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Author's 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. Recent studies have proposed that increasing levels of the neurotransmitter norepinephrine may help to decrease the cognitive impairments associated with Alzheimer's disease and aging. The present study measured the effects that Guanfacine, an alpha-2 noradrenergic agonist, has on memory deficits produced by the acetylcholine antagonist, Scopolamine. Memory ability was assessed using an object recognition task and a socially transmitted food preference task. Following administration of Scopolamine, memory ability was significantly impaired from baseline levels on both memory tasks. Pre-training injection of Scopolamine followed by post-training injection of Guanfacine resulted in memory performance that was equivalent to baseline memory performance on both tasks. Guanfacine administration alone did not improve memory performance, but rather had a trend toward impairing performance. Results from this study indicate that Guanfacine may be effective at improving memory impairments caused by decreased acetylcholine function as seen in Alzheimer's disease.

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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
Locus coeruleus	LC
Medial Septum	MS
Norepinephrine	NE
Object recognition task	ORT
Social transmission of food preference	STFP

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Cognitive deficits seen during aging are known to vary amongst individuals and may be ascribed to a continuum, ranging from little or no cognitive deficits to severe memory impairments like Alzheimer's disease. Although, some people age with no cognitive deficits whatsoever many individuals lose a small part of their cognitive abilities. For example, in many elderly persons a marked decrease in reaction time or paired-associate learning, such as pairing a name to a face or a phone number to a friend, may be present (Shimamura, Berry, Mangels, Rusting, & Jurica, 1995). These deficits are fairly common and are at one end of the spectrum. However, these symptoms can get progressively worse as the other end of the spectrum is approached and many people also experience these more severe deficits. Aged individuals with such deficits may have a hard time remembering simple daily habits, like taking medication or keeping up with personal hygiene. Those individuals with the most severe cognitive deficits are likely sufferers of Alzheimer's disease.

Alzheimer's disease (AD) is a neurodegenerative disease currently affecting nearly 18 million people worldwide and is projected to affect about 34 million people by the year 2025 (World Health Organization, 2006). In patients with Alzheimer's disease, a continual functional decline is often seen. The decline often begins with the patient's inability to remember past events in time. These decreased event remembrances can range from months or years ago to events occurring earlier that day. As the disease progresses, cognitive decline continues with patients losing the ability to perform many activities of daily living. These activities range from basic procedural actions such as eating or bathing to more complex

actions such as shopping or using the telephone (Doody, 2003). Extreme Alzheimer's cases show patients even losing the ability to remember family members and other loved ones. Alzheimer's disease cannot definitively be diagnosed until post-mortem studies can be conducted. Patient death usually occurs 8-10 years after symptomatic onset.

It is obvious that advances in treatment options for age associated cognitive decline and AD are needed in order to improve the lack of memory function associated with them. One potential approach to treatment of Alzheimer's might involve the pharmacological norepinephrine 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 improving long-term memory impairments seen in age associated cognitive decline and AD.

Long-Term Memory

There are many systems of memory that together encompass the concept of long-term memory. Recently, Squire (2004) organized long-term memory into a reliable taxonomy, which accounts for the relevant brain structures that are associated with different systems of memory. This taxonomy breaks long-term memory down into two systems: declarative memory and nondeclarative memory. Nondeclarative memory is an unconscious system of memory that is responsible for extracting common perceptions from several isolated and serial events, allowing one to integrate information from variable contexts. Therefore, nondeclarative memory is associated with procedural skills such as riding a bike. It is also associated with priming and perceptual learning, simple classical conditioning, and nonassociative learning. Declarative memory, which is a conscious system of memory, is

broken down into two parts: episodic memory (events) and semantic memory (facts). It consists of personal information and is often referred to as "self-knowing" (Tulving, 1985). The present study focused on the declarative system of long-term memory.

Declarative memory. The memory taxonomy proposed by Squire (2004) defines declarative memory as recollection-based memory that pertains to facts or personal information from single events in time. The declarative memory system is capable of processing multiple pieces of information and is often impaired in amnesic patients (Squire, 1992). Therefore, it is also likely to be impaired in patients with Alzheimer's disease or aged-associated cognitive deficits. Declarative memory is often associated with many different parts of the brain, but is generally related to the medial temporal lobe, which includes the hippocampus, and the diencephalon. The medial temporal lobe contains specific parts of the brain, each of which have a role in memory regarding acquisition, integration, and recollection.

Cognitive Decline. As aging occurs, it is normal for certain parts of memory to decline; however, in AD the decline is extreme. Persons with mild cognitive impairment may only lose a few bits of semantic knowledge or a few episodic events. While their ability to form new long-term memories may be slightly impaired, they are still able to encode and retain most new information into long-term memory. With AD, the impairments seen in semantic and episodic memories, as well as ability to encode new information to long-term memory, is greatly increased.

In the beginning stages of AD, the decline seen is generally quite mild, yet as the disease progresses, the decline seen becomes more pronounced (see Illustration 1). Nondeclarative memory, specifically procedural memory, usually lasts the longest in AD

patients. Declarative memory, however, is generally the first and primary area of memory affected. Semantic memory declines quite rapidly and patients quickly lose the ability to remember specific facts and general knowledge, such as being able to name common household objects. Episodic memory also declines quite quickly as patients have a harder time remembering past events or pieces of information that are event-based. Short-term memory may decline at a slower pace. Some patients still have an intact digit span and can hold a normal conversation, remembering questions or information given out previously in the conversation. Despite this, ability to encode new long-term memories may still be impaired.

As the effects of the disease reach a maximum, memory ability is hardly functional. Short-term memory is reduced to almost nothing and patients may not be able to hold a conversation that would make sense to a normal person. Patients have lost even more semantic knowledge and episodic memories, which include almost all past information tied to events. Ability to encode long-term memories is largely gone as well. Many AD patients, with extreme symptoms, cannot even recognize names or faces of family members and other loved ones.

Hippocampus

It is widely accepted from previous studies that the hippocampus (HPC) is particularly associated with declarative memory tasks (Astur, Laughlin, Mamelak, Philpott, & Sutherland, 2002; Bohbot, Kalina, Stepankova, Petrides, & Nadel, 1998; Smith & Milner, 1981; Squire, 1992). When intact, the HPC works to provide a means by which new information can be encoded from short-term memory to long-term memory. Studies of amnesic patients show that severe amnesia is marked by significant damage to the HPC. In

general, as damage to the HPC increases so do the cognitive deficits associated with long-term memory and declarative tasks (Scoville & Milner, 1957). This trend follows the age-related cognitive decline continuum; patients with little or no damage to the HPC show mostly intact long-term memory, however, as seen in Alzheimer's patients, individuals with the most damage to the HPC show the most cognitive deficits.

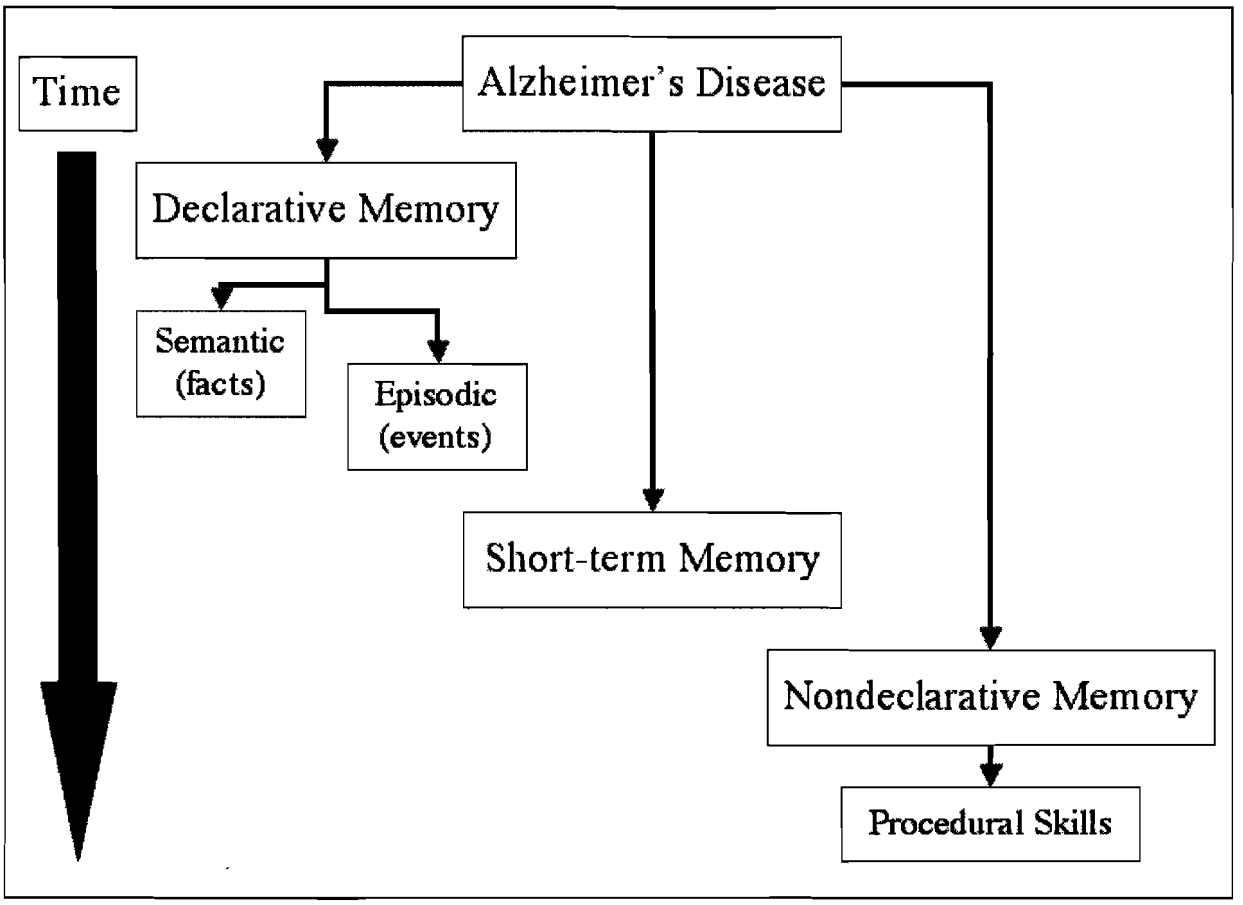

Illustration 1. The memory systems that Alzheimer's disease affects as it progresses. It is important to remember that once AD affects a certain memory system, the deficits seen in that system will continue to increase. For example, once AD impairs episodic memory it will continue to decline leading to instances where patients may not be able to recognize family members. This flowchart is only a generalization; it is not guaranteed that all AD patients will show cognitive impairments in this order.

