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Claire Galloway Date

Investigating the Effects of Specific Acetylcholine Receptor Activation on Hippocampal Function for the Treatment of Alzheimer's Disease

By

Claire R. Galloway
Doctor of Philosophy

Psychology

_____

Joseph R. Manns, Ph.D.
Advisor

_____

Jocelyne Bachevalier, Ph.D.
Committee Member

_____

Robert M. Cohen, MD, Ph.D.
Committee Member

_____

Allan I. Levey, MD, Ph.D.
Committee Member

_____

Elaine F. Walker, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

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Claire R. Galloway

M.A., Emory University, 2013

B.S., Georgia State University, 2011

Advisor: Joseph R. Manns, PhD

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Abstract

Investigating the Effects of Specific Acetylcholine Receptor Activation on Hippocampal Function for the Treatment of Alzheimer's Disease

By Claire R. Galloway

Drugs that selectively increase the activity of M_1 or M_4 muscarinic acetylcholine (ACh) receptors represent potential therapies for memory impairments in Alzheimer's disease (AD), but little is known about how these muscarinic activators influence memory-related neural circuitry *in vivo*. The hippocampus is essential for linking individual items into a spatiotemporal context that supports memory for information about objects in their location, and is disproportionately impacted in AD. Oscillatory synchrony between the CA1 and CA3 subregions of the hippocampus and the spatial fidelity of hippocampal place cells, which fire preferentially within specific locations of a given environment, are useful metrics of hippocampal network activity that relates to memory. Three experiments were conducted in rats to investigate if selectively increasing the activity of M_1 or M_4 can influence hippocampal function in healthy rats, and to test an M_1 agonist as a potential acute therapy for ameliorating hippocampal dysfunction in a new transgenic rat model of AD. In the first experiment (Chapter 2), the results indicated that the activity of CA1 and CA3 of the hippocampus was more synchronous when rats were exploring novel objects, but this increase was similar across drug conditions. In the second experiment (Chapter 3), the results showed that AD rats developed an age-dependent impairment in spatial memory by 9-12 months of age, when non-spatial memory performance of AD rats was still intact. In the third experiment (Chapter 4), the results showed that hippocampal place cells of AD rats had reduced spatial fidelity that was best characterized by decreased signal-to-noise ratio of firing rates, and an M_1 agonist may help improve the signal-to-noise ratio of hippocampal place cells in AD rats. The results of the experiments advance our understanding of how muscarinic drug therapies affect hippocampal function in healthy and AD rats and shed light on the nature of hippocampal dysfunction that underlies memory impairments in Alzheimer's disease.

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This dissertation is dedicated to my grandpa Harold Lee Galloway (1930-2006) and grandma Ruby Mae Harper Galloway (1931-2015). My love for them and frustration over watching them struggle with Alzheimer's disease (one as a patient, one as a caretaker) fueled and directed my academic pursuits since age 16. Moreover, their unconditional love for me and enthusiastic support for every task I ever attempted is a major reason why Claire Bear from the sticks had the audacity to pursue a PhD in the first place.

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Chapter 1

General Introduction

The overall goal of this dissertation is to better understand how activation of acetylcholine (ACh) receptors influences activity in the hippocampus as a potential therapy for treating memory dysfunction in Alzheimer's disease (AD). The hippocampus plays an essential role in declarative memory, which often involves linking individual items to a spatiotemporal context to form the type of everyday memory that includes information about the time and place in which the information was learned (Knierim, 2015). The neuromodulator ACh can influence hippocampal function and is important for declarative memory (Hasselmo, 2006). Both the hippocampus and cholinergic neurons in the basal forebrain are impacted early in the course of AD, a major symptom of which is progressive memory impairments (Braak & Braak, 1995; Jahn, 2013; Whitehouse et al., 1982). The specific objective of this dissertation is to take advantage of the cross-species homology of the hippocampus in humans and rats to study the impact of systemically-administered cholinergic drugs on hippocampal function in healthy rats and hippocampal dysfunction in rats bred to model the neuropathology of human AD. In particular, in several experiments, recently-developed drugs that specifically activate either M_1 or M_4 muscarinic acetylcholine receptor will be administered to rats to determine how the drugs impact memory performance or neural activity in the hippocampus. The following review provides a brief summary of prior studies that motivated the present research.

Memory and the hippocampus

The hippocampus sits within the medial temporal lobe of humans and, in the rodent brain, is a C-shaped structure that reaches from the septal to the temporal pole. The transverse axis of the hippocampus shows a pattern of cell layers that is maintained along the longitudinal axis of the hippocampus (Amaral & Witter, 1989; Bird & Burgess,

2008). The cell layers of the hippocampus are divided into four major subregions: the dentate gyrus (DG), CA3, CA1, and subiculum. CA1 and CA3 are separated by an intermediary cell layer, CA2, but the majority of research has been done in CA3 and CA1 (Chevaleyre & Pisorowski, 2016). The organization and connectivity of the cell layers is highly conserved across many different mammalian species, so investigating hippocampal function in rodents is likely to be meaningful for understanding hippocampal function in humans (Manns & Eichenbaum, 2006).

All the major subregions of the hippocampus are part of a unidirectional circuit that begins with the neighboring entorhinal cortex (EC) projecting into the dentate gyrus, then to CA3, from CA3 to CA1, and out from CA1 to the subiculum (Witter et al., 2000). Although this unidirectional circuit represents a major information pathway through the hippocampus, almost all of the subregions of the hippocampus and EC also share unidirectional or bidirectional connections with one or more of the other subregions. For example, the EC also projects directly to CA3, CA1, and the subiculum (Amaral & Witter, 1989). One prominent model of the functional circuitry of the hippocampus has argued that network states in the hippocampus that bias CA1 pyramidal cells to be most responsive (depolarized) to cortical input from direct EC projections promote the encoding of new information, and network states in the hippocampus that bias CA1 pyramidal cells to be most responsive to CA3 input via Schaffer collaterals promote the retrieval of previously stored information (Hasselmo et al., 2002).

The hippocampus is part of a memory system that consists of its surrounding structures in the medial temporal lobe, including the entorhinal cortex, perirhinal cortex, and parahippocampal cortex (postrhinal cortex in rats; Manns & Eichenbaum, 2006).

Spatial information reaches the hippocampus from parahippocampal projections into the medial EC, and non-spatial information reaches the hippocampus from perirhinal projections into the lateral EC (Manns & Eichenbaum, 2006). Thus, the hippocampus receives both spatial and non-spatial inputs, and is ideally situated to combine that information to support memory for context (e.g. spatial location), individual items (e.g. an object), and combining this information to integrate items within a context (e.g. object-in-location memory; Manns & Eichenbaum, 2006). In line with the clues provided by the neuroanatomical connectivity of the hippocampus, lesions of the hippocampus cause amnesia-like deficits in humans, monkeys, and rats (Squire, 1992). Hippocampal lesions most measurably impact memory for facts and events (declarative memory) in humans and spatial memory in rats, but there is growing consensus that both declarative memory in humans and spatial memory in rats are supported by similar computations in the hippocampus that link individual items into a spatial or temporal context (Knierim, 2015).

Local field potentials in the hippocampus

The hippocampus has markers of neural network activity that relate to successful memory processing. In particular, oscillatory synchrony between CA regions and spiking activity of individual pyramidal cells in the CA regions of the hippocampus may be used as metrics to investigate hippocampal network activity as it relates to normal memory function, memory dysfunction, and memory improvement (Buzsaki & Moser, 2013; Hasselmo et al., 2002; Robitsek et al., 2013; Skaggs et al., 1996; Trimper et al., 2014; Wilson et al., 2003). Oscillations in local field potentials, which reflect the summed activity of excitatory and inhibitory post-synaptic potentials of the neurons being

recorded, provide an important and widely-studied indicator of hippocampal network activity (Buzsaki et al., 2012). Hippocampal oscillations in the theta (6-12 Hz) and gamma (30-90 Hz) frequency ranges are among the oscillatory rhythms most implicated in memory function (Colgin, 2016). Indeed, a large amount of data exists regarding the memory correlates of these oscillations in rats, and thus understanding how hippocampal theta and gamma oscillations are impacted by AD and ACh can be used to understand how both influence memory function and dysfunction at the level of the hippocampal network.

