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NEUROTOXIN INDUCED LESIONS OF ACETYLCHOLINE
AND SEROTONIN AFFERENTS TO THE HIPPOCAMPUS:
MNEMONIC, NEUROCHEMICAL, AND
HISTOPATHOLOGICAL EFFECTS

by

Susan Murtha MSc

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of

Doctor of Philosophy

Department of Psychology

Carleton University
Ottawa, Ontario
July, 1993

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submitted by

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in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Psychology

Thesis Supervisor

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August, 1993

ABSTRACT

The behavioral, biochemical, and histological effects of chemically lesioning the medial septal area and/or the serotonergic pathway to the hippocampus were assessed on 144 Long-Evans adult male rats.

Serotonergic pathways to the hippocampus [fimbria fornix (FF) and cingulate bundle (CB)] were lesioned with the neurotoxin 5,7-DHT (4 μ g free base in 0.4 μ l ascorbic acid/saline solution). The medial septal/vertical diagonal band complex was lesioned with the excitatory amino acid NMDA (n-methyl-d-aspartate, 50 nmoles/0.5 μ l saline/injection).

Approximately 10-15 days post-surgically, the rats were tested in the Morris water maze (MWM), or the radial arm maze (RAM), or the working memory version of the Morris water maze (MWM-WM). 60 rats, 15 VEH (ascorbic acid in saline+saline), 15 5,7-DHT-only (5,7-DHT+saline), 15 NMDA-only (ascorbic acid in saline+NMDA) and 15 DUAL (5,7-DHT+NMDA) had surgery prior to testing in the MWM. 47 rats were pretrained in the RAM, underwent surgery (11 VEH, 12 NMDA, 12 5,7-DHT, 12 DUAL), then underwent 25 days of testing (five blocks of five days). 37 rats were pretrained in the working memory version of the Morris water maze, underwent surgery (9 VEH, 9 5,7-DHT, 9 NMDA, and 10 DUAL) and then 5 blocks of 4 days testing in the maze with delays of 0, 1, 10, 90 minute intervals imposed between the acquisition and test trials.

The behavioral findings can be briefly summarized as

follows: 1) the combined lesion of both cholinergic and serotonergic pathways to the hippocampus produced an impairment on working memory when comparisons were made with the 5,7-DHT-only lesioned rats and control rats. 2) Both the NMDA-only and DUAL lesioned rats recovered their ability to perform the tasks. However, working memory was slow to recover particularly in the DUAL lesioned rats in the RAM. 3) "Recovered" DUAL lesioned rats showed re-emerged but temporary impairments in working memory when a delay was imposed between acquisition and retention testing in the working memory version of the Morris water maze.

The lesion of the medial septum with NMDA and the FF and CB with 5,7-DHT reduced hippocampal ChAT activity and serotonin concentrations, respectively. There was also reduced levels of ACHE staining in the medial septum and the CA1 region of the ventral hippocampus after NMDA treatment. The NMDA injection created substantial damage in the area of the medial septum which suggested that both cholinergic and non-cholinergic cell bodies may have been affected.

In summary, the impairments observed after lesioning the medial septum as well as the serotonergic projections to the hippocampus are task dependent and emerge particularly if the task measures working memory and/or has a delay imposed between acquisition and test trials. This study contributes to our understanding of the mnemonic effects of altered function of cholinergic and serotonergic neurotransmitter

systems in the hippocampus. It also has relevance for the interpretation of the neurochemical pathology of the hippocampus that is observed in Alzheimer's disease.

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List of Abbreviations

5,7-DHT	5,7-dihydroxytryptamine
5-HT	Serotonin
5-HT _{1,2or3}	Serotonin type 1, 2, or 3 receptors
ACH	Acetylcholine
ACHE	Acetylcholinesterase
CB	Cingulate bundle
ChAT	Choline acetyltransferase
HPLC	High performance liquid chromatography
M ₁	Muscarinic type 1 receptor
MS	Medial septal
MWM	Morris water maze
MWM-WM	Morris water maze-working memory version
nBM	nucleus Basalis of Meynert/magnocellularis
NMDA	n-methyl-d-aspartate
PCA	p-chloroamphetamine
PCPA	p-cholorphenylalanine
RAM	Radial arm maze
RSA	Rhythmical slow activity

This project examined the effects of singular versus combined lesions to the cholinergic and serotonergic neurotransmitter systems on memory, specifically the effects of chronically lesioning the pathways which supply acetylcholine (ACH) and serotonin (5-HT) to the hippocampus.

The hippocampus was chosen as the target structure because a great deal of animal and human research supports the hypothesis that this region plays an important role in certain types of memory (see Barnes, 1988, and next section in this paper for review). There is, however, a scarcity of data on the effects of destroying both the cholinergic and serotonergic pathways to the hippocampus on memory. Therefore, the results from this thesis can only add to and possibly clarify the currently available literature.

The Role Of The Hippocampus in Memory

There is a wealth of literature (both human and animal) to suggest that damage to the hippocampal formation will impair the acquisition of certain kinds of information. When discussing the role of the hippocampus in memory in humans, the case study invariably reported in the literature is that of H.M. (see Squire, 1986). In 1953 he underwent surgery involving bilateral resection of the medial temporal lobes (including the hippocampus) in an effort to relieve severe epileptic seizures (reported in Squire 1986). Since his surgery, H.M. has had a deficit in the learning of either

verbal or spatial information. Moreover, H.M.'s impairment appears to be largely on tasks which require working memory (e.g., delayed matching to sample; Olton, 1983). In H.M.'s case, the damage is not solely confined to the hippocampus and therefore it does not prove whether or not damage to the hippocampus alone is sufficient to create memory impairments. Patients with a damaged hippocampus may perform poorly on both spatial and non-spatial memory tasks (Backer Cave, and Squire, 1991). The detailed case history of patient R.B by Zola-Morgan, Squire, and Amaral (1986) revealed, however, that selective damage to the hippocampus (as a result of an ischemic attack) apparently does result in persistent memory deficits. R.B. however, exhibited normal performance on a variety of tests sensitive to cognitive impairments other than memory. Detailed postmortem analysis of his brain revealed complete bilateral depletion of the pyramidal cells in the CA1 field of the hippocampus. Neuropsychological and histological observations from this study and from other similar amnesic cases are consistent with the results obtained from Squire and his colleagues with a model of human amnesia using monkeys (Squire 1992; Squire, 1986; Squire, Zola-Morgan, 1988; Zola-Morgan, Squire, Amaral, 1986).

Different taxonomies of memory form the basis for many theoretical discussions. Distinctions between short-term and long term memory or declarative and procedural memory (Squire and Zola-Morgan, 1988) have been made and are also considered

to be hippocampal dependent or independent. For the purposes of this study the tasks which require spatial working memory (memory which uses spatial cues in the environment to remember choices within a trial) or spatial reference memory (memory which uses spatial cues in the environment to remember choices held constant across trials) are considered hippocampal dependent. These distinctions are commonly applied to maze learning in rats and both may be considered a form of declarative memory (accumulation of facts and data derived from learned experiences; Eichenbaum, Otto, and Cohen, 1992). It should be noted that there are other classifications of memory which are also hypothesized to be hippocampal dependent. These will not be discussed in this thesis (see Eichenbaum, Otto, and Cohen, 1992, for a review).

A large amount of the literature focuses on the effects of destruction of the hippocampus in rodents in order to understand the more complex higher vertebrate memory system. Complete aspiration of the hippocampus has resulted in spatial memory impairments (Morris, Carrud, Rawlins, O'Keefe, 1982; for review see Morris, 1983), disruption in long term memory (primacy component of a serial position task; Kesner, Novak, 1982), and disruption of the rats working memory in the radial arm maze (Jarrard, 1978). Aspiration of the hippocampus (including overlying brain as well as portions of the amygdaloid complex) has been reported not to affect

performance on a non-spatial delayed non-matching to sample task (Rothblat and Kromer, 1991). [In the latter studies the control animals also have the tissue aspirated near the hippocampus (i.e, overlying cerebral cortex), but not the hippocampus itself. If the rats with hippocampal aspirations had been compared to un-operated controls, perhaps they would have shown a deficit in performance on this non-spatial working memory task.] The general message tends to be, however, that aspiration of the hippocampus interferes with performance on spatial/working memory tasks.

While hippocampal aspirations reportedly affect spatial working memory but not non-spatial working memory, the precise role of the hippocampus in memory becomes more difficult to define from the results of research which looks at destroying some of the pathways to this structure. The septo-hippocampal projections have been destroyed by two different methods; 1) fimbria-fornix transections, or 2) electrolytic lesions to the medial septum. Transection of the fimbria/fornix, which provides major projections to and from the hippocampus, affects performance on spatial tasks such as the cone field test (van der Staay, Raaijmakers, Lammers, Tonnaer, 1989), the Morris water maze (Eichenbaum, Stewart, Morris, 1990; Sutherland, Rodriguez, 1989; Nilsson, Shapiro, Gage, Olton, Bjorklund 1987), radial arm maze (Jarrard 1978, see Olton, 1983 for review), and the T-maze (Rothblat and Kromer, 1991). Fimbria-fornix transections do not affect a non-spatial

delayed non-matching to sample task (Rothblat and Kromer, 1991).

Electrolytic lesions of the medial septum (which contains cholinergic cell bodies with terminal fields in the hippocampus) also impair performance on a delayed alternation task (Numen, 1991; Numan and Quaranta, 1990), and retention of working memory after a delay in the Morris water maze (Dokla and Boitana, 1991). Medial septal lesions may or may not impair spatial reference memory on the Morris water maze (Dokla and Boitano, 1991; Nilsson, Strecker, Daszuta, Bjorklund, 1988). Often the impairments observed on the first days of testing are gone by the end of the testing (five days later) on the Morris water maze (Nilsson, Strecker, Daszuta, Bjorklund, 1988). Results for this task may also vary depending on the variable measured (latencies, path lengths during the probe trial, or annulus crossings; Dokla and Boitano, 1991).

In summary, destruction of septo-hippocampal pathways may or may not impair performance on behavioral tasks that measure memory. The likelihood of impairment is dependent on the type of memory assessed, the variables used to measure performance, and whether the animal has received prelesion training prior to testing (see Barnes, 1988 for review; Jarrard, 1978). In general, spatial memory appears to be more affected than non-spatial memory, and working memory (within trial performance) more than reference memory (between trial performance).

Aspiration of the hippocampus, transection of the fimbria/fornix, and electrolytic lesions of the medial septum tend to destroy cholinergic and serotonergic systems as well as any other neurotransmitter pathways passing through the area. Therefore, the variety of results observed when using these methods possibly reflects the effects of destruction of a variety of inputs to the hippocampus. For the purposes of this paper, we wish to determine the effects of selectively manipulating the cholinergic or serotonergic systems, either alone or in combination. These systems and their role in hippocampal function will be discussed next.

Cholinergic Projections To The Hippocampus

Cholinergic cell bodies in the medial septal vertical diagonal band complex show topographical specificity of their terminal fields in the hippocampus (McKinney, Coyle, Hedreen, 1983). The vertical diagonal band and the lateral part of the medial septum (which is primarily cholinergic) project to the ventral hippocampus whereas the medial portion of the medial septum (which also contains non-cholinergic fibres) projects to the dorsal part of the hippocampus (Nyakas, Luiten, Spencer, Traber, 1987; Senut, Menetrey, Lamour, 1989). The two main septo-hippocampal pathways, the fornical and fimbrial fibre tracts reach Ammons horn by invading the CA1 area from both medial and lateral sides (Nyakas, Luiten, Spencer, Traber, 1987). Dense innervation of the stratum oriens and

stratum radiatum in the hippocampal formation results from fibres from the lateral side passing through the pyramidal cell layer of CA2 and CA3 (Fibiger, 1982). In the dentate gyrus, the hilus particularly the polymorph and molecular layer of the granule cell field, also appears to be densely innervated by the cholinergic neurons of the medial septum and vertical diagonal band (Fibiger, 1982; Nyakas, Luiten, Spencer, Traber, 1987).

Serotonergic Projections To The Hippocampus

The serotonergic projections of interest in this study ascend from the dorsal and median raphe nucleus mainly via the median raphe forebrain tract, run along the ventromedial aspect of the medial forebrain bundle and innervate medial forebrain structures such as the cingulate cortex, septum, and hippocampus. In the septum some fibres are distributed to the fornix and these then divide into two pathways the fimbria and the dorsal fornix. Among other structures, the fibres that travel in the dorsal fornix terminate in the subiculum. Those travelling via the fimbria innervate the subfornical organ and pass into the hippocampus proper. These fibres project to the stratum oriens and to the stratum radiatum of CA2-CA4. Fibres also reach the dentate gyrus and innervate the polymorph and molecular layers of the granule cell field. Other fibres from the medial raphe forebrain bundle project via the diagonal tract to the medial nuclei of the septum. A large portion of

these fibres continues dorsally to join the cingulum bundle at the level of the genu of the corpus callosum and extends caudally with this bundle to distribute to the cingulate cortex. They then curve ventrally into the hippocampal complex at the level of the splenium of the corpus callosum, and project through the subiculum into the hippocampus (CA1 between stratum radiatum and stratum moleculare-lacunosum) and dentate gyrus (polymorph layer; Azmitia, 1978).

There appears to be some overlap of the cholinergic and serotonergic projections to the hippocampus. For example, both pathways travel through the septum especially via the fornical and fimbrial pathways. Both also terminate in the stratum oriens and radiatum of the hippocampus proper, and in the granule cell field of the dentate gyrus. One would speculate that destroying the cholinergic cell bodies in the medial septum in addition to lesioning the serotonergic projections in the cingulate bundle and/ or the fimbria/fornix would cause considerable dysfunction of the cell fields in the hippocampus.

Interactions of Cholinergic and Serotonergic Systems of the Hippocampus

Their anatomical overlap suggests a functional interaction on a physiological level between the cholinergic and serotonergic systems. Therefore, it follows that

stimulating or lesioning one system may modulate function of the other.

Lesioning the cholinergic system with a fimbriaectomy, decreased 5-HT₁ (serotonin type 1 receptors) binding in the hippocampus (Quirion and Richard, 1987). These findings are consistent with other research showing that 5-HT₁ receptor sites (which predominate in the frontal cortex, hippocampus, and striatum) have been found on cholinergic terminals (see Cross, 1990 for review; Maura and Raiteri, 1986). It remains to be determined, however, which of the 5-HT₁ receptors (A,B, or,C) are affected after fimbriaectomies (Quirion and Richard, 1987).

Lesions of the cholinergic projections to the cortex and hippocampus have been shown to cause a late appearing increase in 5-HT₂ receptors. After excitotoxic lesion of the basal forebrain (either nucleus Basalis of Meynert or both it and medial septal nuclei), 5-HT₂ receptor density one week after surgery remained unchanged (Wenk and Engisch, 1986), or decreased in the somatosensory area of the neocortex (Quirion, Richard, Dam, 1985). Four months post surgery, however, 5-HT₂ receptor density was increased in the anterior sensorimotor region of the neocortex but not in the hippocampus (Wenk and Engisch, 1986). In addition, the density and activity of serotonergic terminals were decreased in the cortex but not in the hippocampus. These results suggest that four months

following cholinergic basal forebrain lesions, there was a degeneration of cortical serotonergic afferents which induced a localized, neocortical denervation supersensitivity in 5HT₂ receptors (Wenk and Engisch, 1986).

Stimulating serotonergic release with quipazine (a drug which stimulates both pre and post-synaptic nerve terminals) increased levels of acetylcholine in the hippocampus (Samanin, Quattrone, Peri, Ladinsky, Consolo, 1978). The increase in hippocampal cholinergic release produced by quipazine, could be blocked by electrolytically lesioning the serotonergic median raphe nucleus (Samanin, Quattrone, Peri, Ladinsky, Consolo, 1978). This suggests that there is an interaction between the serotonergic and cholinergic system. In support of this, Robinson (1983) observed that lesioning the median raphe nucleus with a neurotoxin 5,7-DHT (5,7-dihydroxytryptamine) did not alter cholinergic turnover rates in the hippocampus, but increased turnover rates of acetylcholine in the cortex. Lesioning the dorsal raphe nuclei, however, increased turnover rates of both hippocampal and cortical ACH (Robinson, 1983). These changes in hippocampal acetylcholine are compatible with the hypothesis that serotonin has a regulatory influence (mainly inhibition) on the cholinergic system, as observed in the cortex (Barnes, Barnes, Costall, Naylor, Tyers, 1989) and in the striatum (Euvrard, Javoy, Herbet, Glowinski, 1977; Gillet, Ammor,

Fillion, 1985).

Intraventricular injections of 5,7-DHT can affect the functional status of muscarinic receptors in the hippocampus or the cortex (Alonso and Soubrie, 1991). Neither the density nor the sensitivity of muscarinic (M_1 and non- M_1) receptors in the hippocampus or cortex are affected by serotonergic denervation. It does appear, however, that destroying serotonergic terminals prevents the up-regulation of muscarinic (non- M_1) receptors otherwise produced by repeated injection of scopolamine (cholinergic antagonist) in rat hippocampus and cortex (Alonso and Soubrie, 1991). It may be that the increased release of acetylcholine observed in serotonergic lesioned rats, reduced the efficacy of scopolamine at non- M_1 receptors, thereby eliminating the compensatory response of these receptors.

In summary, the results from these studies suggest that a straightforward relationship between the cholinergic and serotonergic systems is not often found. Lesioning either neurotransmitter system alone may have some effect on the other - such as altering receptor binding or neurotransmitter release - but it tends to depend on where the lesion is produced, length of time post-lesion the effects are assessed, and how these effects are assessed (for example, receptor binding, receptor sensitivity, turnover rates, and so on). The details of the interaction between the cholinergic and serotonergic system remain to be elucidated.

Hippocampal ACH, 5-HT and the Theta rhythm

The hippocampus produces a regular rhythmical pattern of electrical activity largely controlled by cholinergic neurons in the medial septum (Carlson, 1991; Mitchell, Rawlins, Steward, Olton, 1982). This activity is generally correlated with type I behaviors (a variety of spontaneous motor activities associated with exploration or investigation, such as walking, changing posture, turning or raising the head, and manipulating objects with the forelimbs) and not type II behaviors (reflexive or automatic behaviors such as lying immobile, face-washing, chewing, gnawing, licking, or shivering (Vanderwolf, Baker, Dickson, 1990). Vanderwolf and colleagues have found that ascending serotonergic projections also play a general role in regulating the rhythmical slow activity (theta activity) in the hippocampus (as well as the low voltage fast activity in the cortex), but only during Type I behaviors (1989).

Evidence for the involvement of acetylcholine and serotonin in hippocampal rhythmical slow activity (RSA) comes from studies which manipulate each of these systems alone and together. For example, administering anti-muscarinic drugs such as scopolamine or atropine alone alters some but not all of the hippocampal RSA. The hippocampal RSA which persists after anti-muscarinic treatment is referred to by Vanderwolf and colleagues as atropine-resistant RSA. This atropine-resistant RSA is totally abolished when the rats are

administered a combination of scopolamine or atropine with PCPA (p-chlorophenylalanine) or 5,7-DHT. Blocking or lesioning only the serotonergic system, however, does not alter the spontaneous hippocampal RSA during Type 1 behavior (Vanderwolf, Baker, Dickson, 1990; Vanderwolf, Leung, Eaker, and Stewart, 1989).

These observations suggest that ascending serotonergic fibres may regulate or interact with the electrical activity produced by the cholinergic septohippocampal pathway during Type 1 behaviors. The spontaneous motor activities which define Type I behaviors are essential for performing many maze tasks (Vanderwolf, 1989). It would seem natural to conclude that with the additional lesioning of the serotonergic system, an effect on behavior should occur.

Acetylcholine and Learning/Memory

In humans, interfering with cholinergic transmission by administering drugs (such as scopolamine) that cross the blood brain barrier has resulted in disruption of memory storage and possibly retrieval (Bartus, Dean, Beer, Lippa 1982; Caine, Weingartner, Ludlow, Cudahy, Wehry, 1981; Crow, Grove-White, 1973; Drachman, 1977; Drachman and Leavitt, 1974; Ketchum, Sidell, Crowell, Aghajanian, Hayes, 1973; Petersen, 1977; Safer and Allen, 1971; Sitaram and Weingartner, 1978). In addition, administering physostigmine (an acetylcholinesterase

inhibiter) reversed the memory deficits in subjects who had received scopolamine (Drachman, 1977).

In rats and mice, cholinergic antagonists and agonists can alter performance, but the results are not always consistent. The inconsistent results may be due to different experimental parameters such as drug dosage, when the drug was administered (before or after testing), method of drug administration, and behavioral task. For example, anticholinergic drugs (scopolamine, benztropine, quinuclidinyl benzilate, to name only a few) administered to either rats or mice, interrupted acquisition of an active avoidance task more than retention, but performance on both these measures was significantly impaired compared to controls (Vanderwolf, 1987; Flood, Landry, Jarvik, 1981; Meyers, 1965). In rats, scopolamine also produced an impairment on a cued swim to platform task, and on the Lashley III maze (Vanderwolf, Dickson, Baker, 1990). In addition, rats administered scopolamine reduced choice accuracy or made more working memory errors in the radial arm maze (Peele, Baron, 1988; Stevens, 1981; Watts, Stevens, Robinson, 1981), Morris water maze (Buresova, Bolhuis, Bures, 1986), and in the T-maze (Beninger, Jhamandas, Boegman, El-Defrawy, 1986). Treatment with the antagonist atropine did not affect performance on either reference or working memory in the Morris water maze (Richter-Levin and Segal, 1989). Atropine administration did, however, affect performance on a discrimination task

(Whitehouse, 1964)

Cholinergic agonists (oxotremorine, physostigmine, arecoline, to name only a few) enhanced performance on a passive avoidance task (Altman, Stone, Ogren, 1987), and on an active avoidance T-maze (Flood, Landry, Jarvik, 1981). These positive effects, however, depended on the dose of the drug for the active avoidance task. Both low and high doses of cholinergic agonists did not improve retention (Flood, Landry, Jarvik, 1981).

In order to create a more chronic depletion of acetylcholine, the cells in the basal forebrain (particularly the nucleus Basalis of Meynert/magnocellularis (nBM) and the medial septal (MS) nuclei) have been targeted as a lesion site. There are a number of reasons for creating lesions in this area. First, as mentioned in the previous section on anatomy, mapping studies indicate the basal forebrain to be the primary source of cholinergic projections to the cortex and the hippocampus (see Hagan and Morris, 1988 for overview). Second, there are number of similar behavioral consequences as a result of either a medial septal lesion or a hippocampal lesion (Gray and McNaughton, 1983). Third, the consistent loss of nBM and medial septal neurons observed in the brains of Alzheimer's patients and the corresponding correlation with cognitive dysfunction (Ezrin-Waters, Resch, 1986; Rogers, Brogan, Mirra, 1985) has influenced a large number of researchers to create similar basal forebra'n degeneration in

rats in order to determine the effects on memory.

