for both the RAM and MWM tasks. (Dunnett et al., 1987). Once again, all the articles reviewed used non-specific lesioning techniques. While this does not necessarily invalidate the results, the interpretation should be cautious. These findings were upheld in the 1994 report of Berger-Sweeney, in which saporin was used (Berger-Sweeney et al., 1994).

The potential for a reliable animal model of AD is there, if the work of Berger-Sweeney can be replicated and combined with previous work. Therefore, this study will attempt to replicate and extend the work of Berger-Sweeney et al., by examining the behavioral paradigms that they used and also the radial arm maze. For this study, four groups of rats were compared: 1) rats with saporin-IgG injections into the MSA, 2) the NBM, 3) both the MSA and NBM, and 4) control injections of the vehicle. The injections into the MSA and NBM were chosen

because Berger-Sweeney reported some motor impairments with the ventricular injections. However, it is important to examine the combined effect of depleting both cholinergic systems, so both areas were lesioned individually. Spatial navigation, cued tests, spatial discrimination, and the RAM test were chosen to assess the degree of behavioral deficit of their sensitivity to lesions of the MSA and NBM. The path length, latency to platform and number of errors will be recorded for the MWM tasks. The results for the radial arm maze task will not be reported in this paper. In a recent study by Berger-Sweeney et al. (1994), they reported several intriguing effects of saporin injections on spatial learning in the male rat. In that study male rats were injected with the saporin-IgG complex into the MSA, the NBM and the lateral ventricles and tested using an adapted Morris water maze task. The Morris water maze task usually consists of a spatial navigation task, followed by a probe trial, and a cued task (see below for a more detailed description of the task). The NBM and ventricular injections had the most consistent deficits in the spatial navigation and the probe trial. For the cued task it was only the ventricular group that showed a significant effect increase in latency after the first day. However, both the NBM and ventricular groups showed evidence of perseveration., as shown by the spending a greater amount of time in the original quadrant than the MSA and vehicle groups. A strong correlation was also reported between the amount of cholinergic loss and the resulting behavioral deficits.

Methods

Animals

A total of forty five male Long Evans rats (age 5-7 months) of rats were used for this project. Animals were obtained from the Illinois Wesleyan University animal colony and were housed singly for the duration of the project with a 12 hr light/dark cycle and food and water ad libitum. The animals were randomly placed divided into six different groups (see Table 1).

Group #	# of Animals	Injection site	Vehicle or Saporin
1	8	MSA	Saporin
2	7	MSA	Vehicle
3	8	NBM	Saporin
4	7	NBM	Vehicle
5	8	NBM + MSA	Saporin
6	7	NBM + MSA	Vehicle

Table 1. Table 1 list the number of animals in each group and the location and type of injections administered.

Injections

At the time of surgery, the animals were anesthetized with sodium pentobarbital (50mg/kg), and atropine methylbromide (40mg/kg) which was used to prevent respiratory complications during surgery. The animals were placed into a stereotaxic device (David Kopf Instruments). The coordinates for injections were

empirically determined, using the Paxinos and Watson atlas as a guide. Injections were made into either the NBM (AP: -.8 ML: ±3.6 DV: -5.9), the MSA (AP: +1.8 ML: +1.5 DV: -6.0) or into both areas (all coordinates are relative to bregma). The saporin-IgG complex was purchased from Chemicon (MAB-390) and generously supplied by Dr. Tony Bannon of Abbott Laboratories. The saporin was dissolved in a 0.05% sodium azide solution, at a concentration of .84μg/μl. The vehicle injections were a phosphate buffered solution with the 0.05% sodium azide[3]. Injections were 0.6μl per injection site bilaterally for the NBM and 0.6μl unilaterally for the MSA. The saporin was delivered with a 1.0μl Hamilton syringe, which was lowered slowly into the brain. In order to minimize damage, the injections took place over a 3 min period, with a 5min pause after the injection before raising the needle. After surgery the animals were given a two week recovery period.

Behavioral apparatus

The fiberglass pool, purchased from Advanced Composite Technologies in Champaign, IL, used for the spatial navigation task measured 183 cm in diameter and 90 cm deep. The pool was located in a room with a variety of spatial cues, including a TV monitor, a large washer and dryer and doors. The pool was filled with 26°C water, with a platform placed in the middle of one quadrant. The

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[3]The sodium azide is present to act as a preservative, and has had no effect on cells at 0.05% concentration (Chemicon).

quadrants were arbitrarily labeled N, E, S, and W. The platform had a radius of 6cm and was covered with white cloth to maximize the animals stability. The animals were tracked with a Panasonic black and white video camera and recorded with a VCR.