Theta oscillations in the hippocampus are influenced by intrinsic resonance properties of pyramidal cells, local interneurons, and the disinhibition of hippocampal pyramidal cells by long-range GABAergic and cholinergic inputs from the medial septum onto hippocampal interneurons (Buzsaki, 2002; Buzsaki et al., 2012; Colgin, 2016). These theta oscillations are often broadly synchronized across the hippocampus and connected regions and are thought to help coordinate spike timing and other neuronal oscillations in the service of memory (Buzsaki & Moser, 2013). For example, input to CA1 from EC is strongest at the peaks of the theta oscillation, whereas input to CA1 from CA3 is strongest at the troughs of the theta oscillation, an arrangement that could help the hippocampus alternate between successful encoding of new information from the EC and retrieval of old information from CA3 (Hasselmo et al., 2002; Huerta & Lisman, 1995; Lisman & Jensen, 2013). In addition, several studies have shown that disrupting hippocampal theta with septal lesions disrupts memory performance of rats (Colgin et al., 2009).

Oscillations within the gamma frequency range are driven by the coordinated activity of local inhibitory interneurons and are a general marker of neural activation in many brain regions (Bartos et al., 2007; Fries, 2005). Gamma oscillations in the hippocampus have been further divided into slow gamma (30-55 Hz) and fast gamma (~65-90 Hz). Slow gamma oscillations likely reflect communication between CA3 and CA1, and fast gamma oscillations likely reflect communication between the EC and CA1 (Colgin et al., 2009). Recently, slow gamma synchrony between CA1 and CA3 during novel object exploration was shown to relate to subsequent good memory for objects during an object recognition memory task in rats (Trimper et al., 2014). Taken together, both theta and slow gamma oscillations are important indicators for understanding how neural networks in the hippocampus support memory.

Place cells in the hippocampus

In addition to theta and gamma oscillations, one of the most widely-studied examples of behavioral correlates of hippocampal activity in rats are place cells. Hippocampal place cells were first discovered in the rodent hippocampus and fire preferentially within a specific location of a given environment (the "place field" of the place cell). Place cells form distinct assemblies that allow the hippocampus to form cognitive maps of multiple environments (O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). Place cell activity has been shown to directly support spatial memory. For example, place cell activity before the "choice" portion of a spatial memory task predicted subsequent memory performance in rats (Robitsek et al., 2013), and the ability of place cells to represent changes in the environment correlated with memory performance in rats (Wilson et al., 2003). Although the firing activity of place cells

corresponds most strongly with spatial location, place cell activity can also represent the combination of non-spatial and spatial information that supports object-in-location memory (e.g., Komorowski et al., 2009). Neural activity that relates to spatial location has been found in the hippocampus of non-human primates and humans as well (Hori et all, 2003; Miller et al., 2013; Rolls, 1999). Both the function and dysfunction of place cells have been studied extensively, and there are several well-established metrics to quantify place cell fidelity (Skaggs et al., 1996). Thus, place cells in the hippocampus are useful determine the quality of hippocampal function as it relates to memory across species.

Acetylcholine

ACh is a neuromodulator that acts on receptors which are distributed throughout the peripheral and central nervous systems (Bymaster et al., 2003; Chatzidaki & Millar, 2015). In the synapse, ACh is broken down by acetylcholinesterase into choline and acetate. Choline is taken up into nerve terminals via a high affinity choline transporter where, along with acetyl-CoA, it is catalyzed into ACh by choline acetyltransferase (ChAT). ACh is then translocated into vesicles by vesicular ACh transporter, and upon excitation of the nerve terminal will be released via exocytosis into the synapse (Kummer et al., 2008). Before ACh is broken down in the synapse, ACh can bind to two different classes of receptors on pre and post-synaptic membranes: excitatory, ionotropic nicotinic receptors (nAChRs) and metabotropic muscarinic receptors (mAChRs; Bubser et al., 2012; Kummer et al., 2008). NAChRs consist of five subunits comprised from various combinations of nine α (α2- α10) or three β (β2-β4) subunits that differ in function,

expression levels, and location depending on the particular combination of subunits (Gotti et al., 2009).

There are five distinct subtypes of mAChRs (M_1-M_5). M_1, M_3, and M_5 are coupled to excitatory G_q proteins, and M_2 and M_4 are coupled to inhibitory $G_{i/o}$ proteins (Bubser et al., 2012). Although M_1, M_2, M_3, and M_4 are all highly expressed in the brain (Levey, 1993), M_2 and M_3 are also highly expressed in the PNS and are most implicated in the dose-limiting side effects induced by nonspecific mAChR activation (Bymaster et al., 2003). M_1 and M_4 receptors are expressed in several different regions of the rat brain, including the thalamus, striatum, amygdala, neocortex, and hippocampus. In the hippocampus, M_1 increases the excitability of post-synaptic membranes of cell bodies and dendrites located in the EC, DG, CA3, and CA1 (Levey et al., 1991; Rouse & Levey, 1996; Rouse et al., 1998). In CA1 in particular, M_1 potentiates glutamatergic NMDA receptors and induces plasticity (Dennis et al., 2016; Rouse et al., 1999). M_4 inhibits neurotransmitter release primarily on pre-synaptic terminals in the DG from cell bodies originating in the EC, as well as pre-synaptic glutamatergic terminals from CA3-CA1 Schaffer collaterals (Rouse et al., 1998; Rouse et al., 1999). One functional implication of the pattern of expression of M_1 and M_4 in the hippocampus is that M_1 may act to increase the signal of inputs into CA1, regardless of origin, whereas M_4 may act to decrease interference or "noise" from previously stored information arising from excitatory CA3-CA1 Schaffer collaterals (Dasari & Gulledge, 2011; Shirey et al., 2008). However, there is evidence that M_1 is also important for hippocampal inhibition (e.g. Cea-del Rio et al., 2011), which could conceivably be in the service of reducing noise as well. Thus, M_1 and

M_4 have potential to be used to understand hippocampal function in healthy animals and test for treatment effects in memory disorders.

In line with the dense mAChR expression in the hippocampus, studies of both humans and experimental animals in a variety of memory tasks demonstrate that blocking all mAChRs with scopolamine impairs memory performance (Aigner & Mishkin, 1986; Deutsch & Rocklin, 1967; Drachman & Leavitt, 1974; Ghoneim & Mewaldt, 1975; Meyers et al., 1964; Pazzagli & Pepeu, 1965). On the other hand, activating all mAChRs with broad-acting agonists (e.g. arecholine) improves performance or attenuates memory impairments (Matsuoka et al., 1991; Murray & Fibiger, 1986; Prediger et al., 2006; Rupniak et al., 1989; Smith et al., 1996). Moreover, systemic administration of drugs that selectively activate M_1 and M_4 were shown to improve object recognition memory performance in rats (Galloway et al., 2014). Yet, it is still unclear if and how the memory-enhancing potential of these drugs relates to hippocampal network activity.

The cognitive effects from increasing ACh generally or specific mAChR subtypes may have an inverted-U effect. Chuah and colleagues (2009) found that administering an acetylcholinesterase inhibitor (AChEI) did not improve cognition in rested young adults, but did improve cognition in sleep-deprived young adults. Other studies found that administering an AChEI actually decreased behavioral and neurophysiological measures of cognition in healthy older adults, even as it improved cognition in Alzheimer's disease (AD) patients (Balsters et al., 2011; Bentley et al., 2008). In addition, only rats who performed poorly at baseline benefited from systemic administration of drugs that selectively increased M_1 or M_4 (Galloway et al., 2014). Taken together, it would seem that higher ACh levels or more mAChR activation does not have a simple linear

relationship with cognition, including memory, and the effects of manipulating ACh and ACh receptors may depend on the baseline network function of the individual.

Alzheimer's disease

AD is a progressive, neurodegenerative disease that is currently diagnosed in over 5 million people in the U.S., and this number is projected to increase to 13 million by year 2050 (Alzheimer's Association, 2014). In 2013, AD was the 6th highest cause of death in the U.S., and unlike other major chronic diseases such as HIV, heart disease, and stroke, the number of deaths from AD between 2000-2010 increased dramatically (68% increase; Alzheimer's Association, 2014). AD is the most feared chronic condition of U.S. residents 60 years or older, perhaps because currently there are no known therapies to treat, prevent, or delay AD (Alzheimer's Association, 2014). Thus, the discovery of an effective treatment would greatly improve the quality of life of patients, caregivers, and older adults.

