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Effects of Guanfacine on Mnemonic Processing Following Lesion to Rat Medial Septum: A Novel Treatment Approach to Alzheimer's Memory Type Deficits

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Author Note

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Abstract

Cognitive deficits associated with Alzheimer's disease are known to result from decreases in acetylcholine within the cholinergic system of the medial septal area, which projects to the hippocampus. The significance of the neurotransmitter norepinephrine within this context has not been extensively reviewed. The present study measured the effects that Guanfacine, an alpha-2 noradrenergic agonist, has on memory deficits produced by cholinergic cell lesion in the rat medial septum. Memory processing during pre-lesion, post-lesion, and post-drug administration groups was quantified using a socially transmitted food preference task. Following administration of the cholinergic neurotoxin 192 IgG-saporin, subjects exhibited a significant decrease in memory formation when compared to baseline. Furthermore, following injection of guanfacine into post-lesioned rats, subjects displayed a significant value of memory formation above chance. Memory-related effectiveness of guanfacine seems to be dependent on a baseline number of acetylcholine cells within the medial septum. Consequently, data suggests that guanfacine may carry no more therapeutic worth for patients afflicted by Alzheimer's disease than routinely prescribed acetylcholinesterase inhibitors. However, such conclusions must not be considered definitive by any means, as it is possible that future research might elaborate on possible cellular explanations for the increase in memory formation observed following guanfacine administration in the present examination.

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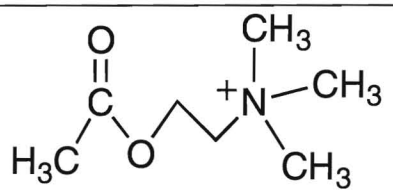
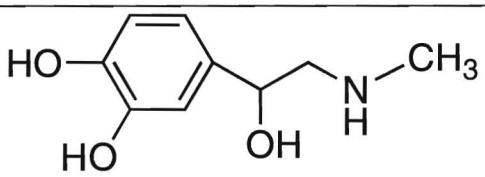
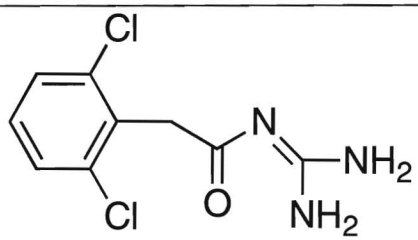
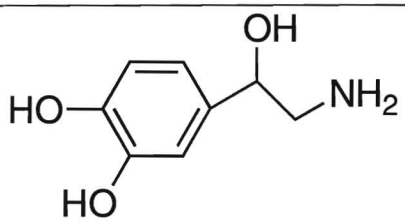
Table of Abbreviations

Name	Abbreviation
Acetylcholine	Ach
Acetylcholine esterase	AChE
Acetylcholine esterase inhibitor	AChE-I
Alzheimer's disease	AD
Choline acetyltransferase	ChAT
Hippocampus	HPC
Immunocytochemistry	ICC
Locus coeruleus	LC
Medial septum	MS
Norepinephrine	NE
Normal goat serum	NGS
Phosphate buffer solution	PBS
Socially transmitted food preference	STFP
Triton-X	TX
Vertical diagonal band	VDB

Table of Molecular Structures

Name	Molecular Structure
Acetylcholine	
Epinephrine	
Guanfacine	
Norepinephrine	

Effects of Guanfacine on Mnemonic Processing Following Lesion to Rat Medial Septum: A Novel Treatment Approach to Alzheimer's Memory Type Deficits

Mnemonic processing encompasses tasks related to the acquisition, integration, and recollection of information from cognition. The hippocampus (HPC) is the most widely accepted brain region associated with such memory (Aggleton, Hunt, & Rawlins, 1986; Astur, Taylor, Mamelak, Philpott, & Sutherland, 2002; O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). Within the HPC, acetylcholine (ACh) neurotransmission is coupled with forming novel memories (Whishaw, 1985; Kesner, 1998; White & MacDonald, 2002; Gold, 2004). The medial septum (MS), anatomically connected to the HPC, is the primary source of ACh to the HPC (Baxter, Vale-Martinez, & Eichenbaum, 2002; Decker & McGaugh, 1991; Izquierdo, Dacunha, Rosat, Jerusalinsky, Ferreira, & Medina, 1992; Myhrer, 2003). Patients diagnosed with Alzheimer's disease (AD) display marked decreases in ACh functioning not seen in the normal aging populace (Bartus, Dean, Beer, & Lippa, 1982). Consequently, impairment of the ACh system and thus the MS are associated with memory deficits within the AD population (Rossor, Iversen, Johnson, Mountjoy, & Roth, 1981).

The interaction of the norepinephrine (NE) neurotransmitter system with the ACh system in memory-related tasks has received much attention recently (Crutcher & Davis, 1981; Decker & McGaugh, 1989; Sirvio, Riekkinen Jr., Valjakka, Pitkanen, Partanen, & Riekkinen, 1990). Decker and colleagues (1989) suggest that a reduction in NE might

further increase memory deficits when coupled with an already degraded cholinergic innervation. The locus coeruleus (LC) is the primary brain area that supplies NE to the HPC (Loughlin, Foote, & Fallon, 1982; MacDonald & Scheinin, 1995; Nakamura & Sakaguchi; 1990). Therefore, a shortage of NE from the LC might substantially contribute to the lack of memory function associated with AD (Bondareff, Mountjoy, & Roth, 1981, 1982; Chanpalay, 1991).

One potential approach to treatment of Alzheimer's might involve the pharmacological NE agonist Guanfacine, which has been shown to increase learning and memory in both rodent models and human studies (Arnsten, Cai, & Goldmanrakic, 1988; Arnsten & Contant, 1992; Jakala, Riekkinen, Sirvio, Koivisto, Kejonen, & Vanhanen, 1999). Accordingly, Guanfacine administration might aid in delaying the onset of hippocampal-based memory AD-like brain impairments. In order to measure the effects that Guanfacine has on mnemonic processing, the present study involves lesioning the medial septum followed by administration of guanfacine within a rat population. Guanfacince is hypothesized to increase memory performance on a socially transmitted food preference task.

Memory Systems

Thirty years ago, a majority of memory systems were conceptualized as specific dichotomies. As a result, much disparity existed within the field of neuroscience when defining such systems. For example, evidence was gathered exclusively for declarative and procedural memory systems (Cohen & Squire, 1980), explicit and implicit memory systems (Graf & Schacter, 1985), as well as memory and habit systems (Mishkin, Malamut, & Bachevalier, 1984). This means that individual memory dichotomies were

gaining support as separate entities, while the field of memory systems should have been integrating all dichotomies within a single more efficient taxonomy. It makes sense that subsequent studies have shown interactions existed between single components of several of the dichotomies, thus discrediting any single memory dichotomy as a reliable model. One such interaction involved a contrast between declarative memory and habit memory in amnesic patients (Knowlton, Mangels, & Squire, 1996). Consequently, there were many confounding interactions between differential brain areas believed to be involved in very specific memory systems.

Recently, Squire (2004) elaborated on a reliable taxonomy, which accounts for relevant brain structures associated with different memory systems (see Figure 1).

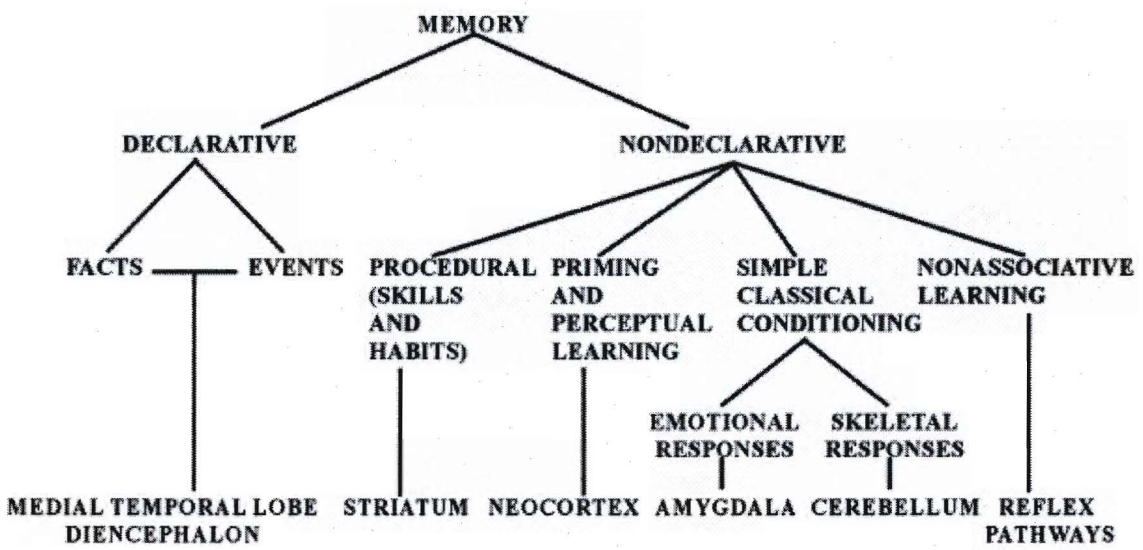


Figure. 1. A taxonomy of mammalian long-term memory systems. The taxonomy lists the brain structures thought to be especially important for each form of declarative and nondeclarative memory. In addition to its central role in emotional learning, the amygdala is able to modulate the strength of both declarative and nondeclarative memory (adapted from Squire, 2004).

This taxonomy seems to be quite reliable and well respected within the literature. The classification divides memory very broadly into "declarative" and "nondeclarative" systems. "Declarative" memory is recollection-based, pertaining to facts and perceptions in regards to single events. This system is capable of processing multiple pieces of information and is impaired in amnesic patients (Squire, 1992). Furthermore, the memory scheme is associated with operating in the medial temporal lobe and diencephalon. "Nondeclarative" memory is responsible for extracting common perceptions from several isolated and serial events, allowing one to integrate information from variable contexts. This scheme is linked with the following brain areas: striatium, neocortex, amygdala, cerebellum, and reflex pathways.

Many of the memory systems within the proposed anatomical taxonomy, which include specific brain regions, have been shown to function both independently and dependently (Packard & McGaugh, 1996; Tulving, Hayman, & MacDonald, 1991). The evolution from single memory dichotomies to a reliable taxonomy has led to the widely accepted discovery that the hippocampus is most definitely involved in declarative memory tasks.

Hippocampus and memory: evidence from human studies

Following establishment of a useful and reliable memory systems model, studies have shown that the hippocampus is involved in mnemonic processing, particularly in "declarative" tasks (Astur, Laughlin, Mamelak, Philpott, & Sutherland, 2002; Bohbot, Kalina, Stepankova, Spackova, Petrides, & Nadel, 1998; Smith & Milner, 1981; Squire, 1992).

Amnesic patients. Human studies investigating the role of the hippocampus in memory processing have revolved around deficits exhibited by amnesic patients. Milner (1962) introduced findings that memory is dependent on the medial temporal lobe in the patient H.M.. The medial temporal lobe anatomically includes the hippocampus. When the medial temporal lobe was removed bilaterally in order to ameliorate epileptic symptoms, the patient H.M. displayed severe amnesia. H.M. displayed severe amnesia in his inability to form any new memories involving factual information. A similar study involving amnesic patients has been implicated in an attempt to integrate the role of the hippocampus in memory processing (Zola-Morgan, Squire, & Amaral, 1986). For example, in the case of patient R.B., the presence of a post-mortem examined bilateral lesion in the CA1 region of the hippocampus is believed to be responsible for marked decreases in memory performance. Similarly, patients with bilateral lesions to the hippocampus have exhibited decreases in maze learning (Corkin, 1965).

Functional magnetic resonance imaging. Functional magnetic resonance imaging (fMRI) studies have provided insight into the function of the hippocampus on declarative memory tasks by increasing visual acuity (Press, Amaral, & Squire, 1989). Imaging has shown shrinkage of brain areas (fimbria, dentate gyrus, hippocampus proper, and subiculum) within the hippocampus of patients suffering from memory deficits (Squire, 1992). After examining symptoms associated with amnesic patients and fMRI studies, it is clear that the hippocampus is predominantly involved in declarative memory tasks.

Hippocampus and memory: evidence from rat studies

Declarative memory impairments seen in rats similarly emphasize the role of the hippocampus. Much of the research involving rats has utilized hippocampal lesioning

techniques in order to produce reliable processing deficits (Aggleton et al., 1986; Sloan, Dobrossy, & Dunnett, 2006; Squire, Clark, West, & Zola, 2001). As seen in human amnesic patients, bilateral lesions to the CA1 region in the rat hippocampus have likewise produced memory dysfunction (Barnes, 1988; Sutherland & Rudy, 1989).

Subsequent studies have shown the importance of the hippocampus in spatial memory tasks, thereby indicating the role of working memory (Bannerman, Yee, Good, Heupel, Iversen, & Rawlings, 1999; Cassel, Cassel, Galani, Kelche, Will, & Jarrad, 1998; Eichenbaum, 1997; Olton, Becker, & Handelmann, 1979). Lesions to the hippocampus have lead to severe deficits in processing spatial information on working memory tasks (Aggleton et al., 1986; Olton & Rawlings, 1982). Working memory in rats is often tested within the context of a delayed-dependency task, meaning that the longer the delay after presentation of items and before recall, the greater the impairment on memory (Dunnett, 1985).

A novel test, the conditional delayed matching and non-matching to position task, has integrated aspects of the delayed-dependency task in order to show that hippocampal lesions positively weaken memory processing (Sloan et al., 2006). Following a review of relevant rodent literature, it is apparent that the hippocampus is involved in memory processing on declarative tasks.