Non-Cued task

The rats were tested four times per day for six days. The water of the pool was obscured with a non-toxic white paint, and the platform was submerged 3 cm below the surface of the water. Each animal was introduced in a pseudo-random order from each one of the four possible starting points, one per quadrant. The animals were given 90 seconds to find the platform. If they exceeded that time, they were gently guided to the platform. Once on the platform, the rats were given a 10 second rest period, in order to orient themselves. The paths were traced off of a television monitor and the latency to the platform was also recorded.

Probe test

After the fourth trial on the sixth day of the non-cued task, a probe test was administered. The probe trial generally considered an accurate test for retention of the spatial task. For the probe task, the platform was removed from the pool. The rats were introduced into the pool once, with each animal starting from the same point, and the path was traced off of the TV monitor. In addition, the time spent in each of the quadrants was recorded.

Cued task

On the following day the cued task began. Each rat was given four trials per days for two days. The platform was placed into the original quadrant, however this time it was 3 cm above the water. In addition, the water was not obscured with the white paint. The rats were introduced once into each quadrant, and the path length and latency to platform were recorded.

Spatial Discrimination.

Two platforms were placed into the pool in opposing quadrants, neither of which had been used for the spatial navigation task (ex., N and S). One platform was a false platform, designed so that when the rat tried to stand on it, it would tip, dumping the rat back into the water. The second platform was secure, so that the rat would be able to easily stand on it. The platforms were made to look almost identical to each other. Each animal was given two trials, one entry from each quadrant not containing a platform. The path was traced, and the latency to the correct platform and the total number of errors (attempts to get on the false platform) were recorded.

Data analysis.

It was clear after the first several tests that the vehicle injected controls did not differ on spatial navigation and discrimination tasks. An ANOVA revealed that none of the controls differed significantly on the spatial navigation and discrimination tests. Consequently those groups were combined in all subsequent data analysis for these two tasks. All data were analyzed using ANOVA's . Post

hoc comparisons were evaluated with Fisher's least-significance test.

Results

Spatial navigation.

Figure 4 illustrates the mean latency to find the platform for the saporin injections and the combined group of injected controls. A two way (4x6; injection by days) mixed design ANOVA was used in the data analysis. There was a main effect of injection $F(3,71)=11.8029$, $p<.0001$, and a main effect of days $F(5,360)=208.54629$, $p<.0001$. In addition there was a significant interaction between days and injection $F(15,71)=7.04237$, $p<.0001$. Post hoc analysis revealed that on day one the MSA saporin group had a significantly longer latency than all the other groups. On days two, three and six, the NBM saporin group had significantly longer latencies than all the other groups. On day four, the NBM saporin group was different from the vehicles and the MSA saporin group, but not the NBM+MSA saporin group. On day five, both the NBM+MSA and NBM saporin groups had significantly longer latencies than the other two groups.

Probe test.

For the probe test, the mean latency in each of the four quadrants was recorded (see figure 5). A three way (3x2x4; injection by quadrant) mixed design ANOVA was used. There was a main effect of injection type $F(1,53)=4.09557$, $p<.05$, and quadrant $F(3,48)=45.12979$, $p<.0001$. In addition, there was an interaction between injection site and quadrant $F(6,162)=2.44473$, $p<.0279$. The

three way interaction between injection site, injection type, and quadrant was not significant at the $p<.05$ level, however the results were close, $F(6,162)=2.515328$, $p=.0509$. Post hoc analysis revealed that every group except for the MSA vehicle injections spent significanlty longer time in quadrant four, the quadrant that originally had the platform.

Cued navigation.

Figure six illustrates the mean latency to platform for the different groups. A three way (3x2x2; injection site by injection type by days) mixed design ANOVA was used to analyze the latencies. Main effects of injection site $F(2,53)=13.04348$, $p<.0001$ and days $F(1,54)=12.47978$, $p<.001$ was revealed. Interactions between injection site and days $F(2,54)=4.58735$, $p<.05$ and injection site, injection type, and days $F(2,48)=3.7573$, $p<.05$ were present. Post hoc analysis revealed that the NBM saporin and NBM vehicle groups had significantly longer latencies than the other groups on day one. On day two, the only significant effects were between the NBM saporin and the MSA saporin and NBM + MSA vehicle.

Spatial discrimination.

For the spatial discrimination task both latency to platform and total number of errors were recorded. A two-way ANOVA was performed, comparing the vehicle injection animals to each other. There was no significant difference between the groups, so for all subsequent analyses they were treated as one group.