AD is characterized by hallmark pathology that consists of beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) of hyperphosphorylated tau (Small & Cappai, 2006). AD involves other signs of neural dysfunction, such as inflammation, oxidative stress, synaptic dysfunction, and cell death in later stages of AD (Querfurth & LaFerla, 2010). The most prevailing theory of AD, the "amyloid cascade hypothesis," proposes that Aβ pathology drives the progression of AD pathology (Hardy & Higgins, 1992). According to this theory, AD pathology begins with abnormal processing of amyloid precursor protein (APP), which subsequently increases the production of toxic Aβ oligomers and extracellular plaques, which in turn trigger tau pathology (Hardy & Higgins, 1992; Hardy & Selkoe, 2002). Tau pathology, by destabilizing microtubules and

forming intracellular NFTs (Querfurth & LaFerla, 2010), may contribute to synaptic dysfunction and cell death in key brain regions, thereby causing progressive memory impairments (Hardy & Selkoe, 2002). Thus, disruptions in the balance in APP processing, Aβ production, or clearance may be the underlying mechanism of AD.

AD cases can be classified into early onset AD and late onset AD. Late onset AD accounts for the large majority of AD cases and mostly occurs after age 65 (Bekris et al., 2010). Although there is no known cause of late onset AD, carriers of the ϵ4 allele of the apolipoprotein E gene have an increased risk for AD (Corder et al., 1993). Yet, only 40-50% of AD patients are carriers of this gene (van Leuven, 2000), and currently the biggest risk factor for late onset AD is age (Kukull et al., 2002). In contrast, early onset AD accounts for a very small population of AD patients (~5%; van Leuven, 2000), and can begin as early as 30 years of age (Bekris et al., 2010). Early onset AD occurs in families with specific genetic mutations (APP, presenilin 1 (PS1), and presenilin 2 (PS2); Wu et al., 2012) that all alter APP metabolism at various stages to ultimately increase Aβ pathology (Bettens et al., 2010).

Animal models of AD

Most transgenic mouse models of AD involve the insertion of human early onset AD genes, and they have been useful for studying how Aβ affects memory and the hippocampus (LaFerla & Green, 2012). For example, many mouse models of AD have corroborated studies with human that found early AD is characterized by hyperexcitation in the hippocampus (Busche & Konnerth, 2015), and abnormal synaptic plasticity in the hippocampus (Morrissette et al., 2009). However, many drug treatments that were effective in reducing Aβ pathology in AD mouse models have not translated into

effective therapies for humans with AD (LaFerla & Green, 2012). A newly developed rat model (TgF344) of AD with early onset AD mutations APP_{Swe} and PS1ΔE9 age-dependently develop Aβ plaques, NFTs, neuroinflammation, and cell death (Cohen et al., 2013). AD rats also show age-dependent memory impairments in spatial and non-spatial memory tasks (Cohen et al., 2013). The similarity between the pathology and cognitive dysfunction of AD rats and human AD highlights the potential to use AD rats to investigate the underlying neural network dysfunction that occurs in AD and meaningfully evaluate new therapies for AD.

Hippocampus and memory in AD

The dementia in Alzheimer's disease encompasses a wide range of cognitive functions including memory loss (Gallagher & Koh, 2011; Lyketsos et al., 2011). In accordance with the disproportional impact on the hippocampus and surrounding structures in the medial temporal lobe, the type of declarative memory that depends on the hippocampus is also disproportionally impaired in AD (Gallagher & Koh, 2011; Jahn, 2013). The hippocampus and surrounding medial temporal lobe structures are the earliest targets of NFTs that occur in AD (Braak & Braak, 1995), and at the gross anatomical level the hippocampus is already atrophied early in the disease process (Stoub et al., 2010). At the functional level, fMRI studies have shown that the hippocampus is hyperactive in the early stages of AD but hypoactive in later stages of AD (Celone et al., 2006; Miller et al., 2008). Aβ also causes abnormalities in synaptic plasticity in the hippocampus (e.g. Shankar et al., 2008). More recently, AD mice were found to have abnormal hippocampal place cell activity (Cacucci et al., 2008; Cheng & Ji, 2013; Zhao et al., 2014). Dysfunction of the hippocampus in AD has been demonstrated at multiple

levels of analysis, and could be a key region to understand the network dysfunction that underlies the cognitive dysfunction in AD.

The cholinergic hypothesis of AD cognitive deficits

A longstanding theory posits that cholinergic dysfunction underlies memory loss symptoms in AD (Bartus et al., 1982). For example, post-mortem studies of AD patients have revealed a disproportionate loss of neuronal cell bodies in the basal forebrain, which provides the main source of ACh to the cortex and hippocampus (Whitehouse et al., 1982). Reduced post-mortem ChAT levels were found in the brains, especially in the hippocampus, of AD patients (Perry et al., 1977), and there is evidence that reduced ChAT levels relate to the severity of AD (Potter et al., 2011). Moreover, the most widely-prescribed FDA-approved drugs for treating AD memory loss increase acetylcholine levels by inhibiting the enzyme acetylcholinesterase, which normally functions within synapses to break down acetylcholine (Anand & Singh, 2013). Although AChEIs do temporarily relieve memory loss symptoms in some AD patients, the nonspecific activation of both nAChRs and mAChRs by AChEIs can cause dose-limiting negative side effects (Hogan, 2007; Levey, 1996; Wallace et al., 2011). Thus, low ACh levels may underlie the memory loss in AD but the lack of specificity of current cholinergic therapies has limited the efficacy of these drugs.

Drugs that selectively activate specific mAChRs may be able to circumvent some of the drawbacks of generally increasing ACh levels. Although both nAChRs and mAChRs show potential to improve memory, there is some evidence that activation of nAChRs can increase Aβ pathology by promoting the amyloidogenic pathway of APP metabolism and increase tau pathology by promoting tau phosphorylation (Ovsepian et

al., 2016). On the other hand, mAChRs, especially M_1, can promote the non-amyloidogenic pathway of APP metabolism and decrease tau pathology by inhibiting the hyperphosphoylation of tau (Ferreira-Vieira et al., 2016; Jiang et al., 2012; Shirey et al., 2009; Tarr et al., 2012). Recently a selective M_1 agonist reduced Aβ pathology in a mouse model of AD and improved the degree to which the activity of hippocampal place cells distinguished between very similar environments in healthy young rats (Lebois, 2014; Lebois et al., 2016). Investigating if and how the M_1 agonist will affect memory performance and hippocampal network function in AD rats may contribute to the development of more effective AD therapies.

Object Recognition Memory Tasks

Many tasks have been used to assess memory in rodents, but variants of the novel object recognition memory task have become increasingly common (Clark & Squire, 2010). These types of recognition memory tasks are well-suited to evaluate hippocampal function in diseased states and in response to drug therapies. Object recognition memory tasks take advantage of the innate novelty preference of rats (Ennaceur and Delacour, 1988), so that memory for a repeated object can be inferred by the decrease in exploration relative to a novel object. The novelty preference also extends to object-location pairings, so that memory for specific object-location pairings can be inferred by a decrease in exploration of a repeated object presented in the same location relative to a repeated object presented in a different location than it initially appeared (e.g. Barker & Warburton, 2011). Object-only and object-in-location recognition memory tasks can be designed so that multiple trials can be completed within one session (e.g. Bass et al., 2014; Galloway et al., 2014), which reduces the likelihood that random variation in

exploration for a given trial will lead to spurious results that are unrelated to the experimental conditions.

The spontaneous nature of object recognition memory tasks makes them particularly useful for evaluating drug effects or testing memory of animal models of disease, as differences between diseased and healthy animals or across drug conditions are not confounded by the inability of an animal to learn the task rules. An additional benefit of the spontaneous nature of the task is that there are no learning effects across sessions, so that multiple sessions in a row can be used to gather more data and evaluate the efficacy of drug treatments without concerns about the task becoming less challenging over time. Object recognition memory tasks also place relatively low demands on physical activity and do not involve anything particularly aversive for rodents, both of which could interact with drug treatments or disease states and complicate the interpretation of behavioral effects.