Amnesic patients. Much of the information collected regarding the involvement of the HPC in declarative memory tasks in humans has been from patients with amnesia. Milner (1962) presented evidence from patient H.M. that the memory is dependent upon the medial temporal lobe. The medial temporal lobe, which includes the HPC, was removed from patient H.M. bilaterally in order to alleviate epileptic seizures. After removal of the medial temporal lobe in H.M., he was found to have severe amnesia and lacked virtually any long-term memory ability. Squire (1992) studied the effects of varying amounts of damage to the HPC in patients. In patients with more complete damage to the hippocampal formation, amnesia can be significant and may span across numerous decades, with sparing of very old memories. Hippocampal damage was also shown to affect both factual information and autobiographical, event-specific information.

Functional magnetic resonance imaging. Functional magnetic resonance imaging (fMRI) has allowed the role of the HPC in declarative memory tasks to be studied more extensively (Press, Amaral, & Squire, 1989). The increase in visual acuity allowed from the fMRI studies has shown shrinkage in brain areas (fimbria, dentate gyrus, hippocampus proper, and subiculum) of the HPC in patients with memory deficits (Squire, 1992). Another study showed that the medial temporal lobe was highly activated in persons while listening to their previously recorded autobiographical memories (episodic events), suggesting that the medial temporal lobe is required for episodic memory (Levine, Turner, Tisserand, Hevenor, Graham, & McIntosh, 2004). These studies have provided even more conclusive evidence that the HPC is predominately involved in declarative memory tasks.

Evidence from rat studies. Memory associated with the rat HPC follows that in humans, generally with more hippocampal damage leading to larger and more profound

cognitive deficits. The long-term declarative memory impairments similarly implicate the role of the HPC (Aggleton, Hunt, & Rawlins, 1986; Opal & Countryman, 2007; Sloan, Dobrossy, & Dunnett, 2006; Squire, Clark, West, & Zola, 2001). Studies have shown that, similar to human amnesic patients, bilateral lesions to the rat HPC have produced memory dysfunction (Barnes, 1988; Sutherland & Rudy, 1989). These studies implicate the extensive role of the HPC in memory formation, specifically with declarative tasks.

Acetylcholine

Acetylcholine (ACh) exists predominately in the peripheral nervous system and in brain tissue. It is synthesized within axon terminals from the precursors choline and acetyl coenzyme A (acetyl CoA) via a one-step reaction. Choline acetyltransferase (ChAT), an enzyme, aids in the joining of choline and acetyl CoA to form the neurotransmitter ACh. After fusing, ACh is released into the synaptic cleft and binds reversibly to receptors on the post-synaptic dendritic membrane. Two types of ACh receptors, muscarinic and nicotinic, exist in the brain. Excess ACh is then broken down for reuptake by the enzyme acetylcholine esterase (AChE).

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, Dean, Beer, & Lippa, 1982). Multiple 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, et al., 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). AD patients, in particular, have shown decreased levels of ChAT in the basal forebrain, which contains the medial septum, a main source of ACh to the HPC (Whitehouse, Price, Clark, Coyle, & DeLong, 1981; Whitehouse, Price, Struble, Clark, Coyle, & Delong, 1982).

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, Bassant, Senut, & Lamour, 1995; Everitt & Robbins, 1997; Sarter & Bruno, 2000). The present study focused on ACh transmission in the hippocampus because the receptors that ACh normally binds to within the HPC were effectively blocked through an injection of scopolamine.

Increased ACh release has been observed in a number of hippocampal-dependent tasks. For example, increased ACh release has been observed in working memory tasks (Fadda, Melis, & Stancampiano, 1996). Increases in ACh have also been found in tasks of visual discrimination (Yamamuro, Hori, Tanaka, Iwano, & Nomura, 1995). In rats, tasks that present a novel stimulus in a novel environment have increased release of ACh (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). These studies provide evidence for the

hypothesis that increased ACh levels are correlated to an increase in learning ability. Further evidence supports the link between decreased ACh levels and a decrease in memory ability.

The "cholinergic hypothesis". Impairments in the cholinergic system have been hypothesized to account for AD-type memory deficits (Bartus et al., 1982; Coyle, Price, & Delong, 1983). Describing a correlation of this magnitude must consider three criteria elaborated by Bartus et al. (1982):

> (i) specific dysfunctions in cholinergic markers should be found in the brains of subjects suffering from age-related memory loss (ii) artificial disruption of central cholinergic function in young subjects should induce behavioral impairments that mimic the cognitive loss found naturally in aged subjects and (iii) appropriately enhancing central cholinergic activity in aged subjects should significantly reduce age-related cognitive deficits. (p. 408)

Enhancement of the cholinergic system. Enhancement of the cholinergic system has been shown to decrease memory deficits (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 (Furushiro, 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, Durai, & Jobe, 1998).

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 HPC 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).

Impairment of the cholinergic system. There is substantial 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 HPC 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).

Deliberate impairments made to the cholinergic system in rat subjects have produced memory deficits similar to those seen in AD subjects (Carli, Luschi, & Samanin, 1997; Decker, Radek, Majchrzak, & Anderson, 1992; Farr, Flood, & Morley, 2000; Gold, 2003; Hagan, Salamone, Simpson, Iversen, & Morris, 1988; Lilliquist, Burkhalter, Lobaugh, & Amsel, 1993; Ohno, Yamamoto, & Watanabe, 1994; Wallenstein & Vago, 2001; Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996). Much of the work dealing with cholinergic impairment within the HPC has been limited to administration of cholinergic antagonists,

such as Scopolamine, or chemical lesions to the medial septum, a brain region where ACh neurons originate.

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).

Decreases in ACh resulting from lesions to the MS/VDB have been associated with decreases in hippocampal-dependent memory ability (Bartus et al., 1982; Opal & Countryman, 2007). For example, decreases in working memory have been found following lesion to the MS/VDB (Decker et al., 1992; Walsh et al., 1996). Also, many studies have shown that lesions to the rat MS not only result in decreased levels of ACh, but also decreased levels of AChE and ChAT in the HPC (Lewis, Shute, & Silver, 1967; Oderfeld-Nowak, Nariewicz, Bialowas, Dabrowska, Wieraszko, & Gradkowska, 1974; Potemska, Gradkowska, & Oderfeld-Nowak, 1975). This provides support for the link between ACh levels and subsequent levels of AChE and ChAT.

Furthermore, a memory-based place navigation task has displayed marked impairment following lesion to the MS/VDB in the rat (Hagan et al., 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 HPC (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.

Scopolamine. Scopolamine amnesia has often been proposed as a pharmacological model for human dementia and for AD (Bartus, Bean, Pontecorvo, & Flicker, 1985 as cited in Bertaina-Anglade, Enjuanes, Morillon, & Drieu la Rochelle, 2006). Scopolamine works by blocking ACh receptors in the brain. Since the HPC is a brain structure central to learning and memory, which contains a large concentration of ACh receptors, it makes sense that scopolamine would induce large memory impairments. These impairments are likely due to an inability to encode new information and store it as a long-term memory (Rogers & Kesner, 2003). Scopolamine is of particular interest for the present study, as it will serve to impair ACh functioning in the HPC and, therefore, also impair memory ability.

The muscarinic receptor antagonist, Scopolamine, has been shown to effectively and temporarily impair memory ability in humans and rats (Bertaina-Anglade et al., 2006; Boix-Trelis, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2006; Fujiwara, Ohgami, Inada, & Wasaki, 1996; Rammsayer, Rodewald, & Groh, 1999; Rogers & Kesner, 2003; Winters, Saksida, & Bussey, 2006). Two specific tasks, which have been impaired by Scopolamine, the social transmission of food preference task and the object recognition task are of particular interest, as these tasks will be utilized as measures of memory ability in the present study.

Acetylcholine esterase inhibitors. Acetylcholine esterase inhibitors (AChE-Is) have been the most promising pharmacological treatment of memory impairments seen in AD

patients. AchE-Is work to increase the availability of ACh in the brain by preventing the hydrolysis of ACh from the enzyme acetylcholine esterase (Prickaerts, Sik, van der Staay, de Vente, & Blokland, 2005; Rogers, 1998). Since ACh receptor activity in the HPC and MS has been shown to increase memory performance, AchE-Is have been utilized in the AD population to aid in delaying memory impairments (Delagarza, 2003; Kumar et al., 1998; Rogers, 1998). Currently, there are four AchE-Is that have been approved by the Food and Drug Administration for use in AD: Tacrine (Cognex), Rivastigamine (Excelon), Donepezil (Aricept), and Galantamine (Reminyl).