Figure seven illustrates the mean latencies for the saporin and vehicle groups. A two way mixed design ANOVA was used to analyze the results. There was a main effect of injection site $F(3,71)=12.00489$, $p<.0001$, and of days $F(3,72)=35.95313$, $p<.0001$. In addition there was an interaction between injection site and days $F(3,71)=7.60044$, $p<.001$. Post hoc analysis with a protected T-test revealed that on day one the NBM saporin and NBM+MSA saporin groups were had significantly longer latencies than the MSA saporin and the vehicle groups. On day two the NBM + MSA saporin group had significantly longer mean latencies than the other groups.

Analysis of the total number of errors summed over the two trials revealed a main effect of injection $F(3,71)=5.89796$, $p<.01$, and of days $F(3,72)=54.98846$, $p<.0001$. In addition, there was an interaction between days and injection $F(3,71)=6.63889$, $p<.0001$. Post hoc analysis revealed that the NBM saporin had significantly more errors than the vehicle and MSA saporin groups. The NBM + MSA saporin group was significantly higher than the MSA saporin group. On day two, the vehicles had significantly more errors than the MSA saporin and the NBM saporin groups.

The path length and swim speed analyses have not been completed.

Discussion

The results reveal that saporin injections into the NBM and NBM + MSA groups create a disruption of the male rats spatial ability. There was an significant

interaction of injection of injection and days on the spatial navigation. The MSA injections did show a deficit in the spatial navigation. The only significant effect during the probe test was in the MSA control injection group. The NBM saporin and NBM vehicle performed significantly worse in the cued task on day one, but only the NBM saporin group was significantly worse on day two. The MSA saporin animals did significantly better than the control injection groups in the spatial discrimination task on day one, and the NBM saporin groups were significantly better than the controls on day two. This result is confusing, and will be analysed further with more sensitive techniques, including swim speed and pathlength analysis.

Medial septal area. The only significant effect was on the first day of testing. On all other days, there was no effect, and it appears that a full recovery of function was made. This contradicts previous findings with non-specific lesions (Hepler et al., 1985; Miyamoto et al., 1987; Messer et al, 1991; Hagan et al., 1988; Kelsey and Landry, 1988), but supports the findings of Berger-Sweeney, et al. (1994).

The probe revealed some confusing results. The MSA vehicle injected animals were the only group that spent equal amounts of time in all four quadrants. At this point this result is difficult to explain, however latencies are just a rough measure of the rat's performance. Another more sensitive test is a measure of annulus crossings. In this analysis, the number of times that the animal crosses

into and out of the previous location of the platform used to be is measured. This has been important in distinguishing between aged animals, and normal animals that otherwise appear normal.

In the spatial discrimination task, the MSA animals took significantly less time and committed less errors before finding the correct platform. This runs opposite of the supposed sensitivity pf the spatial discrimination task to MSA lesions. However, once again, latencies are just one possible measure. The actual path lengths and swim speeds should be analyzed before any conclusions are made. Finally, all of these results depend on histological verification of injection sites, and cholinergic depletion.

Nucleus basalis. The NBM-lesion animals performed significantly worse on the spatial navigation task on days two through six. This supports the findings of Berger-Sweeney, et al. (1994). In the probe test, the NBM saporin and control injection groups spent significantly more time in the quadrant that contained the platform. In order to try to differentiate between these two groups, annulus crossings will be examined.

In addition, the NBM saporin and control injected groups had significantly higher latencies in the cued task. The same caveat that applied for the MSA with regard to path length and swim speed, applies to the NBM animals. One striking result is the mean latency on day one of the cued task for the NBM control animals. It should be noted that this effect is from one animal whose mean latency

was very different from the others in the group, and from the performance of the animal over several days. The difference in the NBM animals needs to be carefully explored. Normally the cued task is a control to insure that the deficits are not a result of non-specific brain damage. The path length will be measured, and the swim speed computed and analyzed. If there is a difference in the swim speed, it would indicate that there is a motoric deficit. The path length will be the most reliable indicator of overall ability.

In the spatial discrimination test, the NBM saporin animals had significantly more errors and longer latencies than the control groups on day one. On day two, however, the NBM saporin group committed no errors, and were no different than the other groups in latency measures.

Combined injections. The injections into the medial septal area and the nucleus basalis generated some spatial deficits. In the spatial navigation task, the combined injection group had significantly longer latencies than the MSA and control injection groups on day five and six. The probe tests results were similar to those for the NBM, and further analysis is required.

In the cued test there was no significant difference between the combined group and the control injections. Though this was expected, analysis of the path length, swim speed, and histological confirmation remains to be completed.

In the spatial discrimination task, the combined group was significantly worse than the controls on both days. This too, needs to be more rigorously

analyzed before definite conclusions are drawn, however it suggests synergy between the MSA and NBM for completing this task.