Object recognition tasks are also easily translated to laboratory tasks for humans. For example, Zola and colleagues (2012) found that a visual paired comparison task, which measured memory for repeated pictures by the relative amount of time participants spent spontaneously looking at repeated versus novel pictures presented on a screen, was able to predict with high accuracy which participants would subsequently develop AD. Importantly, it has also been demonstrated that activity in the hippocampus relates to both object-only and object-in-location recognition memory in rats (Bass & Manns, 2015; Bass et al., 2014; Manns & Eichenbaum, 2009; Trimper et al., 2014). Thus, object-only and object-in-location recognition memory tasks are particularly useful to investigate drug effects and the nature of memory dysfunction, particularly in AD.

Summary

The hippocampus is essential for linking individual items into a spatiotemporal context that supports memory, including memory for individual objects, spatial location, and conjunctive information about objects in their location. Oscillatory synchrony between CA1 and CA3 and spatial fidelity of hippocampal place cells are useful metrics of hippocampal network activity that supports memory. Drugs that selectively increase the activity of M_1 and M_4 mAChRs have potential to improve memory performance and neural function in the hippocampus. AD is marked by memory loss and hippocampal dysfunction, and place cell fidelity is a useful metric for understanding the underlying neural dysfunction that contributes to memory impairments in AD. Activating the mAChR subtype M_1 shows potential to improve memory performance and neural dysfunction in AD. Finally, object recognition memory tasks are well-suited for testing drug therapies and memory performance in animal models of disease.

The following chapters will report three experiments that involved memory and the hippocampus. In the first study (Chapter 2), we investigated if selectively increasing the activity of M_1 or M_4 would improve object recognition memory performance and increase measures of oscillatory synchrony between LFPs recorded from CA1 and CA3 of the hippocampus in healthy rats. In the second study (Chapter 3), we longitudinally assessed AD rats and wild-type (WT) rats on an object-only and object-in-location recognition memory tasks from an age (5 months) at which AD rats were previously shown to have intact memory until they developed object-in-location memory impairments relative to WT rats. In the third study (Chapter 4), we investigated the nature of hippocampal dysfunction in AD rats and if selectively activating M_1 would improve

hippocampal function in AD rats. Hippocampal function was measured by the spatial fidelity of place cells and performance on a memory task that allowed for the assessment of both object-only and object-in-location recognition memory. A final chapter (Chapter 5) summarizes and interprets the results in the context of previous research.

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Chapter 2

M_1 and M_4 Effects on Hippocampal Function

Abstract

Newly developed compounds that are able to target specific receptors of the neuromodulator acetylcholine (ACh) offer opportunities to investigate how ACh receptor subtypes influence memory-related network activity in the brain, particularly in the hippocampus. ACh acting at muscarinic ACh receptors (mAChRs) can improve memory, and the M_1 or M_4 mAChR subtypes hold the most potential for successfully targeting the hippocampus and improving memory function without activating off-target mAChRs in the peripheral nervous system. It was recently shown that synchronous oscillatory activity (which can be mathematically defined as coherence) between two subregions of the hippocampus, CA1 and CA3, in the slow gamma range (~30-55 Hz) likely reflects hippocampal network states that support memory. In order to test if acute, systemic activation or potentiation of drugs that selectively increase the activity of M_4 (the M_4 PAM VU0152100) or M_1 (the M_1 PAM BQCA or M_1 agonist VU0364572) would improve hippocampal function, we administered the M_4 PAM, M_1 PAM, or M_1 agonist to healthy male rats (N=7) prior to completing an object recognition memory task while recording local field potentials from CA1 and CA3 of the hippocampus. We found that rats performed similarly well on the object recognition memory task across most drug conditions. In line with the good memory performance, CA3-CA1 slow gamma coherence increased during novel object exploration similarly across drug conditions, despite decreased CA1 or CA3 power in the experimental drug conditions. The results implicated that acute, systemic administration of drugs that selectively increase the activity of M_1 or M_4 may not provide an additional benefit to memory performance or CA3-CA1 slow gamma coherence in healthy young rats.

Newly developed compounds that are able to target specific receptors of the neuromodulator acetylcholine (ACh) offer opportunities to investigate how specific receptor subtypes influence memory-related network activity in the brain, particularly in the hippocampus. ACh binds to ionotropic nicotinic acetylcholine receptors (nAChRs) and metabotropic muscarinic acetylcholine receptors (mAChRs) throughout the central nervous system (CNS) and peripheral nervous system (PNS; Kummer et al., 2008). Decades of research has shown that mAChRs are important for successful memory performance in several different memory tasks (Aigner & Mishkin, 1986; Deutsch & Rocklin, 1967; Drachman & Leavitt, 1974; Ghoneim & Mewaldt, 1975; Meyers et al., 1964; Pazzagli & Pepeu, 1965), including object recognition memory (De Jaeger et al., 2013; Han et al., 2012; Stanley et al., 2012).

The five distinct mAChR subtypes (M_1-M_5) differ in their potential to be used as treatments to improve memory. M_1-M_4 are highly expressed in the brain, but M_2 and M_3 are most implicated in the dose-limiting side effects induced by nonspecific mAChR activation (Bymaster et al., 2003; Levey, 1996). In the hippocampus, a brain region essential for forming memories of items in a spatiotemporal context, the post-synaptic excitation from M_1 activation may increase the signal of inputs and the pre-synaptic inhibition of M_4 may suppress the noise of previously stored information during encoding of new information (Dasari & Gulledge, 2011). Therefore, increasing the activity of M_1 or M_4 holds the most potential to successfully target brain activity in regions important for memory without activating off-target mAChRs in the PNS.

The conserved orthosteric binding site of mAChRs has hindered the development of pharmacological compounds that were able to target and test the memory effects of

specific mAChR subtypes (Conn et al., 2009). Newly developed compounds that bind to unique alternative (allosteric) binding sites are able to target specific mAChR subtypes (Bridges et al., 2010). Some of these drugs, such as the M_1 allosteric agonist VU0364572, activate the receptor independently of ACh (Lebois et al., 2011). Other drugs increase receptor function by positively modulating or potentiating the effects of ACh at the receptor, such as the M_1 positive allosteric modulator (PAM) BQCA and the M_4 PAM VU0152100 (Brady et al., 2008; Shirey et al., 2009). Importantly, all of these compounds were shown to have central penetrance and improve memory performance in rats (Byun et al., 2014; Chambon et al., 2011; Digby et al., 2012; Ma et al., 2009). One study found that the M_4 PAM, M_1 PAM, and M_1 agonist all improved memory performance of healthy young rats who performed poorly at baseline (Galloway et al., 2014). However, it is still unknown how M_1 and M_4 alter brain network activity in the service of improving memory.

Investigating how the hippocampus reflects brain network changes by drugs selective for M_1 and M_4 opens opportunities to characterize the changes in hippocampal activity that correlate with memory improvement. Synchronous oscillatory activity (often mathematically defined as coherence, which takes into account the consistency of the phase relationship and co-modulation of amplitude between two signals) in the local field potentials (LFPs) between the two major pyramidal layers of the hippocampus, CA1 and CA3, in the slow gamma range (~30-55 Hz) likely reflects hippocampal network states that support memory processing (Colgin et al., 2009). For example, Trimper and colleagues (2014) reported that CA3-CA1 slow gamma coherence selectively increased when rats explored novel objects that they subsequently remembered well, and

improvements in object recognition memory performance in rats from electrical stimulation of the basolateral complex of the amygdala coincided with an increase in CA3-CA1 slow gamma coherence (Manns & Bass, 2016). Moreover, a general mAChR agonist and drugs that increase M_1 activity also increased CA3-CA1 slow gamma coherence of rats during a random foraging task (Lebois et al., 2016). Taken together, it seems likely that the degree of CA3-CA1 slow gamma coherence during an object recognition task may be a useful metric to determine if and how increasing the activity of M_1 or M_4 mAChRs alters hippocampal function.