Acetylcholine neurotransmission and memory formation.

It is clear that an interaction exists between multiple memory systems and the neurotransmitter ACh (Gold, 2004; Kesner, 1998; Whishaw, 1985; White & MacDonald, 2002). Whether ACh modulates memory processing or processing modulates ACh release has been debated (Gold, 2003). However, as a means of identifying memory

activation within the hippocampus and other brain regions (e.g., amygdala, striatium, and hippocampus), ACh transmission has proven quite useful (Dutar et al., 1995; Everitt & Robbins, 1997; Sarter & Bruno, 2000). The present study will focus only in ACh transmission within the hippocampus because the medial septum, the main source of ACh to the hippocampus, is the only region that will be lesioned.

Acetylcholine production and transmission. ACh exists predominantly in the peripheral nervous system and brain tissue. It is synthesized within axon terminals from the precursors choline and acetyl coenzyme A (acetyl CoA) via a one-step reaction (see Figure 2).

Once synthesized, ACh is stored for subsequent utilization in synaptic vesicles and released after the induction of an action potential. When the action potential stimulates the axon, an influx of calcium (Ca^{2+}) ions into the terminal causes vesicles containing ACh to fuse with the outermost internal region of the terminal. After fusing, ACh neurotransmitters are released into the synaptic cleft where they bind reversibly with receptors on the post-synaptic dendritic membrane. Binding causes an influx of sodium (Na^{+}) ions into the post-synaptic cell, resulting in propagation of the action potential. Following Na^{+} entry, receptor-bound ACh is immediately broken down by the enzyme acetylcholine esterase (AChE).

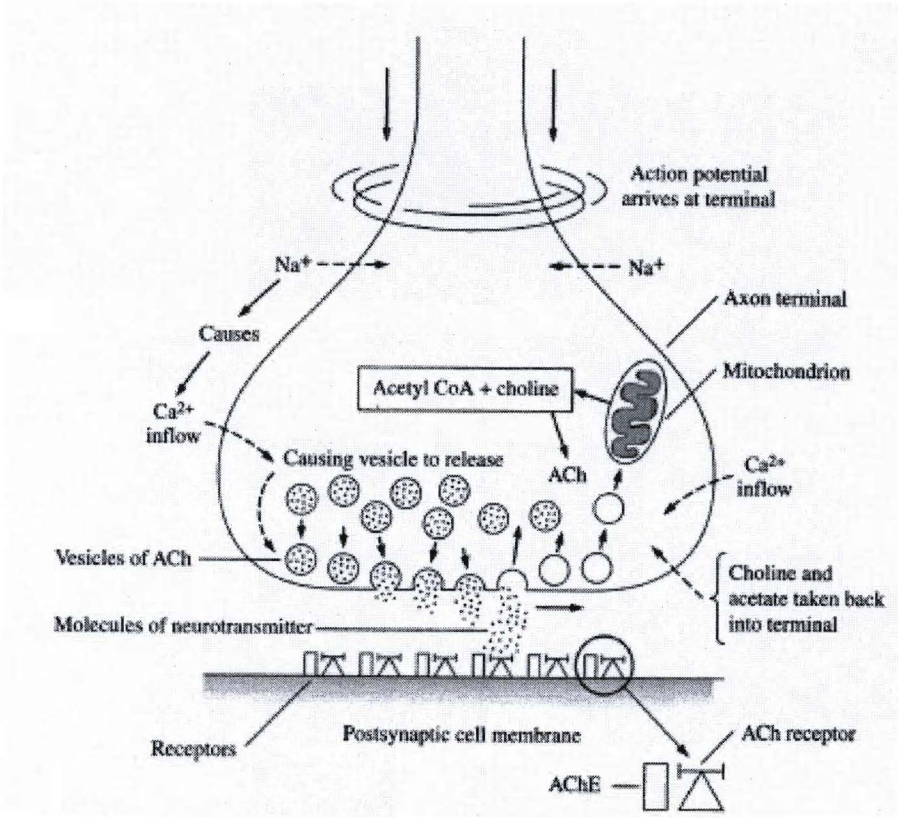


Figure 2. Cholinergic synapse. Acetylcholine is made in the axon terminal from acetyl coenzyme A (acetyl CoA) and choline, stored in vesicles, and released. When the action potential arrives at the terminal, closed calcium channels in the terminal are opened and Ca^{2+} rushes into the terminal, triggering vesicles to fuse with the membrane and release ACh molecules into the synaptic cleft. ACh molecules attach to ACh receptors on the postsynaptic membrane and trigger the opening of Na^{+} channels. ACh is immediately broken down at the receptors by acetylcholine esterase (AChE) into choline and acetate, which are taken back up by the terminal and reused (adapted from Julien, 2005).

Detecting acetylcholine activity. One method of investigating ACh modulation in the hippocampus is by the use of cholinergic agonists and antagonists (Bartus, Dean, Beer, & Lippa, 1982; Coyle, Price, & Delong, 1983). These techniques make it possible to observe the effects of decreasing or increasing ACh levels during memory tasks. For example, cholinergic antagonists, such as scopolamine, have been shown to decrease ACh release when injected into the hippocampus, thereby impairing memory (Carli,

Luschi, & Samanin, 1997; Farr, Flood, & Morley, 2000; Gold, 2003; Lilliquist, Burkhalter, Lobaugh, & Amsel, 1993; Ohno, Yamamoto, & Watanabe, 1994; Wallenstein & Vago, 2001). In contrast, agonists, such as physostigmine, have been shown to increase ACh release in the hippocampus, consequently enhancing memory (Maelicke, Schrattenholz, Samochocki, Radina, & Albuquerque, 2000; Nistri & Di Angelantonio, 2002). Collectively these findings indicate that obstructing ACh neurotransmission leads to impairment, while stimulation of the cholinergic pathway increases memory processing within the hippocampus (Chang & Gold, 2004).

Subsequent methods have been utilized to show that increasing ACh release in the hippocampus increases memory processing. Recently, assessing such increases in ACh in live and awake animals has been made possible due to novel advances in technology (e.g., fMRI, mircodialysis, HPLC). For example, a precursor to ACh synthesis, choline has been shown to increase in uptake within ACh neurons throughout memory tasks (Messier, Durkin, Mrabet, & Destrade, 1990). Additionally, glucose injection into the hippocampus increases ACh release during memory-dependent training tasks in the rat (Ragozzino, Wenk, & Gold, 1994; Ragozzino, Unick, & Gold, 1996; Ragozzino, Pal, Unick, Stefani, & Gold, 1998). Furthermore, pregnenotone, a neurosteriod, increases both ACh release in the hippocampus and performance on tasks of spatial memory (Darnaudery, Koehl, Piazza, Le Moal, & Mayo, 2000).

Acetylcholine modulation. Increased ACh release has been observed in a number of memory related hippocampal-dependent tasks. For instance, Fadda and colleagues (1996) have observed increased ACh release during working memory tasks. ACh release has been shown to increase on tasks of visual discrimination (Yamamuro, Hori, Tanaka,

Iwano, & Nomura, 1995). The presentation of novel stimuli within a novel environment has triggered an increase in ACh transmission in the rat (Aloisi, Casamenti, Scali, Pepeu, & Carli, 1997; Inglis & Fibiger, 1995). Brain-region specific ACh release in the rat has also been observed during performance on a T-Maze task (Chang & Gold, 2003; McIntyre, Pal, Marriot, & Gold, 2003). Although tasks discussed above are associated with memory processing, the aim of each study may not have been to exclusively investigate such outcomes on memory. Nonetheless, these studies were useful in substantiating evidence for the hypothesis that increased ACh levels are correlated to an increase in memory processing. Further evidence supports the link between decreased ACh transmission and decreased memory processing following lesions to the medial septum.

The medial septum: acetylcholine modulation and memory processing

The septal-hippocampal (SH) projection of cholinergic neurons in the medial septum and vertical limb of the diagonal band (MS/VDB) are believed to provide the main source of ACh to the hippocampus (Dutar et al., 1995; Segal & Auerbach, 1997) (see Figure 3). The SH pathway originates within the basal forebrain, particularly the medial septum. The medial septum is divided into the medial septal nucleus and nucleus of the vertical and horizontal limbs of the diagonal band of broca (McKinney, Coyle, & Hendreen, 1983; Dutar et al., 1995).

There is much support for the hypothesis that a decrease in ACh is associated with decreased memory performance. For example, morphine injection into the intraseptal pathway in the hippocampus decreases ACh release and memory processing (Ragozzino

& Gold, 1995). Galanin, a neuropeptide, has been shown to decrease both ACh release and learning on spatial tasks in the rat (Ogren, Kehr, & Schott, 1996).

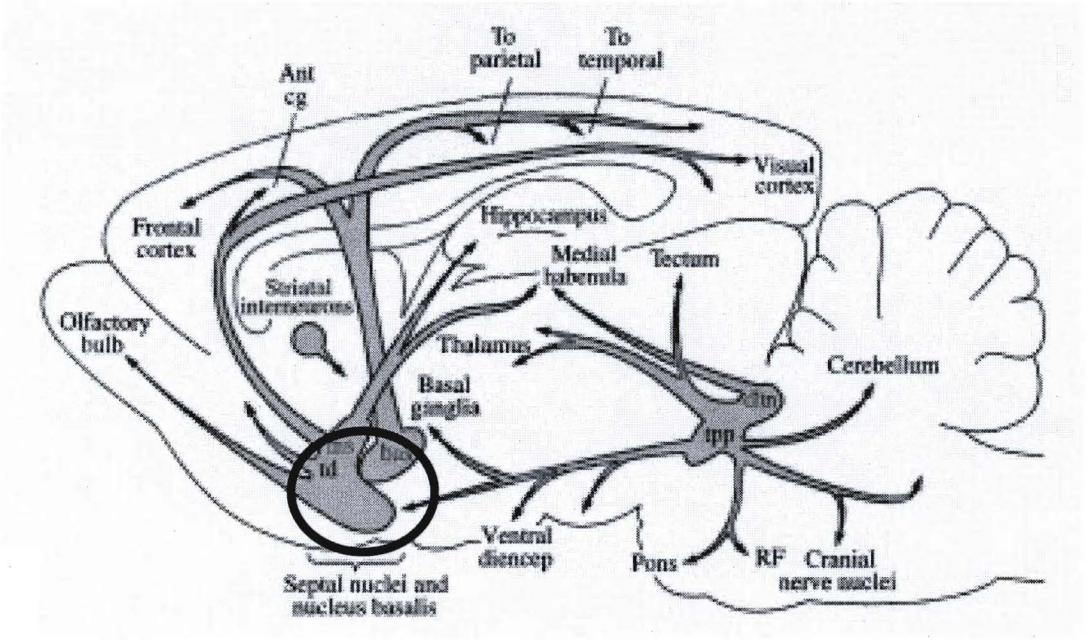


Figure 3. Representation of the cholinergic system in the rat brain. The central cholinergic neurons exhibit two basic organizational schemata: (1) local circuit cells (those that morphologically are arrayed wholly within the neural structure in which they are found) exemplified by the interneurons of the caudate-putamen nucleus; (2) projection neurons (those that connect two or more different regions). Of the cholinergic projection neurons that interconnect central structures, the major subconstellation implemented in this study deals with the forebrain cholinergic complex composed of neurons in the medial septal nucleus (ms) and the nucleus basalis (bas) [circled in figure] and projecting to the entire nonstriatal telencephalon (adapted from Woolf, 1991).

In the present experiment, the medial septal area has been chosen for lesion over other brain regions for several reasons. First, it has been shown through extensive histological analysis that the sole projection of acetylcholine to the hippocampus originates in the medial septum. Since a primary aim of the present study is to examine

the role of the cholinergic system within AD, it is only logical to lesion the medial septum. Second, lesioning the hippocampus directly reduces the ability of the present manipulation to generalize to human populations. This lack of generalization results from the observation that patients with AD possess an intact hippocampus. Lesioning the rat hippocampus directly would not maximally mimic AD within a human

Effects of medial septum/vertical diagonal band lesions. Decreases in ACh resulting from lesions to the MS/VDB have been associated with decreases in hippocampal-dependent memory impairment (Bartus et al., 1982). For example, decreases in working memory have been found following lesion to the MS/VDB (Decker, Radek, Majchrzak, & Anderson, 1992; Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996).

Furthermore, memory-based place navigation task has displayed marked impairment following lesion to the MS/VDB in the rat (Hagan, Salamone, Simpson, Iversen, & Morris, 1988). Similarly, spatial memory performance has decreased in rats following chemical lesion to the intraseptal pathway in the MS/VDB (Janis, Glasier, Fulop, & Stein, 1998; Nilsson, Leanza, Rosenblad, Lappi, Wiley, & Bjoklund, 1992). Memory deficits resulting from lesions to the MS/VDB produce strikingly similar learning and memory impairments as seen in damage to the hippocampus (Hagan et al., 1988; Decker et al., 1992; Janis et al., 1998). It is reasonable to conclude that learning and memory is most likely modulated by the interdependent functioning of ACh within both brain regions.