Destroying the cholinergic cell bodies in the nBM but not the fibres of passage with excitotoxins such as ibotenic acid, N-methyl-D-aspartic acid (NMDA), or kainic acid impaired retention on a passive avoidance task (Bartus, Pontecorvo, Flicker, Dean, Figueiredo, 1986; Berman, Crosland, Jenden, Altman, 1988; Santucci, Kanof, Haroutunian, 1989), impaired working memory on the radial arm maze (Bartus, Flicker, Dean, Pontecorvo, Figueiredo, Fisher, 1985; Beninger, Wirsching, Jhamandas, Boegman, El-Defrawy, 1986; Hepler, Wenk, Cribbs, Olton, Coyle, 1985; Wozniak, Stewart, Finger, Olney, Cozzari, 1989), and impaired reference memory on the radial arm maze (Murray and Fibiger, 1985). Lesioning the nBM with ibotenic acid or kainic acid also impaired choice accuracy in the T-maze (Beninger, Jhamandas, Boegman, El-Defrawy, 1986; Hepler, Wenk, Cribbs, Olton, Coyle, 1985; Salamone, Beart, Alpert, Iversen, 1984). Lesioning the nBM with quisqualic acid also increased the number of working memory errors in the double Y-maze but had no effect on reference memory errors (Biggan, Beninger, Cockhill, Jhamandas, Boegman, 1991). Lesioning the nBM affects the performance on a variety of memory tasks but it does not consistently affect performance in the Morris water maze, a spatial reference memory task (Hagan, Salamone, Simpson, Iversen, Morris, 1988; Riekkinen, Sirvio, Riekkinen, 1990a). It has been proposed that lesions with different excitotoxins may produce a variety of effects depending on the

amount of damage to non-cholinergic neurons in the basal forebrain (Page, Everitt, Robbins, Marston, Wilkinson, 1991).

Lesioning the nBM destroys the cholinergic projections to the cortex (Fibiger, 1982; Johnston, McKinney, Coyle, 1979) and not to the hippocampus (Fibiger, 1982; McKinney Coyle, Hedreen, 1983). A couple of studies have also reported memory impairments on the T-maze, radial arm maze, and on passive/active avoidance tasks after lesioning the hippocampal projections from the medial septum with ibotenic acid (Hepler, Olton, Wenk, Coyle, 1985; Hepler, Wenk, Cribbs, Olton, Coyle, 1985), similar to the memory impairments that occurred after nBM lesions. Riekkinen, Sirvio, and Riekkinen (1990a), also found impairments in the Morris water maze for both medial septal lesioned rats and nBM lesioned rats. As mentioned previously, however, this is not in agreement with Hagan et al. (1988) who found that medial septal lesions but not nBM lesions with ibotenic acid impaired performance on the Morris water maze (Hagan, Salamone, Simpson, Iversen, Morris, 1988). Although some studies show lack of agreement in behavioral deficits between MS and nBM lesioned rats (Mayo, 1989), there remains the fact the MS lesioned rats show behavioral impairments on a variety of spatial and working memory tasks.

Serotonin and Learning/Memory

The research which looks at the effects of 5-HT agonists

or antagonists on learning and memory reveals a variety of inconsistent results (see Altman and Normile, 1988; McEntee and Crook, 1991, for reviews). There are a number of questions that still remain unanswered.

It appears that drugs which affect the serotonergic system may or may not produce an impairment, depending on the conditions of the memory task, the drug used, where the drug has been injected, and the subjects used. For example, enhancing the release of serotonin with high doses of alaproclate (a 5-HT reuptake blocker) in rats significantly worsened performance in the Morris water maze by increasing escape distances (Riekkinen, Jakala, Sirvio, Riekkinen, 1991). But, in mice at high doses, alaproclate and zimeldine (a 5-HT reuptake blocker), enhanced retrieval on a passive avoidance task (Altman, Nordy, Ogren, 1984; Altman, Stone, Ogren, 1987). In addition, a variety of 5-HT_{1A} agonists (LSD, RU 24969, 8-OH-DPAT), decreased the rats efficiency in solving the radial maze (Winter and Petti, 1987). Decreasing the available serotonin with the antagonist methysergide worsened working memory on a continuous non-matching to sample task. The antagonist ketanserin was without effect, however (Sakurai and Wenk, 1990).

Inhibiting the synthesis of serotonin with PCPA did not create impairments on an active avoidance task (Vanderwolf, 1987), the Lashley III maze (a non spatial task), or on a cued swim to platform task (a simpler version of the cued swim to

platform task in the Morris water maze; Vanderwolf, Dickson, Baker, 1990). The percentage of correct responses was also not affected by the administration of PCPA in a delayed non-matching to position task (Jakala, Sirvio, Riekkinen, Riekkinen, 1993).

Vanderwolf (1989) found that producing a chronic lesion in the raphe nuclei with 5,7-DHT (a serotonergic neurotoxin) will impair performance on the cued swim to platform task, but only when the atropine-resistant cerebral activation (both RSA and cortical low voltage fast activity) was decreased or abolished. In addition, impairments in choice accuracy of a delayed spatial alternation T-maze task were observed in rats injected with 5,7-DHT into the median and dorsal raphe, but only when the delay was a minimum of 60 seconds (Wenk, Hughey, Boundy, Kim, Walker, Olton, 1987). Nilsson et al. (1988) found that intraventricular injections of 5,7-DHT did not produce a significant impairment in either latencies or path lengths in the Morris water maze.

The previously mentioned studies reflect the effects of more global (both cortical and hippocampal) depletions of serotonin. When Altman and colleagues administered 5,7-DHT to the two main extrinsic serotonergic sources which arise from the raphe nuclei and project to the hippocampus, the fimbria-fornix and the cingulum bundle, they created a selective lesion of serotonergic terminals in both the dorsal and ventral hippocampus. The lesioned rats performed better in a

Stone 14 Unit T-maze, a complex, positively reinforced spatial discrimination task, than sham operated or un-operated control rats. They made significantly fewer errors and reached criterion in significantly fewer trials (Altman, Normile, Galloway, Ramirez, Azmitia, 1990).

Thus it appears that increasing the available serotonin impairs performance in spatial memory tasks such as the Morris water maze and the radial arm maze, but does not affect performance in a non-spatial passive avoidance task. This difference, however, could be species specific. Decreasing global serotonergic levels has very little or no effect on either spatial or nonspatial tasks. Any impairments that are observed tend to depend on whether or not a delay has been used, and the type of task. Indeed, on one task (Stone T-maze), reduction of hippocampal serotonin improved performance.

There is ample evidence that both acetylcholine and serotonin play important roles in the processes underlying memory. Their precise roles, however, remain difficult to define. Inconsistencies in the behavioral effects may be due to the nature of the intervention used to alter cholinergic or serotonergic functions, and whether or not these interventions have produced global or regional changes. Although these problems exist, there are published experiments in which attempts have been made to look at the effects of

lesion of both the cholinergic and serotonergic systems on different memory tasks. The results of these studies suggests that there may exist a functional interaction between the cholinergic and serotonergic systems in learning and memory. The results of these experiments will be discussed next.

Cholinergic-Serotonergic Interaction and Learning/Memory: Acute Interventions

If increasing the availability of either serotonin or acetylcholine improves performance, then enhancing the release of both these neurotransmitters at the same time may improve performance more than the stimulation of either system alone. Administering the serotonergic and cholinergic agonists (alaproclate and oxotremorine, respectively) enhanced retrieval of a previously learned aversive habit in mice (Altman, Stone, Ogren, 1987). The simultaneous administration of both these drugs shifted the dose response curve such that a 58% reduction in peak dose was needed to achieve the same level of performance as was seen following the administration of either drug alone. Quipazine (purportedly a 5-HT₂ agonist, but may also be an autoreceptor antagonist) did not block the enhancement observed after the administration of both drugs or oxotremorine, although it did block the enhanced performance observed after the administration of alaproclate. Scopolamine completely blocked the facilitation of performance due to the combined treatment, and due to the oxotremorine treatment, but

did not affect the alaproclate treated mice (Altman, Stone, Ogren, 1987). If the combined agonist effects were additive then blocking either one should diminish the effects of the joint administration of these two drugs. This was not the case however, and as a result, Altman and colleagues (1987) proposed that the serotonergic system may modulate the cholinergic system. In other words, the release of serotonin may potentiate the effectiveness of acetylcholine. If the expression of acetylcholine mediates the final retrieval of information, then blocking serotonin would not alter performance of the rats administered both the serotonergic and cholinergic agonists, but blocking acetylcholine would. Whether or not this hypothesis can be applied to other memory tasks is open to speculation.

Contrary to the above, some studies suggest that the enhancement of serotonin has an inhibitory effect on learning and memory functions (Altman, Normile, 1988; Riekkinen, Sirvio, Riekkinen, 1990a). Enhancing the release of serotonin with low doses of alaproclate in combination with blocking acetylcholine with scopolamine in rats, significantly worsened performance in a water maze as compared to those animals administered scopolamine alone (Riekkinen, Jakala, Sirvio, Riekkinen, 1991). In this case, the effects of alaproclate were dose dependent. When administered alone at low doses, it did not affect task performance, but at higher doses it

significantly impaired performance on both the hidden platform and the cued navigation versions of this spatial memory task. The authors suggested that increased amount of synaptic serotonin after high doses of alaproclate may have interfered with sensory processing or motivational factors. Pilocarpine (which reverses the performance decrement in scopolamine treated animals) diminished but did not reverse the decrement in the combined drug-treated animals. The results of this research are opposite to what would be expected if enhancing the release of serotonin improves performance (Altman et al., 1987). That is, enhancing the release of serotonin did not decrease the performance deficits due to cholinergic blockade. In addition, manipulating the cholinergic system did not create the changes in performance that would be expected if this system actually mediated the final retrieval of information. These latter effects may be task dependent, however.

If enhancing serotonin has an inhibitory effect on the processes underlying memory, then blocking or destroying the serotonergic system alone should improve performance on memory tasks (Altman, Normile, Galloway, Ramirez, Azmitia, 1990). When jointly administered with cholinomimetics, there should be an attenuation of performance deficits due to cholinergic blockade alone. This is not the case, however. Blocking the release of serotonin with the antagonist methysergide (5-HT₂

receptor blocker) in addition to antagonizing acetylcholine with scopolamine, impaired performance on a non-spatial non-matching to sample task which assesses working memory. The rats that were given both drugs did not perform any worse than rats administered scopolamine alone, although they did perform worse than rats given methysergide alone (Sakurai and Wenk, 1990). In addition, reducing serotonin synthesis with PCPA in combination with the cholinergic antagonists atropine and scopolamine impaired acquisition of both reference and working memory in the Morris water maze (Richter-Levin and Segal, 1989), impaired performance in an active avoidance task (Vanderwolf, 1987), and impaired performance in the Lashley III maze and the cued swim to platform test (Vanderwolf, Dickson, Baker, 1990). In all cases the dual (serotonergic and cholinergic) lesioned animals performed worse than the rats with only cholinergic lesions or serotonergic lesions. In fact, neither PCPA nor atropine when administered alone affected the rats performance in the Morris water maze (Richter-Levin and Segal, 1989), the Lashley III maze, cued swim to platform task, or the active avoidance task (Vanderwolf, 1987; Vanderwolf, Dickson, Baker, 1990). The administration of scopolamine alone, however, produced an impairment in active avoidance, cued swim to platform, and on the Lashley III maze (Vanderwolf, 1987; Vanderwolf, Dickson, Baker, 1990). These results indicate that regardless of whether the tasks assess spatial or non-spatial memory,

performance tends to worsen in animals administered drugs which globally block both the serotonergic and cholinergic systems. In all cases, however, the performance of the dual lesioned animals is not always worse than the performance of the cholinergic-only lesioned animals. For instance, antagonizing the cholinergic system with scopolamine in combination with PCPA or destroying the serotonergic system with p-chloroamphetamine (PCA) impaired choice accuracy in a non-spatial continuous non-matching to sample task (Sakurai and Wenk, 1990), and discriminative accuracy in a 5-choice serial reaction time task (Jakala, Sirvio, Jolkkonen, Riekkinen, Acsady, Riekkinen, 1992). The rats administered both injections, however, were not significantly different from the rats administered only scopolamine (Jakala, Sirvio, Jolkkonen, Riekkinen, Acsady, Riekkinen, 1992; Sakurai and Wenk, 1990).

Effects Of Chronic Interventions

There is a paucity of research on the effects of lesioning the serotonergic projections to the hippocampus in combination with lesioning the cholinergic projections to the hippocampus. There are a number of studies, however, that have looked at more global depletions of serotonin in combination with cholinergic hippocampal (medial septal) or cortical (nBM) lesions. Cortical and hippocampal depletions of serotonin have been created with intraventricular injections of 5,7-DHT

in combination with a radio frequency lesion to the septum (which reduces choline acetyltransferase activity in hippocampus but not in cortex) in order to determine the effects on learning and memory. By the end of testing the rats with a combined serotonergic and cholinergic lesion took longer to find the platform in the Morris water maze than the cholinergic and serotonergic only depleted groups (Nilsson, Strecker, Daszuta, Bjorklund, 1988). Although there appeared to be an acquisition deficit in the rats with the single lesions, the deficit was gone after 5 days of testing. Nilsson et al. (1988) also found that the dual lesioned rats continued to perform poorly when retested 25 weeks after surgery as compared to operated controls (1988). In another study, rats lesioned with ibotenic acid in the nBM in combination with 5,7-DHT in the raphe dorsalis, were more impaired (had significantly longer escape latency's and path lengths) than the nBM-only lesioned rats in the Morris water maze. Both these groups were more impaired than the rats lesioned with 5,7-DHT alone or control animals. The animals treated with 5,7-DHT-only did not perform any differently than the control animals (Riekkinen, Sirvio, Riekkinen, 1990b). On a nonspatial non-matching to sample task (which assesses working memory), rats lesioned only with ibotenic acid in the nBM as well as rats with the combined treatment of nBM lesion and p-chloroamphetamine (PCA) performed poorly over the first 9 weeks of testing as compared to control animals. The PCA-

only treated animals performed as well as but not better than control animals (Markowska and Wenk, 1991). Although the nBM lesioned and the combined nBM plus PCA treated rats were not significantly different from each other over the first 9 weeks of testing, after 32 weeks of testing the nBM lesioned animals significantly improved their performance as compared to the rats receiving a combined nBM plus PCA treatment (Markowska and Wenk, 1991). The results of this research would suggest that a global serotonergic lesion potentiates performance deficits observed in cholinergic-only lesioned rats in spatial memory tasks but not in non-spatial memory tasks. Regardless of the task, the deficits observed in the dual lesioned rats appears to last long after the cholinergic-only lesioned rats have recovered.

In contrast to the aforementioned studies, rats treated intraperitoneally with PCA in combination with ibotenic acid injected into the nBM did not perform any worse than animals treated with ibotenic acid alone or control animals in a complex spatial discrimination task (Stone T-maze). As mentioned previously, the rats depleted only of serotonin performed better than the three other treatment groups (Normile, Jenden, Kuhn, Wolf, Altman, 1990). Improved performance in the Stone T-maze has also been found after a selective serotonergic depletion in the hippocampus (Altman, Normile, Galloway, Ramirez, Azmitia, 1990). This research

supports the notion that serotonergic lesions enhance performance on a memory task. It would appear that the facilitatory effects of serotonergic lesions are diminished when an additional cortical cholinergic lesion is created. It is unclear, however, why this happened when the cholinergic-only lesion did not impair performance of the rats in this particular maze.

This research suggests that lesioning serotonergic and cholinergic systems together may have three possible outcomes. The addition of the serotonergic lesion may worsen the performance of rats depleted of acetylcholine (Riekkinen, Sirvio, Riekkinen, 1990b). It may improve the performance of cholinergic lesioned rats, but there may be no change in performance in comparison to controls because the cholinergic lesion blocks the improved performance normally observed in rats who are depleted of only serotonin (Normile et al., 1990). Lastly, the combined lesion may prevent the recovery of function on either a non-spatial (Markowska and Wenk, 1991) or a spatial memory task (Nilsson et al., 1988) that is usually observed in cholinergic-only lesioned rats.

Thus, there remains some discrepancy in the literature with respect to the functional interaction between serotonin and acetylcholine lesions. This discrepancy may be due to a variety of reasons. Results may depend on the drugs used to

manipulate the two systems, particularly their pre or post synaptic site of action. Behavioral performance may be affected by whether or not the lesions are selective for the hippocampus or more globally affect the entire neurotransmitter system. Finally, it is possible that some lesions may produce performance deficits on tasks which assess only certain types of memory.

In the experiments outlined below, I assessed the effects of both singular and combined lesions of the serotonergic and cholinergic pathways to the hippocampus on spatial memory (both reference and working memory). Specifically, the following hypothesis regarding the effects of the combined lesions were assessed:

- 1) the combined (DUAL) lesion of both acetylcholine (ACH) and serotonin (5-HT) will produce an impairment on the memory tasks greater than that observed in the cholinergic or serotonergic only lesioned rats.

- 2) the DUAL lesioned rats will not recover their ability to perform the task as quickly as the cholinergic- or serotonergic-only lesioned rats.

- 3) "recovered" DUAL lesioned rats will show persisting impairments in working memory when the limits of their memory are probed, for example by lengthening the interval between acquisition and retention testing.

The first hypothesis was assessed by three experiments. The first experiment examined spatial reference memory with the Morris water maze. The second experiment assessed both spatial working and reference memory in the 12 arm radial maze. On the second task I was also able to test the second hypothesis which concerned recovery of function. The lesions were confirmed by biochemical assays of choline acetyltransferase (used as an indirect measure of acetylcholine depletion) and of catecholamines on selected brain parts obtained from the rats from the first two experiments. A third experiment looked at the effects of these lesions on working memory in the Morris water maze. This third experiment was designed to reassess recovery of function of working memory in addition to testing the third hypothesis. Once the rats had recovered and performed as well as control animals, a delay was inserted between the acquisition and the test trial in order to see if this would reveal a residual deficit in working memory. The brains from this third experiment were sectioned and stained for cresyl violet. In order to determine if any changes had occurred in morphology of the hippocampus a fourth group of rats underwent surgery and eventually their brains were sectioned and stained for acetylcholinesterase.

METHOD

Animals

In total, 144 Long-Evans male retired breeders were obtained from Charles River Laboratories. The rats were approximately 9 months of age when they arrived and weighed between 300-400g. They were housed in pairs in polypropylene tubs (unless otherwise indicated) in a climate controlled vivarium, and were placed on a 12 hr reverse light/dark cycle (lights off at 08.00 h). They were fed ad lib food (unless otherwise specified) and water.

Surgery

All rats were pretreated a minimum of thirty minutes prior to surgery with desmethylinipramine (DMI; Sigma) 10 mg/ml in order to prevent the uptake of 5,7-dihydroxytryptamine (5,7-DHT) into norepinephrine/dopamine containing terminals (Bjorklund, Baumgarten, Rensch, 1975).

The rats were anaesthetized with a halothane/oxygen combination. During induction they received up to 5% halothane and 1.5% oxygen. Once anaesthetized they were maintained at 1.5% oxygen and approximately 2% halothane. All rats received 21.7 mg/kg sodium pentobarbitol (approximately 33% of their body weight) at the end of surgery. This amount of sodium pentobarbitol kept the rats partly sedated for about another hour after surgery. Pilot work in this laboratory revealed

that some of the rats that had received NMDA treatment showed mild tonic-clonic seizure activity within the first few minutes after the halothane had worn off. The administration of sodium pentobarbital has been found to prevent seizure activity (see Prichard, 1980 for overview).

A 5 μ l Hamilton syringe with a 33 gauge (0.20 mm) stainless steel needle was used to deliver the drugs. 0.5 μ l was pulse injected at the rate of 1/10 to 1/15 μ l /minute. The needle remained in position 4 minutes following the last injection. Injections took approximately 8-9 minutes in total.

All injections were made in the skull flat position. The coordinates for the medial septum were obtained from Paxinos and Watson (1986). The coordinates, for the fimbria fornix (FF) and cingulate bundle (CB) were obtained from a procedure outlined by Altman et al. (1990) and correspond to similar stereotaxic coordinates in Paxinos and Watson (1986).

Lesions of the FF and CB were created with the neurotoxin 5,7-DHT (Sigma) at the concentration of 4 μ g free base in 0.4 μ l solution (1 mg/ml ascorbic acid in saline). Injections in the CB were made on a 15 degree angle from vertical, A.P -1.0mm, L.M. $\pm$ 1.3, and D.V. 2.5mm from the skull surface. Injections into the FF were made at the same angle and A.P (-1.0mm) but the needle was lowered D.V. 4.5mm. The FF was injected half the time when the needle was positioned to the left and the other half the time when it was positioned to the right. Pilot

work in this laboratory had revealed that the single injection into the FF depletes about 80% of the serotonin in the hippocampus. A second injection into the FF from the opposite side slightly increased the amount of serotonin depleted, but it also tended to increase the amount of norepinephrine depleted, and added more time to an already lengthy surgical procedure. It was concluded that the second injection was unnecessary because the problems of administering a second injection from the opposite side of the brain appeared to outweigh the benefits of marginally increasing an already large depletion of serotonin.

Lesions in the medial septal/vertical diagonal band complex were created with the excitatory amino acid NMDA at the concentration of 50 nmoles/0.5 μ l saline/injection (pH 7.4). This excitotoxin was chosen because it is considered to be a stable, inexpensive, effective tool for lesioning basal forebrain cholinergic neurons (Santucci, Kanof, Haroutunian, 1989; Stewart, Price, Olney, Hartman, Cozzari, 1986; Wozniak, Stewart, Finger, Olney, Cozzari, 1989). Three injections were made at the midline by gently pulling aside the midsagittal sinus. Two injections were made at A.P. +0.7 mm, one at D.V. 7.0 mm and the second at 6.4 mm from the skull surface. The third injection was made at A.P. +0.2mm, and D.V. 6.5 mm.

For the sake of brevity, rats that received only vehicle injections (ascorbic acid in saline + saline) at the sites mentioned above will be referred to as VEH. Rats treated with

the neurotoxin 5,7-DHT (5,7-DHT + saline) will be referred to as 5,7-DHT or 5,7-DHT-only. Rats treated with the excitatory amino acid NMDA (ascorbic acid in saline + NMDA) will be referred to as NMDA or NMDA-only. Rats treated with both 5,7-DHT and NMDA will be referred to as DUAL lesioned.

Behavioral assessment began approximately 10-15 days after surgery. This time period was chosen to allow for recovery from the surgery, and to allow for the degeneration of neurons to occur following the lesion.