Despite the memory improvements seen with the use of AchE-Is, many problems with this pharmacological treatment of AD exist. First, the inhibition of ACh hydrolysis causes serious side effects in AD patients. Side effects of AchE-Is include: nausea, anorexia, aggression, liver toxicity, and cardiovascular irregularities (Lazartigues, Freslon, Telliogu, Brefel-Courbon, Pelat, Tran, Montastruc, & Rascol, 1998). Second, 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). Finally, 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 levels of ACh continue to decrease, cholinergic AchE-I therapies decrease drastically in effectiveness (Lazartigues et al., 1998; Mann, Yates, & Hawkes, 1982). Additionally, pharmacological treatment of AD symptoms with AchE-Is only remains effect for one to two years at most. The inhibition of AChE works while there is ACh still present in the brain but once ACh is nearly depleted, as AD progresses, the inhibition of AChE has no effect.

Norepinephrine

One reason cholinergic treatments are not more effective in ameliorating deficits in AD patients is that AD is not solely a cholinergic insufficiency. AD is a very complex illness and is likely to induce impairments in multiple neurotransmitter pathways (D'Amato, Zweig, Whitehouse, Wenk, Singer, & Mayeux, 1987; Herrmann, Lanctot, & Khan, 2004).

The norepinephrine (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. The dorsal bundle is of particular interest in the present manipulation because it projects directly into the hippocampus. The locus coeruleus (LC) is the predominant brain structure that provides the main source of NE to the HPC. In fact, fibers projecting to the HPC from the LC pass through the MS, possibly implicating an interaction between ACh and NE (Loy, Milner, & Moore, 1980).

Locus coeruleus. 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 innervates roughly every component of the telencephalon and the diencephalon, the most notable of which is the HPC. 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 include the dentate gyrus of the hippocampus and the substantia nigra pars

reticulata (Herrmann et al., 2004). In general, α-receptors are found in the central nervous system and β-receptors are found in the peripheral nervous system.

Norepinephrine and Alzheimer's disease. While degradation of the ACh pathway has been studied extensively in AD patients, the NE neurotransmitter pathway has been investigated less thoroughly. However, several studies indicate that the NE neurotransmitter pathway may be involved in AD as decreased levels of NE have been noted in AD patients (Bondareff, Mountjoy, & Roth, 1981; Mann, Lincoln, Yates, Stamp, & Toper, 1980; Mann et al, 1982). Therefore, a shortage of NE from the LC might substantially contribute to the lack of memory function associated with AD (Bondareff et al., 1981; Bondareff, Mountjoy, & Roth, 1982; Chanpalay, 1991). These studies provide evidence for the interaction of the ACh and NE neurotransmitter systems in AD patients and memory ability in general.

Marked decreases of noradrenergic neurons in the LC have been well established in patients with age associated cognitive impairments, specifically in AD (Hermann et al., 2004; Hoogendijk, Feenstra, Botterblom, Gilhuis, Sommer, & Kamphorst, et al., 1999; Mann, 1983; Szot, White, Greenup, Leverenz, Peskind, & Raskind, 2005). Furthermore, the biggest decrease in NE neurons has been seen in the center of the LC (Marcyniuk, Mann, & Yates, 1986). This evidence is significant because the central part of the LC is thought to project to the HPC.

Norepinephrine and acetylcholine. The evidence above shows that NE and ACh neurotransmitter systems interact in the modulation of memory formation within the HPC. Decker & McGaugh (1991) have presented anatomical details of such an interaction (see Illustration 2). Results have shown that blockade of either system lead to memory impairments (Sirvio & MacDonald, 1999). Many studies have also shown that ACh and NE

modulate each other's release within the HPC (Azam & McIntosh, 2006; Moroni, Tanganelli, Antonelli, Carla, Bianchi, & Beani, 1983). For example, stimulation of NE receptors in the MS produce increases of ACh in the HPC (Robinson, Cheney, & Costa, 1978). This interaction is especially helpful in explaining the role of NE in the AD population.

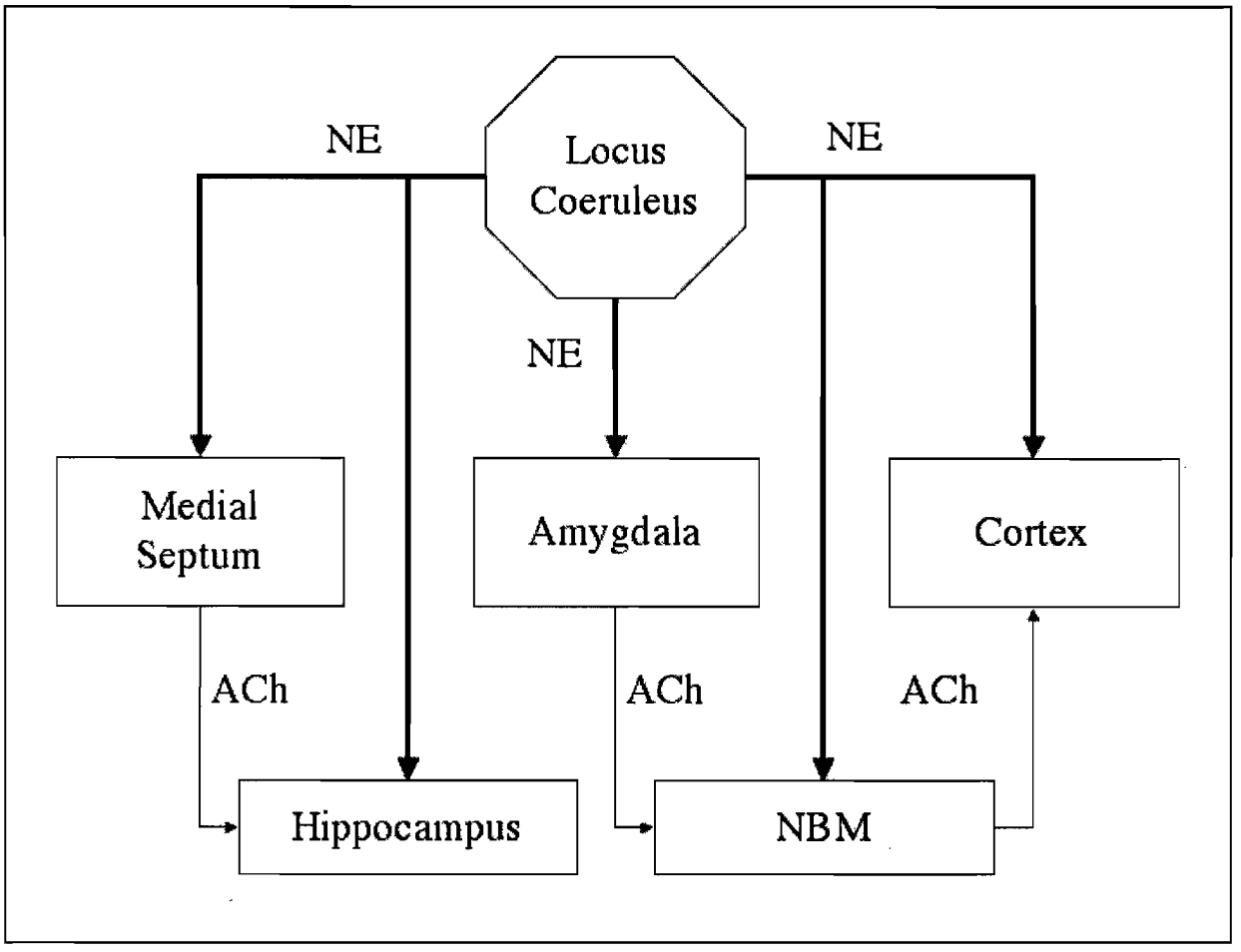

Illustration 2. Hypothetical model of sites at which cholinergic and noradrenergic interactions might occur. Darker lines indicate norepinephrine projections while thin lines indicate acetylcholine projections. NBM refers to the nucleus basilis magnocellularis.

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. In conclusion, pharmacological agents merely targeting the ACh system may be one reason cholinergic therapies within the AD populace are ineffective.

Norepinephrine agonists. 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, et al., 1999; Rama, Linnankoski, Tanila, Pertovaara, & Carlson, 1996; Sirvio, Riekkinen, Vajanto, Koivisto, & Reikkinen, 1991). In the present experiment, one such agonist, Guanfacine, was administered to rats in an attempt to improve memory impairments induced by Scopolamine.

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; Arnsten & Cai, 1993). Guanfacine has shown similar effective results in humans, improving performance on working memory tasks (Jakala et al., 1999).

The NE agonist, Guanfacine, is an exciting, novel pharmacological agent that may be useful in the treatment of AD-type memory deficits. Studies involving the administration of Guanfacine after Scopolamine induced memory impairments on a socially transmitted food preference task and an object recognition task are nonexistent. The present study allowed for new conclusions to be made on the interaction between the NE and ACh transmitter pathways in memory and AD.

Current Study

Object recognition task. The object recognition task (ORT) was also used as a quantification of memory performance in the current study. The object discrimination task has been utilized as a marker for memory performance in numerous studies (Bertaina-Anglade et al., 2006; Bowman, Maclusky, Diaz, Zrull, & Luine, 2006; Silvers, Harrod, Mactutus, & Booze, 2007; Tzavara, Bymaster, Overshiner, Davis, Perry, Wolff et al., 2005). The ORT has often been a reliable and popular method for its ability to test memory performance without putting undue stress on an animal (Silvers et al., 2007). Furthermore, the task places few requirements on the animal and allows the experimenter to directly observe and record behavior. Also, scopolamine has been shown to impair long-term memory formation on the object recognition task (Bertaina-Anglade et al., 2006). Overall, it is a task that is easy to train and test on in one day. In the current study, it also served as a confirmation for memory abilities of rats on the STFP task. Explanation of the ORT that was used in this study can be found under the methods section.