A selective toxin for ACh neurons, 192 IgG-saporin, has been used to chemically lesion the MS/VDB via direct injection. 192 IgG-saporin acts to eliminate ACh neurons

by joining saporin, the ribosome inactivating protein, with a monoclonal antibody to the p75 cholinergic nerve growth factor receptor (see figure 4). This activity decreases ACh

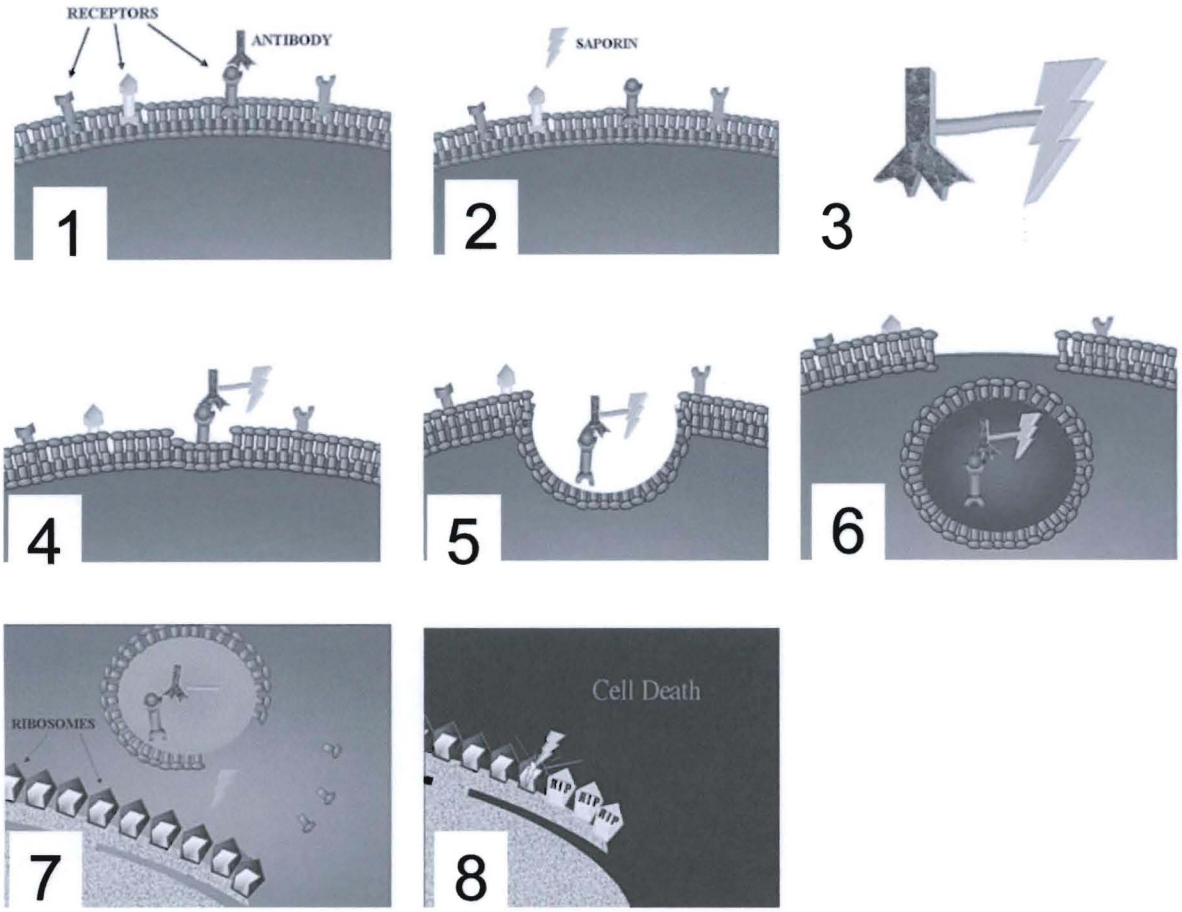


Figure 4. Action of the neurotoxin 192 IgG-saporin: (1) Monoclonal antibody obtained specific to the ACh cell type, (2) Inject saporin, which is lethal inside ACh cell, (3) Saporin binds to the monoclonal antibody, (4) Saporin-antiobody complex targets the p75 nerve growth factor recpetor, (5) The saporin-antibody-p75 receptor compex is internalized into the ACh call, (6) The saporin-antiobody-p75 receptor complex enters the ACh cell completely, (7) The bond between saporin and the antibody-receptor complex is broken, releasing saporin, & (8) Saporin binds, inactivating the ACh ribosome: cell death.

transmission in the hippocampus and provides a neurochemical marker for measuring levels of ACh in the hippocampus (details: see Chang & Gold, 2004; Pottter, Gaughan, & Assouline, 1999). Numerous studies have shown that lesions to the rat MS result in decreases of acetylcholine esterase, choline acetyltransferase (ChAT), and ACh levels in the hippocampus (Lewis, Shute, & Silver, 1967; Oderfeld-Nowak, Nariewicz, Bialowas, Dabrowska, Wieraszko, & Gradkowska, 1974; Potemska, Gradkowska, & Oderfeld-Nowak, 1975). This indicates that decreases in ACh levels lead to decreases in ChAT levels.

The saporin lesion agent is of particular interest because it will be used in this investigation to lesion the rat MS/VDB in an attempt to model the decreased levels of ACh as seen in Alzheimer's disease patients. In addition, 192 IgG-saporin is useful because it only targets ACh neurons, leaving all other neurons undamaged and functional. Since the MS/VDB projects to the hippocampus, lesioning the MS/VDB has been shown to produce substantial deficits in memory processing within the hippocampus (Berger-Sweeny, Stearns, Murg, Floerke-Nashner, Lappi, & Baxter, 2001; Chang & Gold, 2003; Janis et al., 1998; Nagle, Liberatore, Aombon, Pokala, Li, Pokala, & Johnson, 2002; Pokala, Libertore, Zambon, Nagle, Li, Witt-Enderby, & Johnson, 2002; Vale-Martinez, Baxter, & Eichenbaum, 2002).

While 192 IgG-saporin lesions in the MS/VDB have produced memory deficits, presumably due to a loss of ChAT-positive neurons, numerous studies have not replicated such decreases in processing following the same lesion technique (Baxter, Bucci, Wiley, Gorman, & Gallagher, 1995; Baxter, Bucci, Sobel, Williams, Gorman, & Gallagher, 1996; Baxter & Murg, 2002; Bizon, Han, Hudson, & Gallagher, 2003). Results from

these investigations have shown that 192 IgG-saporin does not decrease memory processing on certain memory tasks when injected in the MS/VDB. Importantly, such evidence exists within observations of the same experiments that nearly all of the ChAT-positive neurons in the MS/VDB were eliminated. This seems to suggest that 192 IgG-saporin lesions, while effective in decreasing ChAT-positive neurons, do not effectively decrease mnemonic processing.

Gold (2003) offers several explanations for the negative findings associated with 192 IgG-saporin as discussed above. Firstly, a threshold level of ACh neuronal loss might be necessary to produce marked memory impairments (Leanza, Nilsson, Wiley, & Bjorklund 1995 in Gold, 2003; Wrenn, Lappi, & Wiley, 1999). Secondly, 192 IgG-lesions might not be a definitive measure, which would allow one to make the conclusion that ACh projection from the MS/VDB to the hippocampus is a sufficient contributor to memory deficits as a single causal entity. Perhaps one domain of memory does not require stimulation of the intraseptal pathway. Investigating the effects of lesioning this projection would therefore yield no observed memory deficits. Thirdly, neuronal restructuring might compensate for the loss of ACh function (Crutcher & Davis, 1981; Decker & Mcgaugh, 1989; Olton, 1991). Perhaps sympathetic in-growth from alternative projections of undamaged brain regions might increase ACh production and availability to the hippocampus. Certainly, if ACh is intensively involved in memory formation, the origin of ACh projection is irrelevant.

Alzheimer's disease: decreased memory processing and acetylcholine levels

Alzheimer's disease (AD) is the most widespread neurodegenerative disease affecting nearly two-thirds of all patients diagnosed with dementia (Nussbaum & Ellis,

2003). The general progressive characteristic of AD accounts for irreversible losses of cholinergic neurons and synapses with the hippocampus (Coyle et al., 1983; Davies & Maloney, 1976). Recognizable detection of AD symptoms most often occurs after 60 years of age, although rare diagnosable forms have also occurred before this age. Prevalence rates increase rapidly within normal aging populations, ranging from 1% among 65-69 years of age to 40-50% among 95 years of age with men accounting for slightly less cases per age bracket (Nussbaum & Ellis, 2003). Death usually results within 8-10 years following symptomatic onset. Decreases in memory processing, capacity, and learning capabilities seems to be the most prominent debilitating and maladaptive symptoms. Currently, a definitive diagnosis can only be made after post-mortem brain autopsy. While the cause of AD continues to remain a mystery, senile neuritic plaques and neurofibrillary tangles mark the diagnosable brain tissue (Yamada & Nabeshima, 2000). Recently, similar impairments seen within the cholinergic neurotransmission system have been hypothesized to account for AD-type memory dysfunctions (Bartus et al., 1982; Coyle et al., 1983).

The "cholinergic hypothesis". The "cholinergic hypothesis" has sought to account for memory deficits associated with Alzheimer's disease patients by considering the cholinergic system (Bartus et al., 1982; Coyle et al., 1983). Describing a correlation of this magnitude must consider three criteria elaborated by Bartus et al. (1982): (i) very specific impairments must exist within the cholinergic system of AD patients, substantially more severe when compared to deficits seen in a normal aging populace (ii) deliberate impairment of the cholinergic system in young subjects should produce deficits

similar to those impairments seen in an AD populace (iii) enhancement of the cholinergic system should decrease memory deficits similar to those seen in an AD populace.

Many studies have shown reduced levels of ChAT and AChE in brains of AD patients as compared to a normal-aged sample (Bowen, Sims, Benton, Curzon, Davison, Neary, & Thomas, 1981; Giacobini, 2003; Kuhar, 1976). Giacobini (2003) found that AD patients exhibited decreased AChE levels by 60-80%. It is logical to hypothesize that decreases in AChE levels is a direct result of decreases in ACh neurons because AChE breaks down ACh within the synapse. These reductions indicate that decreased ACh levels might predominately contribute to those AD memory deficits observed (Bowen et al., 1981; Winkler, Thal, Gage, & Fisher, 1998). Particularly, decreased levels of ChAT have been observed in the basal forebrain, which contains the medial septum, in AD patients (Whitehouse, Price, Clark, Coyle, & DeLong, 1981; Whitehouse, Price, Struble, Clark, Coyle, & Delong, 1982). While normal aging is associated with decreases of cholinergic neurons in the basal forebrain these reductions are not nearly as great when compared to neuronal loss in AD patients (Bartus et al., 1981).

Deliberate impairments made to the cholinergic system in young human and rat subjects have produced memory deficits similar to those seen in AD subjects (Carli et al., 1997; Decker et al., 1992; Farr et al., 2000; Gold, 2003; Hagan et al., 1988; Lilliquist et al., 1993; Ohno et al., 1994; Thiel, 2003; Wallenstein & Vago, 2001; Walsh et al., 1996). Much of the work dealing with cholinergic impairment within the hippocampus has been limited to the cholinergic antagonist scopolamine and medial septum lesions using 192 IgG-saporin. Details regarding the magnitude of impairment necessary to produce AD-type deficits using scopolamine and 192 IgG-saporin can be found above in the sections

titled "ACh neurotransmission and memory formation" and "the medial septum: ACh modulation and memory processing" respectively.

Enhancement of the cholinergic system has conversely been shown to decrease memory deficits by decreasing AD-type impairments (Suzuki, Yamatoya, Sakai, Kataoka, Furushiro, & Kudo, 2001). One such method involves the use of ACh precursors. Upon injection into the brain, precursors become converted into choline, thus allowing for increased synthesis of ACh from choline and acetate. One such precursor, lecithin, is correlated to marked increases in performance on a Morris water maze task after administered orally to rats exhibiting AD-type deficits (Suzuki et al., 2001). In addition, rats displaying AD-type memory deficits following scopolamine administration exhibited decreases in impairment after the delivery of lecithin (Furnshiro, Suzuki, Shishido, Sakai, Yamatoya, & Kudo, 1997). Similarly, rats subcutaneously administered the precursor, choline chloride, displayed increases in performance on the same Morris water maze task (Tees & Mohammadi, 1999). While ACh precursors have produced evidence for decreasing memory deficits, beneficial effects are extremely limited and lack effectiveness (Kumar et al., 1998).

Acetylcholine esterase inhibitors. Acetylcholine esterase inhibitors (AChE-I) have shown the most promise in ameliorating memory impairments in AD patients. This class of pharmacological agents increases the availability of ACh, by preventing the hydrolysis of ACh by acetylcholinesterase, thereby increasing stimulation of ACh receptors, (Prickaert et al., 2005; Rogers, 1998). Since ACh receptor activation in the HPC and MS has been shown to increase memory performance, AChE-Is have been utilized in the AD populace (Delagarza, 2003; Kumar, Durai, & Jobe, 1998; Rogers,

1998). Presently, four AChE-Is have been approved by the Food and Drug Administration for use in AD: tacrine (Cognex), rivastigamine (Excelon), donepezil (Aricept), and galantamine (Reminyl).

Several alarming issues have arisen since the introduction of prescription AChE-Is for AD treatment. One problem that exists is that inhibition of ACh hydrolysis produces serious side effects in AD patients. Many common side effects include nausea, anorexia, aggression, liver toxicity, and cardiovascular irregularities (Lazartigues, Freslon, Tellioglu, Brefel-Courbon, Pelat, Tran, Montastruc, & Rascol, 1998). Secondly, AChE-Is have exhibited low levels of efficacy in AD patients because these agents only moderately delay memory impairment (Doody, 2003). One study indicates that only 20% of AD patients will experience a one-year delay in full cognitive impairments (Tariot, Solomon, Morris, Kershaw, Lilienfeld, & Ding, 2000). Rat studies with AChE-Is show only modest improvements in cognitive deficits (for review see: Ohara, Fukaya, Tanaka, & Seno, 1997). Furthermore, AChE-Is do not cure or avert the onset of AD. No single treatment for AD is capable of obtaining such results. As AD progresses and ACh neuronal levels continue to decrease, cholinergic AChE-I therapies decrease drastically in effectiveness (Lazartigues et al., 1998; Mann, Yates, & Hawkes 1982). One reason cholinergic treatments are not more efficient in ameliorating deficits is that AD is not solely a cholinergic insufficiency. Being a very complex illness, AD is likely to induce impairments in multiple neurotransmitter pathways (D'Amato, Zweig, Whitehouse, Wenk, Singer, & Mayeux, 1987; Herrmann, Lanctot, & Khan, 2004).