Morris water maze-reference memory version

60 rats (15 in each lesion condition) had surgery prior to testing in the Morris water maze (MWM).

The water maze consisted of a large opaque white cylindrical pool (150 cm diameter, 45 cm deep) filled to a depth of 30 cm with room temperature water rendered opaque by the addition of powdered skim milk. A transparent plexiglass platform was placed 1-2 cm below the water surface in the middle of one quadrant of the pool (north-east quadrant). The platform was not visible to the rat. The pool was situated in the middle of a room containing many extra-maze cues such as sink, T.V. monitor, and posters. It is thought that the rat uses the external spatial cues to help him locate the hidden platform.

Hidden platform test: The first five days consisted of 4

consecutive trials at the start of which the rats were placed at one of 4 randomly selected locations around the perimeter of the pool (90 degrees apart). The platform remained in the same position, and the rat's latency to locate the hidden platform was recorded for each trial. A maximum of 90 seconds was allotted per trial. If the rat failed to locate the platform within this time, it was placed on the platform for 20 sec. If the rat successfully found the platform within 90 sec, it was allowed to remain on the platform for 20 sec.

No platform test: On the sixth test day, the platform was removed from the pool and the rats were allowed to swim for 60 seconds around the platformless pool. These sessions were videotaped and scored for 1) percentage path length in each quadrant; 2) percentage time in each quadrant; 3) swim speed (total distance/total time); 4) number of annulus crossings within the north-east quadrant (the annulus is defined as covering a space twice the diameter of the platform); and 5) the heading angle. The heading angle was defined as the angle of the rat's swimming direction relative to the platform's location as the rat leaves the starting point, whereby 1 was assigned to a heading angle 0-23°, 2 was assigned to a heading angle 24°-46°, 3 was assigned to a heading angle 47°-70°, and 4 was assigned to a heading angle 70°-90°.

It is conceivable that the latency to find the platform would decrease over the five test days even if the rat is not using spatial cues to find the platform. For example they may

instead use a circular search strategy. The purpose of the platformless trial was to assess spatial memory for platform location. Rats that learn the platform's location using spatial cues will have a heading angle towards the platform, swim proportionately more in the quadrant that had previously contained the platform, and as well have more annulus crossing for this quadrant. Swim speed should not vary between the groups.

Cued navigation test: The seventh test day involved 4 training trials to locate a visible black platform which was raised slightly above the water's surface. The purpose of this test was to assess the rats motivation to escape the water, its physical capacity to swim and its ability to learn a simple cued task. Performance on cued navigation is usually unaffected by damage to the hippocampus.

12 Arm Radial Maze

47 rats were pretrained in the radial arm maze (RAM) for 20 days. Subsequently they underwent surgery, recovery, and 25 days of testing.

All rats were exposed to the novel food reward (Kelloggs Froot Loops) and handled for one-two weeks prior to training in the radial arm maze. During training/testing animals were moved to single housing in stainless steel, wire mesh, hanging cages (20x24x18 cm). Rats were food-deprived to 80-85% of their predeprivation body weight (allowing for a minimum gain

of 3 g per week for normal body growth). During pre- and post-lesion training/testing the rat's daily food ration was provided immediately after each test session.

Apparatus: The 12-arm radial maze was built on a stand 84 cm above the floor. The maze was constructed of dark-grey polyvinyl chloride (Lexan). The arms were 65 cm X 9.5 cm and were spaced equally around the central platform (34 cm in diameter) so that the angle between each arm was approximately 30 degrees. On both sides of each arm a clear sloping barrier (18 cm high at the edge of the central platform and 8 cm at the distal edge) extended 34 cm into the arm. These barriers were flush with the 2.5 cm sides which ran down the entire length of each arm. Circular food wells (2 cm deep, 3.5 cm diameter) were located 6 mm in from the ends of each arm. A white opaque polyvinyl chloride cylinder was placed on the central platform of the maze in order to confine the rats' movements at the beginning of each trial. The maze was centred in an enclosed fluorescent-lit room with a variety of extra-maze cues such as shelves, a door, posters, and the experimenter, which all remained constant. An industrial fan (kept in the same location throughout the experiment) was used to provide masking noise.

One-half a Froot Loop was placed in the food cup at the end of the six arms in the baited set. One quasi-random

pellet-placement-pattern was chosen (baited arms were not all odd or even numbered arms nor more than two arms apart) and remained constant for each rat across all pre- and post-lesion sessions. Reinforcement was not replaced during acquisition training or retention testing. Thus a maximum of six food rewards (and hence six correct choices) could be obtained. Working memory errors were defined as a repeat visit to any of the six baited arms. Reference memory errors were defined as a first time visit to a never baited arm (6 errors in total could be made).

Animals were weighed before each training/test session, then carried to the actual testing room in their home-cage. Rats were placed on the centre of the maze, and their initial movements were confined by a plastic cylinder for no more than ten seconds. The training/test session began when the cylinder was manually removed by the investigator. The maze was wiped clean (with a water-alcohol solution), after each training/test session.

Shaping: Rats were given two days of shaping. On the first day the rats were allowed a twenty minute session to familiarize themselves with the maze. During this session, small pieces of Froot Loops were placed leading up to the cups in the baited arms. On the second day of shaping, the rats were allowed 10 minutes to explore the maze and find the Froot

Loops. This time the reward was placed in the middle of the arm extensions and also adjacent to the food cups.

Prelesion Training: An arm entry was recorded when the rat placed four paws past the mid-way point of the arm. The rat was removed from the maze when all six food rewards had been retrieved or 10 minutes had elapsed, whichever came first. Scoring consisted of 1) number of entries into baited arms (working memory errors). 2) first entry into never baited arms (reference memory errors, maximum of six). 3) The number of correct choices out of the first six arm entries (choice accuracy).

The first day of pre-lesion training commenced on the third day of exposure to the maze. The food reward was placed inside the food cups at the ends of the pattern-appropriate arms. Pretraining ended after a maximum number of 20 days, rats which failed to make five correct choices within the first six arm entries for at least one day were eliminated from the study. The remaining rats were assigned to one of the four experimental groups. Efforts were taken to ensure that all of the groups received rats from a distribution of skill levels.

Maze Rotation occurred on day 21 of pre-lesion training, the maze was rotated 90 degrees counter-clockwise. In this way

we were able to assess the possible salience of any uncontrolled and hence confounding intra-maze cues during prelesion training. The pre-established baiting patterns remained the same in maze rotation as in acquisition training. That is, all baited arms remained constant relative to the distal cues in the testing room throughout the training period. There were no significant differences ($p>0.05$) in number of working memory or reference memory errors or choice accuracy between the average of the last block of prelesion training and the rotation day, suggesting that the rats had learned the task without using intra-maze cues.

Postlesion Testing: After the 20 days of pretraining on the maze, the rats were assigned to one of the four lesion groups (11 vehicle controls, 12 NMDA, 12 5,7-DHT, 12 5,7-DHT+NMDA). Prior to surgery, there were no significant group differences ($p>0.05$) in the average number of working or reference memory errors or choice accuracy. The rats underwent surgery, then post-lesion testing began after an average of 10-15 days of recovery.

The procedure as outlined for the prelesion training period was followed. Except, the rats were not exposed to the two days of shaping at the beginning of the test period. They were observed for 10 minutes a day or until the 6 food rewards had been obtained. All the groups were tested for a minimum of 25 days. At this point both the VEH and 5,7-DHT treated rats

asymptoted in the number of errors they were making. The NMDA and DUAL lesioned rats were tested for another block of five days. The rats behavior was scored for working and reference memory errors and choice accuracy in the radial arm maze.

Morris water maze-working memory version

37 rats were assessed in the working memory version of the Morris water maze (MWM-WM).

The water maze was the same one as described in Experiment I. The procedure, however, differed. A transparent plexiglass platform was placed 1-2 cm below the water surface in the middle of a different quadrant of the pool on each test day. The platform placement was quasi-random, so that over the four days the rat had to locate the platform in four different quadrants. The platform was not visible to the rat. The pool was situated in the middle of a room containing many extra-maze cues such as sink, T.V. monitor, and posters.

Presurgical exposure: The first four days consisted of 2 consecutive trials (T1 and T2) per day, with the platform located in the centre of a different quadrant each day. The rats were placed at one of 2 quasi-randomly selected locations around the perimeter of the pool (90 degrees apart). These locations varied depending on the position of the platform. That is, the two furthest locations from the platform were chosen for each day. For example, if the platform was located in the south-west quadrant then the rats were started either

from the north or the east locations in the pool. Whichever location not used in the first trial, was used in the second trial. This procedure of choosing the furthest starting locations was followed in order to equate the distance the rat had to travel to reach the platform. This procedure also controlled for randomly selecting a start location on the second trial which was shorter in distance and therefore artificially reduced latencies on the second test trial. A maximum of 60 seconds was allotted per trial. If the rat failed to locate the platform within this time, it was placed on the platform for 20 sec. If the rat successfully found the platform within 60 sec, it was allowed to remain on the platform for 20 sec.

The rat's latency to locate the hidden platform was recorded for each trial. Rats that remember where the platform was on the acquisition or first trial (T1) should have reduced platform location latencies on the test or second trial (T2). Working memory can be measured by the savings in latencies between T1 and T2. Because some rats are faster at performing the task than others, percentage of savings $(T1 - T2 / T1 * 100)$ was used as the measure in the analysis.

After the 4 days of pretraining on the maze, the rats were assigned to one of the four lesion groups (10 in the DUAL lesioned group and 9 in the other three groups). There were no significant differences ($p > 0.05$) between the groups in

percentage savings. The rats underwent surgery, then an average of 10-15 days of recovery.

Postlesion Testing: The rats were exposed to an acquisition trial (T1) and one test trial (T2) for 4 days.

The block of 4 days was analyzed for group differences. If there were no differences then a delay was introduced between T1 and T2.

Delay testing: One, 10, and 90 minute delays were used between T1 and T2. Dokla and Boitano (1991) reported that a delay of 5 minutes increased mean latency in rats administered an electrolytic lesion of the medial septum. We wished to see if a delay would worsen the performance of the DUAL lesioned rats as compared to the NMDA-only lesioned rats. Increasingly longer delays were used in this experiment to test the limits of the memory impairment.

Neurochemical Assays

The effects of the 5,7-DHT neurotoxin on catecholamine depletion were determined by high performance liquid chromatography (HPLC) on 43 rats (11 VEH, 5,7-DHT, DUAL, and 10 NMDA-only treated) from the first experiment (those behaviorally tested on the MWM) and on 47 rats (11 VEH, and 12 5,7-DHT, NMDA and DUAL lesioned rats) from the second experiment (those behaviorally tested on the RAM). The effect

of NMDA on cholinergic neurons was assessed by measurement of choline acetyl transferase (ChAT) activity in the brains of a total of 95 rats. ChAT activity was assessed 34-45 days postsurgically in 48 rats who were tested in the MWM, and an average of 61-75 days postsurgically in 47 rats tested in the RAM. These biochemical data may be considered representative of the effects of the surgery.

HPLC Assay: For this assay the rats were decapitated and their brains were dissected on ice into dorsal and ventral hippocampus, caudate, anterior cortex (rostral to rhinal fissure), and posterior cortex (caudal to rhinal fissure). Once dissected, the tissue was rapidly frozen in liquid nitrogen. Tissue was stored at -70°C until it could be subjected to HPLC analysis.

The procedure and equipment were the same as outlined in Pappas et al. (1992) with the exception of the following changes in the mobile phase. The buffer system consisted of 150 mM sodium acetate, 1.5 mM octane sulphonic acid, 1mM EDTA, 10% methanol, and 2% acetonitrile (the aqueous phase was adjusted to a pH of 3.55 prior to the addition of the organic load).

ChAT Activity: The rats were decapitated and their brains were dissected into four brain areas: hippocampus, caudate, anterior cortex (rostral to the rhinal fissure) and posterior

cortex (post-rhinal fissure). Cerebella or boiled cortices from a few animals were used as a tissue blank. ChAT activities for the blank were subtracted from all tissue values. Tissues were stored on ice until homogenized for ChAT assay. The ChAT assay was a modified version of Fonnum (1975).

Following dissection, the tissue was weighed to the nearest 0.1 mg and homogenized in a buffer containing 2.9 g/l EDTA (ethylene diamine tetra-acetic acid, Sigma), 2.0 ml/l Triton X-100 (Sigma), 8.0 mM choline bromide (Sigma) and 0.10 mM physostigmine (Sigma). The buffer was adjusted to pH 7.4. Tissue was extracted in the ratio of 5 ml per gram of tissue. The tissue was homogenized for no more than 10 seconds using a Polytron tissue homogenizer. Following homogenization, the tissue extract was returned to ice and rehomogenized following a 20 second wait when necessary. Duplicate 20 μ l tissue homogenates were used for the assay. The remaining extract was retained for protein analysis using the Bio-Rad protein assay (Bio-Rad, Canada). One ml of unused frontal cortex tissue was boiled in a water bath for 15 minutes to denature the enzyme and was used for a denatured tissue blank.

Analyses were carried out in 500 μ l Eppendorf centrifuge tubes. 20 μ l tissue extracts were added to each tube and 20 μ l of 650 μ M 3 H-Acetyl Coenzyme A was added to initiate the reaction. The reaction was carried out for 15 minutes at 37°C using a thermostatically controlled "dry bath" (Canlab). "Stop" solutions for each sample were prepared in advance in

20 ml scintillation vials. Each "stop" solution contained ca. 10 mg of sodium tetraphenyl boron, 5.0 ml of 10 mM sodium phosphate buffer, pH 7.4, and 2.0 ml of acetonitrile. The reactions were stopped by transferring ca. 500 μ l of "stop" solution to the vial. The resulting solution was then transferred back into the scintillation vial. Ten ml of toluene based scintillation cocktail (EconoFluor, Du Pont), containing 4 g/l of PPO (2,5-Diphenyloxazole, Sigma) and 0.15 g/L of POPOP (1,4-bis-[2-(5-phenyloxazolyl)] benzene, Sigma), was added to the scintillation vial. The resulting suspension was mixed by gently rocking the vial at least six times. This action performs a phase extraction whereby the newly synthesized ^3H -ACh is extracted into the toluene phase, and unreacted ^3H -Acetyl Co-A remains in the aqueous phase. Twenty μ l of 650 μM ^3H -Acetyl Co-A was added to 10 ml of Aquasol Scintillation Cocktail (Du Pont) and counted with the other samples. The radiolabelled products were counted in a Beckman model LS 5000 TD Scintillation counter for a maximum of 2 minutes per sample. Final enzyme activity were expressed as nmole/hour/mg protein.

Histological Assessment

Cresyl Violet staining was carried out on the perfused brains of 37 rats from experiment III (rats behaviorally tested on the MWM-WM) 30-46 days postsurgically. Acetylcholinesterase staining was carried out on an additional

15 rats 51-59 days postsurgically.

Cresyl Violet: Following behavioral testing, the rats were perfused with 10% formalin after an overdose of pentobarbital. The brains were removed and stored in 10% formalin until sectioned. Tissue was cryoprotected by immersing the brains in 10% formalin with 15% sucrose for one day prior to sectioning (Priestley, 1987). The brains were frozen and sectioned coronally at 40 um in a cryostat. Every fifth section throughout the lesion site and the hippocampus was mounted on glass slides. The sections were stained with cresyl violet following a standard procedure. The cresyl violet solution was made up of the following ingredients mixed together and filtered: 5 gms of cresyl violet acetate, 3 ml of 10% acetic acid, and 1000 ml of distilled water. The acetic formalin differentiator was comprised of 4 ml of 37% formaldehyde 0.2 ml glacial acetic acid, and 100 ml of distilled water. The mounted sections were passed through the following solutions for the allotted time:

95% alcohol (ethanol; 2 min.), 95% alcohol (2 min.), 95% alcohol (2 min.), distilled water (2 min.), cresyl violet (1-3 min.), distilled water (wash), 95% alcohol (1 min.), 70% chloroform + 30% absolute ethanol (dunk), 95% alcohol (wash), distilled water (wash), acetic formalin differentiator (1-3 min.), distilled water (wash), 95% alcohol (2 min.), 95% alcohol (3 min.), 100% alcohol (3 min.), xylene (3 min.),

xylene (3 min.), xylene (3 min.). Finally, the slides were coverslipped with permount.

Sections were examined quantitatively in order to assess the extent of the lesions. The sites measured corresponded to the stereotaxic atlas by Paxinos and Watson (1986). The area of the lateral ventricles was measured anterior to the site of the lesions (A/P +1.0mm), at the site of the lesions (A/P +0.7mm, +0.2mm, -1.3), and posterior to the site of the lesions (at the level of the dorsal hippocampus; A/P -2.3). As well, the area of the caudate was measured at the site of the administration of NMDA (A/P+0.7mm, and +0.2mm). Area measurements were obtained from digitized images of the sections with a commercially available software package (Imaging Research, St. Catharines, Ontario). The tissue was also examined qualitatively for the extent of the lesion (such as necrosis).

Acetylcholinesterase (ACHE) stain: The procedure was a modified version of that outlined in Paxinos and Watson (1986). First the slides were rinsed in 100mM sodium acetate buffer (29g sodium acetate to 1L distilled water) for 2-3 minutes. Then they were reacted overnight (minimum 15 hours) in 50 mls of the stock solution + 50 mg acetylthiocholine iodide. The stock solution consisted of adding the following to 1 L of distilled water: 6.8g sodium acetate, 1.0g copper sulphate, 1.2g glycine. This stock solution was lowered to pH

5.00 with hydrochloric acid (6N). After the slides were immersed overnight, the slides were rinsed in 100mM sodium acetate buffer (2-3 minutes). They were developed in 1% sodium sulphide (pH 7.5) for 10 minutes. After which, the slides were rinsed through a sequence of decreasing concentrations of buffer (approximately 50mM, 25mM, 12mM) for 2-3 minutes, and then dehydrated for 5 minutes in 100% alcohol. Lastly, the slides were immersed in xylene for 5 minutes prior to coverslipping with permount.

A section of the dorsal hippocampus (rostral to the ventral hippocampus appearing) was assessed quantitatively for changes in ACHE staining density in both the left and right CA1, CA2, CA3 cell fields (multiple density samples were obtained and averaged for the stratum oriens, pyramidal, stratum radiatum, and molecular lacunosum), and in the dentate gyrus (molecular granule, granule, and polymorph layers). A section of the ventral hippocampus (just after the dorsal hippocampus drops down to join the ventral hippocampus) was assessed quantitatively for changes in ACHE staining density in the left and right CA1 and CA3 cell fields. The medial septum density measurements were obtained from a section closest to the second lesion site (A/P +0.20 mm). The medial septum was defined as an area which excluded structures ventral to the anterior commissure (anterior) (some of the vertical limb and all of the horizontal limb of the diagonal band of Broca), and as well excluded the septal hippocampal

nuclei and lateral septum. Density scores were obtained from a similar size and shape of area for all the rats. Density scores were also obtained for the vertical and horizontal limbs of the diagonal band of broca, septal hippocampal nuclei, and lateral septum. The sections were digitized by means of a camera and run through an IBM computer system. The density measures were obtained from digitized images of the sections taken with commercially available software (Imaging Research, St. Catherines, Ontario).

Summary of experiments:

Experiment I

Surgery (15 VEH, 15 5,7-DHT, 15 NMDA, 15 DUAL)	Recovery (ave. 10 days)	MWM (7 days)	ChAT Activity & HPLC (34-45 days postsurgically)
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Experiment II

Prelesion Surgery training (11 VEH, (20 days) 12 5,7-DHT, 12 NMDA, 12 DUAL)	Recovery (ave. 14 days)	RAM (30 days)	ChAT Activity and HPLC (61- 75 days post- surgically)
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Experiment III

Prelesion Surgery training (9 VEH, (4 days) 9 5,7-DHT, 9 NMDA, 10 DUAL)	Recovery (ave. 15 days)	MWM-WM (20 days)	Histology (30-46 days post- surgically)
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Experiment IV

Surgery (3 VEH, 3 5,7-DHT, 5 NMDA, 4 DUAL)	Recovery	ACHE (51-59 days post- surgically)
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RESULTS

Biochemical and histological data were usually analyzed with one-way ANOVA with the data obtained from each brain part as the dependent variable and the type of lesion as the independent variable. The behavioral data were analyzed with one-way ANOVA, or repeated measures MANOVA depending on whether or not the behavior was measured on one day or over repeated days.

The results for the biochemistry and histology will be presented to characterize the effects produced by the surgery, prior to outlining the behavioral results. Most of the biochemistry and histological results for this thesis were derived after the behavioral testing was completed. It is important to note that decisions determining the design of each successive experiment were based on the results of completed behavioral tests.

Neurochemical Assays

ChAT activity: The data are reported separately for the rats tested in the Morris Water maze and the radial arm maze. The data for the ChAT activity for the 48 rats tested in the Morris water maze were calculated in nmol/h/mg protein. Protein assay failure of the 47 rats tested in the radial arm maze necessitated that the data be reported as nmol/mg tissue. The interpretation of the ChAT activity analysis obtained from RAM tested rats must be made with some caution because of the

lack of availability of protein values. One NMDA injected rat from the MWM experiment had ChAT activity higher than the control mean, therefore this rat's data were dropped from any further analysis.

ChAT activity was submitted to one-way ANOVA's for the hippocampus, caudate, anterior and posterior cortex for the rats tested in the MWM and the RAM. The type of treatment received (VEH, 5,7-DHT, NMDA, or DUAL) was the between group factor. Analysis of ChAT activity in the hippocampus revealed a significant between groups effect for the MWM rats ($p < 0.001$) and for the RAM rats ($p < 0.001$). For the MWM rats, multiple comparisons with Tukey's test indicated that the amount of ChAT activity for the NMDA-only treated rats was significantly reduced to 47% and 48% of the mean values for VEH and 5,7-DHT treated rats, respectively. As well, the DUAL lesioned rats ChAT activity was significantly reduced to 55% and 56% of the mean values for VEH and 5,7-DHT treated rats, respectively (see Figure 1). For the RAM rats, multiple comparisons with Tukey's test indicated that ChAT activity for the NMDA-only treated rats was significantly reduced to 62% and 71% of the mean value for the VEH and 5,7-DHT treated rats, respectively. As well, the DUAL lesioned rats ChAT activity was significantly reduced to 63% and 70% of the mean values for VEH and 5,7-DHT treated rats, respectively (see Figure 2).

Figure 1: ChAT Activity (nmol/h/mg protein) in the hippocampus, caudate, anterior and posterior cortex, for the VEH (n=13), 5,7-DHT (n=12), NMDA (n=11), and DUAL (n=12) lesioned rats tested in the MWM and assayed 34-45 days postsurgically.