Memory improvement from selectively increasing the activity of M_1 and M_4 mAChRs may alter hippocampal oscillations in the theta (6-12 Hz) range as well. Theta is well known for its role in memory by coordinating individual spikes and gamma oscillations (Buzsaki, 2005), and ACh acting through mAChRs regulates hippocampal theta (Buzsaki, 2002; Li et al., 2007). Ablating cholinergic neurons in the medial septum, the major source of cholinergic input to the hippocampus, or blocking mAChRs impaired memory performance and altered hippocampal theta oscillations in rats (Kramis et al., 1975; Masuoka et al., 2006; Yoder & Pang, 2005). Thus, CA3-CA1 theta coherence, in addition to CA3-CA1 slow gamma coherence, may reflect memory-related improvements in hippocampal network activity from drugs that selectively increase the activity of M_1 or M_4.

The goal of this study was to investigate if acute, systemic activation or potentiation of M_4 (M_4 PAM) or M_1 (M_1 PAM or M_1 agonist) mAChRs would improve the performance of rats on an object recognition memory task, and to determine if memory improvements from the M_4 PAM, M_1 PAM, or M_1 agonist would coincide with

an increase in CA3-CA1 slow gamma coherence in the hippocampus of rats as they explore novel objects. We administered the M_4 PAM, M_1 PAM, or M_1 agonist to rats prior to completing an object recognition memory task while recording from CA1 and CA3 of the hippocampus. We found that rats performed similarly well on the object recognition memory task across most drug conditions. In line with the good memory performance, CA3-CA1 slow gamma coherence increased during novel object exploration similarly across drug conditions. However, all of the experimental drugs generally decreased power in the hippocampus, although the M_4 PAM, M_1 PAM, and M_1 agonist did not decrease power uniformly across CA regions, frequency ranges, or behavioral state of the rats (exploring novel objects vs. not exploring novel objects).

Method

Subjects

Adult male Long-Evans rats (*N*=7) were used to evaluate the effects of increasing the activity of M_4 or M_1 on memory performance and hippocampal LFP spectral measures during an object recognition memory task. In the days preceding the testing sessions for the current study, all rats performed various object recognition memory tasks that involved encountering objects on the same circular track used for the current study. The rats were kept on a 12-hour light/dark cycle (testing occurred during the light period), individually housed with free access to water, and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

Drugs

Three different drugs that selectively target either the M_1 or M_4 mAChR subtype were administered. VU0364572 is a bitopic agonist selective for M_1 (M_1 agonist; Lebois et al., 2011), BQCA is a PAM selective for M_1 (M_1 PAM; Shirey et al., 2009), and VU0152100 is a PAM selective for M_4 (M_4 PAM; Brady et al., 2008). Rats received subcutaneous (s.c.) injections of either 1 mg/kg of M_1 agonist, 1 mg/kg of M_1 PAM, or 3 mg/kg of M_4 PAM for experimental drug sessions, or 0.2 ml of 0.9% saline for control sessions. The dose for each drug was determined by a previous study that tested all of these drugs at three different doses in the same behavioral task (Galloway et al., 2014). The order of drug administration was counterbalanced to the extent possible with 3 experimental drug conditions and 7 rats.

The M_4 PAM was formulated as an HCl salt in a solution of Tween-80 (13-17% v/v in nuclease free H_2O). The M_1 PAM was formulated as a sodium salt in a solution of 2-hydroxypropyl-β-cyclodextrin (15% v/v in nuclease free H_2O) and nuclease free H_2O. The M_1 agonist was formulated as an HCl salt in nuclease free H_2O. For all drugs, the pH was titrated to 6.5–8.5 using 1 N NaOH and 1 mol/l HCl. Each drug was chosen for its selectivity for the target mAChR subtype versus the other mAChR subtypes, lack of activity at other off-target proteins (e.g. ion channels, G-protein coupled receptors, etc.), brain penetration, and evidence of *in vivo* efficacy of altering memory performance in mice and rats (Brady et al., 2008; Digby et al., 2012; Galloway et al., 2014; Lebois et al., 2011; Shirey et al., 2009).

Surgery

Sterile tip surgery was performed on each rat to implant a chronic recording assembly with up to 32 tetrodes to record cellular activity in the hippocampus. The

recording assembly was affixed to the skull above a craniotomy centered at 3.5 to 4.5 mm posterior to bregma and 2.0 to 2.5 mm lateral to the central suture so that tetrodes could be independently lowered ventrally into the target regions of the hippocampus. Each tetrode consisted of four 12.5 µm nichrome wires. To facilitate the detection of individual units in the hippocampus, the tip of each tetrode was plated in gold to reduce impedance to 100-200 kΩ at 1 kHz.

Prior to surgery, rats were anesthetized with isoflurane in 1-3% oxygen and injected s.c. with 0.05 mg/kg buprenorphine and 5mL lactated ringers. Nine stainless-steel screws were secured along the ridges of the skull to serve as an anchor for the recording assembly. One additional stainless steel screw, soldered to a wire attached to the recording assembly, served as the ground and was secured to the posterior portion of the skull centered above the cerebellum. The base of the chronic recording assembly was secured to the anchor screws with dental acrylic. During surgery, tetrodes were slowly lowered into the brain so that they were roughly 1mm above the targeted regions. Topical antibiotics were applied to the incision site before the incision was closed with 2-3 stitches. Soon after rats began to ambulate post-surgery, they were given an oral dose of .75mL meloxicam. One day after surgery, rats were given s.c. injections of 0.05 mg/kg buprenorphine and an oral dose of .75mL meloxicam, and two days after surgery rats were given an oral dose of 0.75mL meloxicam only. All rats were given 1 week to recover, and then tetrodes were slowly lowered, 20-80µm at a time, into the target regions of the hippocampus using hallmark electrophysiological cues. To minimize the movement of tetrodes between drug conditions, tetrodes were not raised or lowered from 24 hours prior to the first testing session until the rat was euthanized. After testing was

completed and just prior to euthanasia, small lesions were made at the tips of recording electrodes by passing 20-40 μA of current for 20 seconds each. After euthanasia, brains were sectioned into 40μm-thick coronal slices and stained with a cresyl violet stain to confirm the locations of tetrodes in hippocampal pyramidal layers. For recording CA1 and CA3 local field potentials, for each rat we selected one tetrode with CA1 pyramidal units and one tetrode with CA3 pyramidal units across testing sessions so that the tetrode position within the pyramidal layer was as consistent as possible across testing sessions.

Procedure

Acute drug effects on hippocampal function were measured while rats performed an object recognition memory task that involved completing multiple laps in which novel or familiar objects were placed on the outer edge of a circular track for rats to explore voluntarily. Object recognition memory tasks take advantage of the innate novelty preference of rats (Ennaceur & Delacour, 1988), so that memory for a repeated object is inferred by the reduced time rats spontaneously explore repeated objects relative to novel objects. Prior to surgery, rats were trained to complete laps around a circular track for a small chocolate reward. After they reached a pre-surgery criteria of completing 80 laps around the circular track in 40 minutes, they were implanted with a 32-tetrode chronic recording assembly. Testing typically occurred 1-3 months after surgery. After recovering from surgery, rats were retrained to the pre-test criteria to complete 80 laps in 40 minutes. Rats began testing as soon as they were re-trained to complete laps on the track and the tetrodes reached their targeted subregion in the hippocampus.

To test drug effects on the memory performance and CA3-CA1 oscillatory synchrony of rats, we used the same object recognition task used in a previous

experiment to test the effects of the M_4 PAM, M_1 PAM, and M_1 agonist on memory performance (Galloway et al., 2014). Objects ranged from 10 – 2,000 cm^3 in size and were made of wood, ceramic, metal, or plastic material. Figure 2.1 shows a schematic of the daily testing sessions and object recognition memory task. Each rat completed five sessions on five different days. On each day of testing, rats were injected s.c. with the M_4 PAM, M_1 PAM, M_1 agonist, or vehicle control 30 minutes prior to performing the object recognition memory task. The two control sessions were always session 1 and session 5, and rats were administered one of the experimental drugs in sessions 2-4. Rats were administered the experimental drugs in a partially counterbalanced order (a full counterbalancing was not possible with 3 drugs conditions and 7 rats). The object recognition memory task consisted of a study phase, short delay, and test phase. On each of the 12 laps of the study phase, rats encountered a different novel object affixed to adjustable flaps in one of two locations (10 or 2 o'clock) on the side of the track. After a 5-minute delay, rats encountered 24 objects during the test phase. Half of the objects encountered during the test phase were novel, and half were duplicate repeats of the study phase objects. Repeated objects always appeared in the same location as they had appeared during the study phase. The location in which study and test phase objects appeared was pseudo-counterbalanced so that a rat did not encounter different objects in the same location more than two laps in a row. During the test phase, repeat and novel objects were intermixed so that rats did not encounter novel or repeated objects more than two laps in a row.