One potentially problematic aspect of these results is that it is only in the spatial discrimination task that an amplification of the behavioral deficits are seen in the combined injection site animals. The project by Berger-Sweeney, et al., (1994) reported that those animals receiving lateral ventricular injections had deficits in all the behavioral tasks, though this effect is confounded by the loss of cerebellar purkinje cells, and possibly other cells expressing the p75 NGFr. If the preliminary results of this project hold up, it would suggest that a large degree of the deficits reported can be attributable to non-specific tissue damage.

Pending the further analysis of these results, and the completion of the radial arm maze portion of this project, these preliminary results suggest that saporin-IgG can be used to reliably generate specific cholinergic lesions that generate specific behavioral deficits. Future projects could try to combine either the corticosterone or Aß injections to try to generate an accurate and reliable animal model for Alzheimer disease.

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Figure Captions

Figure 1. Magnetic resonance images of a coronal section of A) normal aged human, and B) a person with AD. The arrows indicate the atrophic hippocampus and parahippocampal gyrus. The cortical atrophy and deep degeneration can also be easily seen (Taken from Duara, 1994).

Figure 2. Figure 2 is a schematic of the human and rat brains, illustrating the major cholinergic projections found in each. (Taken from Nicholls, Martin, and Wallace, 1992; and Fibiger, 1991).

Figure 3. Figure 3 is schematic of the Morris water maze. The maze is divided into four quadrants, with the pool located in the middle of one of them, e.g.. SE.

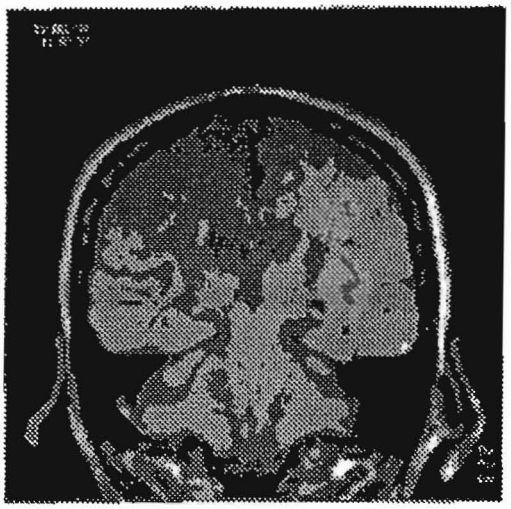
Figure 4. Figure 4 illustrates the mean latency to find the platform for the spatial navigation task. The NBM saporin group is significantly higher on days two through six.

Figure 5. Figure five illustrates the time spent in each quadrant during a 90 second probe test. The platform was originally located in quadrant four.

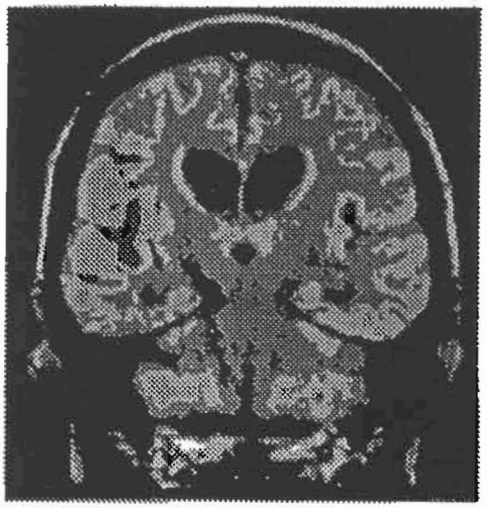
Figure 6. Figure six illustrates the mean latencies of the cued navigation task. The NBM group is significantly higher on days one and two.

Figure 7. Figure seven illustrates the mean latency to find the correct platform for the visual discrimination task for all six groups. The MSA saporin are significantly lower than the other groups.

Figure 8. Figure eight illustrates the total number of errors committed over two trials for the combined control injection groups and the different saporin injection groups.