* NMDA and DUAL significantly less than VEH, $p < 0.05$

** NMDA and DUAL significantly less than 5,7-DHT, $p < 0.05$

ChAT Activity (nmol/h/mg protein)

Figure 2: ChAT Activity (nmol/mg tissue) in the hippocampus, caudate, anterior and posterior cortex, for the VEH (n=11), 5,7-DHT (n=12), NMDA (n=12), and DUAL (n=12) lesioned rats tested in the RAM and assayed 61-75 days post surgically.

*** NMDA and DUAL significantly less then VEH, $p < 0.05$**

**** NMDA and DUAL significantly less then 5,7-DHT, $p < 0.05$**

ChAT Activity (nmol/mg tissue)

There were no other significant group differences for the RAM rats in the caudate, anterior and posterior cortex. There were, however, significant between group effects for the MWM rats in the both the anterior and posterior cortex ($p < 0.001$), but not in the caudate. Multiple comparisons with Tukey's test indicated ChAT activity in both the NMDA and the DUAL lesioned rats was reduced to an average of 70% of both VEH and 5,7-DHT treated rats in the anterior cortex and to an average of 60% in the posterior cortex as compared to both the VEH and 5,7-DHT treated rats (see Figure 1).

Summary of ANOVA's can be found in Appendix D for these and all following statistics.

Monoamine assay: The analyses for the monoamines were done separately for the rats tested in the MWM and the RAM. The reasons for these separate analyses were because the data were derived from different HPLC systems a year apart and one assay was reported in nmol/mg protein while the other was reported in ng/mg tissue (protein assay unavailable).

Serotonin levels for the dorsal and ventral hippocampi, anterior cortex, and posterior cortex for the rats tested in the MWM were submitted to a one-way ANOVA. Both the dorsal and ventral hippocampi revealed significant ($p < 0.001$) group effects (see Figure 3). In the dorsal hippocampus, multiple comparisons with Tukey's test indicated that substantially less serotonin was remaining in the 5,7-DHT treated rats as

Figure 3: Serotonin levels (nmol/h/mg protein) in the dorsal and ventral hippocampus, anterior and posterior cortex, for the VEH (n=11), 5,7-DHT (n=11), NMDA (n=10), and DUAL (n=11) lesioned rats tested in the MWM and assayed 34-45 days postsurgically.

* 5,7-DHT and DUAL significantly less than VEH

** 5,7-DHT and DUAL significantly less than NMDA

compared to VEH and NMDA groups (18% and 16%, respectively) and less was remaining in the DUAL lesioned rats as compared to the VEH and NMDA groups (21% and 18%, respectively). In the ventral hippocampus, multiple comparisons with Tukey's test indicated that significantly less serotonin was remaining in the 5,7-DHT treated rats as compared to VEH and NMDA groups (25% and 22%, respectively) and significantly less was remaining in the DUAL lesioned rats as compared to the VEH and NMDA groups (20% and 18%, respectively). Group differences were also observed in the levels of serotonin in the anterior cortex ($p < 0.05$). In this case, however, no two groups were significantly different from one another. Only when the groups were collapsed across the 5,7-DHT and DUAL and compared to the NMDA-only plus VEH treated rats were significant differences observed (Scheffe's test, $p < 0.05$). There were no significant differences in serotonin levels in the posterior cortex.

Analysis of the serotonin values obtained from the rats tested in the RAM revealed significant between group effects for the dorsal hippocampus ($p < 0.01$) and the ventral hippocampus ($p < 0.001$) (see Figure 4). In the dorsal hippocampus multiple comparisons with Scheffe's test indicated that significantly less serotonin was remaining in the DUAL lesioned rats as compared to VEH (25%). Non-significant, but large depletions of serotonin were found in the 5,7-DHT treated rats as compared to the VEH rats (40% remaining). In the ventral hippocampus, multiple comparisons with Scheffe's

Figure 4: Serotonin levels (ng/mg tissue) in the dorsal and ventral hippocampus for the VEH (n=11), 5,7-DHT (n=12), NMDA (n=12), and DUAL (n=11) lesioned rats tested in the RAM and assayed 61-75 days postsurgically.

* DUAL significantly less than VEH

** 5,7-DHT and DUAL significantly less than VEH and NMDA

Serotonin levels ng/mg tissue

test indicated that significantly less serotonin was remaining in the 5,7-DHT treated rats as compared to VEH and NMDA groups (27% and 26%, respectively) and significantly less was remaining in the DUAL lesioned rats as compared to the VEH and NMDA groups (28% and 27%, respectively). Significant group differences for levels of serotonin in the anterior cortex were not found ($p > 0.05$). The data for the posterior cortex were not available at the time this paper was submitted.

Norepinephrine values were submitted to one-way ANOVA. There were minimal depletions of norepinephrine (see Table I), and as a result there were no significant differences between groups for the dorsal hippocampus, the anterior cortex, or the posterior cortex. There were however, significant changes in the level of norepinephrine in the ventral hippocampus ($p < 0.05$). Tukey's test indicated that the NMDA treated rats had significantly more norepinephrine than the 5,7-DHT (31%) treated and the DUAL (41%) lesioned animals. Although they also had more than the VEH (20%) rats the difference was not significant. There were no significant differences between the 5,7-DHT and DUAL lesioned groups and the VEH controls.

Insert Table I here.

The norepinephrine values for the rats tested in the RAM also indicated minimal depletions but there were no significant differences between groups for the ventral hippocampus or the anterior cortex. Although there was the same tendency for the norepinephrine values to be elevated (31%) in the NMDA treated rats as compared to the 5,7-DHT treated rats in the ventral hippocampus there was no significant between group effect (see Table II). The analysis of the dorsal hippocampus, however, indicated a significant between groups effect ($F_{(3,42)}=3.19$, $p<0.05$). A similar pattern of elevated norepinephrine in NMDA treated rats as compared to the 5,7-DHT and DUAL lesioned rats existed in these data, but no two groups, or any comparison of groups collapsed, were significantly different from each other.

Insert Table II here.

Dopamine values were also submitted to one-way ANOVA for the caudate, anterior and posterior cortex for the MWM tested rats. There were no significant between group differences for any of these brain parts (see Table III). Data for the dorsal and ventral hippocampi were missing because levels were not detectable.

Insert Table III here.

At the time of writing this paper, the dopamine values for the dorsal and ventral hippocampi, and anterior cortex had been assayed from the RAM tested rats. No significant differences were observed between groups. The data for the caudate and posterior cortex were not available.

TABLE I: Norepinephrine levels (mean $\pm$ S.E.M. nmol/h/mg protein) for rats tested in the MWM

<u>Group</u>	<u>BRAIN PART</u>			
	<u>Dorsal Hippocampus</u>	<u>Ventral Hippocampus</u>	<u>Anterior Cortex</u>	<u>Posterior Cortex</u>
VEH (n=8)	66.4 $\pm$ 3.5	30.9 $\pm$ 2.1	73.1 $\pm$ 7.3	35.0 $\pm$ 2.0
5,7-DHT (n=10)	62.4 $\pm$ 4.8	28.4 $\pm$ 1.6 ^{tt}	58.4 $\pm$ 4.9	33.6 $\pm$ 2.0
NMDA (n=9)	70.7 $\pm$ 5.4	37.0 $\pm$ 2.5	74.2 $\pm$ 7.6	31.0 $\pm$ 0.7
DUAL (n=9)	61.5 $\pm$ 5.6	26.2 $\pm$ 1.8 ^{tt}	80.2 $\pm$ 8.7	37.5 $\pm$ 1.7

^{tt} p<0.05 sig. dif. from NMDA

TABLE II: Norepinephrine levels (mean $\pm$ S.E.M. ng/mg tissue) for rats tested in the RAM

<u>Group</u>	<u>BRAIN PART</u>		
	<u>Dorsal Hippocampus</u>	<u>Ventral Hippocampus</u>	<u>Anterior Cortex</u>
VEH (n=11)	0.260 $\pm$ 0.024	0.581 $\pm$ 0.053	0.486 $\pm$ 0.067
5,7-DHT (n=12)	0.168 $\pm$ 0.037	0.491 $\pm$ 0.039	0.718 $\pm$ 0.137
NMDA (n=12)	0.245 $\pm$ 0.014	0.645 $\pm$ 0.050	0.554 $\pm$ 0.098
DUAL (n=11)	0.158 $\pm$ 0.035	0.578 $\pm$ 0.554	0.413 $\pm$ 0.057

TABLE III: Dopamine levels (mean $\pm$ S.E.M. nmol/h/mg protein)
for rats tested in the MWM

<u>Group</u>	<u>BRAIN PART</u>		
	<u>Caudate</u>	<u>Anterior Cortex</u>	<u>Posterior Cortex</u>
VEH (n=8)	148.1 $\pm$ 57.7	196.9 $\pm$ 18.6	113.3 $\pm$ 6.6
5,7-DHT (n=10)	159.2 $\pm$ 66.0	154.3 $\pm$ 15.0	99.8 $\pm$ 7.2
NMDA (n=9)	132.7 $\pm$ 97.5	210.4 $\pm$ 21.7	104.2 $\pm$ 3.4
DUAL (n=9)	236.9 $\pm$ 63.9	191.2 $\pm$ 29.2	115.6 $\pm$ 4.5

Histological Assessment

There were no significant differences between the left and right ventricles for the measurements obtained at the five sites measured (A/P +1.0mm, +0.7mm, +0.2mm, -1.3mm, -2.3mm). Therefore, the area of the lateral ventricles was averaged across the left and right hemispheres and submitted to a repeated measures MANOVA. The data obtained from the five sites measured in each brain became the repeated measure factor, and treatment was the between groups factor (see Figure 5). The analysis indicated a significant Group by Area interaction ($F(18,156)=2.11$, $p<0.01$). This interaction occurred because there were significant differences in the averaged area of the lateral ventricles between groups at the two sites of NMDA injection (A/P +0.7mm, +0.2mm). There were no differences between groups anterior or posterior to the injections of NMDA (A/P +1.0mm, AP-1.3mm, AP-2.3mm).

At the most anterior site of NMDA injection (A/P+0.7mm) the lateral ventricles were larger in the NMDA lesioned rats by 53% and 69%, respectively, as compared to the VEH and 5,7-DHT treated rats. The DUAL lesioned also had larger ventricles than the VEH (42%) and the 5,7-DHT only rats (57%). Multiple comparisons of the means using Scheffe's test indicated that both the DUAL and NMDA treated rats were significantly different ($p<0.05$) from the 5,7-DHT treated rats but not from the VEH. There were also significant differences between all the rats that were injected with NMDA as compared to those who

Figure 5: Average area of the lateral ventricles (sq.mm) from 5 sections anterior to bregma (+1.0mm,+0.7mm,+0.2mm) and posterior to bregma (-1.3mm, -2.3mm) for VEH (n=7), 5,7-DHT (n=8), NMDA (n=8), and DUAL (n=7) rats at 30-46 days postsurgically and after behavioral testing in the MWM-WM.

did not receive NMDA (Scheffe's, $p < 0.05$).

As mentioned, the Area by Group interaction also reached significance at the more posterior site of injection of NMDA (A/P+0.2mm). The NMDA treated rats had ventricles 55% and 52% larger than the VEH and 5,7-DHT treated rats, respectively. The DUAL lesioned animals had lateral ventricles 51% and 48% larger than the VEH and 5,7-DHT treated rats, respectively. Scheffe's test indicated significant group differences between the NMDA and the VEH and the 5,7-DHT treated rats ($p < 0.05$). There were no significant differences between the DUAL lesioned rats and either the VEH or the 5,7-DHT treated rats. There were, however, significant differences between all animals that received NMDA versus those that did not receive NMDA (Scheffe's test $p < 0.01$).

The area of the caudates for the two sites of injection of NMDA were also analyzed for left and right differences. At the most anterior site (A/P+0.7mm) a significant side main effect was found ($F_{(1,32)} = 8.64$, $p < 0.01$). There were no interactions with side or side main effects at the more posterior injection site. Collapsed across all groups at the anterior site, A/P+0.7mm, the left side was 3% larger than the right side. Such a small difference in the left and right side at this anterior site could have been created simply from blocking and mounting the brain unevenly on the pedestal. This effect was considered to be spurious. Therefore, the area of the caudate was averaged across the left and right hemispheres

and also submitted to a repeated measures MANOVA for the two sites where NMDA was injected (A/P +0.7 and +0.2). There were no interactions or main effects.

In general, the examination of the sections indicated that the injection of NMDA into the septum created a hole, extending ventrally from the corpus callosum to the anterior commissure, as well as from at least the anterior site of NMDA injection (sometimes just caudal to the crossing of the corpus callosum) up to and including the posterior site of the second NMDA injection (prior to the crossing of the anterior commissure). In many cases the structures missing were along the site of the injection pathway (particularly at the most anterior site of injection of NMDA) such as the septal hippocampal nuclei, lateral septal intermediate (LSi) nuclei, and the medial septum. For example, at the more anterior site of injection of NMDA (A/P +0.7mm), 46% of the NMDA injected rats were missing at least three-quarters of the septal hippocampal nuclei. Twenty-eight percent of the NMDA injected rats were missing at least three-quarters of the LSi nuclei. Thirty-two percent of all the rats receiving NMDA were missing some of their medial septum. None of the VEH or 5,7-DHT treated rats were missing these nuclei. The lateral septal dorsal (LSd) nucleus was the most damaged by the surgery. At section A/P +0.7mm, 93% of the rats administered NMDA were missing at least three-quarters of the LSd, 47% of the rats not receiving the NMDA injection were also missing the LSd.

This structure is fairly small and adjacent to the lateral ventricles. It is possible that the injection procedures caused ventricular dilation and damaged the lateral septal dorsal nuclei. It is just as possible that the age of the rat may also have contributed to the ventricular dilation. As there were no young unoperated controls in this study however, it is difficult to ascertain what exactly contributed to the loss of the LSd.

Density scores from the ACHE stained sections for the areas measured were expressed as a percentage of the density score from the superficial grey layer of the superior colliculus. Although the same recipe and procedure for the ACHE stain was used for all the rats, there were invariably differences between different boats of slides or between tissue stained on different days. Therefore this calculation was done in order to control for sporadic differences between staining intensity. The superficial grey layer of the superior colliculus stains consistently dark for ACHE and there were no group differences in staining density for this area ($p > 0.05$). As there were also no significant differences between VEH and 5,7-DHT treated rats or NMDA and DUAL lesioned rats, the analysis was performed on two groups (those that received NMDA vs those that didn't).

The percentage density scores for the medial septum, and vertical and horizontal limbs of the diagonal band of Broca,

were obtained from a section closer to the posterior site of injection of NMDA where there was less tissue missing. The data from these sites were each submitted to one-way ANOVA. A significant between groups effect was found ($F_{(1,13)}=11.5$, $p<0.01$) for the medial septum. The rats that received NMDA in the medial septum had 27% reduced ACHE staining as compared to the rats that did not receive NMDA as a treatment (see Figure 6). There were no significant differences between groups for the vertical and horizontal limbs of the diagonal bands. The means, however, for the density scores for the vertical limb of the diagonal band indicate that the NMDA treated rats had on average about a 12% reduction in staining as compared to the control rats. The density scores for the horizontal limb indicate approximately a 3% reduction in staining for the NMDA treated rats.

The density scores for the septal hippocampal nuclei and the lateral septal nuclei were not analyzed. Although the values were obtained in control tissue, 6 of the 9 NMDA treated rats were missing this tissue.

Figure 6: Percentage ACHE density scores of the medial septum for rats that received NMDA treatment (NMDA and the DUAL lesioned, n=9) and compared to control rats (VEH and 5,7-DHT treated, n=6) approximately 51-59 days postsurgically.

Analysis of percentage density scores for the dorsal hippocampus revealed no significant differences between the left and right hemispheres, therefore the data were averaged across left and right sides. These data were then submitted to repeated measures MANOVA for the oriens, radiatum, pyramidal, molecular layers of CA1, CA2, CA3 in the hippocampus, and the molecular, granule, and polymorph layers of the dentate gyrus (see Figure 7). There were no significant differences between the groups for any of the cell fields measured in the dorsal hippocampus. There was a tendency, however, for the ACHE stain to be reduced approximately 10% in the rats that received NMDA as a treatment.

Figure 7: Upper and Middle: Percentage ACHE density scores in the stratum oriens (Or), pyramidal (Py) radiatum (Ra) and molecular lacunosum (moLa) for CA1 (upper), and CA3 (middle) cell fields of the dorsal hippocampus for rats that received NMDA treatment (NMDA and the DUAL lesioned, n=9) and compared to control rats (VEH and 5,7-DHT treated, n=6) approximately 51-59 days postsurgically.

Lower: Percentage ACHE density scores in the molecular granular (molGr), granule (Gr), and polymorph (Po) layers in the Dentate gyrus of the dorsal hippocampus for rats that received NMDA treatment (NMDA and the DUAL lesioned, n=9) and compared to control rats (VEH and 5,7-DHT treated, n=6) approximately 51-59 days postsurgically.

ACHE Density Score - Dorsal Hippocampus

ACHE density scores for the ventral hippocampus were also assessed for left and right hemisphere differences. There were no side differences in CA1 cell fields. The percentage density scores were collapsed across left and right side and submitted to a repeated measure MANOVA, with the cell fields (stratum oriens, pyramidal, radiatum, and molecular lacunosum) as the within subject factor. A significant group main effect was found ($F_{(1,13)} = 5.96, p < 0.05$). The group by cell field interaction failed to reach significance ($F_{(3,39)} = 2.63, p = 0.06$). Overall, the rats that received medial septal injections of NMDA had density scores reduced by 15% as compared to the control rats (see Figure 8). There were significant differences between staining the left and right side of the CA3 region of the ventral hippocampus. Consistently the right side stained about 8% darker than the left side. The data for the left and right hemispheres were submitted to a repeated measures MANOVA with cell fields and side as the repeated measures factors. There was only the significant side main effect ($F_{(1,13)} = 11.28, P < 0.01$), no other main effects or interactions reached significance (see Figure 8 for CA3 ACHE density scores collapsed across left and right sides).

Figure 8: Percentage ACHE density scores in the stratum oriens (Or), pyramidal (Py) radiatum (Ra) and molecular lacunosum (moLa) for CA1 (upper), and CA3 (lower) of the ventral hippocampus for rats that received NMDA treatment (NMDA and the DUAL lesioned, n=9) and compared to control rats (VEH and 5,7-DHT treated, n=6) approximately 51-59 days postsurgically.

ACHE Density Score – Ventral Hippocampus

In summary, the lesion of the medial septal neurons with NMDA and the fimbria fornix and cingulate bundle with 5,7-DHT reduced CHAT activity and serotonin concentrations, respectively. The pretreatment of the rats with DMI protected the noradrenergic and dopaminergic terminals against the toxic effects of 5,7-DHT. In addition, the NMDA-only treatment increased the level of norepinephrine in the ventral hippocampus, but only in the rats who had their brains dissected and prepared for HPLC 34-45 days postsurgically. There was also reduced levels of ACHE staining in the medial septum and the CA1 region of the ventral hippocampus. There was, however no significantly reduced levels of ACHE staining in the dorsal hippocampus, or the CA3 region of the ventral hippocampus. The NMDA injection created substantial damage in the area of the medial septum which suggested that both cholinergic and non-cholinergic cell bodies may have been affected.

Behavioral Results

In general, all of the animals recovered quickly from the surgery. Consistent with the literature (Carlson, 1986), some (exact numbers are not known but it was definitely not all) of the medial septal lesioned rats showed a marked hyperresponsivity to being touched. In addition some of the NMDA-only treated rats and the DUAL lesioned rats tampered with the sutures, and continuously reopened their wounds. In

these few cases, the rats were monitored throughout the test period and their wounds were treated daily with antiseptic wound powder. Sometimes the rats were given something to distract them from scratching their heads (such as a paper towel to rip up, or a poly-vinyl-chloride pipe to explore).

MWM-reference memory version

Hidden platform test: Latencies to find the hidden platform in the Morris water maze were averaged over the 4 trials a day for 5 days (see Figure 9). The repeated measures variables were the five days and group was the between subjects variable. There were no significant group by day interactions, or group main effects. The group effect approached significance ($F_{(3,55)}=2.61$, $p=.06$), however. Inspection of Figure 9 suggests a tendency for longer platform location latencies in the rats that received NMDA as a treatment. There was a significant days main effect ($F_{(4,220)}=134.21$, $p<0.0001$), which suggests that all groups improved their performance over the five days.