Social transmission of food preference task. In order to quantify memory performance, a socially transmitted food preference task (STFP) was 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 (Countryman & Gold, 2007).

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, Baxter, & Eichenbaum, 2002). For example, an observer rat having been presented cocoa flavored chow via a demonstrator rat will exhibit preference to this flavored food for periods extending over one month (Clark, Broadbent, Zola, & Squire, 2002). This suggests that the rat has formed a declarative memory for cocoa-flavored chow. In relation to the present study, administration of scopolamine prior to training on STFP task has been shown to impair long-term memory formation (Boix-Trelis et al., 2006).

Studies have shown increases in CREB phosphorylation and c-Fos expression in the HPC during a STFP task (Countryman & Gold, 2007; 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-like" memory formation. Explanation of the STFP task that was used in this study can be found under the methods section.

Summary and implications. After a review of the literature it is clear that the hippocampus is extensively involved in declarative memory tasks. The decrease in neurotransmitter acetylcholine has been shown to impair memory formation within the hippocampus. Reductions in acetylcholine levels within the hippocampus and medial septum have been observed in Alzheimer's disease patients. Scopolamine is an effective method by which to block acetylcholine receptors in the hippocampus and impair memory performance. Reductions in levels of the norepinephrine neurons in the center of the locus coeruleus have been associated with Alzheimer's disease. Numerous studies have, therefore, implicated the possible interaction of the acetylcholine and norepinephrine neurotransmitter systems in age associated cognitive impairments and Alzheimer's disease. Norepinephrine agonists, particularly the alpha-2 noradrenergic agonists have shown to increase performance on a number of memory-related tasks in aged animals and humans. However, few studies have examined the effects of norepinephrine agonists on Alzheimer's-like memory impairments. Furthermore, no studies have investigated the effect of Guanfacine on long-term declarative memory after induced memory impairments by the administration of Scopolamine. Therefore, the present study measured the effects that two different doses of Guanfacine, a high dose and a low dose, had on memory performance in an object recognition task and the effects that a low dose of Guanfacine had on memory performance in a socially transmitted food preference task following administration of Scopolamine to the rat.

Hypotheses. Numerous hypotheses exist for the current study (see Table 1). The main hypotheses for the present study include implications of Scopolamine administration on

memory ability compared to saline treated rats. The implications of Guanfacine, a high dose and low dose, on memory ability in both scopolamine and saline treated rats are also listed. Overall, it is predicted that Scopolamine will effectively impair memory ability on both the STFP task and the ORT and that Guanfacine will reverse these memory impairments. Also, it is hypothesized that rats administered saline and treated with Guanfacine will have higher memory ability than at baseline.

Table 1

Summary of hypotheses

1. Rats administered Scopolamine prior to training on the STFP task and the ORT and treated with saline after training will show significant memory impairments during recall.
2. Rats administered Scopolamine prior to training on the STFP task and the ORT and treated with a low dose of Guanfacine after training will perform better during recall than rats administered Scopolamine and treated with saline.
3. Rats administered Scopolamine prior to training on the STFP task and the ORT and treated with a high dose of Guanfacine after training will perform better during recall than both rats administered Scopolamine and treated with a low dose of Guanfacine and rats administered Scopolamine and treated with saline.
4. Rats administered saline prior to training on the STFP task and ORT will perform better than baseline during recall when treated with Guanfacine after training on the STFP task and ORT.

Methods

Subjects

Subjects for this study were twenty-four Long-Evans male rats, purchased from Harlan (Indianapolis, IN). During the course of the experiment, twelve rats served as demonstrator rats (d-rats) and twelve rats served as subject rats (s-rats/experimental rats). Rats were maintained on a 12 hour light-dark schedule and in a humidity and temperature-controlled environment. Throughout this study, rats were pair-housed, based on d-rat or s-rat status, in 42 x 24 x 27 cm plastic bottom cages.

Food and water were available *ad libitum* except during the five days prior to each socially transmitted food preference training session. During this time, rats were placed on a 22-hour food deprivation schedule with *ad libitum* water. All 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. Following arrival in the lab and prior to any behavioral testing, all rats were allowed to habituate to surroundings for one week, throughout which they were handled for five minutes a day.

Object recognition task

An object recognition task was first utilized following similar procedures as Tzavara et al. (2006). S-rats were trained on the task and were then tested on the task following a 3-hour delay. Each rat was placed in the center of a 64 x 33 x 41 cm wooden observation box containing two identical objects (noted as object A) at either end of the box. Each s-rat was allowed to explore the objects for 3 minutes and the time spent interacting with each object, designated right or left, was recorded. For this task, interacting was defined as sniffing at, whisking at, gnawing at, or looking at the object from no more than 2 cm away. Behavior

not oriented to the objects (accidental sitting, standing on the object, or touching the object while passing by) was not quantified. Following the 3-hour delay period, s-rats were returned to the box one by one. Each rat was placed in the same center location of the box as in the training session. For the testing trial, the box contained a familiar object (object A) and a novel object (object B). To account for right or left side biases, object A and object B were counterbalanced and randomly switched to the opposite side of the box between every few test subjects. The amount of time spent interacting with each object, either familiar or novel, was recorded for three minutes. Different combinations of objects (wooden blocks, Lego blocks, cups, small tin flower pots with marbles, etc.) were used for each training/testing session (see Figure 3).

Social transmission of food preference task

The social transmission of food preference (STFP) task followed similar procedures as those utilized by Countryman, Orlowski, et al., (2005). D-rats and s-rats were placed on 23-hour food deprivation and were only allowed to eat in one-hour blocks five days prior to training on the task. Water was continually available *ad libitum* throughout all food deprivation and testing procedures. On the day prior to training, each d-rat and each s-rat were situated in opposite sides of an approximately 42 x 24 x 27 cm plastic bottom interaction cage. The cage was equipped with corncob bedding and a wire-mesh screen dividing the apparatus into half. Each d-rat was housed on one side of the screen and each s-rat was housed on the other side.

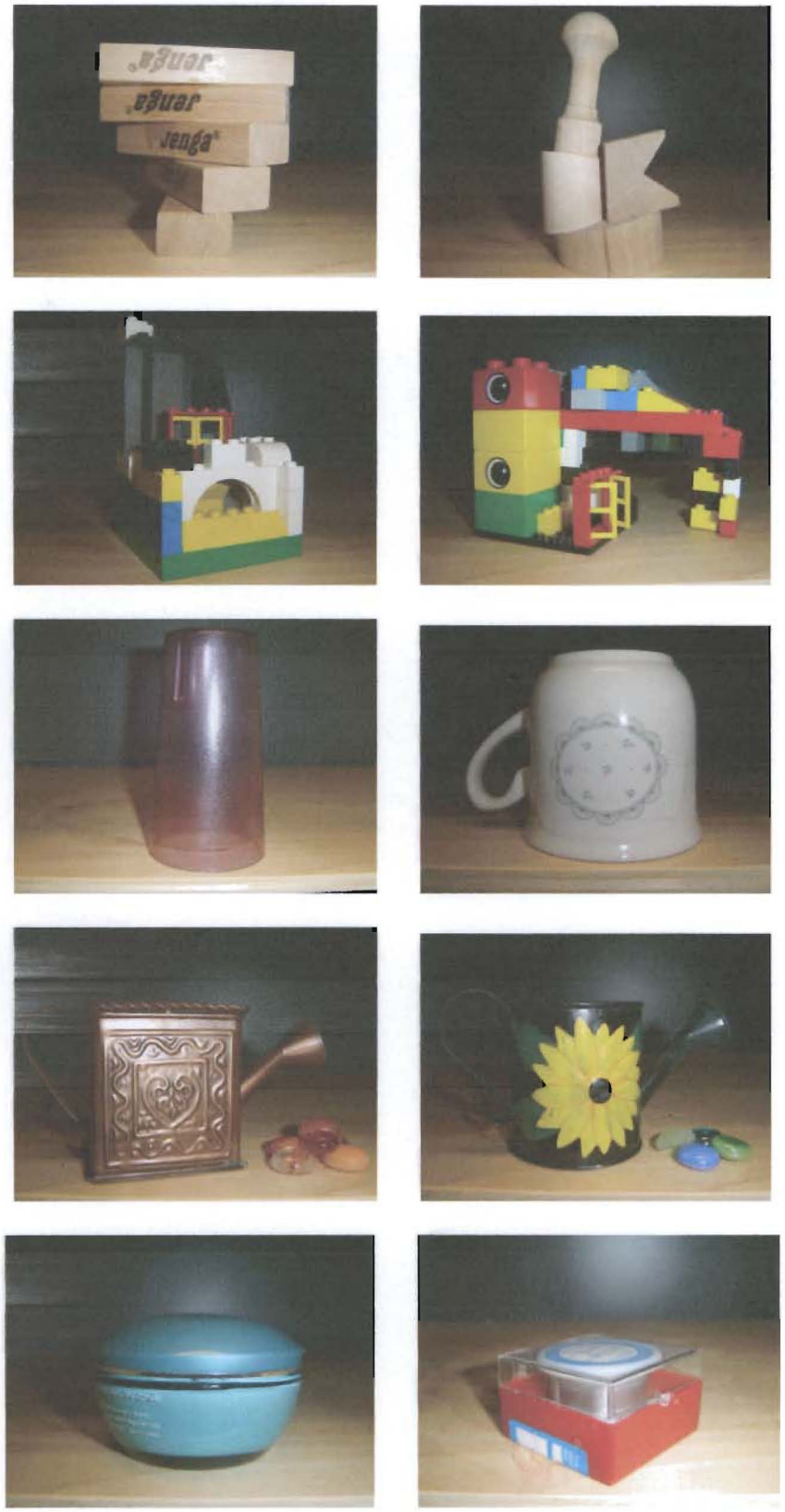

Figure 3. Pairs of objects used for object recognition task.