Norepinephrine and Alzheimer's disease

While degradation of the ACh pathway has been studied extensively, the NE transmitter pathway has been investigated less. Several studies indicate that decreases in NE neurotransmitter levels have been noted in AD patients (Bondareef et al., 1981; Mann, Lincoln, Yates, Stamp, & Toper, 1980; Mann et al., 1982). The NE system contains two projection orientations: (1) the ventral noradrenergic bundle, forming a main part of the lateral tegmental system and (2) the ascending dorsal noradrenergic bundle of the locus coeruleus system (see Figure 5). The dorsal bundle is of particular interest in the present manipulation because it projects directly into the hippocampus.

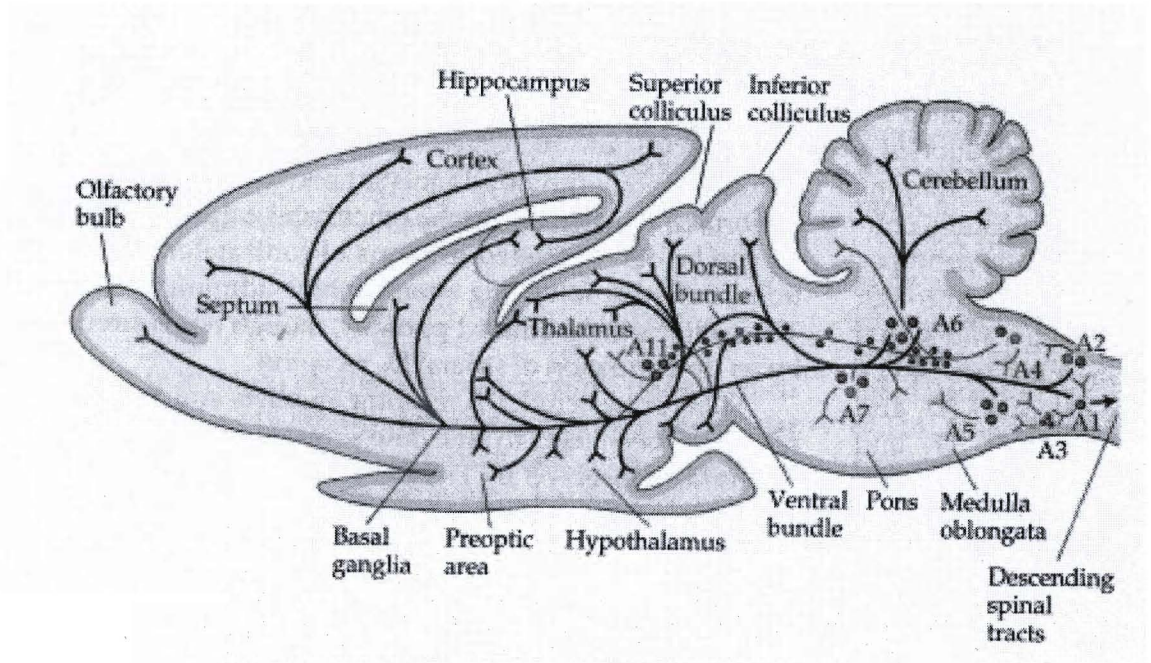


Figure 5. The noradrenergic system in the rat brain. Projections of the noradrenergic neurons are shown in a sagittal view. Clusters of cell bodies are shown as dots and are differentiated by the number prefixed by the letter A, according to the scheme of Dahlstron and Fuxe (adapted from Cotman & McGaugh, 1980).

Cell loss in the locus coeruleus (LC) has been noted in AD patients (Forno, 1978; Friedman, Adler, & Davis, 1999; Hoogendijk, Feenstra, Botterblom, Gilhuis, Sommer, & Kamphorst, et al., 1999; Marcyniuk, Mann, & Yates, 1986). The LC is the predominant brain region that provides the main source of NE to the HPC. While LC anatomy is beyond the scope of this paper it is useful to understand the projections of the LC.

Locus coeruleus anatomy. The LC is the largest noradrenergic nucleus in the human and rat brain, containing approximately 15,000 NE neurons per hemisphere in humans and 1,600 neurons per hemisphere in rats (Coull, 1994; Foote, Bloom, & Aston-Jones, 1983; Rogawski, 1985). It is located next to the fourth ventricle in the pontine region of the brainstem (see Figure 5). The LC innervates roughly every component of the telencephalon and diencephalon; most notably the hippocampus, neocortex, amygdala, septum, thalamus, and hypothalamus. The HPC obtains NE innervation entirely from the LC. The LC contains alpha (α) and beta (β) receptor types. The β-receptor type is further divided into β_1, β_2, and β_3-receptor subtypes. β-receptors have been isolated in the heart, spleen, bladder, and other major organs. The α-receptor type is divided into α_{1a}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, and α_{2c}-receptor subtypes. Areas of high-affinity α_2-receptor binding includes the dentate gyrus of the hippocampus and the substantia nigra pars reticulata.

Norepinephrine neuronal sprouting. Many studies have indicated that NE neuronal sprouting within the HPC occurs after lesion to the medial septum area (Crutcher & Davis, 1981; Crutcher & davis,1981; Madison & Davis, 1983). In fact, fibers projecting to the HPC from the LC pass through the MS (Loy, Milner, & Moore, 1980). Considering the decrease in ACh neurons after MS lesion, NE axons within the

MS might trigger NE in-growth in compensation for decreased ACh MS projections. This hypothesis is supported by the observation that after a MS lesion, NE fibers within the HPC increase substantially in number (Crutcher & Davis, 1981; Madison & Davis, 1983; Stenevi & Bjorklund, 1978). It might be the case that NE sympathetic fibers replace damaged ACh neurons, thereby compensating for ACh by increasing NE projection to the HPC. The compensation for ACh neurons could account for cholinergic denervarion of the HPC. For this to be a logical conclusion, post-synaptic NE stimulation must produce similar effects to those seen in post-synaptic cholinergic receptor stimulation. Results in support of this concept are few and must be studied in further detail. Based on extensive histological examinations, possible sites of interaction between the noadrenergic and cholinergic transmitter systems have been proposed (see Figure 6).

The compilation of evidence presented above shows that NE and ACh neurotransmitter systems interact in the modulation of memory formation within the HPC. Decker and colleagues (1991) have presented anatomical details of such an interaction (see Figure 6). Results have been shown that blockade of either system produces memory impairments (Sirvio & MacDonald, 1999). Additionally, ACh and NE have been shown to modulate each other's release within the HPC (Azam & McIntosh, 2006; Moroni, Tanganelli, Antonelli, Carla, Bianchi, & Beani, 1983). Furthermore, stimulation of NE receptors in the MS produce increases of ACh in the HPC (Robinson, Cheney, & Costa, 1978).

One study by Kruglikov (1982) provided further evidence that memory is modulated by NE and ACh collectively. Normal animals were given scopolamine and

exhibited decreases in memory on an avoidance task. Next, rats that had been administered the scopolamine were given a LC lesion. The combination of the LC lesion and scopolamine produced much larger impairments. Conclusively, pharmacological agents merely targeting the ACh system may be one reason cholinergic therapies within the AD populace are ineffective.

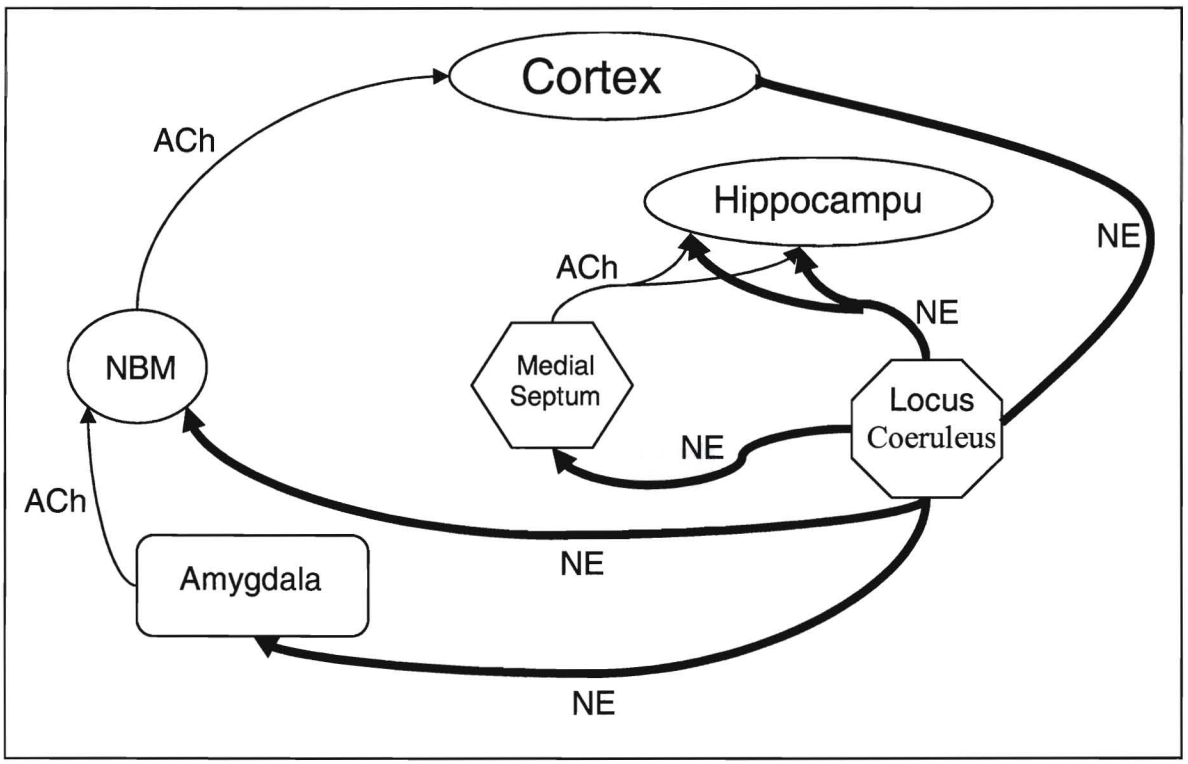


Figure 6. Hypothetical model of sites at which cholinergic and noradrenergic interactions might occur. Darker lines indicate norepinephrine projections while thin lines indicate acetylcholine projections.

Norepinephrine agonists and Alzheimer's disease treatment

Considering the nature of learning and memory tasks, in which NE potentially interacts with the cholinergic system, NE agonists might be useful in reducing memory deficits observed within Alzheimer's patients. Several studies have indicated that alpha-2

receptor subtype adrenergic agonists improve memory on a variety of tasks (Arnsten et al., 1988; Arnsten & Goldman-Rakic, 1990; Arnsten & Leslie, 1991; Arnsten & Contant, 1992; Arnsten & Cai, 1993; Jakala, Riekkinen, Sirvio, Koivisto, Kejonen, & Vanhanen, 1999; Rama, Linnankoski, Tanila, Pertovaara, & Carlson, 1996; Sirvio et al., 1991). One such agonist, Guanfacine, is particularly relevant because it will be administered in the present experiment to post-lesioned rats.

Guanfacine has been shown to increase memory performance in numerous animal and human studies. For example, Guanfacine has been shown to improve delayed response performance in aged monkeys (Rama et al., 1996). In addition, low doses of Guanfacine have improved performance in aged rats on a spatial navigation task (Sirvio et al., 1991). Furthermore, Guanfacine has improved memory in aged monkeys on tasks involving the utilization of working memory (Arnsten et al., 1988; Arsten & Cai, 1993). Guanfacine has shown similar effective results in humans, improving performance on working memory tasks (Jakala et al., 1999).

Applied together, Guanfacine is an exciting novel pharmacological agent that be useful in the treatment of AD-type memory deficits. Studies involving the administration of Guanfacine after MS lesions on a socially transmitted food preference task are nonexistent. The present study will therefore allow for new conclusions and hypotheses to be made on the interaction between the NE and ACh transmitter pathways in memory.

Quantification of memory: socially transmitted food preference task

In order to quantify memory performance a socially transmitted food preference task will be utilized. The STFP task is a nonspatial and spontaneous learning task. There are several benefits to using the STFP task in order to measure memory formation within

the current study. First, all food preferences are acquired in one training period with the demonstrated food, allowing for time points to be defined for acquisition and recall of the food preference. Second, food preferences, when transmitted socially, persist for several weeks, which will allow for sufficient testing time periods. Third, rats acquire the food preference through undemanding circumstances, which do not encompass spatial abilities, visual acuity, or exhausting locomotor activity; presence of which confounds interpretations of surgery-related variation in learning. Lastly, quantification of food preference memory is calculated as a preference for one of two foods, not total volume consumed during the task. This eliminates the possibility of surgery-related variations in motivation of the rats to eat as a universal characteristic of appetite.