A resolution test chart featuring various patterns of vertical and horizontal lines. The patterns are arranged in a grid-like fashion, with some patterns having numerical values associated with them. The values include 1.0, 1.1, 1.25, 1.4, 1.6, 1.8, 2.0, 2.2, 2.5, 2.8, 3.2, 3.6, 4.0, 4.5, 5.0, 5.6, 6.3, 7.1, 8.0, 9.0, 10, 11.2, 12.5, 14, 16, 18, 20, 22.5, 25, 28, 32, 36, 40, 45, 50, 56, 63, 71, 80, 90, 100, 112, 125, 140, 160, 180, 200, 225, 250, 280, 320, 360, 400, 450, 500, 560, 630, 710, 800, 900, 1000, 1120, 1250, 1400, 1600, 1800, 2000, 2250, 2500, 2800, 3200, 3600, 4000, 4500, 5000, 5600, 6300, 7100, 8000, 9000, 10000, 11200, 12500, 14000, 16000, 18000, 20000, 22500, 25000, 28000, 32000, 36000, 40000, 45000, 50000, 56000, 63000, 71000, 80000, 90000, 100000, 112000, 125000, 140000, 160000, 180000, 200000, 225000, 250000, 280000, 320000, 360000, 400000, 450000, 500000, 560000, 630000, 710000, 800000, 900000, 1000000, 1120000, 1250000, 1400000, 1600000, 1800000, 2000000, 2250000, 2500000, 2800000, 3200000, 3600000, 4000000, 4500000, 5000000, 5600000, 6300000, 7100000, 8000000, 9000000, 10000000, 11200000, 12500000, 14000000, 16000000, 18000000, 20000000, 22500000, 25000000, 28000000, 32000000, 36000000, 40000000, 45000000, 50000000, 56000000, 63000000, 71000000, 80000000, 90000000, 100000000, 112000000, 125000000, 140000000, 160000000, 180000000, 200000000, 225000000, 250000000, 280000000, 320000000, 360000000, 400000000, 450000000, 500000000, 560000000, 630000000, 710000000, 800000000, 900000000, 1000000000, 1120000000, 1250000000, 1400000000, 1600000000, 1800000000, 2000000000, 2250000000, 2500000000, 2800000000, 3200000000, 3600000000, 4000000000, 4500000000, 5000000000, 5600000000, 6300000000, 7100000000, 8000000000, 9000000000, 10000000000, 11200000000, 12500000000, 14000000000, 16000000000, 18000000000, 20000000000, 22500000000, 25000000000, 28000000000, 32000000000, 36000000000, 40000000000, 45000000000, 50000000000, 56000000000, 63000000000, 71000000000, 80000000000, 90000000000, 100000000000, 112000000000, 125000000000, 140000000000, 160000000000, 180000000000, 200000000000, 225000000000, 250000000000, 280000000000, 320000000000, 360000000000, 400000000000, 450000000000, 500000000000, 560000000000, 630000000000, 710000000000, 800000000000, 900000000000, 1000000000000, 1120000000000, 1250000000000, 1400000000000, 1600000000000, 1800000000000, 2000000000000, 2250000000000, 2500000000000, 2800000000000, 3200000000000, 3600000000000, 4000000000000, 4500000000000, 5000000000000, 5600000000000, 6300000000000, 7100000000000, 8000000000000, 9000000000000, 10000000000000, 11200000000000, 12500000000000, 14000000000000, 16000000000000, 18000000000000, 20000000000000, 22500000000000, 25000000000000, 28000000000000, 32000000000000, 36000000000000, 40000000000000, 45000000000000, 50000000000000, 56000000000000, 63000000000000, 71000000000000, 80000000000000, 90000000000000, 100000000000000, 112000000000000, 125000000000000, 140000000000000, 160000000000000, 180000000000000, 200000000000000, 225000000000000, 250000000000000, 280000000000000, 320000000000000, 360000000000000, 400000000000000, 450000000000000, 500000000000000, 560000000000000, 630000000000000, 710000000000000, 800000000000000, 900000000000000, 1000000000000000, 1120000000000000, 1250000000000000, 1400000000000000, 1600000000000000, 1800000000000000, 2000000000000000, 2250000000000000, 2500000000000000, 2800000000000000, 3200000000000000, 3600000000000000, 4000000000000000, 4500000000000000, 5000000000000000, 5600000000000000, 6300000000000000, 7100000000000000, 8000000000000000, 9000000000000000, 10000000000000000, 11200000000000000, 12500000000000000, 14000000000000000, 16000000000000000, 18000000000000000, 20000000000000000, 22500000000000000, 25000000000000000, 28000000000000000, 32000000000000000, 36000000000000000, 40000000000000000, 45000000000000000, 50000000000000000, 56000000000000000, 63000000000000000, 71000000000000000, 80000000000000000, 90000000000000000, 100000000000000000, 112000000000000000, 125000000000000000, 140000000000000000, 160000000000000000, 180000000000000000, 200000000000000000, 225000000000000000, 250000000000000000, 280000000000000000, 320000000000000000, 360000000000000000, 400000000000

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

Figure 9: Latency to find the hidden platform in the Morris water maze (4 trials a day for 5 days) for the VEH (n=15), NMDA (n=14), 5,7-DHT (n=15), and DUAL (n=15) treated rats obtained 7-12 days postsurgically.

No platform test: Percentage path lengths (path length in quadrant/total path length*100), and percentage times (time in quadrant/total time*100) for each quadrant were each submitted to repeated measures MANOVA. The percentage values (path length and time) for each quadrant (north-east, south-east, south-west, north-west) were the within subject factor. A significant group by percentage path length was found ($F_{(9,165)}=2.12, p<0.05$). Further analysis revealed that the groups differed in the amount of path length for the north-east quadrant (where the platform had been). Post-hoc comparisons with Scheffe's test indicated that the percentage of path length in the north-east quadrant was less for the DUAL lesioned animals as compared to the 5,7-DHT-only lesioned rats ($p<0.05$; see Figure 10).

The VEH, 5,7-DHT, and NMDA-only treated groups also indicated significant differences ($p<0.001$) between the percentage of path length for each quadrant. Multiple comparisons with Scheffe's tests revealed that both the VEH and 5,7-DHT treated rats swam longer distances in the north-east quadrant (where the platform had been) as compared to the south-west and the north-west quadrant (see Figure 10). The NMDA treated rats swam significantly longer distances in the north-east quadrant as compared to the distances swum in the north-west quadrant. The DUAL lesioned rats, however, did not show a significant differences in percentage path length between the quadrants ($p>0.05$).

Figure 10: % Path length in the North-East (NE), South-East (SE), South-West (SW), and North-West (NW) quadrant for the VEH (n=15), NMDA (n=14), 5,7-DHT (n=15), and DUAL (n=15) treated rats obtained 13-18 days postsurgically.

* $p < 0.05$, DUAL significantly different from 5,7-DHT treated.

There were no interactions or between group effects for the percentage of time spent in each quadrant ($p > 0.05$). There was a significant repeated measures main effect (percentage of time spent in each quadrant, $F_{(3,162)} = 68.71$, $p < 0.001$; see Figure 11). All the groups spent more time in the north-east quadrant than in the south-west or north-west quadrants (Scheffe's, $p < 0.05$).

Number of annulus crossings (see methods section for definition), swim speed (a measure of motor ability), and total path length (a measure of activity) were each submitted to one-way ANOVA's. The number of annulus crossings in the north-east quadrant was not significantly different between groups. Swim speed and total path length were also not significantly different between groups.

Figure 11: % Time in the North-East (NE), South-East (SE), South-West (SW), and North-West (NW) quadrant for the VEH (n=15), NMDA (n=14), 5,7-DHT (n=15), and DUAL (n=15) treated rats obtained 13-18 days postsurgically.

Inspection of the heading angle used by the groups suggested that there was a tendency for more of the NMDA and the DUAL lesioned rats (10/14 and 12/15, respectively) to use a heading angle furthest away (between 47° and 90°) from a more direct path to the platform (between $0-46^{\circ}$) as compared to the VEH treated rats (5/15). These data were analyzed with nonparametric statistics (Chi Square test) comparing the frequency of rats who were categorized as using a heading angle between 47° and 90° with those who used a heading angle between 0 and 46° . The Chi Square indicated that the angle of approach varied significantly between the VEH and both the NMDA and DUAL treated rats ($df=2$, Chi Square = 7.77, $p<0.05$).

Cued Black Platform test: The latencies for the black platform test were submitted to a one-way ANOVA. There were no significant group differences ($p>0.05$).

In summary, it appears that all groups were capable of locating the hidden platform. On the no-platform test day, the time swum in the quadrant where the platform had been did not significantly differ among the groups. The distance swum by the DUAL lesioned animals in the quadrant where the platform had been was less than the distance swum by the 5,7-DHT treated rats, however. Rats who learn to find the hidden platform will tend to preferentially swim in the area where

the platform was when they are assessed with a no-platform test. The results here indicated that perhaps the DUAL lesioned animals did not remember as well as the other groups the platform location between test days. If this were true, then platform location latency of this group on the first trial of each test day should have been longer. MANOVA of the latencies for the first trial over the five days failed to reach significance ($F_{(3,55)} = 2.61, p=0.061$). The DUAL lesioned rats, however, on average took longer to find the platform (44 sec.) as compared to the NMDA (39 sec.), 5,7-DHT (35 sec.) or VEH (32 sec.) rats.

The analysis of heading angle and the observation that the DUAL lesioned rats swam equivalent distances in each of the quadrants supports the conclusion that this group did not locate the platform like the other groups. In addition to - or perhaps instead of - forgetting where the hidden platform had been on the no platform test day, the DUAL lesioned rats may have used a different search strategy to find the platform, perhaps a non-spatial search strategy (such as circling the pool). Thus by searching the pool in circles beginning at the wall and decreasing to the middle, the lesioned rats could become as efficient at finding the hidden platform over the test days as did the single lesioned and control rats.

The lack of difference between groups on total path length, swim speed and cued black platform test suggests that all groups were equally capable of performing this task, and

no group was any more motorically active then any other group.

In conclusion, the NMDA- only and the DUAL lesioned rats did not differ from control and 5,7-DHT-only rats in latency for finding the location of the hidden platform. Analysis of the no platform test suggested that the DUAL lesioned rats showed inferior memory for platform location and/or were using search strategies that did not involve spatial mapping.

This version of the Morris water maze is considered to be largely a measure of reference memory. The information is useful for many trials over the entire experiment in that the animal learns that the platform is always hidden in the same position. Therefore, the question arose whether or not the lesioned rats would perform worse in a task which assesses working rather than reference memory. According to Olton (1983), rats with hippocampal lesions tend to perform poorly on tasks which require working memory whereby stimulus information is useful for only one trial of an experiment. The next behavioral test was designed to assess the effects of the single NMDA versus combined NMDA+5,7-DHT lesion on both working and reference memory using the 12 arm radial maze.

Radial Arm maze (RAM)

Working memory errors (repeat arm entries into a baited arm) were averaged across five blocks of five days each (25 days of testing; see Figure 12) and submitted to a repeated

measure MANOVA. The five blocks were the repeated measures variable. There was no group by block interaction ($p > 0.1$), but there was a significant group main effect ($F_{(3,43)} = 23.10, p < 0.001$). Further comparisons of the group means collapsed across the five blocks indicated that the DUAL lesioned rats made significantly more working memory errors than the VEH (Scheffe's test, $p < 0.05$), but not the 5,7-DHT, or NMDA treated rats. Although the NMDA-only treated rats on average made more errors than the VEH rats, this difference failed to reach significance. None of the other groups were significantly different from each other. A significant effect ($p < 0.05$) with the Scheffe's test was also obtained by comparing the average working memory errors made by all the rats that had received NMDA (NMDA-only and DUAL lesioned) to all the rats that did not receive NMDA (VEH and 5,7-DHT lesioned).

Analysis of working memory errors also indicated a significant block effect ($F_{(4,172)} = 14.37, p < 0.0001$). This suggested that all the groups improved their performance over the five blocks.

Once the performance of the VEH and 5,7-DHT treated rats reached asymptote they were no longer tested in the RAM. The NMDA and DUAL lesioned rats, however, were tested for an additional five days to see if there were still group differences. The average working memory errors for days 26-30

Figure 12: Working memory errors (repeat arm entries into the 6 baited arms) for the VEH (n=11), NMDA (n=12), 5,7-DHT (n=12), and DUAL (n=12) treated rats measured in the RAM approximately 15 days postsurgically for 25 days (5 blocks).

(block 6) for the NMDA and DUAL lesioned rats were compared with a one-way ANOVA to the average working memory errors for block 5 of the VEH and 5,7-DHT treated rats. The analysis indicated a significant between groups effect ($F_{(3,41)}=8.00$, $p<0.001$). Multiple comparisons with Scheffe's test indicated that the DUAL lesioned rats made significantly ($p<0.05$) more errors (2.9 ± 0.7) than the VEH (0.2 ± 0.1) and the 5,7-DHT treated rats (0.4 ± 0.2). The NMDA-only treated rats did not make significantly more errors (1.9 ± 0.6) than the VEH or 5,7-DHT treated rats. Generally, all rats that received NMDA made significantly more errors than those that did not receive NMDA (Scheffe's, $p<0.05$).

Reference memory errors (first entry into non-baited arms) were averaged across five blocks of five days each (25 days total; see Figure 13) and submitted to a repeated measure MANOVA. The five blocks were the within subject variable. The group by block interaction was significant ($F_{(12,172)}=2.00$, $p=0.05$). Further analysis revealed that the interaction was significant because the groups differed in block 1 but not on any other block ($F_{(3,43)}=4.76$, $p, 0.01$). During block 1 the DUAL lesioned rats made significantly more reference memory errors than the VEH controls ($p<0.05$). There were no significant differences between all the rats that received NMDA and all those who did not. No other differences were found between the groups during this or any other block.

Figure 13: Reference memory errors (first arm entry into the 6 non-baited arms) for the VEH (n=11), NMDA (n=12), 5,7-DHT (n=12), and DUAL (n=12) treated rats measured in the RAM approximately 15 days postsurgically for 25 days (5 blocks).

Choice accuracy (the number of choices correct out of the first six arm entries) was analyzed for five blocks of five days with a repeated measures MANOVA. There was a significant group by block interaction ($F_{(12,172)} = 2.42, P < 0.01$). Further analysis revealed that this interaction occurred because there were significant differences between groups for blocks 1 ($F_{(3,43)} = 3.86, p < 0.05$) and 2 ($F_{(3,43)} = 3.10, p < 0.05$) but not blocks 3, 4, or 5 (see Figure 14). In block 1 the DUAL lesioned rats made significantly fewer correct choices as compared to the VEH rats (Scheffe's, $p < 0.05$). No other two groups differed significantly. A significant difference was found when the choice accuracy values for the rats that received NMDA were averaged and compared to those that did not receive NMDA. The rats that were treated with NMDA made fewer correct responses in the first six arm choices than the non-NMDA treated rats (Scheffe's test, $p < 0.05$). In block 2, no two groups were significantly different. Comparisons of the NMDA with the non-NMDA treated rats indicated that the rats receiving NMDA as a treatment made less correct responses in the first six arm entries than those that did not receive NMDA (Scheffe's test, $p < 0.05$).

Figure 14: Choice Accuracy (total correct choices out of the first six choices) for the VEH (n=11), NMDA (n=12), 5,7-DHT (n=12), and DUAL (n=12) treated rats measured in the RAM approximately 15 days postsurgically for 25 days (5 blocks).

In summary, the longest lasting and perhaps the most profound difference between groups was the increased working memory errors of those rats administered NMDA (particularly the DUAL lesioned rats). Since these rats had varying extents of ChAT reduction, it was of interest to determine if the loss of ChAT activity correlated with working memory impairment. Therefore, average working memory errors for each block of five days were correlated with ChAT activity in the hippocampus, caudate, anterior and posterior cortex. There were neither significant correlations nor noticeable tendencies for either a negative or positive correlation for any of the groups in any trial block (see Appendix B, for tables of correlations).

In the RAM, patterns of arm choices were observed in some of the rats. Therefore, biases towards choosing a particular type of turn (0° to 180°) were assessed. When leaving an arm, the rat had the option of either reentering the arm (0° turn) or entering any of the remaining 11 arms. In the twelve arm radial maze, if the rat enters the arm adjacent to the arm just exited (either to the left or to the right) then he has made a 30° turn, if it turns into an arm two away from the one he just exited then it's a 60° turn, and so on to a 180° turn which is six arms away. Therefore, the chosen arm entry (0° to 180° apart) represented turn angle. The total number of entries for each turn angle was then calculated. As the

frequency of arm choice was elevated in the DUAL and NMDA treated rats by their increased total number of errors/arm entries, the data were transformed to percentage turn angle i.e., number of arm entries for each turn angle (0° to 180° turns)/total number of turns*100. These data were calculated for a sample of days (1, 2, 10, 11, 19, and 20 (see Figure 15)) and were then submitted to separate MANOVA's for each test day. Percentage turn angle for each of the six possible choices was the within subjects factor. Significant group by percentage turn angle interactions were found for day 1 ($F_{(18,258)}=2.11$, $P,0.01$), day 2 ($F_{(18,258)}=1.82$, $p<0.05$), and day 10 ($F_{(18,258)}=1.95$, $p<0.05$). Basically, these interactions occurred because on day 1 VEH rats made more 120° turns than the DUAL lesioned (Scheffe's test, $p,0.05$), on day 2 the VEH and NMDA treated rats made significantly more 180° turns than the DUAL lesioned rats, and on day 10 some groups made more 60° and 120° turns (see Figure 15). Multiple comparisons indicated that no two groups were significantly different (Scheffe's test, $p>0.05$) on Day 10. No other group by percentage directional turn interaction occurred after day 10.

The only significant main effect was the repeated measures variable for percentage turn angle. While it was significant for all the test days assessed, none of the angle of turns was favoured significantly more often than another (Scheffe's, $p>0.05$). There was a tendency for most of the rats to favour choosing arms that were 60° or 90° apart.

Figures 15, Upper: Percentage turn angle for VEH (n=11), 5,7-DHT (n=12), NMDA (n=12), and DUAL (n=12) lesioned rats obtained from the RAM for day 1 (left) and 2 (right).

Middle: Percentage turn angle for VEH (n=11), 5,7-DHT (n=12), NMDA (n=12), and DUAL (n=12) lesioned rats obtained from the RAM for day 10 (left) and 11 (right).

Lower: Percentage turn angle for VEH (n=11), 5,7-DHT (n=12), NMDA (n=12), and DUAL (n=12) lesioned rats obtained from the RAM for day 19 (left) and 20 (right).

In summary, the lesions severely affected working memory more than reference memory. There was a general worsening of performance for the NMDA treated rats particularly in working memory errors and choice accuracy. The greatest decrement in performance was observed in the group of rats given the combined lesion of the septum and the serotonergic inputs to the hippocampus. The effects of the DUAL lesion were particularly noticeable during the first week of testing when only the DUAL lesioned rats made more reference memory errors and had reduced choice accuracy as compared to the VEH rats. Differences between groups on these two factors disappeared after the first week of testing, although the DUAL lesioned rats tended to show an increased number of working memory errors throughout and at the end of testing as compared to the VEH treated rats. However, the NMDA-only treated rats were not significantly different from the control group on any of the factors measured.

The RAM data tends to support the results obtained from the reference memory version of the MWM. It should be recalled that the Morris water maze path length results were obtained on average 15 days post-surgically, and the DUAL lesioned rats showed an impairment by swimming less in the quadrant where the platform had been as compared to the 5,7-DHT treated rats. In the RAM, the DUAL lesioned rats showed increased reference memory errors as compared to the VEH rats,

but only during the first week of testing which also occurred about 15 days post-surgically.

The results from the RAM testing also indicated that NMDA treatment tended to affect working memory more profoundly than reference memory. The addition of the 5,7-DHT treatment to the NMDA treatment tended to cause a longer lasting impairment over the test period. It seemed important to determine if the working memory deficit observed in the NMDA-treated rats would also be observed for other tasks which measure spatial working memory. In addition, it was of interest to determine if the DUAL lesioned rats again would be the most impaired on such a task.

The next experiment examined working memory performance in the Morris water maze, and the effects of imposing a delay between the acquisition and test trials. We hypothesized that NMDA treated rats would perform poorly on this task and that imposing the delay would interfere with memory for the platform location more severely for the DUAL lesioned group than for the NMDA-only lesioned group.

MWM-working memory (MWM-WM)

The percentage savings were averaged over the 4 days and submitted to one-way ANOVA. There were no significant group differences at either the no delay or the one minute delay ($p > 0.05$, see Figure 16).

Figure 16: Percentage savings at a no delay (upper) and 1 min delay (lower) for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL (n=10) lesioned rats measured in the MWM-WM for a block of 4 days, 12-27 days postsurgically.

At the ten minute delay there were significant group differences ($p < 0.05$). Multiple comparisons indicated that the NMDA and DUAL lesioned rats had significantly lower percentage savings scores (see Figure 17) as compared to VEH rats, but they were not significantly different from each other. A further block of 4 days testing with the 10 minute delay abolished any significant group difference (see Figure 17). No significant group differences were observed when the delay was increased to 90 minutes ($p > 0.5$) (see Figure 18).

In summary, it appears that when there was no delay between trials on this working memory version of the MWM, differences were not observed between the control and NMDA or DUAL lesioned rats. It was only when a lengthy delay (in this case 10 minutes) was imposed between the two trials that the rats administered NMDA performed worse than the control rats. The DUAL lesioned rats were no more impaired than the NMDA-only injected rats. After repeated exposure to a particular delay condition, all the rats were capable of remembering the platform location between trials.

Figure 17: Percentage savings at a delay of ten minutes for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL (n=10) lesioned rats measured in the MWM-WM for block one (upper) and block two (lower) beginning 17-32 days postsurgically.

- * NMDA significantly worse than VEH
- ** DUAL significantly worse than VEH

Figure 18: Percentage savings at a delay of 90 minutes for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL (n=10) lesioned rats measured in the MWM-WM for a block of 4 days, beginning 26-41 days postsurgically.

DISCUSSION

Neurochemical and Histological Effects of NMDA

The results of the neurochemical analysis indicated that the injection of NMDA into the medial septum caused moderate decreases in ChAT activity in the hippocampus - regardless of the postsurgical time period - similar to that reported in the literature for ibotenic acid (Hagan, Salamone, Simpson, Iversen, Morris, 1988; Hepler, Olton, Wenk, Coyle, 1985; Hepler, Wenk, Cribbs, Olton, Coyle, 1985; Riekkinen, Sirvio, Riekkinen, 1990) quisqualic acid (Decker, Radek, Majchrzak, Anderson, 1992), and radiofrequency lesions (Decker, Radek, Majchrzak, Anderson, 1992; Hepler, Olton, Wenk, Coyle, 1985).

In this study, significant decreases in ChAT activity were also found in both the anterior and posterior cortex of rats assessed 34-45 days postsurgically, but not 61-75 days postsurgically. Depletions of ChAT activity in the anterior cortex are usually not observed in medial septal lesioned rats (Hepler, Olton, Wenk, Coyle, 1985; Riekkinen, Sirvio, Riekkinen, 1990) although they have been reported (Hagan, Salamone, Simpson, Iversen, Morris, 1988). Decreased ChAT activity in the anterior cortex is more often found in rats with lesions of the nBM (Hagan, Salamone, Simpson, Iversen, Morris, 1988; Hepler, Olton, Wenk, Coyle, 1985; Hepler, Wenk, Cribbs, Olton, Coyle, 1985; Riekkinen, Sirvio, Riekkinen). Reduced ChAT activity in the posterior (post rhinal fissure) cortical tissue after medial septal lesion may

be rarely noticed because it is often not measured. Our posterior cortical tissue sample probably also contained the occipital cortex, and neurons in the horizontal limb of the diagonal band project to the occipital cortex (McKinney, Coyle, Hedreen, 1983). Excitotoxic lesions of the medial septum have been found to cause a modest reduction of ChAT in the occipital cortex (McKinney, Coyle, Hedreen; 1983.) and in the posterior cortex (Ugan, Salamone, Simpson, Iversen, Morris, 1988). In the current study, it is possible that because of the quantity of NMDA injected ($0.5\mu\text{l}/3$ injections), the effects of the excitotoxin extended beyond the boundaries of the vertical limb to the horizontal limb of the diagonal band, thereby reducing ChAT activity in the posterior cortex.

In the cortex, gradual recovery in ChAT activity has been found to occur beginning about 3 months post NBM lesion (Berman, Crosland, Jenden, Altman, 1988). Although this may support our findings for the lack of reduced ChAT activity in the anterior cortex for the rats assessed 61-75 days post-surgically, caution must be executed when interpreting these data because the tissue protein contents were unavailable for this assay.

The administration of NMDA into the medial septum reduced ACHE staining in the medial septum, and the ventral hippocampal CA1 cell fields, but not in the dorsal hippocampus. Such an observation is not unusual as there are

reportedly more cholinergic projections from the lateral part of the medial septum to the ventral hippocampus than from the medial part of the medial septum (which contains many non-cholinergic projections) to the dorsal hippocampus (Nyakas, Luiten, Spencer, Traber, 1987).