During training on the STFP task, d-rats were taken from the interaction apparatus and placed into an isolated room. In this location, d-rats were allowed to eat for 30 minutes from approximately 10 g of flavored rat chow. Ground rat chow was mixed with specific flavors in accordance with a food pair chart (see Table 2). D-rats were permitted to drink water whenever food was present. After 30 minutes passed, d-rats were returned to their individual interaction cage and allowed to interact for 30 minutes with s-rats through the wire-mesh divider in a training/learning session. Immediately following the 30-minute interaction period, d-rats and s-rats were returned to home cages and fed unflavored rat chow. After 5 days in home cages, s-rats were taken to a novel room and tested for food preference. Following food preference testing, d and s-rats were returned to normal food and water available *ad libitum*.

The testing portion of the food preference task took place in a 42 x 24 x 27 cm plastic bottom cage. Two food cups were available, one on either side of the cage. The cups contained approximately 10 g each of separate flavored rat chow (see Table 2). S-rats were placed in the middle of the cage so that they were equidistant from each food cup. Each s-rat was permitted to eat from the food cups for one hour with water available for the entire hour. Following the one-hour feeding period, the amount of remaining food for each flavor was weighed. The amount of each flavored food consumed during the food preference task was used to calculate the percentage of demonstrated food consumed according to the following criteria: (1) (Demonstrated food offered – Demonstrated food remaining) + (Novel Food offered – Novel food remaining) = Total food consumed; (2) [Demonstrated food consumed (g)/Total food consumed (g)] x 100 = Percentage of demonstrated food consumed.

The STFP task was administered four times in the current study. For each of these administrations, a different food pairing was used (see Table 2). Each food pair served to train and test all twelve s-rats. The first six s-rats tested will be demonstrated one of the flavored rat chows from a food pair and the next six s-rats will be demonstrated the opposite flavored rat chow in the food pair.

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

Table 2. Food pairs. This is a representation of food pairs used throughout the STFP task. Either FOOD A or FOOD B in a certain food pair was demonstrated to d-rats. S-rats were then 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 is presented during each STFP trial period.

Procedural Timeline

A procedural timeline that encompassed five experiments (treatment conditions) for the ORT (see Table 3) and four experiments (treatment conditions) for the STFP (see Table 4) assessed memory ability for all s-rats. First, baseline measures of s-rats were collected. Afterwards, three experiments effectively investigated the effects of Scopolamine and Guanfacine administration on the memory ability of s-rats on the ORT and two experiments investigated the effects for the STFP task. S-rats were divided into two groups for each

experiment. Both groups of s-rats received the same doses of Scopolamine and Guanfacine, both a high and low dose for the ORT and a low dose for the STFP task, but during different experiments. This served as an effective counterbalancing method. The final experiment (treatment condition) for the ORT and STFP task investigated the effects of Guanfacine alone on memory performance by utilizing saline instead of Scopolamine. Saline injected rats received the overall most effective dose of Guanfacine, either high or low, administered in the previous experiments for the ORT and received the low dose of Guanfacine in the STFP task since the high dose was not tested.

A few implications of Scopolamine and Guanfacine should be discussed further. The dose of Scopolamine and saline given in all experiments were 1 mg/kg. The high and low doses of Guanfacine were 0.375 mg/kg and 0.125 mg/kg, respectively. All drugs were administered to s-rats via injection into the intraperitoneal cavity. Since Scopolamine and Guanfacine act on separate neurotransmitter pathways, it is thought that no interaction effects occurred. Also, the half-lives of Scopolamine and Guanfacine are short enough that no carryover effects are thought to have occurred between experiments. The details of the experiments are discussed below.

In each of the experimental treatment conditions that require pharmacological agents (therefore, excluding baseline conditions) Scopolamine (or saline in the final experiments) was administered to s-rats five minutes prior to training on the ORT and the STFP task. Training for the ORT refers to the placement of rats in the box for exposure to the "familiar" objects. The STFP task training refers to the 30-minute interaction period between d- rats and s-rats. Guanfacine, either high or low dose, (or saline) was administered to s-rats 30 minutes after training in both the ORT and STFP task.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Group 1	Baseline	Scopolamine + Saline	Scopolamine + Guanfacine (Low)	Scopolamine + Guanfacine (High)	Saline + Guanfacine (High)
Group 2	Baseline	Scopolamine + Guanfacine (Low)	Scopolamine + Guanfacine (High)	Scopolamine + Saline	Saline + Guanfacine (High)

Table 3. Procedural Timeline. Representation of administered drugs for each group of s-rats during each experimental condition of the ORT. Each experiment includes training (first listed drug) and testing (second listed drug) on the task. High/Low denotes whether Guanfacine administered is a high dose or a low dose.

	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Group 1	Baseline	Scopolamine + Saline	Scopolamine + Guanfacine (Low)	Saline + Guanfacine (Low)
Group 2	Baseline	Scopolamine + Guanfacine (Low)	Scopolamine + Saline	Saline + Guanfacine (Low)

Table 4. Procedural Timeline. Representation of administered drugs for each group of s-rats during each experiment of the STFP task. Each experiment includes training (first listed drug) and testing (second listed drug) on the STFP.

Results

Object Recognition Task

A repeated measures (rm) ANOVA was used to determine the amount of time rats spent with the novel object under each of the drug conditions (baseline, Scopolamine + saline, Scopolamine + low dose Guanfacine, Scopolamine + high-dose Guanfacine, and Saline + high dose Guanfacine). The rm ANOVA revealed that the time rats spent with the novel object differed significantly across drug treatments, $F(4, 44) = 3.3060$, $p < .05$. Fisher's LSD was performed to determine where the differences occurred. Results showed that Scopolamine + saline caused memory impairment as compared to baseline. Also, when Guanfacine (high or low dose) was administered in conjunction with Scopolamine, memory impairments were reversed in comparison to treatment with Scopolamine alone, all p values $< .05$ (see Figure 1). The saline + Guanfacine treatment condition did not significantly differ from any of the other treatment conditions, all p values $> .05$ but indicated a moderate decrease in memory performance from baseline, Scopolamine + low dose Guanfacine, Scopolamine + high dose Guanfacine conditions, and better memory performance than the Scopolamine + saline condition (see Figure 4). T-tests for independent samples were conducted in order to compare the results of the object recognition task, across conditions, to the 50% chance level. Baseline measures as well as Scopolamine + low dose Guanfacine and Scopolamine + high dose Guanfacine conditions were shown to differ significantly from chance levels, $t(11) = -7.254$, $t(11) = -3.064$, $t(11) = -2.254$, all p values $< .05$. Scopolamine + saline and Saline + high dose Guanfacine conditions were not shown to differ significantly from chance levels.

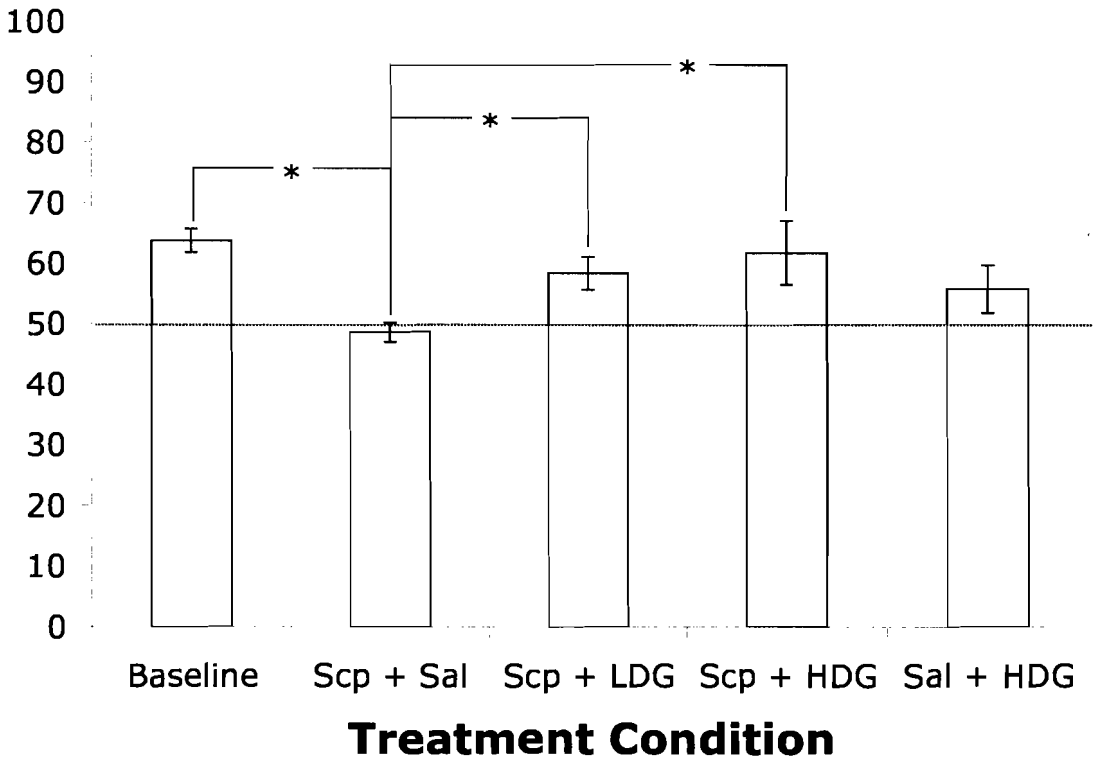

Figure 1. Object Recognition Task Results. Dotted line represents chance levels and asterisks represent significance.