Existing literature indicates that observer rats exhibit a food preference after being exposed to a demonstrator rat that has recently consumed a flavored rat chow (Galef & Whiskin, 2003; Vale-Martinez, Baxer, & Eichenbaum, 2002). For example, an observer rat having been presented cocoa flavored chow via a demonstrator rate will exhibit preference to this flavored food for periods extending over one month (Clark, Broadbent, Zola, & Squire, 2002). This indicates that the rat has formed a declarative memory for coco-flavored chow. In addition, lesions administered prior to training have been shown to impair long-term memory formation and lesions administered after training have produced temporally graded retrograde amnesia for the STFP task. (Wincour, 1990; Wincour, McDonald, & Moscovitch, 2001; Clark et al., 2002).

Studies have shown increases in CREB phosphorylation and c-Fos expression in the hippocampus during a STFP task (Countryman & Gold, *in press*; Countryman, Orlowski, Brightwell, Oskowitz, & Colombo, 2005). It is also known that CREB

phosphorylation and c-Fos expression are both paramount in long-term memory formation (Brightwell, Smith, Countryman, Neve, & Colombo, 2005; Countryman, Kaban, & Colombo, 2005). Consequently, increases in either CREB or c-Fos can be used as a marker for long-term memory processing. This is very significant evidence indicating that the STFP task can be used to quantify declarative memory formation. Explanation of the STFP task used in this experiment can be found under the methods section.

Summary and implications for the present study

After a review of the literature it is clear that mnemonic processing requires the integration of multiple memory systems. The hippocampus is extensively involved in declarative memory tasks. The neurotransmitter acetylcholine has been shown to facilitate memory formation within the hippocampus. The medial septum is anatomically connected to the hippocampus and is the main source of acetylcholine to the hippocampus. Reductions in acetylcholine levels within the hippocampus and medial septum have been observed in Alzheimer's disease patients. Reductions in levels of the norepinephrine neurotransmitter have been associated with Alzheimer's disease. Norepinephrine sympathetic in-growth within the hippocampus has been observed following lesion to the medial septum, suggesting that norepinephrine and acetylcholine are modulated by one another. Norepinephrine agonists, particularly the alpha-2 noradrenergic agonist Guanfacine, have shown to increase performance on a number of memory-related tasks in aged animals and humans. Until recently Guanfacine has been implicated in the treatment of AD-type memory deficits. However, no studies have investigated the effect of Guanfacine after a chemical lesion to the medial septum on a

socially transmitted food preference task. Therefore, the present study will measure the effects that Guanfacine has on memory processing of a socially transmitted food preference task following chemical lesion to the medial septum in the rat brain.

Methods

Subjects

Thirty Long-Evans male rats (purchased from Harlan), at first weighing 250 g, served as both as demonstrator (d) (n=20) and observer (o) (n=10) rats throughout the course of the experiment. Rats were maintained on a 12 h light-dark cycle in a humidity and temperature-controlled (approx. room temp.) environment. Over the course of the experiment, d and o-rats were pair-housed in 42 x 24 x 27 cm plastic bottom cages.

Food and water were available *ad libitum* except during the 5 days before and after each training session. During this time, rats were placed on a 22 h food deprivation schedule with *ad libitum* water. Rats were housed and handled according to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, D.C., 1996) and Illinois Wesleyan University IACUC.

Surgery

Surgeries of o-rats were done in the morning or afternoon hours. In the case of morning surgeries, food pellets were removed from the cage the night before surgery to increase the effectiveness of the anesthesia. In the case of afternoon surgeries, food was detracted from the cage in the morning of surgery to increase the effectiveness of the anesthesia. All anesthetics were injected into the abdomen of o-rats. Surgeries required that o-rats be administered approximately 0.3 ml/kg ketamine/xylazine anesthetic

solution, containing 8 mg of ketamine and 1.2 mg xylazine, under aseptic conditions. Supplemental injections of pure ketamine solution were injected if animals exhibited corneal, hindlimb, tail reflexes, excessive whisker movement or rapid respiratory o-rates. Body temperature was regulated and maintained over the course of the procedure using heating pads, in order to protect against heat loss. Instruments were autoclaved before surgery. Prior to surgery, o-rats were handled for approximately 5 separate times for five minutes each.

During operations, o-rats received stereotaxic surgery, in which the medial septal/vertical diagonal band (MS/VDB) was lesioned with 192-IgG-saporin. Once anesthetized and secured, o-rat scalps were cleaned with betadine scrub. A midline incision exposed the skull and the skull surface was leveled along the bregma-lambda axis. Sterile, autoclaved cotton-tip applicators were utilized while removing blood and debris. Following the incision, surgical clips were attached to the faschia, expanding the opening to the skull.

The MS/VDB surgery required that two holes be drilled in the skull at the stereotaxic coordinates AP +0.4 and ML $\pm$1.0 mm from Bregma (Paxinos & Watson, 1998 in Vale-Matinez et al., 2002). Chemical lesions were made using a microinjector and a 28-gauge needle on a Hamilton syringe. The syringe was loaded with 0.15 µg/µl of 192 IgG-saporin (Sigma-Aldrich, St. Louis, MO) in sterile phosphate-buffered saline to be used with lesion o-rats or with sterile phosphate-buffered saline to be used with control o-rats. Saporin injections were made at depths DV -7.8 and -7.0 mm from the skull surface (see Figure 7 for infusion coordinates). 192-IgG-saporin/saline solutions were distributed at a rate of 0.05 µl /min to a total volume 0.3 µl at each of the DV -7.8

mm sites. Another 0.2 µl injection of solution was distributed at each of the DV -7.0 mm sites at a rate of 0.05 µl /min. The needle remained stationary for 9 min following each 0.3-µl injection and for 6 min following each 0.2 µl injection, thereby ensuring that the solution did not diffuse into the track of the needle. Bone wax was spread over the holes to ensure closure on drill sites.

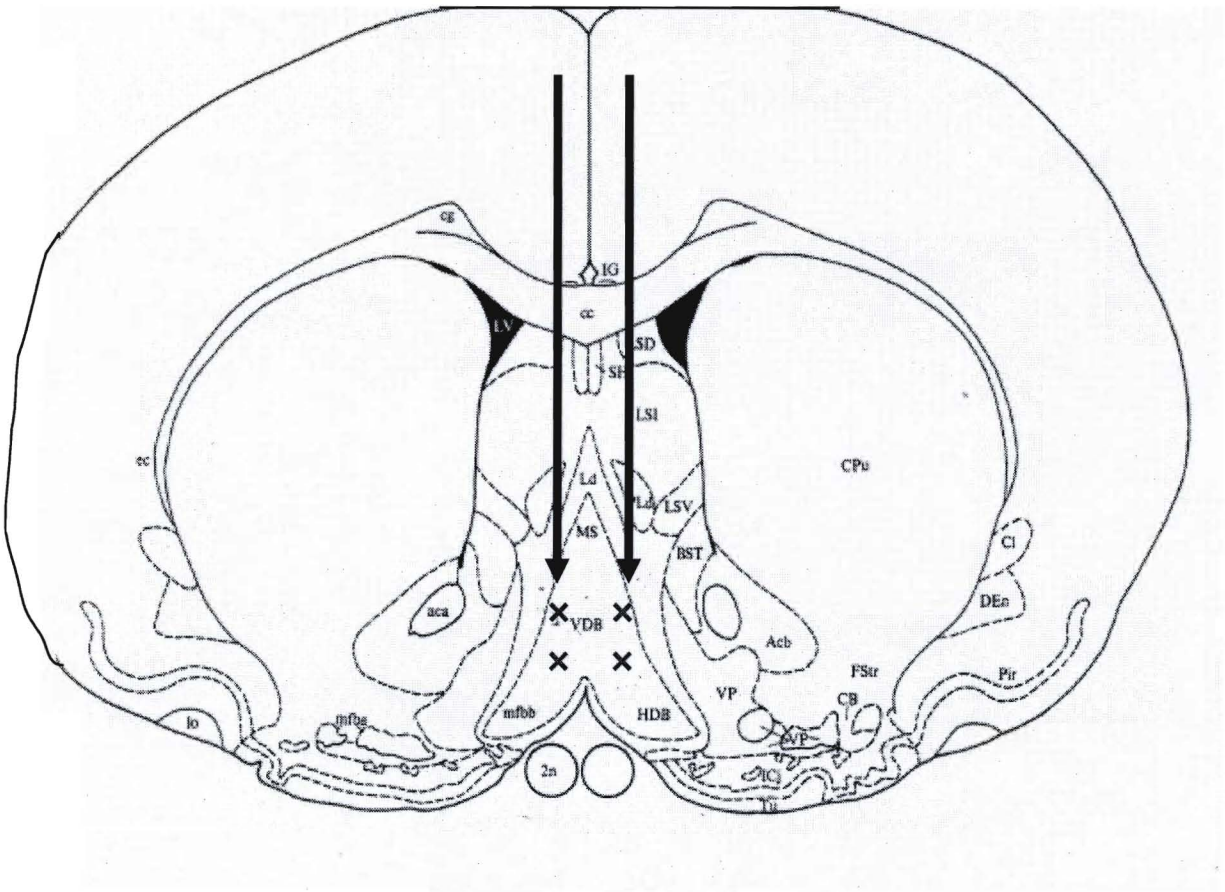


Figure 7. Injection coordinates for saporin infusion into the medial septum. Arrows indicate injection syringes. Cross hashes indicate coordinates of injection; anterior posterior $\pm$ 0.4mm, medial lateral $\pm$ 1.0mm, and dorsal ventral -7.8mm and -7.0mm.

Three staples were used to close the skin. O-rats received injections of 0.2 mg/kg Rimadyl in order to minimize pain. After surgery, o-rats were placed into cages and returned to normal *ad libitum* food schedule. Animal body weight, locomotor action, and

posture were monitored for one full week following surgery. During this time, o-rats were handled daily for 3 min per day over the last five days of recovery. Animals were allowed to recuperate for one week before task testing, being handled 3 min per day for 5 days. Two rat did not survive surgery and an additional eight rats did not recover from surgery. After all surgeries were completed group size for saporin lesioned rats was 7 rats and there were 4 sham lesion rats.

Pharmacological administration

O-rats were administered a single dose of guanfacine (approximately 0.3 mL) (Sigma-Aldrich, St. Louis, MO) following post-lesion testing (see post-lesion group section), in order to determine the effects this pharmacological agent has on mnemonic processing. Guanfacine is a specific alpha-2 agonist, targeting receptors in the locus coeruleus (LC) brain region. Guanfacine was administered systemically, into the abdomen, in a single dose. Prior to administration, 10 mg guanfacine was diluted with 80 mL saline.

Behavioral procedure

The behavioral procedure utilized a repeated measures design, consisting of a socially transmitted food preference (STFP) task, was used in order to assess non-spatial hippocampal-dependent memory in the o-rats. Behavioral procedures were similar to those utilized by Countryman, Orlowski, et al. (2005). Upon arrival in the lab, d and o-rats were allowed to habituate to surroundings for one-week. Following habituation, d and o-rats were handled for 5 min each day for 5 days and placed on a 22 h food deprivation schedule. Both d and o-rats underwent the same STFP task except with different food pairings, as described below in the pre-lesion group, post-lesion group, and

post-drug administration group sections. During the STFP task, d and o-rats were situated in opposite sides of an approximately 42 x 24 x 27 cm plastic bottom interaction cage. The cage was equipped with corn cob bedding and a wire screen that was placed in the middle of the apparatus. Following training, d-rats and o-rats were returned to home cages. Five days after training, o-rats were tested for food preference in a novel room. Details of the STFP task are provided below because minor differences in the task pertain to each food preference-training group.

The food preference test took place in a 42 cm 24 cm 27 cm plastic bottom cage. Two food cups were attached to opposite ends of the cage. Cups contained approximately 15 g each of separate flavored rat chow (cinnamon and coca; thyme and turmeric; marjoram and coriander, cumin and oregano, ginger and nutmeg), see Table 1. O-rats were placed so that they were straddling a ledge in the center of the cage facing a wall. The ledge prevented food from mixing. O-rats were permitted to eat from the food cups for one-hour with water available for the entire hour. The amount of the remaining food was weighed 2-days following the end of the one-hour feeding period to ensure dryness. The amount of each flavored food, which was consumed during the food preference task, was calculated according to the following criteria: (1) (Demonstrated Food 1 offered – Demonstrated food 1 remaining) + (Novel food 2 offered – Novel food 2 remaining) = Total Food Consumed; (2) [Demonstrated Food Consumed (g)/Total Food Consumed (g)] x 100 = Percentage of Demonstrated Food Consumed.

Pre-lesion assessment of food preference

The pre-lesion assessment of food preference was used to determine a baseline of mnemonic processing on a socially transmitted food preference task. The pre-lesion

group contained 20 rats. Twenty-four hours prior to training, d-rats and o-rats were placed into interaction cages and food deprived for 22 hrs. For training, d-rats were taken from the interaction apparatus and placed into an isolated room. In this location, d-rats were permitted to eat for 30 min from approximately 10 g of flavored rat chow. Rat chow was mixed in with specific flavors, in accordance with a food pair chart (see Table 1). D-rats were permitted to drink water whenever food was present. After 30 min had passed, d-rats were taken back to the interaction cage and allowed to interact with o-rats for 30 min through the wire-mesh divider in a training/learning session. Directly following the 30 min interaction period, d-rats and o-rats were returned to home cages and fed unflavored rat chow. After 5 days in home cages, o-rats were taken to a novel room and tested for food preference. The food preference task adhered to the guidelines listed in the behavioral procedure section. Following food preference testing, d and o-rats were returned and given food and water *ad libitum*.