Analyses

Each session was digitally recorded by a video camera mounted to the ceiling of the testing room, and object exploration for the study and test phase was scored by an experimenter who was blind to the drug condition. Memory performance was measured as a discrimination index (DI) calculated from the raw exploration of rats during the test phase: DI = mean novel / (mean novel + mean repeated). A DI of 0.50 reflects chance performance, and a DI of 0.66 indicates that rats explored novel objects twice as much as repeated objects and reflects good performance (Clark & Squire, 2010). The DI, as opposed to raw exploration times, allows for comparisons of memory performance across drug conditions that may change the general disposition of rats to explore objects voluntarily.

LFPs were recorded at a sample rate of 1500 Hz and filtered at 1-400 Hz with the NSpike data acquisition system (nspike.sourceforge.net). The position of rats was recorded by a digital video camera mounted to the ceiling of the testing environment at a 30 Hz sampling rate (30 frames per second). After testing, the rat's location on each frame was calculated using custom MATLAB software, or manually by a trained experimenter, using information emitted from red and green light emitting diodes that were attached to the recording assembly during testing.

LFP data was analyzed with an open source library of functions (Chronux; Bokil et al., 2010). In order to reduce bias and variance from choosing any one taper method, we used multi-taper fast fourier transform to calculate coherence and power for the theta and slow gamma frequency bands (Bokil et al., 2010). To reduce the assumption that oscillatory data is stationary (Mitra & Pesaran, 1999), spectral estimates of the LFPs were calculated in time windows of 0.5 seconds. For the slow gamma frequency band, we used

a frequency half bandwidth of 6 Hz, so that the spectral estimates for each frequency was averaged across +/- 6 Hz and 5 tapers. For the theta frequency band, we used a frequency half bandwidth of +/- 2 Hz and 1 taper. The number of tapers for the different frequency range was determined by the formula 2TW – 1, with T = time of each window of samples (0.5 seconds) and W = frequency bandwidth range (slow gamma = 6 Hz; theta = 2 Hz).

Run speeds were calculated by the frame-to-frame change in the x/y coordinates of the rat within a session. Run speeds when rats were not exploring objects were split into five equal bins (1-12, 13-23, 24-34, 35-45, 46-56 cm/sec) to distinguish between oscillatory changes related to movement as opposed to memory encoding. The 1-12 cm/sec bin run speed bin was considered stationary. The number of 0.5-second sweeps in each run bin for different rats and different run bins was variable, and a low number of sweeps can upwardly bias spectral estimates. Thus, we found the minimum number of 0.5-second sweeps for each rat over all sessions and run bins. Then for each run bin within a session for a given rat, we subsampled from the total sweeps in that run bin 500 different times, and calculated spectral estimates using that rat's minimum sweep number of randomly scrambled sweeps. The final coherence or power value for one run bin of one session of one rat reflects the mean across all 500 subsamples.

CA3-CA1 coherence was used to quantify oscillatory synchrony between these regions. The coherence scores reflect both the consistency of the phase relationship and covariance in amplitude between CA3 and CA1 (Mitra & Pesaran, 1999). Coherence was averaged within each sample and then across trials, and Fisher transformed. Similarly to Trimper and colleagues (2014), CA1 power and CA3 power were calculated in order to determine if any potential changes in CA3-CA1 coherence also corresponded to changes

in power from either subregion. Power estimates of CA1 and CA3 were log10 transformed and multiplied by 10 to convert from bels to decibels. To allow for statistical comparison, both coherence and power were bias corrected. Analyses focused specifically on coherence and power within the theta (7-9 Hz) and gamma frequency (30-55 Hz) bands, as these bands were previously found to relate to task performance (Trimper et al., 2014). For every analyses, the control condition values represent the mean calculations of the combined LFP data from both control sessions.

Results

In general, the rats performed well on the memory task across most conditions. Figure 2.2 shows the average DI for each drug condition. The results are shown for the mean DI across rats. A one-way (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) repeated measures ANOVA on DIs confirmed that the DIs were similar across drug conditions. The exception to the good memory performance was in the M_1 agonist condition, in which a preplanned within-subjects contrast showed that there was a statistical trend for rats to have lower DIs relative to control ($F(1,6)=4.776$, $p=0.072$). Rats were also found to perform similarly across drug conditions when the exploration times for 'familiar' objects that were not explored during the study phase were excluded from the DI calculations, and when raw exploration times during the study phase for each session for each rat were included as covariates (data not shown). Thus, the administration of these three muscarinic activators did not appear to improve memory performance in the present study, in contrast to the findings of a previous study using the same drugs (Galloway et al., 2014).

In light of the possibility that measures of oscillatory synchrony may be more sensitive than memory performance to detect experimental drug effects on hippocampal function, the effects of experimental drugs on oscillatory synchrony in the hippocampus were also analyzed. Measures of neural synchrony (coherence between CA1 and CA3) and spectral estimates (power within CA1 and CA3) between drug conditions are shown across a broad (3-100 Hz) frequency range, but power and coherence within theta and gamma were averaged so that drug effects on theta and slow gamma coherence and power could be tested statistically. The data were analyzed separately for moments of object exploration and for locomotion across a range of running speeds, as exploration and running speed both influence hippocampal oscillations (Ahmed & Mehta, 2012; Jewajee et al., 2008). In an effort to show the potential effect of M_1 agonism, all p values reported from preplanned within-subjects contrasts were calculated with Tukey's LSD, and alpha levels were not adjusted for possible inflation from multiple comparisons. Results from CA1 and CA3 power are reported before the results for CA3-CA1 coherence. For both power and coherence, the results during novel object exploration are reported before the results from when rats were not exploring novel objects. As a broad overview: administration of the muscarinic drugs influenced hippocampal power but not coherence.

CA1 and CA3 power

The M_4 PAM decreased CA3 slow gamma power when rats were exploring novel objects. As a measure of hippocampal function, we analyzed how experimental drugs affected theta and slow gamma power within CA1 and CA3. Figure 2.3 shows average CA1 (top panels) and CA3 (bottom panels) broadband power values of each

frequency bin within each drug condition. The results are shown for the mean power in decibels across run speeds (cool to warm indicate stationary to very fast run speeds) or novel object exploration (shown in blue). In order to better understand how drugs affected theta and slow gamma power, Figure 2.4 shows average CA1 (top panels) or CA3 (bottom panels) power in each drug condition relative to control within the theta (left panels) or slow gamma (right panels). The results are shown for the mean power values at each run speed or novel object exploration (far right of each subplot). The experimental drugs generally decreased hippocampal power when rats were not exploring novel objects.

A three-way 2 (region: CA1 or CA3) x 2 (frequency: slow gamma or theta) x 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) ANOVA with repeated measures on overall hippocampal (CA1 and CA3) power across theta and slow gamma during novel object exploration revealed that theta power was higher than slow gamma power (F[1,6]=1144.425, p=0.000), and the difference in theta power and slow gamma power differed between CA1 and CA3 (F[1,6]=94.617, p=0.000). Preplanned within-subjects contrasts showed that the M_4 PAM generally decreased hippocampal power across theta and slow gamma during exploration relative to control (F[1,6]=9.824, p=0.020). There was a trend for the M_1 PAM to generally decrease hippocampal power across theta and slow gamma relative to control (F[1,6]=4.128, p=0.088), and a trend for the M_1 PAM effects on hippocampal power to differ between theta and slow gamma (F[1,6]=5.261, p=0.062). The M_1 agonist did not have a general effect on hippocampal power across theta and slow gamma during exploration. The results suggested that the M_4 PAM

decreased hippocampal power across slow gamma and theta during exploration, and the M_1 PAM may affect theta and slow gamma power differently.