Generally, researchers who have found overall reduced staining in the hippocampal cell fields have tended to use a qualitative assessment when defining the reduction in ACHE in the hippocampus (Crutcher, Kesner, Novak, 1983; Kesner, Crutcher, Measom, 1986). In the present study, the dorsal hippocampus appeared to have reduced staining, but the quantitative analysis of the density scores from the digitized images did not indicate a significant difference between the control and NMDA injected rats. Considering that projections from the medial septal diagonal band innervate the CA1 and CA3 stratum oriens and radiatum, and the hilus of the dentate gyrus (Mosko, Lynch, Cotman, 1973), it was surprising that a more severe reduction in ACHE staining did not occur.

In the hippocampus, the absence of significant decreases in ACHE staining in all cell fields measured does not eliminate the possibility that damage had occurred as a result of the NMDA treatment. It is possible that sprouting resulted due to the partial lesion to the septohippocampal pathway. Others have shown that 8 weeks following a partial fimbriaectomy there is an increase in cholinergic fibre density in the dorsal hippocampal subiculum and the ventral

hippocampus (Blaker, Armstrong and Gage, 1988), as well as an increase in cholinergic ChAT activity in the dorsal, medial and ventral hippocampus (Gasser and Dravid, 1987). As a result, acetylcholinesterase staining would not necessarily be reduced in these areas. It should also be noted that Lapchak, Jendon and Hefti (1991) have recently reported that incomplete fimbrial lesions failed to alter endogenous choline and acetylcholine in the hippocampus. They suggested that residual cholinergic neurons were able to upregulate their capacity to synthesize and store acetylcholine in vivo, thus compensating for lesion induced losses of cholinergic neurons. Although they did not measure acetylcholinesterase, perhaps upregulation of acetylcholine resulted in no change of the level of the enzyme - ACHE - which normally breaks it down.

One interesting finding here was the significant increase in norepinephrine in the ventral hippocampus in NMDA-only injected rats as compared to the 5,7-DHT-only treated rats or the DUAL lesioned animals. Other researchers have shown that as a result of electrolytic medial septal lesions, peripheral noradrenergic fibres originating from the superior cervical ganglion grow into the hippocampus (Ayyagari, Harrell, Parsons, 1991). This ingrowth of the superior cervical ganglion, however, is most often found in the dentate gyrus and the CA3 region of the dorsal hippocampus (Ayyagari, Harrell, Parsons, 1991; Harrell, Peagler, Parsons, 1990;

Harrell, Barlow, Davis, 1983). It may be however that the increase in norepinephrine in the ventral hippocampus observed here for the NMDA-only treated rats does not reflect the same mechanism for superior cervical ganglion ingrowth as has been observed in the dorsal hippocampus after electrolytic lesion of the medial septum. One may speculate that damage to non-cholinergic systems produced the effect on norepinephrine in the electrolytic lesioned rats. If this were so, then transection of the septohippocampal pathway should also increase hippocampal norepinephrine. This does not, however, occur (Gasser, and Dravid, 1987).

Finally, it should be noted that the significant difference in norepinephrine was found only for the MWM tested rats and only between the NMDA-only treated rats who showed a slight increase and both the 5,7-DHT and DUAL lesioned groups, who both showed a slight decrease in norepinephrine. Although a similar pattern of results for the ventral hippocampus existed in the rats behaviorally tested in the RAM, these results were not significant. It would be of particular interest to determine why the addition of the 5,7-DHT neurotoxin in the DUAL lesioned rats prevents the subsequent increase in norepinephrine caused by the NMDA lesion.

Histologically, the administration of NMDA into the medial septum produced a general shrinkage of the septal neuropil with a profound loss of neuronal tissue and a

concomitant increase in the size of the ventricles. Similar damage to the septum has been found with electrolytic or radiofrequency lesions (Decker, Radek, Majchrzak, 1992; Numan and Quaranta, 1990; Numan, 1991; Worsham, Hamilton, 1973). These lesions, however tend to produce more damage extending both anterior and posterior to the site of the lesion. Damage restricted to the medial septum and the vertical limb has been described by researchers who used ibotenic acid (Hepler, Olton, Wenk, Coyle, 1985; Riekkinen, Sirvio, Riekkinen, 1990) and quisqualic acid (Decker, Radek, Majchrzak, Anderson, 1992). It is important to note that many of the studies available do not always provide extensive coverage of histological damage. Instead, many support their behavioral results by reporting effects on measures such as ChAT activity and ACHE staining. The results from those that do provide histology (including this study) suggest that at high enough concentrations, the excitatory amino acids may be damaging other neurons as well as the cholinergic cell bodies in the medial septal/diagonal band of Broca region. Future research should look at the behavioral effects of agents which reportedly create more selective damage of the cholinergic cell bodies in the medial septum, such as colchicine - an agent which disrupts axonal transport (Barone, Nanry, Mundy, McGinty, Tilson, 1991; Ginn and Peterson, 1992). As well, it is important to further assess NMDA's specificity (or lack thereof) for cholinergic cell bodies in the medial septum by

observing the effects of varied concentrations on ChAT immunoreactivity and on other neurotransmitter/neuromodulator systems .

Neurochemical Effects of 5,7-DHT

The injection of 5,7-DHT into the fimbria fornix (FF) and the cingulate bundle (CB) resulted in a significant and substantial depletion of serotonin in the dorsal and ventral hippocampus. The rat brains assessed for monoamine levels 34-45 days postsurgically also had significantly reduced serotonin in the anterior cortex, although this was much smaller than the reduction in the hippocampus. At 61-75 days postsurgically, there was no reduction in the anterior cortex. Azmitia and his colleagues have shown that microinjection of 5,7-DHT in the FF and CB reduced concentrations of serotonin in the dorsal and ventral hippocampus but not in the cortex (Altman, Normile, Galloway, Ramirez, Azmitia, 1989). Similar injections made with a glass micropipette ($<100\text{ }\mu\text{m}$) into the FF with doses of 5,7-DHT ranging up to $5\text{ }\mu\text{g}$, selectively reduced serotonergic uptake in the dorsal hippocampus (Williams and Azmitia, 1981). In the current study, the injection cannula was larger than the glass micropipette described by Azmitia and his colleagues (Altman et al., 1989) and therefore it is possible that diffusion of the 5,7-DHT outside of the FF and/or the CB may have occurred. The 33 gauge (0.20 mm) stainless steel needle used here was at least

twice the outside diameter of the tip of the glass micropipette used by Azmitia and coworkers. The larger needle was used because glass micropipettes are difficult to make, break often, and plug up easily. It may be that some selectivity of the serotonergic lesion was sacrificed in the interest of obtaining a more robust cannula which could be used for the multiple injections required for the surgery. This does not explain however, the fact that serotonin levels were not reduced in the anterior cortex of the rat brains assessed for monoamine levels 61-75 days postsurgically. This group of rats also had lesser reductions of serotonin overall. Perhaps regeneration of serotonergic axons or upregulation of serotonin synthesis by undamaged axons had occurred in the anterior cortex of these rats at the later time period. In support of this, it has been observed that regeneration of serotonin innervation can occur after 5,7-DHT lesions of the brain (Wiklund, Bjorklund and Nobin, 1978). Finally, it should be emphasized that the depletion of the anterior cortex was very modest and had disappeared by 61-75 days postsurgically.

The results from the HPLC also indicated that the 5,7-DHT injections were selective for serotonergic terminals and had generally no effect on norepinephrine or dopamine in any of the brain parts measured.

In summary, the biochemical and histological results would suggest that at the concentration used, NMDA destroyed

both cholinergic and possibly non-cholinergic neurons in the medial septum. ChAT activity was reduced in the hippocampus and ACHE staining was reduced in the medial septum and the ventral hippocampal CA1 cell fields for all the rats receiving NMDA. The administration of 5,7-DHT into the FF and the CB created a substantial depletion of serotonin in both the dorsal and ventral hippocampus.

Damage to the septo-hippocampal pathways should interfere with memory. The question remains, however, whether or not the addition of damage to the serotonergic projections to the hippocampus interacts with and changes the way the memory is altered by the septal lesions. The results from the behavioral testing will be discussed next.

MWM - Reference Memory Version

Out of all the variables measured in the Morris water maze (latencies to find the hidden platform, path length and time in the quadrant where the platform had been, annulus crossings, heading angle, speed, cued navigation) only two differentiated between the rats that received NMDA and those that did not. One of the measurements - heading angle - suggested that all the rats that had received NMDA were using a different search strategy to find the hidden platform than using the cues in the room to guide their search. The second variable - path length - differentiated between the 5,7-DHT-

only treated rats and the DUAL lesioned rats. In this case, the DUAL lesioned rats showed a reduced path length in the quadrant where the platform had been as compared to the 5,7-DHT treated rats. This suggested that these rats had some difficulty remembering between test days where the platform had been. However, they appeared to have adopted a search strategy that they could use to help them find the platform. This is supported further by the fact that the DUAL lesioned rats did not show significantly different percentage path lengths for the four quadrants, whereas all the other groups had a higher percentage of path length in the quadrant where the platform had been.

Others have also reported that rats with lesions or blockade to both the cholinergic and serotonergic systems have been found to perform worse on the MWM as compared to the cholinergic-only and serotonergic only depleted rats. Increased escape latencies (Nilsson, Strecker, Daszuta, and Bjorklund, 1988; Richter-Levin and Segal, 1989) as well as reduced annulus crossings (Nilsson, Strecker, Daszuta, and Bjorklund, 1988) have been found. Indeed, Nilsson and colleagues reported that the combined septal lesioned plus 5,7-DHT treated rats did not indicate the use of any search strategy whereas the septal-only lesioned animals were using a non-spatial search strategy (1988).

It is not unusual to find lack of consistency between the measurements obtained from the Morris water maze. Some of the

measurements may not be sensitive to the effects of the lesion, or may be sensitive to extraneous factors. For example, unlike path length, the time spent in the quadrant where the platform had been may not indicate differences between groups because time is more sensitive to interfering factors. An animal who floats in one spot for a while would not change its proportional path length in a quadrant, but its time there would increase. In the literature, a variety of results have been shown after testing medial septal lesioned rats in the MWM. Injections of ibotenic acid into the medial septum have produced significant changes in variables such as latencies to find the platform, annulus crossings, and quadrant swim times as compared to controls (Hagan, Salamone, Simpson, Iversen, Morris, 1988; Riekkinen, Sirvio, Riekkinen, 1990). Electrolytic lesions have increased escape latencies but had no effect on heading angle, or time in the quadrant where the platform had been (Dokla and Boitano, 1991). Radiofrequency lesions or injections of quisqualic acid into the medial septum increased latencies to find the platform but only on the first days of testing, and had no effect on the time spent in the quadrant where the platform had been (Decker, Radek, Majchrzak, 1992; Nilsson, Strecker, Daszuta, Bjorklund, 1988).

As well as different measures, the different methods used for running the MWM may be responsible for the inconsistent results of lesions. Sometimes the rats received two trials a

day for 10-12 days (Hagan, Salamone, Simpson, Iversen, Morris, 1988; Riekkinen, Sirvio, Riekkinen, 1990), sometimes they were habituated to the maze for one day before testing began (Hagan et al., 1988), and sometimes they received four trials a day, but the trials were divided up with a 3-4 minutes wait between the third and fourth trial (Decker et al., 1992). Differences in results may also be due to the variety of techniques used to create the lesions and whether or not the damage was localized to cholinergic cell bodies or more global.

Interestingly, although the combined administration of 5,7-DHT with the medial septal lesion worsened the performance of our rats on the MWM, the 5,7-DHT only treatment did not alter performance as compared to VEH rats. Altman and his colleagues have found that a global reduction of serotonin in the brain (Altman and Normile, 1988) as well as serotonergic deafferentation of the hippocampus (Altman et al., 1990) will actually enhance performance of rats in a positively reinforced spatial discrimination task such as the Stone 14 unit T-maze. Although both the MWM and Stone T-maze are spatial tasks it is important to note that they vary in the way the rat has to solve the task. The Stone T-maze has both a working and reference memory component - the correct arm choices remain constant across trials, but the rat has to keep track of where he has been and where he has to go with respect to its location (Altman and Normile, 1988). The MWM is typically used as a reference memory task, the rat only has to

remember where the hidden platform is with respect to spatial cues. Rats depleted of only serotonin do not show behavioral impairments on reference memory tasks in general (Altman and Normile, 1988), a result consistent with the lack of effect found here for MWM performance.

It has been suggested that the serotonergic and cholinergic inputs to the hippocampus may mediate processes other than memory, including so-called Type I behaviors (i.e., spontaneous activity such as postural changes, turning of the head, and walking; Vanderwolf, Baker, Dickson, 1990). If this were the case, then one would expect to find the DUAL lesioned rats deficient in performance on the cued platform test or even swim speed. Neither of these were affected by the DUAL lesion, however.

Radial Arm Maze

In the radial arm maze, working memory (the ability to remember within a trial) was more affected than reference memory (the ability to remember between trials) for all rats that received the administration of NMDA into the medial septum, but especially so in the rats that received both the NMDA and the 5,7-DHT treatment. Administered alone, the 5,7-DHT treatment had no effect on performance in this task, but when it was added to the NMDA lesion it increased the number of working memory errors as compared to the VEH control rats. By the end of testing in the RAM the NMDA-only treated rats

were no longer performing worse than the control rats, but the DUAL lesioned rats were. In addition, the number of reference memory errors was affected only during the first week when the rats who received the combined lesion (5,7-DHT+NMDA) made significantly more. Choice accuracy (number of correct choices out of the first six arm entries) which would reflect both reference and working memory errors was decreased as well during the first week for the DUAL lesioned rats as compared to the control group.

It would appear then that the DUAL lesioned rats were more impaired than the NMDA-only or 5,7-DHT-only treated rats. It is difficult to integrate this result with other experiments because most have compared the effects of combined versus single serotonergic and cholinergic lesions by lesioning the nBM (not the medial septum) in combination with global brain depletions of serotonin (Markowska and Wenk, 1991). Alternatively, they have involved the use of cholinergic antagonists with serotonergic toxins (Sakurai and Wenk, 1990). The one study which is most similar in its treatments only tested the rats in the Morris water maze (Nilsson et al, 1988) and those results were consistent with our findings here for reference memory.

The literature also supports our observations that working memory was more affected than reference memory in the NMDA lesioned rats. For example, it has been reported that electrolytic medial septal lesions produce a deficit on an

alternation task which is attenuated when the working memory component of the task is modified to reference memory (Numan and Quaranta, 1990). This supports our finding that working memory is more affected than reference memory in medial septal lesioned rats.

Other researchers have also found a recovery of function in the radial arm maze following electrolytic medial septal lesions (Crutcher, Kesner, Novak, 1983). It has been speculated that the recovery was retarded by sprouting of peripheral sympathetic noradrenergic axons into the hippocampus (Ayyagari, Harrell, Parsons, 1991; Harrell, Barlow, Davis, 1983; Harrell, Peagler, Parsons, 1990). It is unknown whether a similar peripheral sympathetic sprouting occurred in the NMDA-only lesioned rats in this study. One may speculate that the elevation of norepinephrine in the ventral hippocampus of NMDA-only treated rats may have been due to such sympathetic sprouting. However, peripheral sprouting cannot explain why the DUAL lesioned rats did not recover as well as the NMDA-only treated rats, since they failed to show the elevation of norepinephrine in the hippocampus.

The finding in this study that the 5,7-DHT treatment did not affect reference or working memory in the RAM is consistent with results reported by Altman and Normile (1988). These researchers found that global reductions of serotonin with PCA administration had no effect on the performance of

rats in an 8 arm radial maze regardless of whether or not they measured working or reference memory. However, these researchers have observed an improvement in performance in rats with 5,7-DHT induced deafferentation of the hippocampus on the Stone 14 unit T-maze (Altman et al., 1990). It remains to be determined how these two tasks differ such that the serotonergic lesion may affect performance on one task but not the other. One possibility is that the Stone T-maze mainly assesses egocentric learning (i.e., the rats ability to remember left/right turns) whereas the RAM assesses allocentric spatial learning (i.e., the rats ability to use cues in the environment to solve the maze).

Although the 5,7-DHT treatment alone did not appear to worsen or improve behavioral performance in this study, the addition of 5,7-DHT to the NMDA treatment impaired performance slightly more than did the NMDA-only treatment. Thus, destroying these two projecting systems to the hippocampus created a behavioral impairment which was worse than what was observed in rats with only a lesion to the septohippocampal pathway. Vanderwolf's research would suggest that the combined treatment of destroying the "pacemaker cells" in the medial septum (Vanderwolf et al., 1990) plus the lesioning of the serotonergic pathways to the hippocampus would seriously impair performance in the RAM by interfering with behaviors that play a role in spontaneous activity (Type I behaviors). This could explain why the DUAL lesioned rats made both

reference and working memory errors during the first week of behavioral testing in the RAM, but it does not explain, however, why only working memory errors were affected over the long term in these rats.

One question which arises from these results is whether the increased errors in the rats which were administered NMDA reflects deficits in working memory or some other consequence of the lesion such as perseveration or hyperresponsivity in the first few weeks after the lesion. Alternatively, were the NMDA treated rats using unusual algorithms (patterns of arm entry) to solve the maze which initially caused many errors but allowed them to improve their performance over time?

Numan and Quaranta (1990) have reported that an electrolytic medial septal lesion increased perseveration in an operant alternation task but only when a delay was introduced. It is unlikely that perseveration caused the poor performance of the NMDA-injected rats in the current study however, because reference memory errors would also have been elevated throughout the test period and they were not.

Another alternative is that due to hyperreactivity - an established consequence of septal lesions (Carlson, 1986) - the NMDA treated rats may have become more hyperactive and rushed from one arm to the next without regard for spatial stimuli (showing "ballistic" tendencies) thereby increasing the number of errors made. In fact, it has been observed

previously in medial septal lesioned rats that they lack response inhibition on certain behavioral tasks such as a two way active avoidance task (Gray and McNaughton, 1983). The lack of response inhibition may produce an overall increase in motoric activity into all the arms. It does appear that blocking the rats immediate response to entering an arm eliminates any response tendencies, however, it does not improve choice accuracy in fimbria-fornix lesioned rats (Olton and Werz, 1978). In addition, in this study arm entries in the radial arm maze were scored only when the rat went past the midway point of an arm. This would have eliminated the errors whereby rats hurriedly entered an arm then exited again without going down to the end of the arm. Thus, although the lesioned rats may have initially entered incorrect arms due to a "ballistic" tendency the stringent criterion for arm entry probably minimized errors due to this tendency. Finally, it should also be noted that the NMDA lesioned rats did not show a similar lack of response inhibition into the reference memory arms, nor did they show an increase in motoric activity in the elevated plus maze (total arm entries, open/total arm entries; see Appendix A and Figure 19 for details). Thus, it seems unlikely that hyperactivity/hyperreactivity was the cause of their increased errors in the radial arm maze.

The analysis of patterns of arm entries used (data from percentage turn angle) in the maze would suggest that all the rats regardless of their treatment used a similar algorithm to

solve the maze throughout the test period. They all tended to enter every second or third arm (60° or 90° turns, see Appendix C for a further explanation and interpretation of why they may have used an algorithm). The rats may have incorporated an algorithm with spatial cues to solve the maze. In fact, some rats may have relied on the algorithm more than the spatial cues depending on the damage to the hippocampus. Finally, some rats may have been unable to change their response patterns because they developed a strong win-stay tendency, because of acquired reward associations (Ridley, Aitken, Baker, 1989) with the baited arms on the RAM. Thus while the rats administered NMDA did not appear to use different algorithms for solving the maze, they may not have adjusted their behavior and thus benefited from the use of a pattern whereby they only entered the baited arms once.

In summary, it does not appear that the deficit in working memory on the RAM can be explained by perseveration, hyperresponsivity, or patterns of arm entry.

MWM-Working Memory Version with Delay

It was important to see if the effects on working memory in the RAM could also be observed on a different spatial working memory task. The percentage savings scores for the 5,7-DHT-only, NMDA-only and DUAL lesioned rats did not vary significantly from the control group during the no delay or one minute delay condition. It was only when a 10 minute delay

was introduced between the first and second trial that both NMDA-only and DUAL lesioned rats performed worse than the 5,7-DHT and VEH rats. Dokla and Boitano (1991) found that electrolytically medial septal lesioned rats also had increased latencies to reach the platform in the working memory version of the MWM after a five minute delay. In the present study, the impairment disappeared after the second week of testing at the 10 minute delay, and was not apparent again even at 90 minute delay. It is important to note that this working memory version of the Morris water maze involved four day blocks of testing the rats two trials a day to a platform location in one of the four quadrants. Although the platform location changed daily, there were only four different locations to choose from. Therefore by the second block of testing with the 10 minute delay the rats were being exposed to each of the four different platform locations in the maze for the fourth time (13-16th days of testing). It is possible that by this time all rats had adopted a strategy to solve the maze such as spending less time searching the outer edge of the pool and spending more time in the inner area where the platforms tended to be located (Volpe, Hendrix, Park, Towle, Davis, 1992). If testing will be done for a length of time, future studies should consider always choosing random locations in the pool, not one of only four locations in the centre of each quadrant.

The data from the working memory version of the Morris

water maze can be obtained over a shorter period of time than the RAM. Therefore, as a behavioral test it may be a preferred choice for some types of research. Using this version of the maze, it would be interesting to assess in future studies the effects of administering selective cholinergic and serotonergic agonists and antagonists on the DUAL lesioned rats.

The behavioral findings can be summarized as follows: 1) the combined lesion of both cholinergic and serotonergic pathways to the hippocampus produced an impairment on working memory when comparisons were made with the 5,7-DHT-only lesioned rats and control rats, but not with the NMDA-only treated rats. 2) Both the NMDA-only and DUAL lesioned rats recovered their ability to perform the tasks. However, working memory was slow to recover particularly in the DUAL lesioned rats in the RAM. 3) "Recovered" DUAL lesioned rats showed re-emerged but temporary impairments in working memory when a delay was imposed between acquisition and retention testing in the Morris water maze.

Relevance of Current Research to Alzheimer's Disease

A number of findings have spurred researchers to speculate that specific subcortical nuclei and their corresponding neurotransmitter system may play a critical role in the decline of memory and other cognitive functions in

patients diagnosed with probable Alzheimer's disease. For example, basal forebrain nuclei show neuronal degeneration (Jellinger, 1987a,b;; Rogers, Brogan, Mirra, 1985; Whitehouse and Kellar, 1987; Whitehouse, Price, Struble, Clark, Coyle, Delong, 1982) and reduced levels of ChAT. A corresponding reduction in ChAT and acetylcholinesterase has also been observed in the cortex and hippocampus of patients diagnosed with Alzheimer's Disease (Esiri, Pearson, Steele, Bowen, Powell, 1990; Jellinger, 1987a; Whitehouse, Kellar, 1987; Whitehouse, Martino, Marcus, Zweig, Singer, Price, Kellar, 1988).