Post-lesion group assessment of food preference

The post-lesion assessment of food preference was used to determine the effects that a 192 IgG-saporin medial septal (MS) lesion had on mnemonic processing. The 10 surviving o-rats (3 sham and 7 saporin lesioned) were handled 5 min each day and placed on a 22 h food deprivation schedule for 5 days prior to the behavioral test. O-rats were then moved into interaction cages for 24 hrs before training begins. After 24 hrs, o-rats were tested on the same STFP task and tested for food preference, according to the procedure described in the pre-lesion section, except using a different food pair (see Table 1 for food pairs). Following testing for food preference, o-rats were returned to

their home cages for 1 week, while being monitored on a 22 h food deprivation schedule, until the post-drug administration assessment of food preference.

	FOOD A	**FOOD B**
PAIR 1	Cinnamon (1%)	Cocoa (1%)
PAIR 2	Marjoram (1%)	Coriander (1%)
PAIR 3	Nutmeg (0.8%)	Ginger (1%)
PAIR 4	Thyme (2%)	Turmeric (1%)
PAIR 5	Oregano (1%)	Cumin (1%)

Table 1. Food pairs. Representation of food used throughout the STFP task. If FOOD A demonstrated to d-rats, then o-rats presented both FOOD A and FOOD B during testing of preference. Demonstrated food was counterbalanced within food pair groups in order to ensure that novel food was presented during each STFP trial period.

Post-drug administration assessment of food preference

The post-lesion drug administration assessment of food preference was used in order to determine the effects that guanfacine had on mnemonic processing following a chemical lesion to the MS/VDB. The same 11 o-rats were administered guanfacine 30 min prior to training in the dose according to the protocol described in the pharmacological administration section described above. Following administration, o-rats were tested on the same STFP task and for food preference, according to the procedure described in the pre-lesion section, except using a different food pair.

Histology and immunohistochemistry

Following behavioral testing, o-rats received an overdose of ketamine/xylazine. Each subject was prepared for transcardial perfusion with a solution containing 0.9% saline and then 4% buffered paraformaldehyde. Following fixation, brains were removed and placed in a 20% sucrose solution. Brains were sectioned at 50μm with a Cryocut 1800 (Reichert-Jung) cryostat. Individual brain sections were collected in a cryopreservative until staining for choline acetyltransferase (ChAT).

A modification of the staining procedures used by Chang and Gold (2004) and Countryman, Orlowski, et al., (2005) was used to identify ChAT positive cells in the medial septum (MS). MS sections were washed 4 consecutive times for 10 min each in 0.05M phosphate buffer solution (PBS). Following washing, sections were blocked for 15 min in a solution containing 1% normal goat serum (NGS), 1% H_2O_2, and 0.4% TX in 0.05 M PBS. Next, sections were washed for 10 min in 2% NGS and 0.2% TX in 0.05M PBS. Free-floating sections were incubated in small plastic wells at room temperature for 48 hrs in a solution containing anti-ChAT rabbit primary polycolonal antibody (1:100; Santa Cruz), 1% NGS, 0.2% TX in PBS. Sections were then washed 4 times for 12 min each in 0.05M PBS. Following washing, sections were incubated for 1 hr in a solution containing the biotinylated goat anti-rabbit secondary antibody (1:400; Santa Cruz), 1% NGS, 0.4% TX in PBS. Sections were washed 3 times for 5 min each in 0.05M PBS. Following washing, sections were processed with avidin-biotinylated horseradish peroxidase complex (ABC Elite Kit, Vector Laboratories), 0.2% TX in PBS for 45 min. Sections were washed 4 times for 15 min each in 0.05M PBS. Next, sections were

visualized using diaminobenzidine (DAB Substrate Kit, Vector Laboratories) for 3 minutes. Following incubation, sections were washed 3 times for 8 min each in 0.01M PBS. After washing, sections were mounted, set to dry, and protected with cover slips.

ChAT stained tissue sections were imaged using a Nikon Y-FL Eclipse E600, Nikon Digital Sight DS-SM camera interfaced with an Intel Pentium 4-PC computer. ChAT positive cells in the medial septum were quantified using Scion Image, a pc equivalent program to NIH Image. Cells were identified and counted using protocols previously published (Countryman, Orlowski, et al., 2005; Countryman, Kaban, et al., 2005).

Results

Preliminary comments.

Following surgical lesion, subjects within the "post-lesion" group and "post-lesion plus guanfacine" group displayed a significant decrease in total average food consumed, measured in grams.

Subjects within the present manipulation exhibited a statistically significant formation of a food preference when compared to chance prior to saporin lesion and following guanfacine administration (after lesion) on the STFP task. In contrast, subjects did not exhibit a statistically significant memory formation of food preference following lesion. Furthermore, following lesion, subjects exhibited a statistically significant decrease in memory formation when compared to pre-lesion subjects on the STFP task. In contrast, subjects administered guanfacine (following lesion) did not exhibit a

statistically significant increase in memory formation when compared to post-lesion subjects on the STFP.

Histological analysis revealed a trend toward significance between decreases in the number of ChAT-positive cells within the "sham" group and the number of ChAT-positive cells within the saporin-administered group. Further analysis revealed a trend toward significance between increases in memory formation within the guanfacine-treated group (following lesion) and increases in the number of ChAT-positive cells.

Behavioral performance: socially transmitted food preference task.

A repeated measures analysis of variance (rm-ANOVA) was performed to examine differences in total food consumed at each of the three tests time points (pre-lesion, post-lesion, and post-lesion + guanfacine). A significant difference in total food consumption across the test time points was found, $F(2,14) = 5.57$, $p < 0.05$. In order to determine statistical significance between specific test time point groups, Fisher's post-hoc test was performed. This statistical test revealed that pre-lesion rats consumed significantly more food when compared to the post-lesion average total grams of food consumed ($p < .05$). Similarly, pre-lesion rats consumed significantly more total food when compared to average total grams of food consumed post-lesion + guanfacine (see Figure 8).

Next, a rm-ANOVA was performed to determine differences in the percentage of demonstrated food consumed at each of the three test time points (pre-lesion, post-lesion, and post-lesion + guanfacine). No significant difference between the percentages of demonstrated food consumed as a function of test time points was found, $F(2,10) = 3.08$, $p = 0.09$ (see Figure 9). However, due to an *a priori* hypothesis that the percentage of

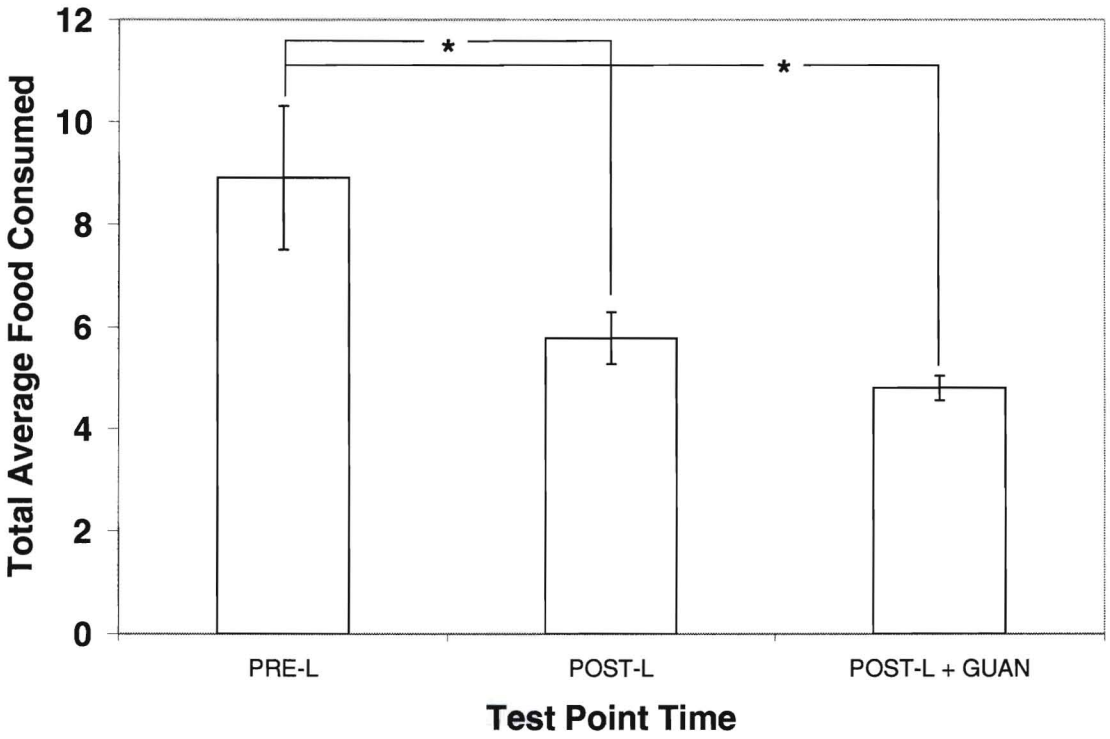


Figure 8. Total food consumed (grams) (mean ± s.e.m.) plotted as a function of test point time. Rats ate significantly less food at test after lesions to the medial septum were performed. PRE-L, pre-lesion; POST-L, post-lesion; post-L + GUAN, post-lesion + guanfacine. * $p < 0.05$.

demonstrated food consumed would decrease following 192 IgG-saporin administration and increase following guanfacine administration, Fisher's post-hoc test was performed. This statistical test revealed a significant decrease in the percentage of demonstrated food consumed from the pre-lesion to the post-lesion time point , $p < 0.05$.

Furthermore, t-tests revealed that "PRE-L" [$t(29) = 8.36, p < 0.05$] and "POST-L + GUAN" [$t(10) = 2.36, p < 0.05$] groups consumed statistically more of the demonstrated food than the 50% chance level. When compared to chance, the "POST-L" group did not consume statistically more of the demonstrated food [$t(10) = 1.75, p = 0.11$] (see Figure 9).

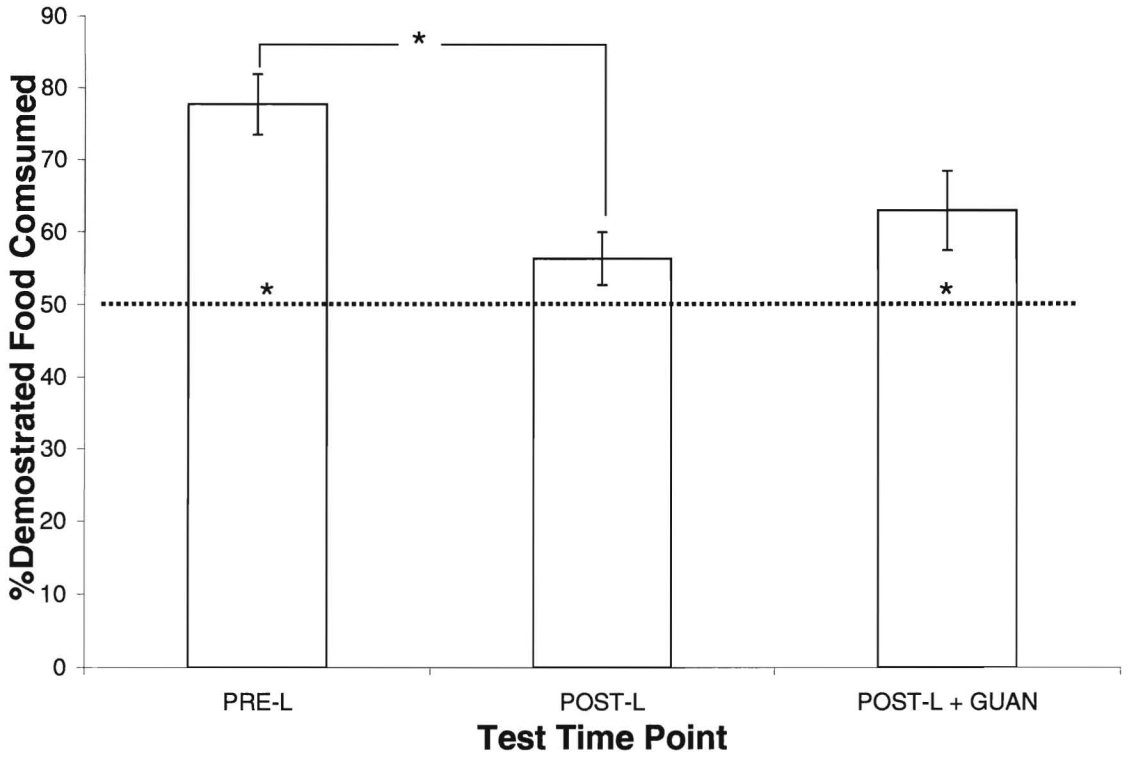


Figure 9. Percentage of demonstrated food consumed (mean ± s.e.m.) plotted as a function of test time points. Dotted horizontal line equivalent to chance (50%). * $p < 0.05$.

Immunocytochemistry (ICC).

In order to examine the neurotoxic effects of saporin, ChAT positive cells located within the medial septum were quantified using microscopic digital photo analysis. An illustrative representation of digital pictures taken in order to quantify ChAT positive cells is displayed in Figure 10. Analysis indicated the absence of a significant decrease in ChAT positive cells when considered as a function of treatment (sham vs saporin lesion), $F(1,8) = 3.31, p = 0.10$. While not significant, a considerable decrease in ChAT positive cells was apparent following administration of saporin (see Figure 11). In order to examine effect size, eta squared was calculated. Eta squared represents the proportion of

variance of the dependent variable that is explained by the independent variable. Eta sq = *0.29*.