Social Transmission of Food Preference Task

A repeated measures ANOVA was used to determine the percentage of demonstrated food consumed by rats under each drug treatment condition (baseline, Scopolamine + saline, Scopolamine + low dose Guanfacine, and Saline + low dose Guanfacine). The rm ANOVA revealed that the percentage of demonstrated food consumed by rats significantly differed across treatment conditions, $F(3, 33) = 8.8616$, $p < .05$. Fisher's LSD was performed to determine where the differences occurred. Results of this analysis showed that Scopolamine + saline and saline + low dose Guanfacine conditions caused memory impairments compared to baseline and Scopolamine + low dose Guanfacine conditions, all p values $< .05$ (see Figure

2). Independent samples t-tests were conducted in order to compare the results of the social transmission of food preference task, across conditions, to chance levels. Baseline and Scopolamine + low dose Guanfacine conditions were shown to differ significantly from chance, t (11) = -12.95 and t (11) = -2.83, both p values < .05. The Scopolamine + saline and saline + low dose Guanfacine conditions were not shown to differ significantly from chance levels.

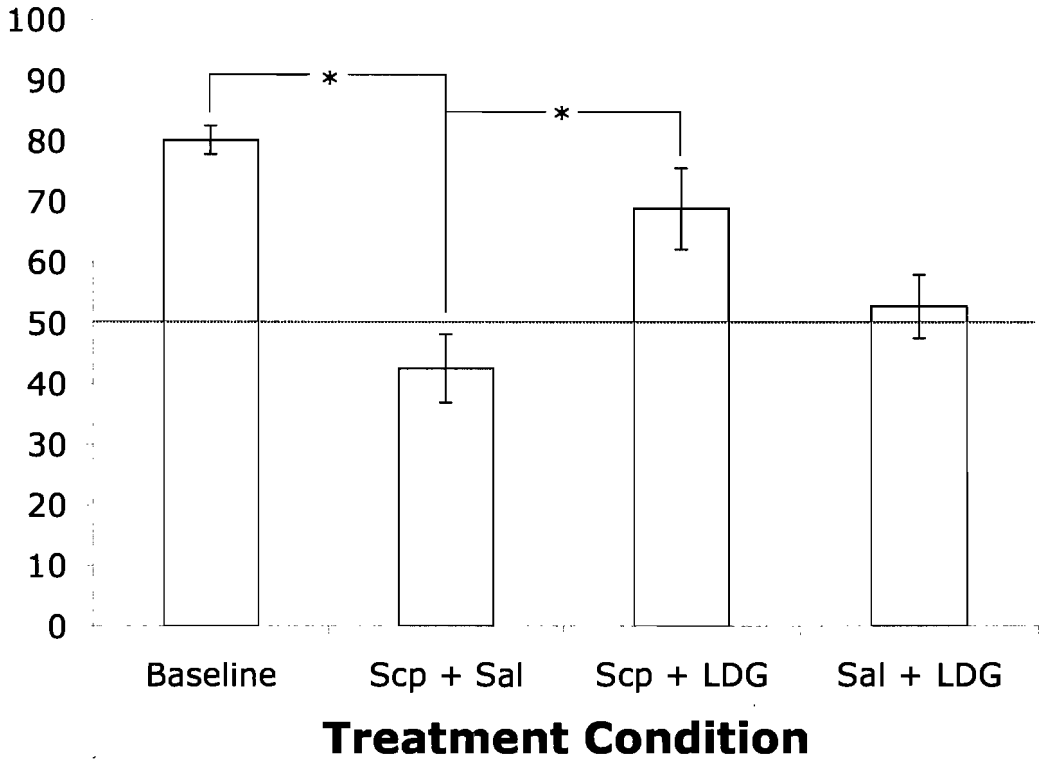

Figure 5. Social Transmission of Food Preference Task Results. Dotted line represents chance levels and asterisks represent significance.

A repeated measures ANOVA was conducted to determine the total amount of food eaten by rats across treatment conditions (baseline, Scopolamine + saline, Scopolamine + low dose Guanfacine, and saline + low dose Guanfacine) during food preference testing. A

significant effect was found, where the total amount of food eaten by rats differed significantly across conditions, $F(3, 33) = 3.4679$, $p < .05$. Fisher's LSD was conducted to determine the differences occurred. Results showed that significantly lower amounts of total food were consumed by rats under the Scopolamine + saline, Scopolamine + low dose Guanfacine, and saline + low dose Guanfacine conditions compared to the baseline condition, all p values $< .05$ (see Figure 3).

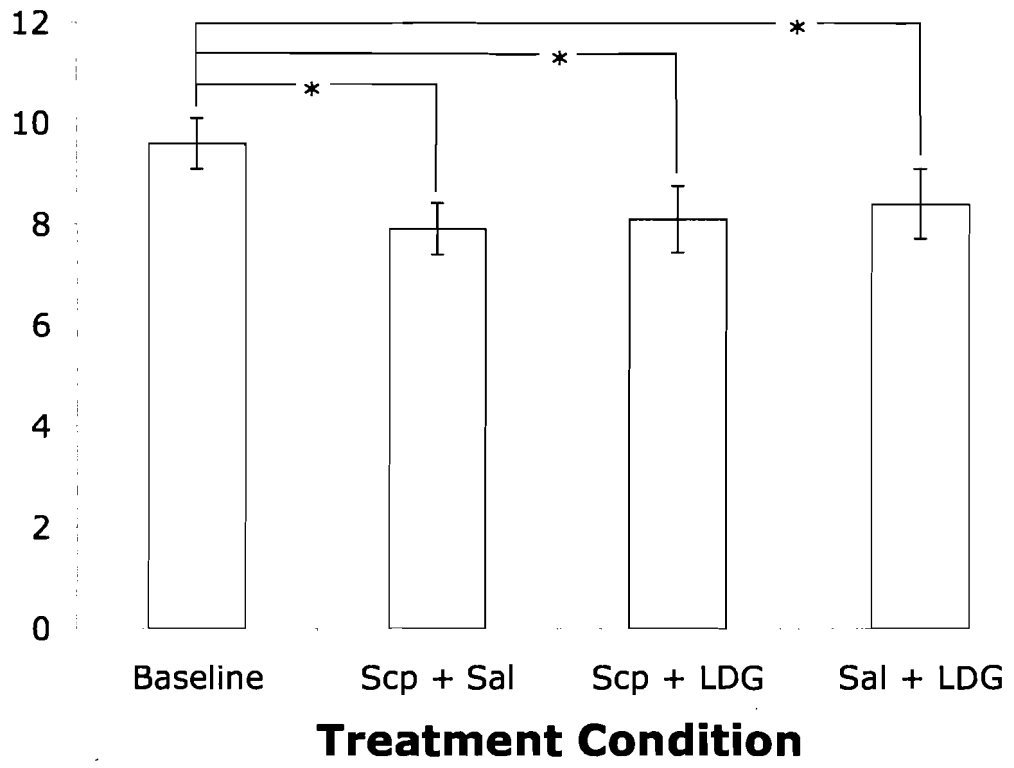

Figure 3. Total Amount of Food Consumed. Asterisks represent significance.

Discussion

Administration of the cholinergic antagonist, Scopolamine, was utilized in the present study in order to investigate changes on two hippocampal-dependent memory tasks: the object recognition task and the social transmission of food preference task. Therefore, reductions in performance on both memory tasks were expected following injection of Scopolamine prior to training. Following injection of Scopolamine and training on each of the tasks, injection of the norepinephrine agonist, Guanfacine, was employed in order to examine any increased memory performance from memory-impaired levels. Guanfacine administration was expected to increase memory performance by compensating for decreased acetylcholine levels within the hippocampus.

Summary of Results

Object recognition task. Rats injected with Scopolamine prior to training on the ORT and treated with saline after training on the tasks showed impaired memory performance compared to baseline conditions. In the ORT, quantification of memory ability showed that rats treated with Scopolamine and saline explored a novel object 48% of the time compared to baseline levels when rats spent 63% of the time exploring the novel object. This indicates that rats performed no better than chance. Rats injected with Scopolamine prior to training and treated with a low dose of Guanfacine after training showed an improvement in memory ability during testing. Rats in this condition spent 58% of the time with a novel object compared to the 48% observed under the Scopolamine and saline condition. When rats were treated with a high dose of Guanfacine instead of a low dose, memory ability was still significantly better compared to the Scopolamine and saline condition. A high dose of Guanfacine resulted in slightly better performance on the ORT (61% compared to 58%) but

this improvement was not significantly different than the Scopolamine + low dose Guanfacine condition. Under the final condition, which investigated the effects of a high dose of Guanfacine alone, no significant findings were found. Despite the lack of significance, rats performed at a lower level during testing than in baseline conditions as well as the other two Guanfacine conditions but still performed better than in the Scopolamine and saline condition.

Social transmission of food preference task. In the STFP task, rats injected with Scopolamine prior to training and saline after training showed impaired memory performance compared to baseline conditions. Quantification of memory performance showed that of the total amount of food rats in the Scopolamine and saline condition consumed, only 42% was the demonstrated food compared to the 80% that was demonstrated food in baseline conditions. This indicates that rats performed no better than chance. When rats treated with Scopolamine prior to training were treated with a low dose of Guanfacine after training memory performance was observed to be better than in the Scopolamine and saline condition. Rats in this condition consumed 69% of the demonstrated food compared to the 42% consumed in the Scopolamine and saline condition. However, performance was not as high as in baseline conditions. In the final condition, in which saline was administered prior to training and a low dose of Guanfacine was administered after training, no significant effects were found. It should be noted that despite lack of significance, rats did perform at a lower level than in baseline and Scopolamine + Guanfacine conditions. This is a point that will later be discussed further. Additionally, during the STFP task it was found that rats consumed significantly less amounts of total food under all drug treatment conditions compared to baseline conditions.