A



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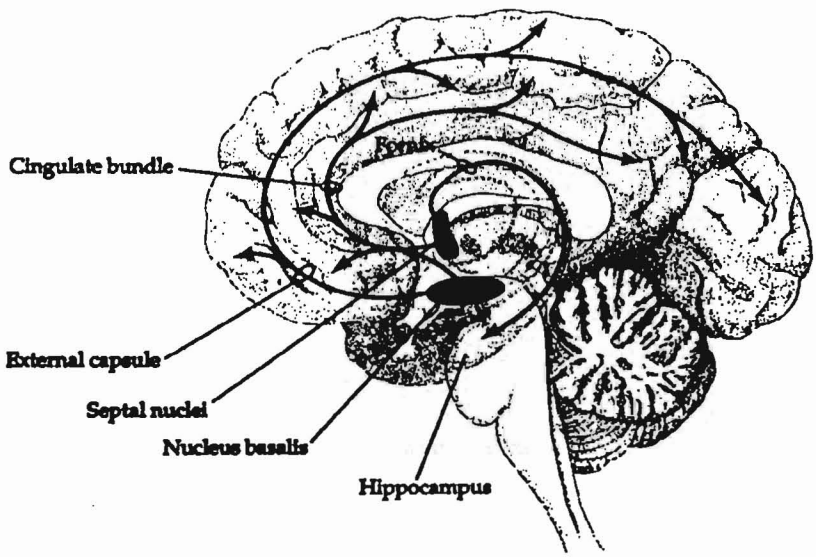
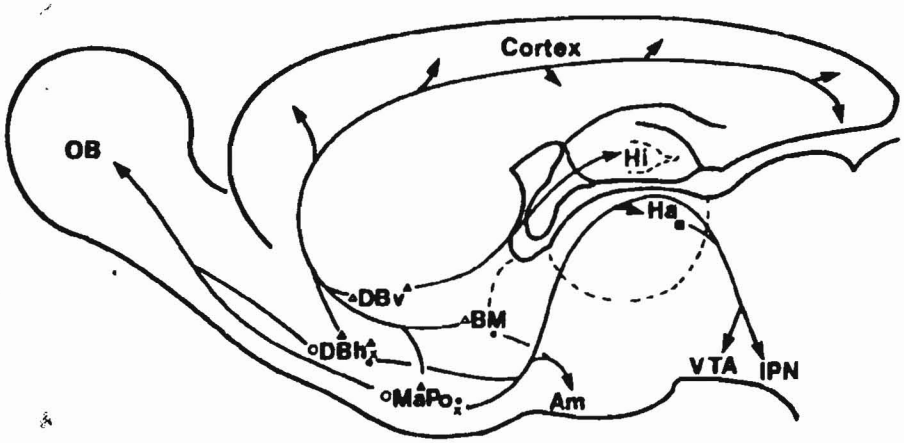
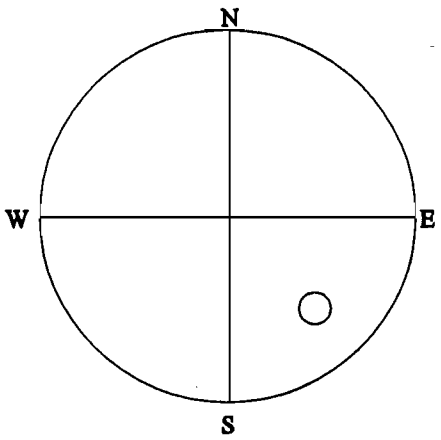
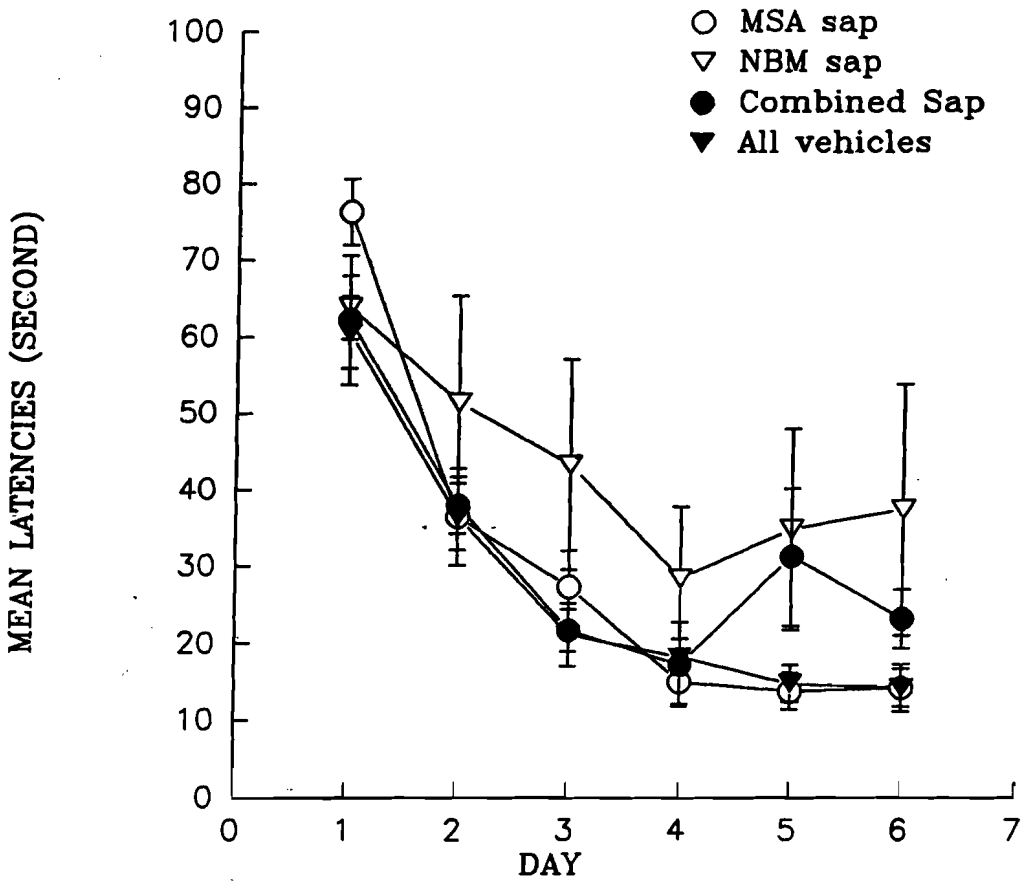