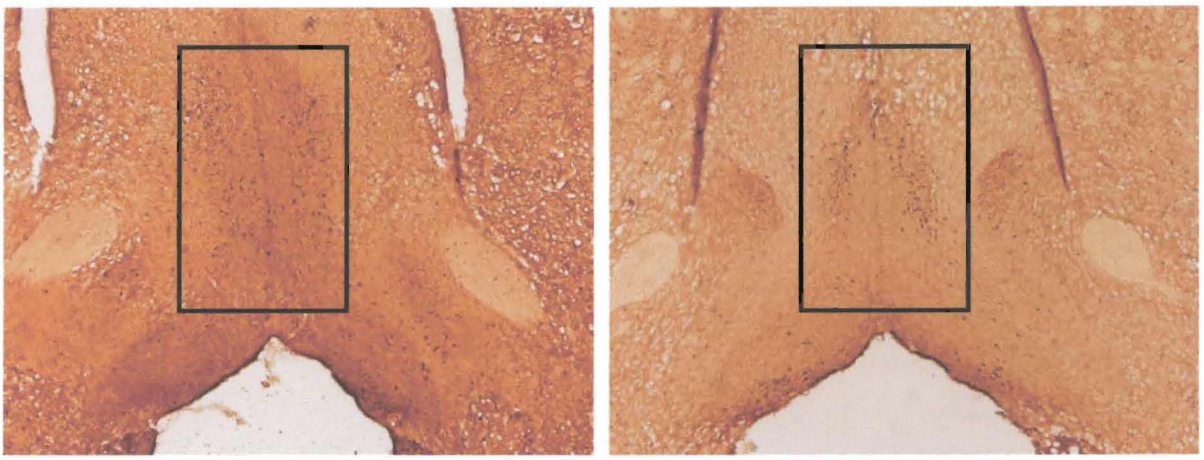


Figure 10. Representative images of a brain infused with saline, sham operated (left column) and a brain infused with IgG-saporin (right column). Notice the lighter staining and decrease in number of ChAT positive cells.

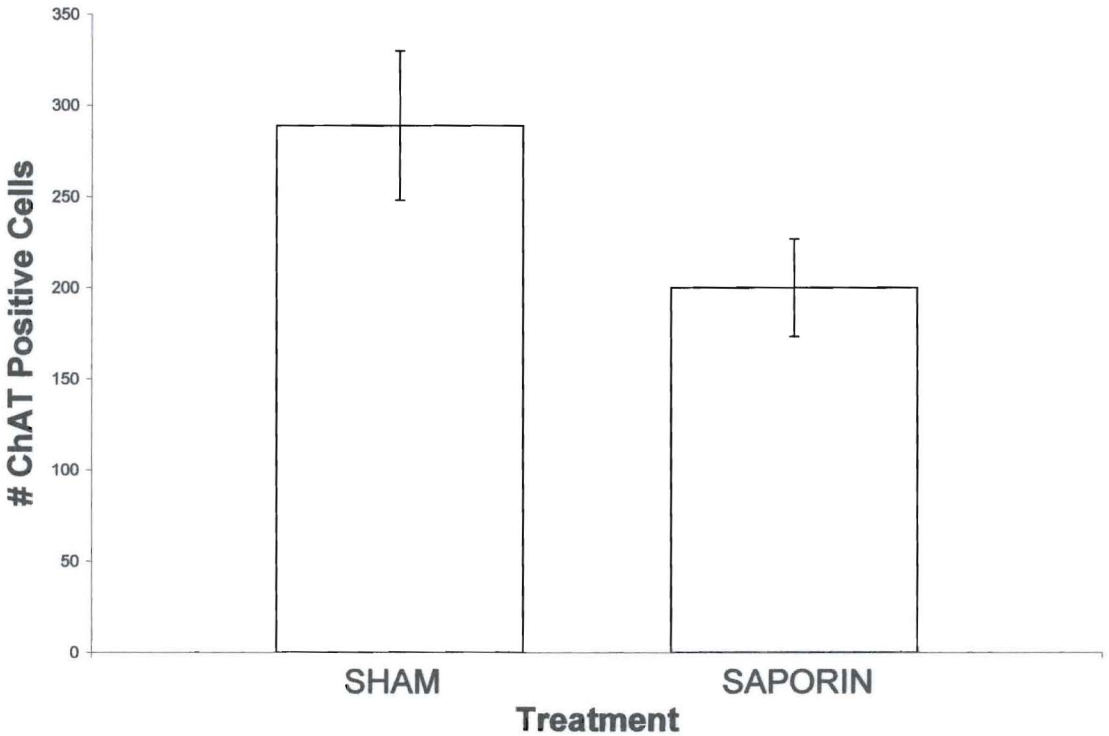


Figure 11. Number of ChAT positive cells (mean $\pm$ s.e.m.) plotted as a function of sham and saporin treatment, $F(1,8) = 3.31$, $p = 0.10$.

A decrease in ChAT cell count was expected to reveal a positive correlation with memory performance. Therefore, a correlation was computed in order to determine if a relationship existed between ChAT positive cells and post-lesion percentage of total demonstrated food consumed. Analysis revealed the absence of correlation between the number of ChAT positive cells within the medial septum and the post-lesion percentage of demonstrated food consumed, $r = -0.20$, $p = 0.657$.

However, following further statistical analysis, a trend toward significance was revealed when considering the number of ChAT positive cells plotted as a function of total demonstrated food consumed in respect to the post-lesion + guanfacine time point, $r = .63$, $p = 0.12$ (see Figure 12). Power analysis with power level set at 0.80 and $r = .63$ indicated that n = 17 would be necessary for a significant correlation.

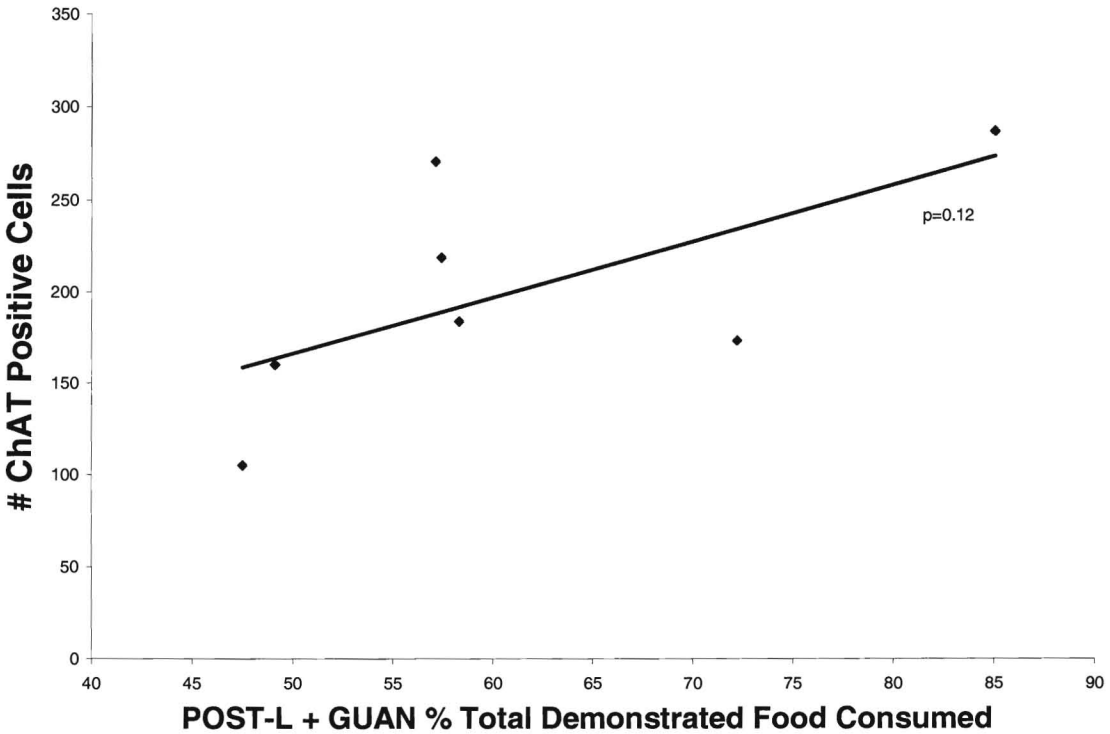


Figure 12. Number of ChAT positive cells plotted as a function of post-lesion + guanfacine percentage of total demonstrated food consumed, $p = 0.12$. Computation of a trendline indicates a trend toward a significant correlation.

Discussion

Administration of the cholinergic lesioning agent 192 IgG-saporin into the rat medial septum was utilized in the present study in order to investigate changes on a hippocampal memory-processing socially transmitted food preference task. Therefore, reductions in performance on the STFP task were expected following microinfusion of saporin. Following saporin infusion, systemic injection of the norepinephrine agonist guanfacine into the rat was employed in order to examine changes on the same STFP task. Guanfacine administration was expected to increase memory performance by compensating for decreased acetylcholine levels within the hippocampus.

Behavioral effects of 192 IgG-saporin

Subjects were trained to acquire a STFP and tested for memory 5 days later. Since the task is hippocampus-sensitive and such processing has been shown to rely on ACh transmission, reductions in medial septum ACh levels were expected to lead to decreased behavioral performance. Performance on the STFP task in the present study significantly decreased following the administration of saporin. Such decreases in processing on the STFP task are quantified by calculating the percentage of demonstrated food consumed. This value refers to the amount of memory for the demonstrated food the rat population displayed (see method section for detailed STFP procedure).

Observers in the "pre-lesion" group consumed on average 77% of the demonstrated food. This value is logical because transmission of acetylcholine within the hippocampus is assumed to be unaltered at this time point. It is important to note that acetylcholine neurotransmission is positively correlated with memory processing. Furthermore, the existence of such a high percentage indicates that memory formation of

the demonstrated food did in fact occur and that the long-term memory trace is maintained for periods extending beyond 5 days. Results of this nature are congruent with previous research on a STFP task indicating the persistence of the memory trace at long delays (Clark et al., 2002, Countryman, Kaban, et al., 2005). Vale-Martinez et al. (2002) indicates similar findings that control subjects consumed on average 85% of demonstrated food during the STFP task when tested for preference 5 days following interaction with demonstrators.

It is important to note that memory for the food preference was assessed after 5 days for several reasons in the present study. First, previous research indicates that memory of a food preference can last for periods extending one month following demonstration (Clark et al., 2002). This persistence of memory is relevant to the present study in that it validates the amount of time between demonstration and assessment of preference. If subjects display memory over a one-month period, certainly memory will be displayed after a 5-day period.

Secondly, research indicates that aged animals begin to display decreases in memory for food preference by 72 hours on the STFP task (Countryman & Gold, *in press*). The same data indicate that by one week over 50% of aged rats are unable to maintain a memory for the demonstrated food. This information is applicable to the present study due to the lapse of time that passes before the onset of forgetting. If aged rats maintain memory for a socially transmitted food preference for up to 72-hrs, then the test for memory deficits due to lesions of the medial septum should be beyond this time point. Also, Vale-Martinez and colleagues (2002) have demonstrated that memory deficits for this task following IgG-Saporin lesions are apparent after 5 days. Even more

relevant is the fact that subjects in the present manipulation are assumed to represent the effects of aging – decreased cholinergic cells as a result of chemical lesion. Consequently, based on previous research, it is not surprising that subjects displayed memory for food preference following a 5-day period.

Observers in the post-lesion group consumed on average 56% of the demonstrated food; just 6% above chance. Similarly, this value is consistent with the notion that decreases in acetylcholine neurotransmission, as a result of saporin administration, are responsible for decreases in memory formation of the demonstrated food. Vale-Martinez et al. (2002) present results that are consistent with the current findings of decreased processing following saporin infusion. Specifically, following administration of saporin into the medial septum/vertical diagonal band of Broca, percentage of total demonstrated food consumed by observers, when tested for food preference after 5 days of interaction with demonstrator, decreased by 45% when compared to pre-lesion percentages of total demonstrated food consumed.

Furthermore, the lack of a significant value of percentage of demonstrated food consumed above chance when considering the post-lesion group is indicative of an absence of long-term memory formation. This conclusion is expected because saporin has been shown to decrease acetylcholine levels in the hippocampus when injected into the medial septum, thereby decreasing memory function (Gold, 2004). Berger-Sweeney et al. (2001) indicate similar decreases in swim Morris water maze performance following saporin lesion; a task applied in order to examine memory effects.

Surgery effects

Surgery was performed in order to infuse saporin (via an automated microinfusion device) directly into the medial septal brain region (see methods section for detailed procedure). Following surgical procedures, "post-lesion" and "post-lesion plus guanfacine" administered subjects exhibited significant decreases in total average food consumed per test point, measured in grams. Findings of this nature are congruent with previous literature, which indicate that subjects having undergone surgery consume less total food on average as a side effect of surgical methods (Brightwell, et al., 2005; Countryman, Kaban, et al., 2005). Therefore, significant decreases in total average food consumed per STFP trial are not generally alarming and do not affect quantification measures of memory formation within this paradigm.

Behavioral effects of guanfacine

Guanfacine is an alpha-2 norepinephrine agonist known to increase memory processing following administration in healthy human populations and aged non-human primates (Arnsten et al., 1988; Arnsten & Cai, 1993; Jakala et al., 1999). Therefore, increases in memory formation following training on the STFP task subsequent to saporin injection were hypothesized. Subjects in the present study did not exhibit a significant increase in memory formation on the STFP task from the "post-lesion" time test point to the "post-lesion plus guanfacine" test time point. This means that subjects in the guanfacine treatment group did not consume significantly more food when compared to subjects in the post-lesion group.

However, rats in the post-lesion plus guanfacine treatment group did exhibit a significant increase above chance in the percentage of demonstrated food consumed.

Guanfacine treatment group rats exhibited a 63% demonstrated food consumed value; 13% above chance. This means that guanfacine-treated subjects did consume significantly more demonstrated food within the "post-lesion plus guanfacine" STFP task test time point.