Effects of Scopolamine on Memory

Object recognition task. In line with previous research, it was expected that Scopolamine would impair memory performance on the ORT in rats. Numerous studies support the findings of the current study (Azmi, Norman, Spicer, & Bennett, 2006; Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006; Dodart, Mathis, & Ungerer, 1997; Rutten, Prickaerts, & Blokland, 2006; Winters et al., 2006). However, contrary to the current study, one study investigated the effects of Scopolamine on the ORT via direct infusion into the brain (Winters et al., 2006). The current study utilized a methodology in which Scopolamine was injected via the intraperitoneal cavity. Various studies have found similar memory impairing effects of Scopolamine on the ORT via this route (Azmi et al., 2006; Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006, Rutten et al., 2006).

Numerous differences exist in the methodologies of research that supports Scopolamine-induced memory impairments on the ORT. Three major ways in which methodologies differ is in the dose of Scopolamine administered, the time it was administered prior to training, and the time delay used in the ORT. Scopolamine has been shown effective at inducing memory deficits at doses of 0.1 mg/kg (Bertaina-Anglade et al., 2006), 0.3 mg/kg (Bertaina-Anglade et al., 2006; Dodart et al., 1997), 0.63 mg/kg (de Bruin & Pouzet, 2006), 1 mg/kg (Bertaina-Anglade et al., 2006, Dodart et al., 1997), and 3 mg/kg (Dodart et al., 1997). Additionally, Winters et al. (2006) effectively impaired memory performance on the ORT using a 10-mg/ml concentration of Scopolamine infused over 2 minutes to each hemisphere of the brain for a total of 1 μl per hemisphere. The current study utilized a dose of 1 mg/kg of Scopolamine to effectively impair memory on the ORT. It is

clear that a wide variety of Scopolamine doses are effective at inducing memory impairments on the ORT.

The current study utilized a three-hour time delay in between training and testing on the ORT. One study has found similar results in regards to the decline of memory performance from Scopolamine after a three-hour delay between training and testing on the ORT (Dodart et al., 1997). Other studies utilizing Scopolamine and the ORT have shown declined memory performance at delay intervals of one hour (Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006; Rutten et al., 2006), four hours (de Bruin, et al., 2006), up to 20 hours (Winters et al., 2006), and 24 hours (de Bruin et al., 2006). In all cases, rats with Scopolamine-induced memory deficits showed significantly lower memory ability compared to rats without Scopolamine-induced deficits on the same time delay. Additionally, most studies were consistent with the current study in that Scopolamine was administered 30 minutes prior to training on the ORT (Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006; Rutten et al., 2006).

Social transmission of food preference task. No studies have investigated the effects of Scopolamine injected via the intraperitoneal cavity on memory performance in a STFP task. It was expected that Scopolamine would induce significant memory impairment during the testing phase of the STFP based on previous findings of Scopolamine on similar hippocampal-dependent memory tasks (Azmi et al., 2006; Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006; Dodart et al., 1997; Rutten et al., 2006; Winters et al., 2006). One study has utilized the STFP and found similar declined memory ability from Scopolamine administration as the current study; however, the methodology used is significantly different. Boix-Trelis et al. (2007) infused Scopolamine directly into the prelimbic cortex, a component

of the medial prefrontal region. The drug was bilaterally injected at a dose of 20 μg per site prior to training. Rats showed a severe impairment in performance on the STFP task measured in two retention sessions, both immediately and 24 hours after training (Boix-Trelis et al., 2007). In accordance with the findings of the current study, Scopolamine did significantly impair memory performance on the STFP task in both studies.

In light of the results from Boix-Trelis et al. (2007) it is possible that some of the effects seen on memory performance in the current study may stem from blocking muscarinic cholinergic receptors in the prefrontal cortex. Since the current study injected Scopolamine via an intraperitoneal route, there is no way to verify exactly what muscarinic cholinergic receptors were being blocked in various areas of the brain. Various methods, such as chemical lesion or injection of a cholinergic muscarinic antagonist, of reducing cholinergic transmission in the prefrontal cortex and associated areas have been used to show decreased memory performance on working memory tasks and visual attention tasks (Chudasama, Dalley, Nathwani, Bougher, & Robbins, 2004). Similarly, the areas of the prefrontal cortex have been implicated in several functions necessary for relational learning such as behavioral flexibility (Dias, & Aggleton, 2000; Ragozzino, Detrick, & Kesner, 1999; Ragozzino, Kim, Hassert, Minniti, & Kiang, 2003), working memory and attention (Dalley, Cardinal, & Robbins, 2004), and the organization and expression of adaptive behavior in novel circumstances (Gisquet-Verrier & Delatour, 2006).

The current study attempted to impair cholinergic transmission in the hippocampus by blocking muscarinic cholinergic receptors in order to mimic acetylcholine deficiencies seen in AD. Results of the STFP, a hippocampal-dependent memory task, suggest that cholinergic receptors in the hippocampus were effectively blocked. However, previous

studies have shown declined memory performance on the STFP from decreased acetylcholine transmission in the prefrontal cortex (Boix-Trelis et al., 2007). Such findings indicate a possibility that some of the memory deficits observed in the current study could be due to similar effects. In such a case, the study would still be considered and effective model of Alzheimer's disease since it is generally known that a decrease in ACh function exists in AD as well.

It is still probable that much of the impairment seen on the STFP task in the current study is due to muscarinic cholinergic blockages in the hippocampus and not the prefrontal cortex. Many studies have shown that the hippocampus and related areas are necessary for the delayed recall of the STFP task (Bunsey & Eichenbaum, 1995; Clark, Broadbent, Zola, & Squire, 2002; Countryman et al., 2005; Roberts & Shapiro, 2002; Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001). In line with this previous research, the current study utilized a five day delay in between training and testing on the STFP task. This lengthy delay, which highly implicates long-term memory and thus the hippocampus, is indicative of reduced cholinergic transmission in the hippocampus.

The only other study to use Scopolamine-induced memory deficits on the STFP task used a shorter inter-trial delay than the current study. Boix-Trelis et al. (2007) utilized a 24 hour delay to show the negative effects of Scopolamine on memory. Since the current study tested a delay of five days, it was expected that similar impaired memory performance would be found. Many studies, using other memory tasks, have also utilized relatively short delay periods to test the effects of Scopolamine (Azmi et al., 2006; 2006Beninger, Jhamandas, Boegman, & el-Defrawy, 1986; Bertaina-Anglade et al., 2006; de Bruin & Pouzet, 2006; Dodart et al., 1997; Rutten et al., 2006; Winters et al., 2006). The current study was unique

in the length of delay between trials but was still consistent with studies that utilized a four or five day inter-trial delays to test the effects of Scopolamine on memory performance in the Morris Water Maze task (Chalas & Conway, 1996; Herrera-Morales, Mar, Serrano, & Bermudez-Rattoni, 2007)

Total Amount of Food Consumed

The results of the STFP task indicated that rats consumed significantly less food during testing periods of all drug treatment compared to baseline conditions. The decreases in the total amount of food consumed may be attributed to two variables: the age of the rats at drug treatment conditions compared to baseline and possible stress caused by injections of Scopolamine, Guanfacine, and saline. The latter of the two hypotheses doesn't seem to be as credible as injections were only administered five minutes prior to training and 30 minutes after training. With the length of delay between training and testing periods being five days, it is not likely that the injections caused stress to be carried over into the testing and food consumption time period. However, this is still a plausible contributor to the decrease in amount of total food consumed. Additionally, it is not plausible that any side effects of Scopolamine or Guanfacine contributed to the decrease in the total amount of food consumed as over the five day delay, the majority of each of these drugs would have cleared the rats' system.

The age of the rats seems to be the most likely cause of the decrease in total food consumed during drug treatment conditions. Baseline measures were conducted three months in advance of the drug treatment conditions while rats were still reaching full adult growth. It is plausible that rats ate more food during baseline testing due to the increase in amount of growth and body weight that was still occurring. Prior to drug treatment

conditions, rats had developed into adults and no longer required the amount of food they previously required to continue growing. This hypothesis is supported in two ways (see Figure 6): (1) rats still ate a substantial amount of food in the drug conditions and (2) the total amount of food consumed during each of the drug treatment conditions was relatively identical indicating that, perhaps, rats only needed to consume that much food in order to feel full.

Ability of Guanfacine to Reverse Scopolamine Impairments

The current study investigated the ability of the norepinephrine agonist, Guanfacine, to reverse Scopolamine-induced memory impairments on the ORT and the STFP task. No previous study has tested the ability of Guanfacine to reverse Scopolamine-induced memory impairments on any memory tasks. Previous research has focused on acetylcholine agonists to reverse Scopolamine impairments on tasks such as the 8-arm radial arm maze task and the ORT. Studies have shown that Rolipram (Egawa, Mishima, Matsumoto, Iwasaki, Iwasaki, & Fujiwara, 1997; Rutten et al., 2006), Pilocarpine (Levin & Torry, 1996; Riekkinen, Sirvio, Valjakka, Miettinen, & Riekkinen, 1991), Nebracetam (Iwasaki, Matsumoto, & Fukiwara, 1992), and glucose, amphetamine, epinephrine, Physostigmine, and Oxotremorine (Stone, Walser, Gold, & Gold, 1991) all reverse the effects of Scopolamine on various memory tasks.