Next, we conducted separate analyses on the drug effects on power across theta and slow gamma within CA1 and CA3. There was not an overall drug difference in CA1 power or CA3 power across theta and slow gamma during novel object exploration. Two-way 2 (frequency: theta or slow gamma) x 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) ANOVAs with repeated measures on CA1 or CA3 power across theta and slow gamma showed that theta power was higher than slow gamma power in CA1 (F[1,6]=528.390, p=0.000) and CA3 (F[1,6]=925.379, p=0.000). Preplanned within-subjects contrasts found that the M_4 PAM decreased CA3 power across theta and slow gamma relative to control (F[1,6]=11.452, p=0.015), and there was a trend for the M_4 PAM to decrease CA1 power across theta and slow gamma (F[1,6]=4.404, p=0.081). Thus, the general decrease of hippocampal oscillatory power across theta and slow gamma for the M_4 PAM condition across regions was driven largely by the decrease in CA3 power.

In parallel to the region-specific analyses, we conducted separate analyses on the drug effects across CA1 and CA3 within theta and slow gamma power. There was no main effect of drug in either frequency range, but slow gamma power was higher in CA3 than CA1 (F[1,6]=16.595, p=0.007). Preplanned within-subjects contrasts on theta and slow gamma power across CA1 and CA3 revealed a trend for the M_4 PAM to decrease slow gamma power across CA1 and CA3 (F[1,6]=5.978, p=0.050), and there was a trend for both the M_4 PAM and the M_1 PAM to decrease theta power across CA1 and CA3 (M_4 PAM: F[1,6]=4.461, p=0.079; M_1 PAM: F[1,6]=4.822, p=0.071). The results did not

indicate that any of the experimental drugs generally decreased slow gamma power across CA1 and CA3.

Finally, CA1 theta power, CA1 slow gamma power, CA3 theta power, and CA3 slow gamma power were analyzed separately with one-way (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) ANOVAs with repeated measures on power during novel object exploration. There was no overall drug effect for CA1 theta power, CA1 slow gamma power, or CA3 theta power, but there was a main effect of drug for CA3 slow gamma power ($F[3,18]=3.362$, $p=0.042$). Preplanned within-subjects contrasts confirmed that were was no effect of any experimental drug relative to control for CA1 power in either frequency range during novel object exploration. Although there was no overall drug effect for CA3 theta power, preplanned within-subjects contrasts did reveal a trend for the M_4 PAM to decrease CA3 theta power relative to control ($F[1,6]=5.145$, $p=0.064$). The overall drug effect in CA3 slow gamma power seemed to be driven by the decreased power in the M_4 PAM condition relative to control ($F[1,6]=9.696$, $p=0.021$). Taken together, the most robust drug effect when rats were exploring novel objects was for the M_4 PAM to decrease power, most strongly for CA3 slow gamma power. There was a statistical trend for the M_1 PAM to decrease theta power across CA1 and CA3, but the effect was not apparent within CA1 theta power or CA3 theta power. None of the analyses indicated that the M_1 agonist decreased power, across or within theta and slow gamma or CA1 and CA3, when rats were exploring novel objects.

All of the experimental drugs generally decreased power when rats were not exploring novel objects. All of the experimental drugs decreased hippocampal power when rats were not exploring novel objects in at least one particular frequency range or

region. A four-way 2 (region: CA1 or CA3) x 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 2 (frequency: theta or slow gamma) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVA with repeated measures on hippocampal power across theta and slow gamma revealed that generally, hippocampal power across theta and slow gamma was not the same across drug conditions (F[3,18]=6.831, p=0.003), theta power was higher than slow gamma power (F[3,18]=673.735, p=0.000), and the difference between CA1 and CA3 power differed between theta and slow gamma power (F[1,6]=161.911, p=0.000). The effect of run speed on hippocampal power across theta and slow gamma differed between CA1 and CA3 (F[4,24]=24.464, p=0.000) and theta and slow gamma (F[4,24]=18.329, p=0.000), and there was also a trend for a three way interaction between drug, frequency, and run speed (F[12,72]=1.866, p=0.053). Preplanned within-subject contrasts revealed that all of the experimental drugs decreased hippocampal power across theta and slow gamma relative to control (M_4 PAM F[1,6]=9.497, p=0.022; M_1 PAM (F[1,6]=13.617, p=0.010; M_1 agonist F[1,6]=10.420, p=0.018). There was also a trend for the effects on hippocampal power across theta and slow gamma of the M_1 PAM relative to control to differ between theta and slow gamma and across different run speeds (F[1,6]=4.355, p=0.082), and a trend for the effects of the M_1 agonist on hippocampal power across theta and slow gamma relative to control to differ between theta and slow gamma (F[1,6]=4.441, p=0.080). These results suggest that the M_4 PAM, M_1 PAM, and M_1 agonist may affect hippocampal power differently across CA regions, frequency ranges, and run speeds when rats are not exploring novel objects.

All of the experimental drugs, especially the M_1 PAM and M_1 agonist, decreased CA1 power when rats were not exploring novel objects. Separate analyses

were conducted for CA1 power and CA3 power across theta and slow gamma. A three-way 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 2 (frequency: theta or slow gamma) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVA with repeated measures on power across theta and slow gamma showed that CA1 power across theta and slow gamma was generally different across drug conditions when rats were not exploring novel objects ($F[3,18]=6.831$, $p=0.003$), and the effects of drugs on CA1 power across theta and slow gamma differed between theta and slow gamma ($F[3,18]=3.944$, $p=0.025$). CA1 theta power was higher than CA1 slow gamma power ($F[1,6]=674.507$, $p=0.000$), and there was a 3 way interaction between drug, frequency ranges, and run speed on CA1 power across theta and slow gamma ($F[12,72]=2.066$, $p=0.030$). CA1 power across theta and slow gamma was also different across run speeds ($F[4,24]=6.808$, $p=0.001$), but the effects of different run speeds on CA1 power across theta and slow gamma differed between theta and slow gamma ($F[4,24]=11.519$, $p=0.000$). Preplanned within-subjects contrasts revealed that CA1 power across theta and slow gamma was significantly decreased relative to control in the M_1 PAM ($F[1,6]=20.829$, $p=0.004$) and the M_1 agonist condition ($F[1,6]=21.016$, $p=0.004$), and there was a trend for lower CA1 power across theta and slow gamma in the M_4 PAM condition relative to control ($F[1,6]=4.797$, $p=0.071$). The effect of the M_1 PAM on CA1 power across theta and slow gamma relative to control was different for theta and slow gamma ($F[1,6]=7.405$, $p=0.035$). The results indicated that increasing M_1 activity with the M_1 PAM or M_1 agonist decreased CA1 power across theta and slow gamma relative to control when rats were not exploring novel objects.

Separate analyses for CA1 slow gamma and CA1 theta power when rats were not exploring novel objects showed that the M_1 PAM and M_1 agonist decreased power in both frequency ranges. Two-way 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVAs with repeated measures revealed that there was a main effect of drug on CA1 theta power (F[3,18]=7.091, p=0.002) and CA1 slow gamma power (F[3,18]=3.226, p=0.047). CA1 theta power and CA1 slow gamma power also differed across run speeds (theta F[4,24]=10.457, p=0.000; slow gamma F[4,24]=5.687, p=0.002). Preplanned within-subjects contrasts showed that CA1 power decreased in both frequency ranges for the M_1 PAM (theta F[1,6]=10.639, p=0.017; slow gamma F[1,6]=9.992, p=0.020) and M_1 agonist (theta F[1,6]=15.481, p=0.008; slow gamma F[1,6]=10.526, p=0.018). There was a trend for the M_4 PAM to decrease CA1 slow gamma power relative to control (F[1,6]=4.960, p=0.068), but not CA1 theta power. The results indicate that when rats are not exploring novel objects, the M_1 PAM and M_1 agonist decreased CA1 theta power and CA1 slow gamma power. The results also revealed that the effects of run speed are different for CA1 theta power than CA1 slow gamma power when rats are not exploring novel objects.

The M_4 PAM and the M_1 PAM decreased CA3 slow gamma power when rats were not exploring novel objects. A three-way 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 2 (frequency: theta or slow gamma) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVA with repeated measures showed that CA3 power across theta and slow gamma was generally different across drug conditions (F[3,18]=3.951, p=0.025), and CA3 theta power was higher than CA3 slow gamma power (F[1,6]=348.795, p=0.000). In contrast to CA1, there was not a significant difference

between drug effects between CA3 theta power and CA3 slow gamma power (F[3,18]=0.533, p=0.653). As with CA1 power, CA3 power across theta and slow gamma was different across run speeds (F[4,24]=7.706, p=0.000), and the effects of different run speeds on CA3 power across theta and slow gamma differed between theta and slow gamma (F[4,24]=20.931, p=0.000). There were no statistically significant differences in CA3 power across theta and slow gamma between any of the experimental drugs relative to control, although in the M_4 PAM condition there was a trend for a three way interaction between drug, frequency, and run speed (F[1,6]=5.269, p=0.061).