Theoretical considerations: noradrenergic sympathetic sprouting. Previous literature indicates that administration of guanfacine (an alpha-2 adrenergic agonist) in the absence of a cholinergic lesion has led to increases in memory performance in aged monkeys and human populations (Arnsten et al., 1988; Arsten & Cai, 1993; Jakala et al., 1999). In addition, Sirvio et al. (1991) found increases in performance on a spatial navigation task within a rat population following guanfacine administration. Collectively, guanfacine seems to improve memory performance, at least partly among aged animal subjects. Consider aged animals, which consistently exhibit decreased levels of ACh within the hippocampus. It might be the case that increased stimulation of alpha-2 adrenergic receptors, which project to the hippocampus, could improve memory formation in the presence of decreased ACh neurons.

Of interest, following lesion to the cholinergic system, multiple studies have indicated sympathetic noradrenergic sprouting into the hippocampus (Crutcher & Davis, 1981; Harrell et al., 2001; Harrell et al., 2005; Madison & Davis, 1982). Increased noradrenergic axonal sprouting might be a reasonable compensatory mechanism in response to ACh loss. This is logical to assume due to the fact that norepinephrine receptor stimulation in the absence of ACh loss already has been shown to stimulate increases in memory performance (Arnsten et al., 1988; Arsten & Cai, 1993; Jakala et al., 1999). It is plausible to conclude that stimulation of alpha-2 adrenergic receptors within

the locus coeruleus (the primary brain region, which projects NE to the hippocampus) by guanfacine may have increased delivery of NE to the hippocampus at a greater rate due to increased NE sprouting. The increase in delivery of NE to hippocampus following guanfacine administation may have compensated for decreases in ACh. Such compensation, therefore, may have led to the significant increases in values of percentages of total average demonstrated food consumed, indicative of increased memory performance.

One important implication to consider when taking into account NE axonal sprouting is the time window immediately following the saporin lesion and the initial administration of guanfacine. One study by Harrell et al. (2001) indicated that a minimum of 8 weeks must pass following saporin infusion in order to visualize NE ingrowth via histofluoresence. In the present study, guanfacine injection took place only 3 weeks following saporin administration. This is certainly an impetus in concluding that increased NE delivery via increased NE sprouting to the hippocampus actually occurred. It might be the case that NE axonal ingrowth did not commence prior to guanfacine injection. While considering this limitation in current methodology, it is important to recognize that studies indicating a minimum amount of time passage before NE ingrowth following saporin lesions to the MS are few and far between. In the present study, NE fiber ingrowth was not quantified, therefore conclusions gathered on the issue rely solely on the interpretation of behavioral and histological data.

Immunohistochemistry: analysis of saporin within the medial septum

In the present study, the relation between the number of choline acetyltransferase (ChAT) positive cells within the medial septum and memory performance was

investigated. ChAT is a good quantitative measure of acetylcholine neurons within tissue locales because ChAT is the enzyme that catalyzes the synthesis of acetylcholine. Therefore, high numbers of ChAT positive cells within the medial septum are indicative of large numbers of acetylcholine neurons.

Since decreases in acetylcholine neurons have been correlated with decreases on memory-related tasks, it was necessary in the present study to perform similar histological analysis. Comparable studies that manipulated the medial septal area with saporin indicate greater reductions (approximately 60-70%) in ChAT-positive cells than the present manipulation (Berger-Sweeney et al., 2001; Leanza et al., 1995). The current analysis produced approximately a 35-40% reduction in ChAT-positive cells. Incomplete lesion to the medial septum with saporin is likely to have left intact a higher number of ChAT-positive cells and thus the lack of a significant reduction from the sham to saporin treatment group. This lack of a significant reduction in ChAT-positive cells is not entirely important due to the presence of a large effect size between the sham and saporin groups. Increasing the number of infusion sites of saporin within the medial septum will likely produce larger decreases in ChAT-positive cell bodies.

In addition, statistical analysis of the data indicates the absence of a significant correlation between the number of ChAT positive cells and percentage of total demonstrated food consumed from the post-lesion group. Lack of a significant value indicates that memory performance did not necessarily rely on a certain number of acetylcholine neurons. That is, decreases in the number of acetylcholine neurons were not coupled with decreases in memory processing. This observation is not particularly surprising as extensive previous research analyzing correlations between cell counts and

memory performance has failed to present any significant values (Countryman, Orlowski, et al., 2005; Countryman, Kaban, et al., 2005; Countryman & Gold, *in press*).

Not expectantly, on a purely statistical level, the present histological analysis is in direct conflict with performance on the behavioral STFP task. Data indicated that decreases in memory processing on the STFP task occurred following saporin lesion. Statistically, this decrease in performance should have been correlated with decreases in ChAT positive cell numbers. One explanation for the lack of significance relates directly to small control and manipulation group numbers (n=3 and n=7, respectively). Low numbers of subjects within these groups led to large values of standard error within such groups. It is very plausible that increasing the number of subjects within each group will be advantageous in reaching a statistically significant value.

Contrary to the above findings, further analysis of data revealed a trend toward significance when considering the amount of ChAT positive cells plotted as a function of percentage of total demonstrated food consumed at the "post-lesion plus gaunfacine" time point. As the number of ChAT positive cells increased across subjects, memory performance similarly increased on the STFP task. This finding is extremely relevant because it suggests that a certain baseline number of ACh neurons within the medial septum must be present in order for guanfacine to produce effects on memory. Effectiveness of guanfacine seems to be dependent on a particular number of acetylcholine neurons.

Guanfacine as a pharmacological agent: Alzheimer's disease

Within the context of Alzheimer's disease, the observation that guanfacine is dependent on a specific basal number of acetylcholine cell numbers within the medial

septum is quite relevant. Note that Alzheimer's disease is correlated with gradual degeneration of acetylcholine neurons within the hippocampus; those cells responsible for the efficiency of memory performance (Bartus, 2000). Spontaneous and rapid deterioration of ACh neurons within an individual experiencing decreases in memory acquisition and recall is usually employed as diagnostic marker for dementia of the Alzheimer's type. Health care professionals, in the midst of suspecting AD, utilize the term "dementia" because AD remains diagnosable exclusively post-mortem via autopsy.

In addition, the onset of dementia of the Alzheimer's type is particularly alarming because the loss of ACh neurons in AD is terminal. This means that increasing reductions in ACh cells ultimately contribute to death. Such imminent and unavoidable cell death has been proclaimed as the single greatest limiting factor when considering pharmacological treatment methods for dementia of the Alzheimer's type (Delagarza, 2003).

One such treatment method falls under the category of acetylcholinesterase inhibitors (AChE-I). Note that pharmacological agents of this genre exert beneficial therapeutic effects by inhibiting acetylcholinesterase (AChE), the enzyme responsible for degrading ACh neurotransmitters following post-synaptic binding. Inhibition of AChE leads to a decrease in hydrolysis of ACh thereby permitting increased ACh receptor stimulation. Ultimately increases in receptor activation presumably lead to increased memory performance, mainly during recall (Rogers, 1998).

Increases in memory performance seem to be largely dependent on the number of active and surviving ACh neurons, since ACh neurons are responsible for transmission of information during information acquisition, storage, and recall. This observation alone is

evidence of the limiting application of AChE-Is in that AChE can only be inhibited to the extent by which ACh cell survival exists. AChE-Is therefore do not protect against ACh cell death, they merely aid in the preservation of memory processing as long as a certain basal number of ACh neurons continue to function.

The present study is harmonious with observations of the limiting nature of AChE-Is. More specifically, the behavioral effects of guanfacine, while not statistically significant (however, approaching significance), seem to be dependent on a certain number of surviving ACh cells within the medial septum. As a result, the norepinephrine agonist guanfacine possesses similar drawbacks associated with AChE-Is. Most likely, utilization of guanfacine in the treatment of dementia of the Alzheimer's type will only exert therapeutic effects for as long as ACh cells survive.

Before entirely abandoning guanfacine as a plausible treatment approach to AD, the present significant increase in percentage of demonstrated food consumed within the "post-lesion plus guanfacine" treatment group is extremely relevant. Taking into perspective this value, it is likely that memory formation occurred following guanfacine injection. Since a similar significant value was not observed in the "post-lesion" group it is reasonable to assume that guanfacine likely contributed to a large degree in the onset of memory formation. It is probable that guanfacine may have restored the subjects' ability to form novel memories.

Future research

Restoration of memory formation following the administration of guanfacine, an alpha-2 norepinephrine agonist, in the present experiment contributes to the relevance of an animal model for Alzheimer's disease. Identifying the rat as a valid animal in which

to manipulate treatment approaches to AD is most likely the greatest result of the present study. Additionally, observations involving the reliance of guanfacine on a basal ACh neuron level lead to the conclusion that norepinephrine agonists might suffer from similar limitations associated with AChE-Is. In the seemingly never-ending quest to uncover safe, effective, and neuronal-protective pharmacological agents, future research must utilize precise methodology.

As a result of the present experiment, future analysis must attempt to uncover the relationship between the adrenergic system and the cholinergic system within the STFP behavioral paradigm. Current results indicate that following administration of guanfacine, memory formation increased in the post-lesion group. However, whether NE axonal sprouting into the hippocampus was responsible for the return of memory formation could not be determined due to the absence of available NE histoflourescent staining materials. Obtaining such materials would allow one to measure the prevalence of NE sprouting into the hippocampus by comparing levels to controls.

Measurements of NE axonal in-growth might then be analyzed with levels of surviving ACh neurons within the medial septum following saporin infusion. This would allow one to investigate the relationship between NE compensatory sprouting into the hippocampus in reaction to ACh cell death within the medial septum. Based on previous research, this would permit one to make comments on the compensatory hypothesis of interacting noradrenergic and cholinergic systems in animal subjects who display substantial Alzheimer's memory-type deficits.

In addition, future research should examine the relationship between NE sprouting, ACh levels, and behavioral performance on the STFP task. This would allow

one to collectively evaluate the modulation of these components in a statistical analytical program, inspecting for correlations. It is likely that interactions exist between the ACh system, NE system, and a behavioral STFP-type paradigm as supported by previous research.

Once relationships have been revealed between modulation of the cholinergic and adrenergic systems following ACh cell loss, studies involving NE agonists might take place. One stipulation of entering into such trials should rely on the observation that NE axonal sprouting occurs in compensation for ACh loss within the hippocampus. Following norepinehprine agonist administration (after saporin infusion) it would be beneficial to measure norepinephrine levels within the hippocampus via high performance liquid chromatography (HPLC). Norepinephrine levels might then be correlated to ACh levels within the hippocampus, measured via HPLC as well. One might expect decreased ACh levels within the hippocampus and increased NE levels due to the previously published compensatory nature of NE axonal sprouting into the hippocampus (Crutcher & Davis, 1981; Crutcher & davis,1981; Madison & Davis, 1983). Norepinephrine and acetylcholine levels might then be correlated to behavioral performance on the STFP task, indicating increases in memory formation as a result of excess NE stimulation within the hippocampus following saporin lesion to the medial septum.

Finally, the present investigation likely suffered from small control and manipulation group numbers. Low subject population numbers indisputably lead to a decrease in statistical power and large standard error values and as a result to a decrease in significant correlations between experimental and control groups. Future work should

ensure the survival of subject populations beyond the ones reported within this study. A power analysis examining the correlation between ChAT-positive cells and the post-lesion plus Guanfacine-treated group, indicated that a subject population of 17 would be needed to attain a significant correlation.

Final comments

Since the onset of the "cholinergic hypothesis", considerable evidence has been obtained in support of impairment of the cholinergic system within Alzheimer's diseased patients (Bartus et al., 1982). First, in the context of learning and memory tasks, predominant modulation of memory processing by acetylcholine has been shown to occur within the hippocampus (Dutar et al., 1995). Numerous studies indicate that in human populations, Alzheimer's disease (AD) is marked by a lack of cholinergic transmission within the hippocampal region (Bartus et al., 1982; Rossor et al., 1981). The medial septal area, which exhibits neuronal projection to the hippocampus, has been implicated as the principal source of acetylcholine to the hippocampus (Vale-Martinez et al., 2002). Consequently, lesioning the medial septum has produced widespread memory deficits via decreased availability of acetylcholine transmitters to the hippocampus (Potter et al., 1999).

Undoubtedly, the adrenergic and cholinergic systems are clearly linked within the context of information acquisition and subsequent memory formation. One study by Kruglikov (1982) indicated that impairment did not exist on an avoidance memory task following administration of the cholinergic antagonist scopolamine. However, when scopolamine was administered in conjunction with locus coeruleus lesions, drastic impairment resulted. Another study by Harrell, Peagler, & Parsons (1990) presented data

that increases in impairment were exhibited by rats on a radial maze apparatus following administration of the beta-adrenergic antagonist propranolol in conjunction with medial septum lesions. Application of propranolol following lesion to the medial septum significantly worsened already decreased learning and memory performance. Moreover, numerous observations have been made that stimulation of the alpha-2 adrenergic receptor within the locus coeruleus likely accounts for increases on memory-related tasks (Arnsten et al., 1988; Arsten & Cai, 1993; Jakala et al., 1999).

Final conclusions

In light of previous theoretical framework, the present study reports three meaningful conclusions: (1) following infusion of saporin into the medial septum, memory formation for a socially transmitted food preference decreased significantly, (2) following administration of guanfacine after saporin infusion, memory formation for a socially transmitted food preference increased significantly, and (3) memory formation for a socially transmitted food preference following guanfacine administration is approaching significance when positively correlated to the number of surviving ACh cells following saporin infusion.

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