Nonetheless, the pathology that occurs in the brain of patients diagnosed with Alzheimer's disease cannot be attributed to degeneration of one neuronal system. Some reasons for this are as follows: 1) The degree of atrophy of the brain is greater than would be expected if only a selective degeneration of cholinergic neurons occurred (Winblad, Bucht, Fowler, Wallace, 1986). In fact, degeneration of other neurotransmitter systems has also been found. For example, reduced levels of serotonin and its metabolite 5-hydroxyindoleacetic acid have been observed in the cortex (D'Amato, Zweig, Whitehouse, Wenk, Singer, Mayeux, Price, Snyder, 1987; Palmer, Francis, Benton, Sims, Mann, Neary, Snowden, Bowen, 1987; Arai, Kosaka, Iizuka, 1984; Cross, Crow, Ferrier, Johnson, Bloom, Corsellis, 1984; Herregodts, Bruyland, De Keyser, Solheid, Michotte, Ebinger, 1989) and the

hippocampus (Gottfries, Adolfsson, Aquilonius, Carlsson, Eckernas, Nordberg, Oreland, Svennerholm, Wiberg, Winblad 1983). 2) Degeneration of one neurotransmitter system does not appear to be a sufficient condition to produce the decline in cognitive functions observed in Alzheimer's Disease (Woolfe, Jacobs, Butcher, 1989). Germane to this is the observation that replacing the loss of a neurotransmitter (such as acetylcholine) does not attenuate or halt the progressive decline in cognitive functions in Alzheimer's disease (Kumar and Calache, 1991).

The effects due to the degeneration of more than one neurotransmitter system remain, however, unknown. Whether these effects are additive or synergistic, it is plausible that as different neurotransmitter systems are damaged they may interact thereby creating the cascade of events which result in the progressive degeneration of memory and other cognitive functions observed in Alzheimer's disease.

The hippocampus shows loss of cholinergic and serotonergic markers in patients diagnosed with Alzheimer's disease (Mann, 1988; Esiri, Pearson, Steel, Bowen, Powell, 1990). Furthermore, memory loss is the most consistent and profound cognitive symptom of Alzheimer's disease. For example, patients with Alzheimer's disease show reduced working memory, an increased rate of forgetting from working memory, and impaired secondary or long term memory (Becker, 1988). This thesis looked at the effects of singular vs

combined lesions to the cholinergic and serotonergic neurotransmitter systems on memory, specifically the effects of chronically lesioning the pathways which supply acetylcholine and serotonin to the hippocampus. The fact that the DUAL lesioned rats in this study appeared to be more severely impaired than the NMDA-only treated rats does suggest that this combined lesion of these hippocampal afferents models the basis for the severe memory disturbance observed in Alzheimer's disease. However, these lesions did not cause a progressive degeneration or prevent recovery of function. Rather, there was recovery of function, although this was delayed in the DUAL lesioned rats. Thus, rather than creating a model of brain dysfunction in Alzheimer's disease, this thesis instead helps to explain further the role that these two projection systems play in behavioral tasks which measure memory.

REFERENCES

- Alonso, R., Soubrie, P. (1991). Effects of serotonergic denervation on the density and plasticity of brain muscarinic receptors in the rat. Synapse, 8, 30-37.
- Altman, H.J., Nordy, D.A., Ogren, S.O. (1984). Role of serotonin in memory: Facilitation by alaproclate and zimeldine. Psychopharmacology, 84, 496-502.
- Altman, H.J., Normile, H.J. (1988). What is the nature of the role of the serotonergic nervous system in learning and memory: Prospects for development of an effective treatment strategy for senile dementia. Neurobiology of Aging, 9, 627-638.
- Altman, H.J., Normile, H.J., Galloway, M.P., Ramirez, A., Azmitia, E.C. (1990). Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. Brain Research, 518, 61-66.
- Altman, H.J., Stone, W.S., Ogren, S.O. (1987). Evidence for a possible functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. Behavioral and Neural Biology, 48, 49-62.
- Arai, H., Kosaka, K., Iizuka, R. (1984). Changes of biogenic amines and their metabolites in postmortem brains from patients with Alzheimer-type dementia. Journal of Neurochemistry, 43, 388-393.
- Ayyagari, V., Harrell, L.E., Parsons, D.S. (1991). Interaction of neurotransmitter systems in the hippocampus: A study of some behavioral effects of hippocampal sympathetic ingrowth. The Journal of Neuroscience, 11(9), 2848-2854.
- Azmitia, E.C. (1978). The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In L.L. Iversen, S.D. Iversen, S.H. Snyder (Eds.), Handbook of Psychopharmacology, (Vol 8, pp233-262). New York: Raven Press.
- Backer Cave C., Squire, L.R. (1991). Equivalent impairment of spatial and nonspatial memory following damage to the human hippocampus. Hippocampus, 1(3), 329-340.
- Barnes, C.A. (1988). Spatial learning and memory processes: The search for their neurobiological mechanisms in the rat. Trends in Neuroscience, 11(4), 163 -169.

Barnes, J.M., Barnes, N.M., Costall, B., Naylor, R.J., Tyers, M.B. (1989). 5-HT₃ receptors mediate inhibition of acetylcholine release in cortical tissue. Nature, 338, 762-763.

Barone, S., Nanry, K.P., Mundy, W.R., McGinty, J.F., Tilson, H.A. (1991). Spatial learning deficits are not solely due to cholinergic deficits following medial septal lesions with colchicine. Psychobiology, 19(1), 41-50.

Bartus, R.T., Dear, R.L., Beer, B., Lippa, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. Science, 217, 408-417.

Bartus, R.T., Flicker, C., Dean, R.L., Pontecorvo, M., Figueiredo, J.C., Fisher, S.K. (1985). Selective memory loss following nucleus basalis lesions: Long term behavioral recovery despite persistent cholinergic deficiencies. Pharmacology, Biochemistry, and Behavior, 23, 125-135.

Bartus, R.T., Pontecorvo, M.J., Flicker, C., Dean, R.L., Figueiredo, J.C. (1986). Behavioral recovery following bilateral lesions of the nucleus basalis does not occur spontaneously. Pharmacology, Biochemistry, and Behavior, 24, 1287-1292.

Becker, J.T. (1988). Working memory and secondary memory deficits in Alzheimer's disease. Journal of Clinical and Experimental Neuropsychology, 10(6), 739-753.

Beninger, R.J., Jhamandas, K., Boegman, R.J., El-Defrawy, S.R., (1986). Effects of scopolamine and unilateral lesions of the basal forebrain on T-Maze spatial discrimination and alternation in rats. Pharmacology, Biochemistry, Behavior, 24, 1353-1360.

Beninger, R.J., Wirsching, B.A., Jhamandas, K., Boegman, R.J., El-Defrawy, S.R. (1986). Effects of altered cholinergic function on working and reference memory in the rat. Canadian Journal of Physiology and Pharmacology, 64, 376-382.

Berman, R.F., Crosland, R.D., Jendon, D.J., Altman, H.J. (1988). Persisting behavioral and neurochemical deficits in rats following lesions of the basal forebrain. Pharmacology, Biochemistry, and Behavior, 29, 581-586.

Biggan, S.L., Beninger, R.J., Cockhill, J., Jhamandas, K., Boegman, R.J. (1991). Quisqualate lesions of rat NBM: Selective effects on working memory in a double Y-maze. Brain Research Bulletin, 26, 613-616.

Bjorklund, A., Baumgarten, H.G., Rensch, A. (1975). 5,7-

dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. Journal of Neurochemistry, 24, 833-835.

Blaker, S.N., Armstrong, D.M., Gage, F.H. (1988). Cholinergic Neurons within the rat hippocampus: Response to fimbria-fornix transection. The Journal of Comparative Neurology, 272, 127-138.

Buresova, O., Bolhuis, J.J., Bures, J. (1986). Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. Behavioral Neuroscience, 100(4), 476-482.

Caine, E.D., Weingartner, H., Ludlow, C.L., Cudahy, E.A., Wehry, S. (1981). Qualitative analysis of scopolamine-induced amnesia. Psychopharmacology, 74, 74-80.

Carlson, N.R. (1991). Physiology of Behavior (4th ed.). Boston, London, Toronto: Allyn and Bacon.

Cross, A.J. (1990). Serotonin in Alzheimer-type dementia and other dementing illnesses. In P.M. Whitaker-Azmitia and S.J. Peroutka (Eds.), The Neuropharmacology of Serotonin, (Vol 600, pp 405-417). New York: The New York Academy of Sciences.

Cross, A.J., Crow, T.J., Ferrier, I.N., Johnson, J.A., Bloom, S.R., and Corsellis, J.A.N. (1984). Serotonin receptor changes in dementia of the Alzheimer type. Journal of Neurochemistry, 43, 1574-1581.

Crow, T.J., Grove-White, I.G. (1973). An analysis of the learning deficit following hyoscine administration to man. British Journal of Pharmacology, 49, 322-327.

Crutcher, K.A., Kesner, R.P., and Novak, J.M. (1983). Medial septal lesions, radial arm maze performance, and sympathetic sprouting: A study of recovery of function. Brain Research, 262, 91-98.

D'Amato, R.J., Zweig, R.M., Whitehouse, P.J., Wenk, G.L., Singer, H.S., Mayeux, R., Price, D.L., Snyder, S.H. (1987). Aminergic systems in Alzheimer's disease and Parkinson's disease. Annals of Neurology, 22(2), 229-236.

Decker, M.W., Radek, R.J., Majchrzak, M.J., Anderson, D.J. (1992). Differential effects of medial septal lesions on spatial-memory tasks. Psychobiology, 20(1), 9-17.

Dokla, C.P., Boitano, J.J. (1991). Physostigmine fails to attenuate spatial working and reference memory deficits in the Morris water maze in rats with medial septal lesions. Society

for Neuroscience Abstracts, 17, abstract # 55.14.

Drachman, D.A. (1977). Memory and cognitive function in man: Does the cholinergic system have a specific role? Neurology, 27, 783-790.

Drachman, D.A., Leavitt, J. (1974). Human memory and the cholinergic system. Archives of Neurology, 30, 113-121.

Eichenbaum, H., Otto, T., Cohen, N.J., (1992). The hippocampus-What does it do?. Behavioral and Neural Biology, 57, 2-36.

Eichenbaum, H., Stewart, C., Morris, R.G.M. (1990). Hippocampal representation in place learning. The Journal of Neuroscience, 10(11), 3531-3542.

Esiri, M.M., Pearson, R.C.A., Steele, J.E., Bowen, D.M., Powell, T.P.S. (1990). A quantitative study of the neurofibrillary tangles and the choline acetyltransferase activity in the cerebral cortex and the amygdala in Alzheimer's disease. Journal of Neurology, Neurosurgery, and Psychiatry, 53, 161-165.

Euvrard, C., Javoy, F., Herbet, A., Glowinski, J. (1977). Effect of quipazine, a serotonin-like drug, on striatal cholinergic interneurons. European Journal of Pharmacology, 41, 281-289.

Ezrin-Waters, C., Resch, L. (1986). The nucleus basalis of Meynert. Canadian Journal of Neurological Science, 13, 8-14.

Fibiger, H.C. (1982). The organization and some projections of cholinergic neurons of the mammalian forebrain. Brain Research Reviews, 4, 327-388.

Flood, J.F., Landry, D.W., Jarvik, M.E. (1981). Cholinergic receptor interactions and their effects on long-term memory processing. Brain Research, 215, 177-185.

Fonnum, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. Journal of Neurochemistry, 24, 407-409.

Gasser, U.E., Dravid, A.R. (1987). Noradrenergic, serotonergic, and cholinergic sprouting in the hippocampus that follows partial or complete transection of the septohippocampal pathway: Contributions of the spared inputs. Experimental Neurology, 96, 352-364.

Gillet, G., Ammor, S., Fillion, G. (1985). Serotonin inhibits acetylcholine release from rat striatum slices: Evidence for

a presynaptic receptor-mediated effect. Journal of Neurochemistry, 45, 1687-1691.

Ginn, S.R. and Peterson, G.M. (1992). Studies related to the use of colchicine as a neurotoxin in the septohippocampal cholinergic system. Brain Research, 590, 144-152.

Gottfries, C-G., Adolfsson, R., Aquilonius, S-M., Carlsson, A., Eckernas, S-A., Nordberg, A., Orelund, L., Svennerholm, L., Wiberg, A., Winblad, A.B. (1983). Biochemical changes in dementia disorders of Alzheimer type (AD/SDAT). Neurobiology of Aging, 4, 261-271.

Gray, J.A., and McNaughton, N. (1983). Comparison between the behavioral effects of septal and hippocampal lesions: A review. Neuroscience and Biobehavioral Reviews, 7, 119-188.

Hagan, J.J., Morris, R.G.M. (1988). The cholinergic hypothesis of memory: a review of animal experiments. In L. Iversen, S. Iversen, and S. Snyder (Eds.), Psychopharmacology of the Aging Nervous System (pp 237-324). New York: Oxford University Press.

Hagan, J.J., Salamone, J.D., Simpson, J., Iversen, S.D., Morris, R.G.M. (1988). Place navigation in rats is impaired by lesions of medial septum and diagonal band but not nucleus basalis magnocellularis. Behavioral Brain Research, 27, 9-20.

Harrell, L.E., Barlow, T.S., Davis, J. (1983). Sympathetic sprouting and recovery of spatial behavior. Experimental Neurology, 82, 379-390.

Harrell, L.E., Peagler, A., Parsons, D.S. (1990). Adrenoreceptor antagonist treatment influences recovery of learning following medial septal lesions and hippocampal sympathetic ingrowth. Pharmacology Biochemistry and Behavior, 35, 21-28.

Hepler, D.J., Olton, D.S., Wenk, G.L., Coyle, J.T. (1985). Lesions in nucleus basalis magnocellularis and medial septal area of rats produce qualitatively similar memory impairments. The Journal of Neuroscience, 5(4), 866-873.

Hepler, D.J., Wenk, G.L., Cribbs, B.L., Olton, D.S., and Coyle, J.T. (1985). Memory impairments following basal forebrain lesions. Brain Research, 346, 8-14.

Herregodts, P., Bruylant, M., De Keyser, J., Solheid, C., Michotte, Y., Ebinger, G. (1989). Monoaminergic neurotransmitters in Alzheimer's disease. Journal of the Neurological Sciences, 92, 101-116.

- Jakala, P., Sirvio, J., Jolkkonen, J., Riekkinen, P., Acsady, L., Riekkinen, P. (1992). The effects of p-chlorophenylalanine-induced serotonin synthesis inhibition and muscarinic blockade on the performance of rats in a 5-choice serial reaction time task. Behavioral Brain Research, 51, 29-40.
- Jakala, P., Sirvio, J., Riekkinen, P., Riekkinen, P. (1993). Effects, of p-Chlorophenylalanine and methysergide on the performance of a working memory task. Pharmacology, Biochemistry, and Behavior, 44, 411-418.
- Jarrard, L.E. (1978). Selective hippocampal lesions: Differential effects on performance by rats of a spatial task with preoperative versus postoperative training. Journal of Comparative and Physiological Psychology, 92(6), 1119-1127.
- Jellinger, K. (1987a). Neuropathological substrates of Alzheimer's disease and Parkinson's disease. Journal of Neural Transmission, (Supple.), 24, 109-129.
- Jellinger, K. (1987b). Quantitative changes in some subcortical nuclei in aging, Alzheimer's disease, and Parkinson's disease. Neurobiology of Aging, 8, 556-561.
- Johnston, M.V., McKinney, M., Coyle, J.T. (1979). Evidence for a cholinergic projection to neocortex from neurons in basal forebrain. Proceedings of the National Academy of Science, 76(10), 5392-5396.
- Kesner, R.P., Crutcher, K.A., Measom, M.O. (1986). Medial septal and nucleus basalis magnocellularis lesions produce order memory deficits in rats which mimic symptomatology of Alzheimer's disease. Neurobiology of Aging, 7, 287-289.
- Kesner, R.P., Novak J.M. (1982). Serial position curve in rats: Role of the dorsal hippocampus. Science, 218, 173-175.
- Ketchum, J.S., Sidell, F.R., Crowell, E.B., Jr., Aghajanian, G.K., Hayes, A.H., Jr. (1973). Atropine, scopolamine, and ditran: Comparative pharmacology and antagonists in man. Psychopharmacologia, 28, 121-145.
- Kumar, V., Calache, M. (1991). Treatment of Alzheimer's disease with cholinergic drugs. International Journal of Clinical Pharmacology, Therapy, and Toxicology, 29(1), 23-37.
- Lapchak, P.A. Jenden, D.J., Hefti, F. (1991). Compensatory elevation of acetylcholine synthesis in vivo by cholinergic neurons surviving partial lesions of the septohippocampal pathway. The Journal of Neuroscience, 11(9), 2821-2828.

Mann, D.M.A. (1988). Neuropathology and neurochemical aspect of Alzheimer's disease. In L. Iversen, S. Iversen, and S. Snyder (Eds.), Psychopharmacology of the Aging Nervous System (pp 1-67). New York: Oxford University Press.

Markowska, A.J., Wenk, G.L. (1991). Serotonin influences the behavioral recovery of rats following nucleus basalis lesions. Pharmacology Biochemistry and Behavior, 38, 731-737.

Maura, G., Raiteri, M. (1986). Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release. European Journal of Pharmacology, 129, 333-337.

Mayo, W. (1989). Comparative study of two types of cholinergic lesion in rats. Archives of Gerontology and Geriatrics, (Suppl. 1), 91-98.

McEntee, W.J., Crook, T.H. (1991). Serotonin, memory, and the aging brain. Psychopharmacology, 103, 143-149.

McKinney, M., Coyle, J.T., Hedreen, J.C. (1983). Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. The Journal of Comparative Neurology, 217, 103-121.

Meyers, B. (1965). Some effects of scopolamine on a passive avoidance response in rats. Psychopharmacologia (Berl.), 8, 111-119.

Mitchell, S.J., Rawlins, J.N.P., Steward, O., Olton, D.S. (1982). Medial septal area lesions disrupt theta rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. The Journal of Neuroscience, 2(3), 292-302.

Morris, R.G.M. (1983). An attempt to dissociate "spatial-mapping" and "working-memory" theories of hippocampal function. In W. Seiffert (Ed.), Neurobiology of the Hippocampus (pp 405-432). New York: Academic Press.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O'Keefe, J. (1982). Place navigation impaired rats with hippocampal lesions. Nature, 297, 681-683.

Mosko, S., Lynch, G., Cotman, C. (1973). The distribution of septal projections to the hippocampus of the rat. Journal of Comparative Neurology, 152, 163-174.

Murray, C.L., and Fibiger, H.C. (1985). Learning and memory deficits after lesions of the nucleus basalis magnocellularis: reversal by physostigmine. Neuroscience, 14(4), 1025-1032.

Nilsson, O.G., Shapiro, M.L., Gage, F.H., Olton, D.S., Bjorklund, A. (1987). Spatial learning and memory following fimbria-fornix transection and grafting of fetal septal neurons to the hippocampus. Experimental Brain Research, 67, 195-215.

Nilsson, O.G., Strecker, R.E., Daszuta, A., Bjorklund, A. (1988). Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. Brain Research, 453, 235-246.

Normile, H.J., Jenden, D.J., Kuhn, D.M., Wolf, W.A., Altman, H.J. (1990). Effects of combined serotonin depletion and lesions of the nucleus basalis or magnocellularis on acquisition of a complex spatial discrimination task in the rat. Brain Research, 536, 245-250.

Numan, R. (1991). Medial septal lesions impair performance on a preoperatively acquired delayed alternation task. Brain Research Bulletin, 26, 449-453.

Numan, R., Quaranta, J.R., Jr. (1990). Effects of medial septal lesions on operant delayed alternation in rats. Brain Research, 531, 232-241.

Nyakas, C., Luiten, G.M., Spencer, D.G., Traber, J. (1987). Detailed projection patterns of septal and diagonal band afferents to the hippocampus in the rat with emphasis on innervation of CA1 and dentate gyrus. Brain Research Bulletin, 18, 533-545.

Olton, D.S. (1983). Memory functions and the hippocampus. In W. Seiffert (Ed.), Neurobiology of the Hippocampus (pp 335-373). New York: Academic Press.

Olton, D.S. and Werz, M.A. (1978). Hippocampal function and behavior: spatial discrimination and response inhibition. Physiology and Behavior, 20, 597-605.

Page, K.J., Everitt, B.J., Robbins, T.W., Marston, H.M., Wilkinson, L.S. (1991). Dissociable effects on spatial maze and passive avoidance acquisition and retention following AMPA- and ibotenic acid-induced excitotoxic lesions of the basal forebrain in rats: differential dependence on cholinergic neuronal loss. Neuroscience, 43(2/3), 457-472.

Palmer, A.M., Francis, P.T., Benton, J.S., Sims, N.R., Mann, D.M.A., Neary, D., Snowden, J.S., Bowen, D.M. (1987). Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. Journal of Neurochemistry, 48, 8-15.

Pappas, B.A., Murtha, S.J.E., Park, G.A.S., Condon, K.T.,

Szirtes, R.M., Laventure, S.I., and Ally, A. (1992). Neonatal brain dopamine depletion and the cortical and behavioral behavioral consequences of enriched postweaning environment. Pharmacology, Biochemistry, and Behavior, 42, 741-748.

Paxinos, G., Watson, C. (1986). The rat brain in stereotaxic coordinates, (2nd ed.). New York: Academic Press.

Peele, D.B., Baron, S.P. (1988). Effects of selection delays on radial maze performance: Acquisition and effects of scopolamine. Pharmacology, Biochemistry, and Behavior, 29, 143-150.

Petersen, R.C. (1977). Scopolamine induced learning failures in man. Psychopharmacology, 52, 283-289.

Pellow, S., Chopin, P., File, S. E., Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience, 14, 149-167.

Prichard, J.W. (1980). Phenobarbital: Proposed mechanisms of antileptic action. In G.H. Glaser, J.K. Penry, and D.M., Woodbury, D.M., (Eds.), Antileptic Drugs: Mechanisms of Action (pp 553-562). New York: Raven Press.

Priestley, J.V. (1987). Immunocytochemical techniques for the localization of neurochemically characterized nerve pathways. In A.J. Turner and H.S. Bachelard (Eds.) Neurochemistry: A Practical Approach (pp 65-108). Oxford, England: Information Printing Ltd.

Quirion, R., Richard, J. (1987). Differential effects of selective lesions of cholinergic and dopaminergic neurons on serotonin-type 1 receptors in rat brain. Synapse, 1, 124-130.

Quirion, R., Richard, J., Dam, T.V., (1985). Evidence for the existence of serotonin type-2 receptors on cholinergic terminals in rat cortex. Brain Research, 333, 345-349.