No previous studies have investigated the ability of norepinephrine agonists to reverse the effects of Scopolamine. The current study is unique in that it investigated a specific alpha-2 receptor subtype adrenergic agonist, Guanfacine, in relation to its ability to reverse Scopolamine-induced memory deficits in the ORT and the STFP task. However, several studies have shown that norepinephrine agonists, specifically those of the alpha-2

subtype have improved memory performance on a number of memory tasks (Arnsten et al., 1988; Arnsten & Goldman-Rakic, 1990; Arnsten & Leslie, 1991; Arnsten & Contant, 1992; Arnsten & Cai, 1993; Jakala, et al., 1999; Rama et al., 1996; Sirvio et al., 1991).

One particular study is similar to the current study in that Guanfacine was investigated as a way to improve memory performance on the STFP task in rats with a chemical lesion to the medial septal area. Opal & Countryman (2007) surgically gave a chemical lesion to the medial septal area in rats using 192 IgG-saporin. 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 significant memory formation ability above chance levels. Results of this study were consistent with the current study regarding the effects of Guanfacine as a means to improve memory performance on the STFP task.

Effects of Guanfacine Alone

The results of the current study indicated that when Guanfacine was administered alone (to rats with no Scopolamine-induced memory deficits), there was a decrease in memory performance on the ORT and the STFP compared to conditions in which Guanfacine was administered to rats with Scopolamine-induced memory deficits. Although the decreases observed in both tasks were not significant, the findings warrant explanation and discussion.

One explanation for this decrease in memory performance is that an excess of norepinephrine is detrimental when acetylcholine functioning is at normal levels. Perhaps the increase in norepinephrine above normal levels is only beneficial to memory performance

when it is compensating for decreased acetylcholine functioning in the hippocampus. With both systems intact, excess norepinephrine may cause too much stimulation in the brain and lead to the inability of rats to focus their attention on behavioral tasks at hand. One study with healthy male participants supports this hypothesis (Mueller, Clark, Lam, Moore, Murphy, Richmond, et al., 2005). Guanfacine was found to have no effects on executive and memory functions. Furthermore, negative effects on blood pressure and trend effects on backward digit span and reaction time indicated a mild impairment due to Guanfacine administration (Mueller et al., 2005).

Implications of Findings: Guanfacine as an Alzheimer's Treatment

The findings of the present study suggest that Guanfacine may be effective at improving memory impairments caused by decreased acetylcholine function as seen in Alzheimer's disease. The ability of this alpha-2 adrenergic agonist to reverse memory impairments caused by the muscarinic cholinergic antagonist, Scopolamine, establishes Guanfacine as a potentially effective treatment for memory impairments stemming from age-associated cognitive impairments or Alzheimer's disease. Current AChE-I treatments for Alzheimer's disease are only effective for a short period of time. When the benefits of such treatments subside, no other alternative treatment for the associated impaired memory ability exists. Treatment with Guanfacine may be an option beyond that of AChE-Is.

One of the biggest steps that must be taken in order to establish Guanfacine and other noradrenergic drugs as potential treatment for memory impairment associated with Alzheimer's disease is to bridge the gap between animal research and human research. Many animal studies, including the current study, have shown that Guanfacine is able to improve previously lowered memory ability (Arnsten et al., 1988; Arsten & Cai, 1993; Rama et al.,

1996; Sirvio et al., 1991). For the most part, these studies did not intentionally impair memory, but rather used aged monkeys as test subjects. Few studies have administered Guanfacine to a human population, specifically Alzheimer's patients. The few studies that have generally conclude that Guanfacine was ineffective at improving memory impairments (Crook, Wilner, Rothwell, Winterling, & McEntee, 1992; McEntee, Crook, Jenkyn, Petrie, Larrabee, & Coffey, 1991).

The current study may explain some of the conflicting findings between previous animal and human research with Guanfacine. Previous studies, which conclude that Guanfacine had no significant effect on learning and memory (McEntee et al., 1991) and that noradrenergic intervention alone is unlikely to be effective in AD (Crook et al., 1992), differ significantly in the way that Guanfacine was administered. These studies administered Guanfacine to patients meeting the criteria for age-associated memory impairment (McEntee et al., 1991) and patients in the early stages of cognitive deterioration (Crook et al., 1992). The diagnosis of these patients as mildly cognitively impaired likely means that acetylcholine levels are still mostly intact, or at least they haven't begun to deteriorate to the extent as seen in someone with a more advanced case of AD. This is supported by the results of one recent study, which shows that in early stages of Alzheimer's disease ACh function is relatively normal (Ellis, Villemagne, Nathan, Mulligan, Gong, Chan, et al., *in press*).

The current study mimicked more advanced stages of AD in rats by blocking the majority of ACh transmission in the hippocampus. The improved results on impaired memory performance may be due, in part, to the lack of ACh functioning at the time of memory formation. In this respect, perhaps Guanfacine and noradrenergic treatment are only effective at reversing memory impairments when ACh functioning is severely impaired.

This fits with results of the current study. When Guanfacine was administered alone, memory ability on the ORT and STFP task tended to decrease compared to conditions in which Guanfacine was utilized to reverse Scopolamine-induced memory impairments. Results of previous studies testing Guanfacine on humans seem to be more aligned with this finding of the current study. Overall implications of this hypothesis suggest that Guanfacine may be beneficial at reducing memory impairment associated with advanced stages of AD.

Limitations

In order to improve future research it is beneficial to outline potential limitations of the current study. First, baseline levels of memory were taken approximately three months prior to any experimental testing. It is not likely that baseline memory levels would change substantially over such a period of time; however, it does remain a potential limitation. In future research it may be beneficial to have another baseline condition after all experimental manipulations are complete in order to serve as a confirmation for baseline memory levels. Secondly, there is no real AD in rats. The plaques and tangles associated with AD in humans never develop in rats. Rats serve as a beneficial animal model of AD in humans but AD cannot fully be represented in such a model.

Another limitation of the current study is that there was no cellular confirmation of what brain structures Scopolamine affected. Results on each of the memory tasks suggest that most of the memory impairment would be due to the effects of Scopolamine blocking receptors in the HPC. Despite this, decreased ACh function caused by Scopolamine in other brain areas, such as the prefrontal cortex, could have accounted for some of the memory deficits in the current study. Even if this were the case, the model utilized in the current study would still serve as an effective one because of the known effects of ACh function in

both the HPC and prefrontal cortex in AD. However, effects in relation to other brain structures are not accounted for. A final limitation is that, despite the promising findings of memory improvement for patients with AD, cell death in the brain will continue to naturally occur. It is likely that such a treatment would only be effective for an additional year or two at most.

Future Research

Reversal of Scopolamine-induced memory impairment following the administration of Guanfacine, in the present experiment, contributes to the effectiveness of an animal model for AD in humans. Identifying the rat as a valid animal in which to manipulate treatment approaches to AD is one of the strengths of the present study. In the seemingly never-ending quest to uncover safe and effective pharmacological agents for AD, future research must utilize precise methodology.

As previously mentioned, bridging the gap between animal studies, which currently test the ability of drugs such as Guanfacine to reverse or reduce memory impairment, and human studies is an important step for future research to consider. By testing these pharmacological agents on humans, more information can be ascertained regarding the benefits of memory ability in relation to AD. In line with previously discussed implications, it would be beneficial for future research to test the hypothesis that Guanfacine is effective at reducing memory impairments associated with severe and advanced cases of AD in humans. However, more research should be done with animal models prior to clinical trials in a human population.

In order to fully test the ability of Guanfacine to reverse memory impairments, various hippocampal-dependent tasks should be utilized using varying inter-trial delays.

Research that shows the effects of the drug Guanfacine on other hippocampal-dependent memory tasks with varying inter-trial delays might further implicate the effectiveness of Guanfacine at reducing memory impairments as seen in AD. Furthermore, testing the administration of Guanfacine on varying levels of ACh functioning may give a clearer understanding of when in the stages of AD Guanfacine may offer therapeutic support.

Final Comments

Since the onset of the "cholinergic hypothesis," considerable evidence has been obtained in support of impairment of the cholinergic system seen in 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, AD is marked by a lack of cholinergic transmission within the hippocampal region (Bartus et al., 1982; Rossor et al., 1981). Recently, it is been shown that such decline in ACh function may only be significant in severe and advanced stages of AD (Ellis et al., *in press*). 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, lesions to the medial septum have produced widespread memory deficits via decreased availability of acetylcholine to the hippocampus (Potter et al., 1999; Opal & Countryman, 2007). Additionally, Scopolamine has been shown to impair memory performance by blocking the hippocampal receptors available for ACh functioning (Rogers & Kesner, 2003).

Clearly, the adrenergic and cholinergic systems are 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) reported 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 important on memory-related tasks (Arnsten et al., 1988; Arnsten & Cai, 1993; Jakala et al., 1999).

Findings in the current study support the link between cholinergic and noradrenergic neurotransmitter systems in relation to memory ability. It is not known by what mechanisms Guanfacine acts to improve memory ability. We suggest that Scopolamine works to prevent consolidation of information attained during memory tasks. In this sense, information may be encoded, but the longer, more involved process of memory consolidation may be blocked. Therefore, it is likely that administration of Guanfacine helps to increase consolidation of any information encoded and still retained from training on the memory tasks. This increased consolidation is thought to account for the improved memory ability, seen on the two memory tasks, in rats administered Scopolamine prior to training on the tasks.

Conclusion

The current study reports three meaningful findings: (1) Scopolamine significantly impairs memory performance on two hippocampal-dependent memory tasks: the object

recognition task (ORT) and the social transmission of food preference task (STFP), (2) the administration of low dose of Guanfacine and a high dose of Guanfacine significantly improves memory impairments caused by Scopolamine on the ORT, and (3) a low-dose of Guanfacine (high dose not tested) significantly improves memory impairments caused by Scopolamine on the STFP task. Together, these findings significantly contribute to our knowledge of memory impairment and improvement, with regards to ACh and NE, and should be encouraging to those who study pharmacological treatment of memory deficits.

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