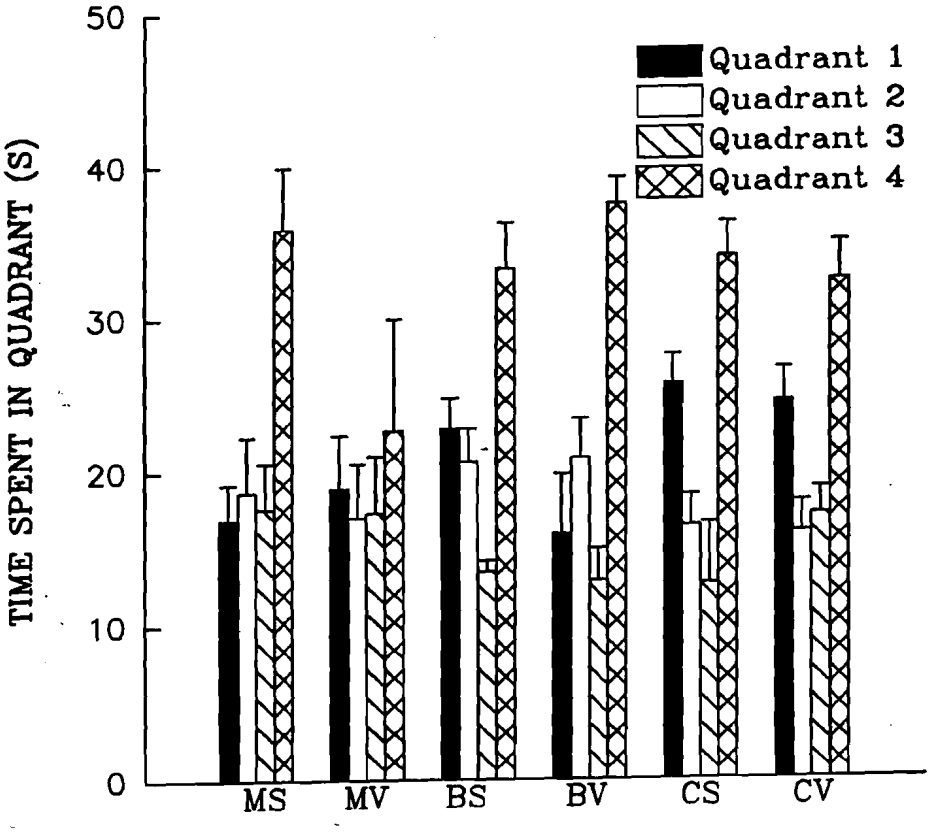
- ○ OLFACTORY BULB
- ● AMYGDALA
- △ CORTEX
- ▲ HIPPOCAMPUS
- x VTA-IPN