Richter-Levin, G. Segal, M. (1989). Spatial performance is severely impaired in rats with combined reduction of serotonergic and cholinergic transmission. Brain Research, 477, 404-407.

Ridley, R.M., Aitken, D.M., Baker, H.F. (1989). Learning about rules but not about reward is impaired following lesions of the cholinergic projections to the hippocampus. Brain Research, 502, 306-318.

Riekkinen, P., Jr., Jakala, P., Sirvio, J., Riekkinen, P.

(1991). The effects of increased serotonergic and decreased cholinergic activities on spatial navigation performance in rats. Pharmacology Biochemistry and Behavior, 39, 25-29.

Riekkinen, P., Jr., Sirvio, J., Riekkinen, P. (1990a). Similar memory impairments found in medial septal-vertical diagonal band of Broca and nucleus basalis lesioned rats: are memory defects induced by nucleus basalis lesions related to the degree of non-specific subcortical cell loss? Behavioral Brain Research, 37, 81-88.

Riekkinen, P., Jr., Sirvio, J., Riekkinen, P. (1990b). Interaction between raphe dorsalis and nucleus basalis magnocellularis in spatial learning. Brain Research, 527, 342-345.

Robinson, S.E. (1983). Effect of specific serotonergic lesions on cholinergic neurons in the hippocampus, cortex, and striatum. Life Sciences, 32, 345-353.

Rogers, J.D., Brogan, D., Mirra, S.S. (1985). The nucleus basalis of Meynert in neurological disease: A quantitative morphological study. Annals of Neurology, 17, 163-170.

Rothblat, L.A., Kromer, L.F. (1991). Object recognition memory in the rat: the role of the hippocampus. Behavioral Brain Research, 42, 25-32.

Safer, D.J., Allen, R.P. (1971). The general effects of scopolamine in man. Biological Psychiatry, 3, 347-355.

Sakurai, Y., Wenk, G.L. (1990). The interaction of acetylcholinergic and serotonergic neural systems on performance in a continuous non-matching to sample task. Brain Research, 519, 118-121.

Salamone, J.D., Beart, P.M., Alpert, J.E., Iversen, S.D. (1984). Impairment in T-maze reinforced alternation performance following nucleus basalis magnocellularis lesions in rats. Behavioral Brain Research, 13, 63-70.

Samanin, R., Quattrone, A., Peri, G., Ladinsky, H., Consolo, S. (1978). Evidence of an interaction between serotonergic and cholinergic neurons in the corpus striatum and hippocampus of the rat brain. Brain Research, 151, 73-82.

Santucci, A.C., Kanof, P.D., Haroutunian, V. (1989). Infusion of NMDA into the nucleus basalis of Meynert, frontal cortex or lateral ventricle in rats: effect on memory and cholinergic brain neurochemistry. Pharmacology, Biochemistry, and Behavioral, 33, 485-488.

Senut, M.C., Menetrey, D., Lamour, Y. (1989). Cholinergic and peptidergic projections from the medial septum and the nucleus of the diagonal band of Broca to dorsal hippocampus, cingulate cortex and olfactory bulb: A combined wheatgerm agglutinin-apohorseradish peroxidase-gold immunohistochemical study. Neuroscience, 30(2), 385-403.

Sitaram, N., Weingartner, H. (1978). Human serial learning: Enhancement with arecholine and choline impairment with scopolamine. Science, 201, 274-276.

Squire, L.R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. Psychological Reviews, 99,(2), 195-231.

Squire, L.R. (1986) Mechanisms of memory. Science, 232, 1612-1619.

Squire, L.R., Zola-Morgan, S. (1988). Memory: brain systems and behavior. Trends In Neuroscience, 11,(4), 170-175.

Stevens, R. (1981). Scopolamine impairs spatial maze performance in rats. Physiology and Behavior, 27, 385-386.

Stewart, G.R., Price, M., Olney, J.W., Hartman, B.K., Cozzari, C. (1986). N-methylaspartate: an effective tool for lesioning basal forebrain cholinergic neurons of the rat. Brain Research, 369, 377-382.

Sutherland, R.J., Rodriguez, A.J. (1989). The role of the fornix/fimbria and some related subcortical structures in place learning and memory. Behavioral Brain Research, 32, 265-277.

van der Staay, F.J., Raaijmakers, W.G.M., Lammers, A.J.J.C., Tonnaer, J.A.D.M. (1989). Selective fimbria lesions impair acquisition of working and reference memory of rats in a complex spatial discrimination task. Behavioral Brain Research, 32, 151-161.

Vanderwolf, C.H. (1987). Near-total loss of 'learning' and 'memory' as a result of combined cholinergic and serotonergic blockade in the rat. Behavioral Brain Research, 23, 43-57.

Vanderwolf, C.H. (1989). A general role for serotonin in the control of behavior: studies with intracerebral 5,7-dihydroxytryptamine. Brain Research, 504, 192-198.

Vanderwolf, C.H., Baker, G.B., Dickson, C. (1990). Serotonergic control of cerebral activity and behavior: Models of dementia. In P.M. Whitaker-Azmitia and S.J. Peroutka (Eds.), The Neuropharmacology of Serotonin, (Vol 600, pp 367-

383). New York: The New York Academy of Sciences.

Vanderwolf, C.H., Dickson, C.T., Baker, G.B. (1990) Effect of p-chlorophenylalanine and scopolamine on retention of habits in rats. Pharmacology, Biochemistry, and Behavior, 35, 847-853.

Vanderwolf, C.H., Leung, L.W.S., Baker, G.B., Stewart, D.J. (1989). The role of serotonin in the control of cerebral activity: studies with intracerebral 5,7-dihydroxytryptamine. Brain Research, 504, 181-191.

Volpe, B.T., Hendrix, C.S., Park, D.H., Towle, A.C., Davis, H.P. (1992). Early post-natal administration of 5,7-dihydroxytryptamine destroys 5-HT neurons but does not affect spatial memory. Brain Research, 589, 262-267.

Watts, J., Stevens, R., Robinson, C. (1981). Effects of scopolamine on radial performance in rats. Physiology and Behavior, 26, 845-851.

Wenk, G.L., Engisch, K.L. (1986). [³H]Ketanserin (serotonin type 2) binding increases in rat cortex following basal forebrain lesions with ibotenic acid. Journal of Neurochemistry, 47(3), 845-850.

Wenk, G., Hughey, D., Boundy, V., Kim, A., Walker, L., Olton, D. (1987). Neurotransmitters and memory: role of cholinergic, serotonergic, and noradrenergic systems. Behavioral Neuroscience, 101(3), 325-332.

Whitehouse, J.M. (1964). Effects of atropine on discrimination learning in the rat. Journal of Comparative and Physiological Psychology, 57(1), 13-15.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., DeLong, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science, 215(5), 1237-1239.

Whitehouse, P.J., Kellar, K.J. (1987). Nicotinic and muscarinic cholinergic receptors in Alzheimer's disease and related disorders. Journal of Neural Transmission (Suppl.), 24, 175-182.

Whitehouse, P.J., Martino, A.M., Marcus, K.A., Zweig, R.M., Singer, H.S., Price, D.L., Kellar, K.J. (1988). Reductions in acetylcholine and nicotine binding in several degenerative disease. Archives of Neurology, 45, 722-724.

Wiklund, L., Bjorklund, A., Nobin, A. (1978). Regeneration of serotonin neurons in the rat brain after 5,6-

dihydroxytryptamine-induced axotomy. Annals of the New York Academy of Sciences, 305, 370-384.

Williams, J.H., and Azmitia, E.C. (1981). Hippocampal serotonergic re-uptake and nocturnal locomotor activity after microinjections of 5,7-DHT in the fornix-fimbria. Brain Research, 207, 95-107.

Winblad, B., Bucht, G., Fowler, C.J., Wallace, W. (1986) Beyond the transmitter based approach to Alzheimer's disease. In T. Crook, R. Bartus, S. Ferris, and S. Gershon (Eds.) Treatment Development strategies for Alzheimer's disease (pp. 67-97). Madison Connecticut: Mark Powley Associates.

Winter, J.C., Petti D.T. (1987). The effects of 8-Hydroxy-2-(di-n-propylamino)tetralin and other serotonergic agonists on performance in a radial maze: A possible role for 5-HT_{1A} receptors in memory. Pharmacology, Biochemistry, and Behavior, 27, 625-628.

Woolfe, N.J., Jacobs, R.W., Butcher, L.L. (1989). The pontomesencephalotegmental cholinergic system does not degenerate in Alzheimer's disease. Neuroscience Letters, 96(3), 277-282.

Worsham, E., Hamilton, L.W. (1973). Acquisition and retention of avoidance behaviors following septal lesions or scopolamine injections in rats. Physiological Psychology, 1(3), 219-226.

Wozniak, D.F., Stewart, G.R., Finger, S., Olney, J.W., Cozzari, C. (1989). Basal forebrain lesions impair tactile discrimination and working memory. Neurobiology of Aging, 10, 173-179.

Zola-Morgan, S., Squire, L.R., Amaral, D.G. (1986). Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. The Journal of Neuroscience, 6(10), 2950-2967.

APPENDIX A

Behavioral Testing: Elevated Plus Maze

The 37 rats who were tested in the MWM-WM were also tested on the elevated-plus maze an average of 15 days postsurgically. This maze has largely been used to assess the effects of anxiolytics (Pellow, Chopin, File, Briley, 1985), but it may also be used in order to see if the lesioned rats differ in general activity (Pappas et al., 1992). The elevated-plus maze was adapted from a design of Pellow et al., (1985). It consisted of two open arms (50 X 10 cm) opposite one another and two opposing, enclosed (50 X 10 X 40 cm) arms that were perpendicular to the open ones. The arms were elevated 50 cm above the floor. The maze was constructed of opaque white plexiglass. The maze was situated in the middle of an illuminated room. For testing, each rat was placed in the centre "neutral zone" of the maze and allowed to freely move for five minutes. The number of open and closed arm entries were recorded. The total number of arm entries were considered a measure of activity. The ratio of open to total arm entries may be considered a measure of anxiety in so far as rats treated with anxiolytic drugs tend to show a higher ratio of open arm entries (Pellow et al., 1985).

Results: Total arm entries, ratio of open/total arm entries, and ratio of closed/total arm entries were submitted to one-way ANOVA's. There were no significant differences between any

of the groups for these factors. The NMDA treated rats had, however, 50% fewer total arm entries than the VEH or 5,7-DHT treated rats (see Figure 19). In addition, both the NMDA and the DUAL lesioned groups had twice as many Open/total arm entries than the VEH treated rats (see Figure 19).

In summary, these results suggested that the NMDA treated rats did not increase their motoric activity or make them more anxious.

Figure 19, Upper: Total arm entries in the elevated-plus maze for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL (n=10) lesioned rats obtained approximately 11 days postsurgically.

Middle: Open/Total arm entries in the elevated-plus maze for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL(n=10) lesioned rats.

Lower: Closed/Total arm entries in the elevated-plus maze for the VEH (n=9), 5,7-DHT (n=9), NMDA (n=9), and DUAL(n=10) lesioned rats.

APPENDIX B

Correlations between ChAT activity and working memory errors in the radial arm maze for the five blocks of testing.

VEH	<u>WME B11</u>	<u>WME B12</u>	<u>WME B13</u>	<u>WME B14</u>	<u>WME B15</u>
HIPPO	0.03	0.20	-0.59	0.26	0.03
CAUD	-0.21	0.46	0.32	0.31	0.33
ANTCTX	-0.19	0.28	-0.03	0.10	0.42
POSTCTX	-0.03	0.02	-0.54	-0.12	-0.10
5,7-DHT	<u>WME B11</u>	<u>WME B12</u>	<u>WME B13</u>	<u>WME B14</u>	<u>WME B15</u>
HIPPO	0.06	0.05	0.37	0.30	-0.37
CAUD	0.16	0.20	0.13	0.32	-0.39
ANTCTX	0.54	0.18	-0.05	0.06	0.03
POSTCTX	0.56	0.07	-0.18	-0.19	-0.09
NMDA	<u>WME B11</u>	<u>WME B12</u>	<u>WME B13</u>	<u>WME B14</u>	<u>WME B15</u>
HIPPO	-0.05	-0.26	0.34	0.37	0.34
CAUD	0.23	-0.41	0.01	-0.14	0.03
ANTCTX	-0.21	0.04	-0.22	-0.14	-0.25
POSTCTX	-0.39	0.53	-0.09	0.13	-0.18
DUAL	<u>WME B11</u>	<u>WME B12</u>	<u>WME B13</u>	<u>WME B14</u>	<u>WME B15</u>
HIPPO	-0.29	0.10	0.20	-0.17	0.15
CAUD	0.48	0.04	0.18	0.16	0.62
ANTCTX	0.17	0.25	0.18	0.32	0.24
POSTCTX	0.38	-0.13	-0.18	0.21	0.45

APPENDIX C

Explanation of patterns in the RAM

In this study, the pattern of arm entry observed in the 12 arm radial maze may be explained as a result of the baited arm pattern. Arms 2, 4, 5, 7, 9, 11 were baited. By entering every second arm and then at some point shifting an arm over, the rats could get all the food rewards. An arm entry pattern of entering every second arm would have increased choice accuracy as well as uniformly decreasing all errors, but only if the rats were lucky enough to begin in an odd numbered arm. That is, if the rat began with an odd numbered arm, choice accuracy would be on average 4 and reference memory errors would not have dropped below 2. Interestingly, on the last block of testing, choice accuracy was on average 4 and reference memory errors were on average not less than 2. Although the average performance of the rats seems to support the notion that these animals may have been using an algorithm to solve the maze, at least one-third of the rats were capable of making fewer than 2 reference memory errors by the end of testing. This suggests that not all the rats were only using the algorithm to solve the maze.

In addition, if an algorithm was used, working memory errors would then become representative of how many times the same pattern of arm entries was repeated until the rat shifted to another arm to start the pattern over again. Perhaps what was tested in the radial arm maze was the rats ability to

prevent or inhibit themselves from repeatedly entering the same arms due to a particular response pattern, regardless of whether or not they remember being there. If the lesioned rats were not capable of using spatial cues to solve the maze then they may have developed a rule (go into every second arm). Flexibility in their response may be hindered by a strong win-stay tendency. This has been observed on tasks with acquired reward associations (Ridley, Aitken, Baker, 1989). Note however, that the successful use of an algorithm depends on the rats choosing an odd arm entry first. Looking at just the last day of block 5 (25th day of testing) all the rats who received NMDA randomly chose an odd or even numbered arm as their first arm entry. The rats that did not receive NMDA (control rats) chose an odd numbered arm as their first arm entry 65% of the time. This would suggest that more of the non NMDA treated rats are orienting themselves to spatial cues in the room; whereas, the NMDA treated rats are not and randomly choosing an odd or even arm. The inability to orient themselves properly in the maze and inflexibility in responding may combine to produce a poor performance in the RAM for the NMDA and DUAL lesioned rats. For example, on the last test day 86% of the VEH and 5,7-DHT treated rats who chose the odd arm entry first tended to make a total number of working memory errors less than or equal to 2. On the other hand, only 33% of the rats administered NMDA who chose an odd arm entry first made working memory errors less than or equal

to 2. Therefore, although both the DUAL and NMDA-only treated rats do not appear to use different algorithms for solving the maze as compared to the VEH and the 5,7-DHT only treated rats, they do not adjust their behavior so that they only enter the arms with food once.

APPENDIX D
Summary of ANOVA's

Table 1
Summary of ANOVA's for ChAT activity

MWM

Brain part	Source	d.f.	F-value	Prob
Hippocampus	Group Error	3 44	31.77	.000*
Caudate	Group Error	3 44	2.72	.055
Anterior Cortex	Group Error	3 44	9.15	.0001*
Posterior Cortex	Group Error	3 44	11.16	.000*

* $p < 0.05$

RAM

Brain part	Source	d.f.	F-value	Prob
Hippocampus	Group Error	3 42	7.09	.0006*
Caudate	Group Error	3 42	1.05	.376
Anterior Cortex	Group Error	3 42	0.96	.419
Posterior Cortex	Group Error	3 42	0.34	.797

* $p < 0.05$

Table 2
Summary of ANOVA's for Serotonin

MWM

Brain part	Source	d.f.	F-value	Prob
Dorsal Hippocampus	Group	3	32.87	.0000*
	Error	39		
Ventral Hippocampus	Group	3	67.91	.0000*
	Error	39		
Anterior Cortex	Group	3	3.18	.0348*
	Error	38		
Posterior Cortex	Group	3	2.03	.1255
	Error	38		

* $p < 0.05$ RAM

Brain part	Source	d.f.	F-value	Prob
Dorsal Hippocampus	Group	3	4.32	.0096*
	Error	42		
Ventral Hippocampus	Group	3	17.45	.0000*
	Error	42		
Anterior Cortex	Group	3	0.99	.4048
	Error	43		
Posterior Cortex	Group	not available		
	Error			

* $p < 0.05$

Table 3
Summary of ANOVA's for Norepinephrine

MWM

Brain part	Source	d.f.	F-value	Prob
Dorsal Hippocampus	Group	3	0.64	.5965
	Error	31		
Ventral Hippocampus	Group	3	5.26	.0044*
	Error	33		
Anterior Cortex	Group	3	1.81	.1646
	Error	32		
Posterior Cortex	Group	3	2.59	.0694
	Error	32		

* $p < 0.05$ RAM

Brain part	Source	d.f.	F-value	Prob
Dorsal Hippocampus	Group	3	3.19	.0331*
	Error	42		
Ventral Hippocampus	Group	3	1.67	.1883
	Error	40		
Anterior Cortex	Group	3	0.86	.1503
	Error	43		
Posterior Cortex	Group		not available	
	Error			

* $p < 0.05$

Table 4
Summary of ANOVA's for Dopamine

MWM

Brain part	Source	d.f.	F-value	Prob
Caudate	Group Error	3 33	0.40	.7551
Dorsal Hippocampus	Group Error		not available	
Ventral Hippocampus	Group Error		not available	
Anterior Cortex	Group Error	3 32	1.31	.2882
Posterior Cortex	Group Error	3 32	1.73	.1816

RAM

Brain part	Source	d.f.	F-value	Prob
Caudate	Group Error		not available	
Dorsal Hippocampus	Group Error	3 42	0.03	.9917
Ventral Hippocampus	Group Error	3 40	0.34	.7982
Anterior Cortex	Group Error	3 43	2.10	.1145
Posterior Cortex	Group Error		not available	

Table 5
Summary of ANOVA's for Area measurements

Brain part	Source	d.f.	F-value	Prob
Lateral Ventricles	Group	3	4.15	.016*
	Area	6	1714.77	.000*
	GxA	18	2.11	.008*
	Error	156		
Caudate (A/P +7.0mm)	Group	3	0.37	.7751
	Error	32		
Caudate (A/P +2.0mm)	Group	3	0.51	.6773
	Error	32		

* $p < 0.05$

Table 6
Summary of ANOVA's for density scores

Brain part	Source	d.f.	F-value	Prob
Medial Septum	Group	1	11.51	.0048
	Error	13		
Vertical Diagonal Band	Group	1	1.12	.3083
	Error	13		
Horizontal Diagonal Band	Group	1	0.07	.7878
	Error	13		
Dorsal Hippocampus				
CA1	Group	1	0.61	.448
	Site	3	87.87	.000*
	GxS	3	0.90	.451
	Error	39		
CA3	Group	1	0.94	.349
	Site	3	84.80	.000*
	GxS	3	0.53	.667
	Error	39		
Dentate Gyrus	Group	1	1.38	.262
	Site	2	35.88	.002*
	GxS	2	0.50	.613
	Error	26		
Ventral Hippocampus				
CA1	Group	1	5.96	.030*
	Site	3	41.25	.000*
	GxS	3	2.63	.063
	Error	39		
CA3	Group	1	3.23	.096
	Site	3	149.75	.000*
	Side	1	11.28	.005*
	GxSite	3	2.51	.073
	GxSide	1	.0	.957
	GxSxS	3	.12	.948
	Error	39		

* $p < 0.05$

Table 7
Summary of ANOVA's for MWM

Measure	Source	d.f.	F-value	Prob
Trial 1 Latencies	Group	3	2.61	.061
	Days	4	134.21	.000*
	GxD	12	0.41	.960
	Error	220		
Average Latencies	Group	3	2.70	.055
	Days	4	230.31	.000*
	GxD	12	.51	.906
	Error	220		
Path length in quadrant	Group	3	.84	.479
	Path	3	43.52	.000*
	GxP	9	2.12	.030*
	Error	165		
Time in quadrant	Group	3	.73	.613
	Time	3	68.71	.000*
	GxT	9	1.01	.436
	Error	162		
Cued Platform	Group	3	0.85	.4725
	Error	55		
Annulus Crossing	Group	3	0.90	.4471
	Error	56		
Swim speed	Group	3	0.50	.6821
	Error	54		

* $p < 0.05$

Table 8
Summary of ANOVA's for RAM

Measure	Source	d.f.	F-value	Prob
Working memory errors	Group	3	23.10	.000*
	Blocks	4	14.37	.000*
	GxB	12	1.54	.115
	Error	172		
Block 6	Group	3	8.00	.0003*
	Error	41		
Reference memory errors	Group	3	0.88	.461
	Blocks	4	33.80	.000*
	GxB	12	2.00	.027*
	Error	172		
Choice accuracy	Group	3	1.94	.137
	Blocks	4	49.00	.000*
	GxB	12	2.42	.006*
	Error	172		
Direction of arm entry Day 1	Group	3	2.54	.069
	Direction	6	29.87	.000*
	GxD	18	2.11	.006*
	Error	258 ^t		
Day 2	Group	3	4.47	.008*
	Direction	6	30.01	.000*
	GxD	18	1.82	.023*
Day 10	Group	3	0.35	.790
	Direction	6	27.21	.000*
	GxD	18	1.95	.013*
Day 11	Group	3	0.65	.589
	Direction	6	23.06	.000*
	GxD	18	0.78	.728
Day 19	Group	3	0.21	.889
	Direction	6	23.51	.000*
	GxD	18	0.52	.949
Day 20	Group	3	0.77	.517
	Direction	6	23.23	.000*
	GxD	18	1.50	.089

* $p < 0.05$,

^t df for error same for all days assessed.

Table 9
Summary of ANOVA's for MWM-WM

Measure	Source	d.f.	F-value	Prob
% Savings no delay	Group Error	3 33	0.63	.5996
1 min. delay	Group Error	3 33	2.39	.0858
10 min. delay block 1	Group Error	3 33	3.44	.0279*
10 min. delay block 2	Group Error	3 33	1.35	.2730
90 min. delay	Group Error	3 33	1.89	.1509

* $p < 0.05$

END

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FIN