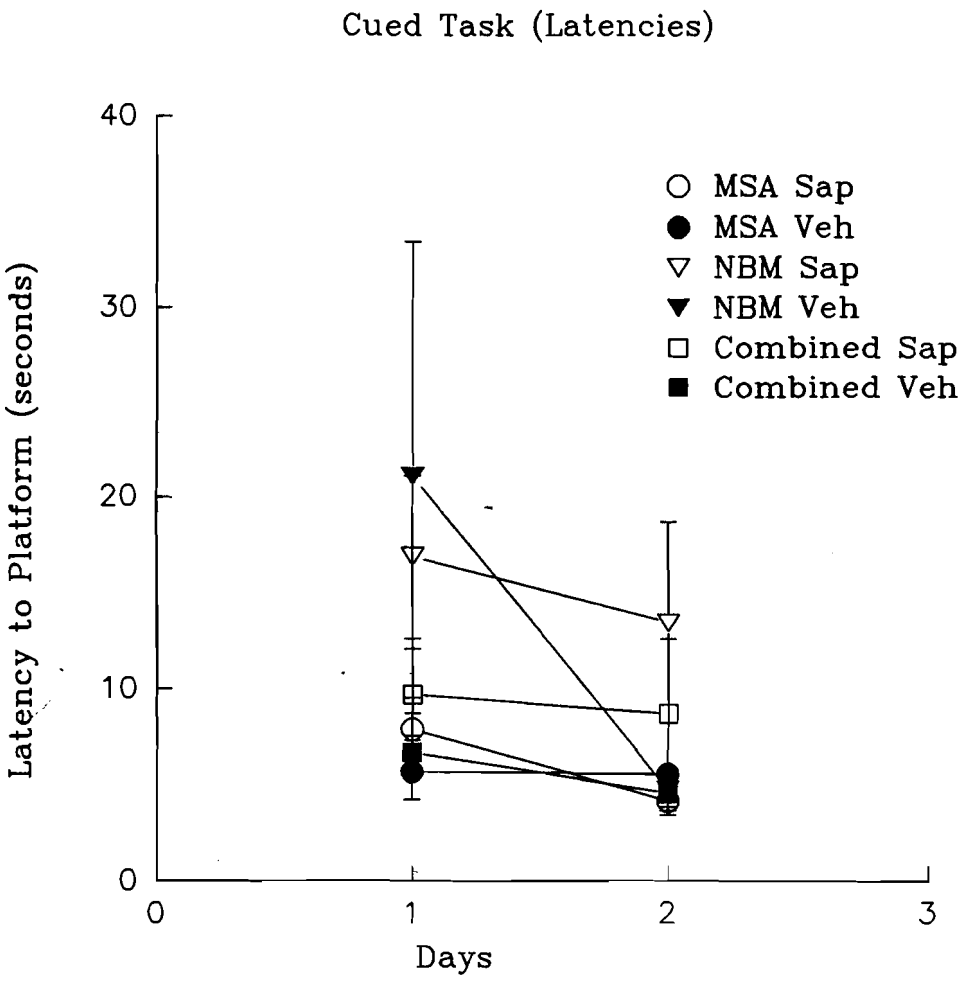


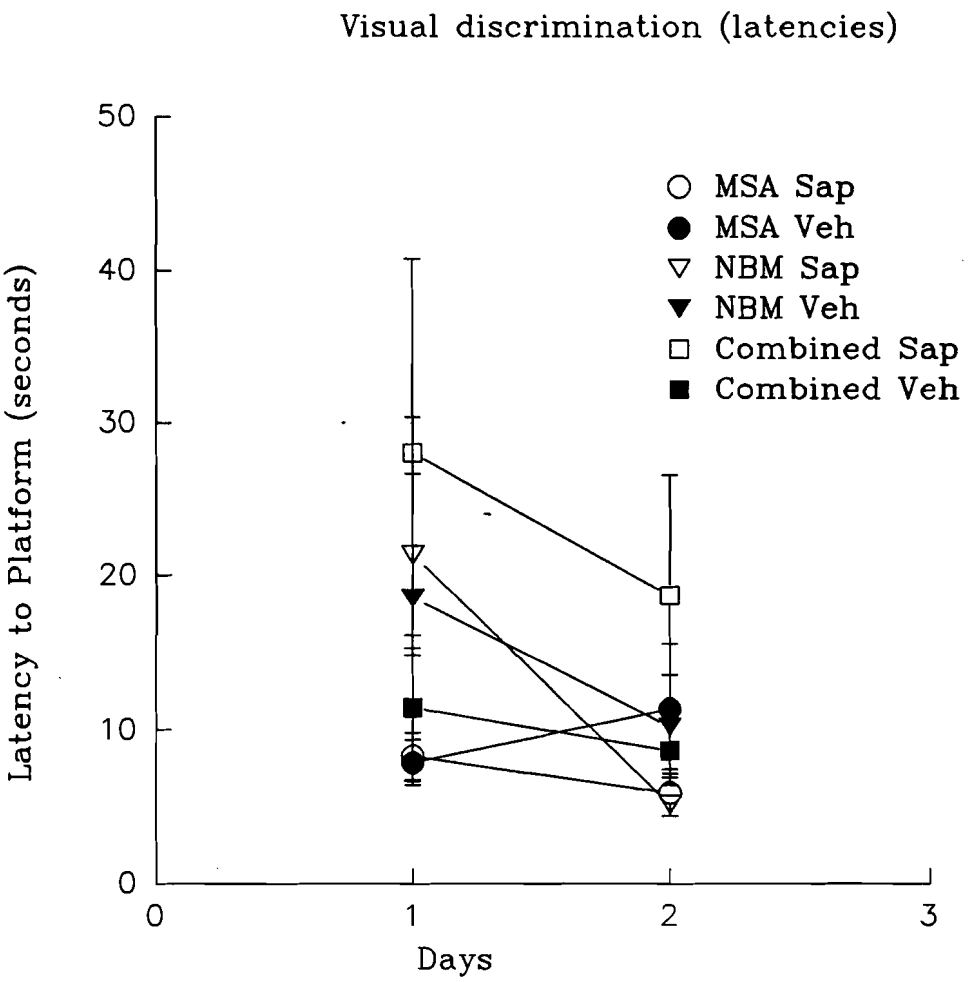

N
W
E
S

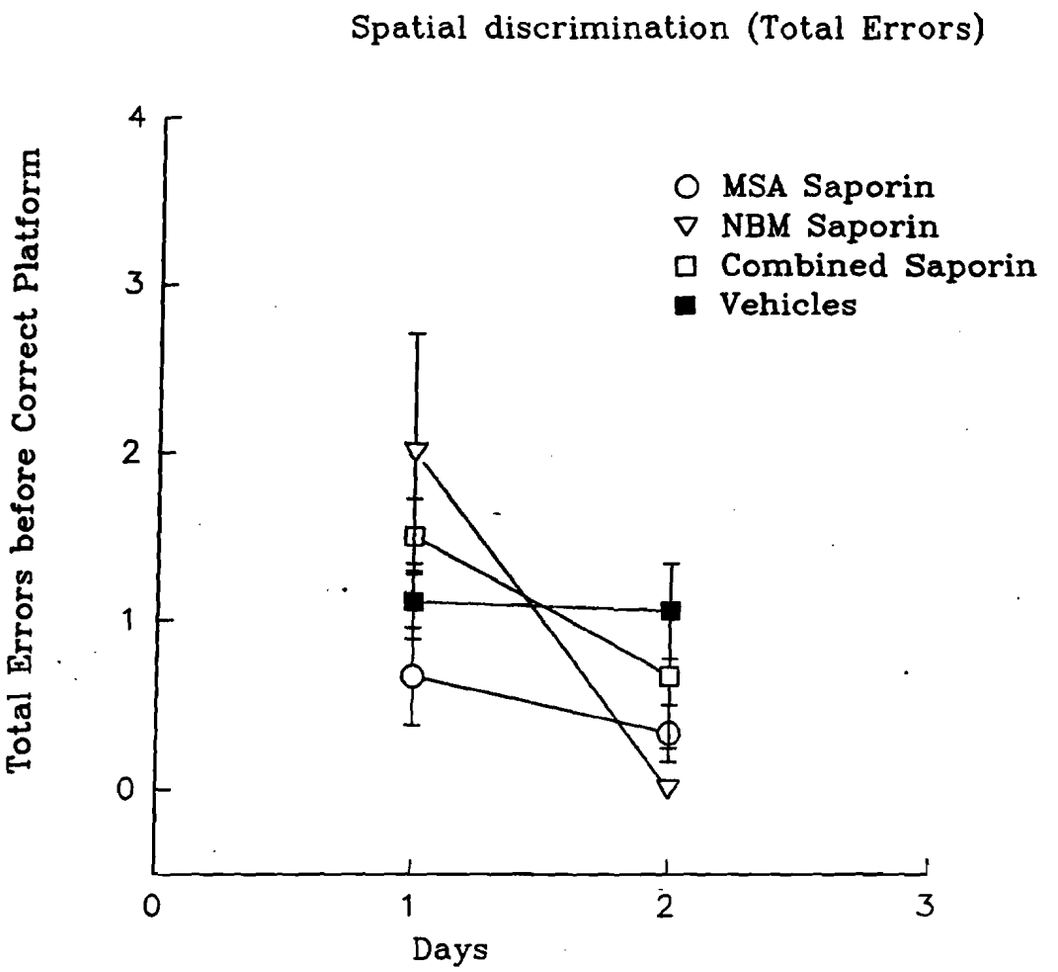
MEAN LATENCY TO PLATFORM FOR NON-CUED TASK




TIME SPENT IN QUADRANT (S)
50
40
30
20
10
0
Quadrant 1
Quadrant 2
Quadrant 3
Quadrant 4
MS
MV
BS
BV
CS
CV


Cued Task (Latencies)
Latency to Platform (seconds)
40
30
20
10
0
0
1
2
3
Days
MSA Sap
MSA Veh
NBM Sap
NBM Veh
Combined Sap
Combined Veh


Visual discrimination (latencies)
Latency to Platform (seconds)
50
40
30
20
10
0
0
1
2
3
Days
MSA Sap
MSA Veh
NBM Sap
NBM Veh
Combined Sap
Combined Veh


Spatial discrimination (Total Errors)
Total Errors before Correct Platform
4
3
2
1
0
0
1
2
3
Days
MSA Saporin
NBM Saporin
Combined Saporin
Vehicles