When CA3 power was analyzed separately for the theta and slow gamma frequency ranges, two-way 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVAs with repeated measures revealed that CA3 slow gamma power was different across drug conditions (F[3,18]=5.125, p=0.010), and there was a trend for CA3 theta power to differ across drug conditions (F[3,18]=2.621, p=0.082). Both CA3 theta power and CA3 slow gamma power differed across run speeds (theta F[4,24]=2.806, p=0.048; slow gamma F[4,24]=64.878, p=0.000). Unlike CA1 theta power, there was a trend for the drug effects on CA3 theta power to differ across run speeds (F[12,72]=1.809, p=0.063). Preplanned within-subjects contrasts revealed that the M_1 PAM significantly decreased CA3 slow gamma power relative to control (F[1,6]=11.917, p=0.014), and there was a trend for the effects of the M_1 PAM on CA3 slow gamma power to differ across run speeds (F[1,6]=4.001, p=0.092). There was also a trend for the M_1 PAM to decrease CA3 theta power relative to control (F[1,6]=5.022, p=0.066). Although the M_1 agonist did not generally decrease CA3 theta power relative to control, there was a trend for the effects

of the M_1 agonist on CA3 theta power to differ across run speeds (F[1,6]=5.955, p=0.050). The M_4 PAM significantly decreased CA3 slow gamma power (F[1,6]=11.079, p=0.016). The results revealed that although the M_4 PAM may not decrease CA3 power across theta and slow gamma, the M_4 PAM did specifically decrease CA3 slow gamma power. The M_1 PAM decreased CA3 slow gamma power but the decrease did not reach statistical significance in CA3 theta power, and the M_1 agonist did not decrease CA3 theta power or CA3 slow gamma power.

Taken together, when rats were not exploring novel objects the general difference in hippocampal power across drug conditions showed a very different pattern of results when the effect of each drug relative to control was analyzed separately between CA regions and frequency ranges. The M_1 PAM showed the most consistent effect on power across regions and frequency ranges, and significantly decreased CA1 theta power, CA1 slow gamma power, and CA3 slow gamma power. The M_1 agonist decreased both CA1 theta power and CA1 slow gamma power, but did not decrease CA3 power in either frequency range. The M_4 PAM decreased CA3 slow gamma power.

CA3-CA1 Coherence

CA3-CA1 coherence differed between running and exploring objects but not across drug conditions. In agreement with past studies (Trimper et al., 2014), novel object exploration coincided with an increase in CA3-CA1 slow gamma coherence. Figure 2.5 shows average CA3-CA1 broadband coherence values within each drug condition. The results are shown for the mean coherence values across run speeds (cool to warm indicate stationary to very fast run speeds) or novel object exploration (shown in blue). In all drug conditions, CA3-CA1 slow gamma coherence was higher when rats

were stationary and exploring novel objects relative to when rats were stationary but not exploring novel objects. Although the experimental drugs generally decreased hippocampal power, the power decreases did not translate into changes in CA3-CA1 coherence across drug conditions. Figure 2.6 shows average CA3-CA1 coherence values within each drug condition within the theta or slow gamma frequency range. The results are shown for the mean coherence values at each run speed or novel object exploration (far right of each subplot) for either theta (both supblots to the left) or slow gamma (both supblots to the right). The top panels show absolute coherence values, and the lower panels show the difference of each experimental drug relative to control. CA3-CA1 coherence in both slow gamma and theta was similar across drug conditions, but there was a robust effect of run speed on CA3-CA1 coherence in both frequency ranges.

There was no overall drug effect within or across frequency ranges on CA3-CA1 coherence when rats were exploring novel objects. A two-way 2 (frequency: theta or slow gamma) x 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) mixed ANOVA with repeated measures on CA3-CA1 coherence across theta and slow gamma did not indicate that CA3-CA1 coherence in general was different across drug conditions, although preplanned within-subjects contrasts revealed a trend for the M_1 PAM to affect CA3-CA1 coherence across theta and slow gamma differently within the theta and slow gamma frequency ranges ($F[1,6]=4.399$, $p=0.081$). One-way (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) ANOVAs with repeated measures for each frequency range showed no overall drug effect for either CA3-CA1 theta coherence or CA3-CA1 slow gamma coherence, but preplanned within-subjects showed a trend for the M_1 PAM to increase CA3-CA1 theta coherence relative to control (theta $F[1,6]=4.482$, $p=0.079$). Thus, the

results showed that CA3-CA1 slow gamma coherence and CA3-CA1 theta coherence was similar between each of the experimental drug conditions relative to control during novel object exploration.

CA3-CA1 slow gamma and theta coherence was not different across drug conditions, but did differ across run speeds, when rats were not exploring objects. The results indicated that run speed had a strong influence on CA3-CA1 theta coherence and CA3-CA1 slow gamma coherence when rats were not exploring objects. A three-way 4 (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 2 (frequency: theta or slow gamma x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVA with repeated measures on CA3-CA1 coherence across theta and slow gamma , followed by two-way (drug: control, M_4 PAM, M_1 PAM, or M_1 agonist) x 5 (run speed: 1-12, 12-23, 23-34, 34-45, 45-56) mixed ANOVA with repeated measures on CA3-CA1 coherence within theta and slow gamma did not indicate that CA3-CA1 coherence differed across drug conditions when rats were not exploring novel objects. There was a trend for the M_4 PAM condition to increase CA3-CA1 slow gamma coherence relative to control (F[1,6]=4.464, p=0.079). The run speeds of rats strongly influenced CA3-CA1 coherence across theta and slow (F[4,24]=3.714, p=0.017), and the effect of run speed differed between drug conditions (F[12,72]=2.067, p=0.030) and between frequency ranges (F[4,24]=12.865, p=0.000). There was also a main effect of run speed within CA3-CA1 theta coherence (F[4,24]=16.401, p=0.000) and CA3-CA1 slow gamma coherence F[4,24]=6.584, p=0.001), but there was not an interaction between run speed and drug condition when each frequency range was analyzed separately. The results suggest that the general tendency for CA3-CA1 theta coherence to increase with higher run speeds and for CA3-

CA1 slow gamma coherence to decrease with higher run speeds was similar across drug conditions. The difference in the effect of run speed between different frequency ranges highlights the importance of including run speed as a variable when comparing spectral estimates in rats performing non-stationary tasks.

The results from both behavioral states showed that the M_4 PAM had the strongest effect on CA3 slow gamma power, both when rats were exploring novel objects and when they were not. The M_1 PAM decreased CA1 theta power, CA1 slow gamma power, and CA3 slow gamma power when rats were not exploring novel objects, but there was no evidence the M_1 PAM decreased power in any CA region or frequency range when rats were exploring novel objects. The M_1 agonist decreased CA1 theta and CA1 slow gamma power when rats were not exploring novel objects, but there was no evidence that the M_1 agonist decreased power within any region or any frequency range when rats were exploring novel objects. Finally, decreased hippocampal power in any drug condition did not seem to appreciably change CA3-CA1 slow gamma coherence or CA3-CA1 theta coherence whether rats were or were not exploring novel objects.

Discussion

The current study investigated how oscillatory synchrony and power in the hippocampus reflected brain network changes in rats performing an object recognition memory task after acute and systemic administration of drugs that selectively increased the activity of M_1 or M_4. We found that CA3-CA1 slow gamma coherence increased when rats were exploring novel objects, more so than when they were stationary but not exploring novel objects. However, the main findings related to muscarinic drug administration were unexpected, and raise several interesting points of discussion that are

organized around the following themes: 1) drugs that selectively increase M_1 or M_4 mAChRs did not increase CA3-CA1 slow gamma coherence above control, 2) selectively increasing M_1 or M_4 mAChRs did decrease power in the hippocampus, but the power decreases did not change overall CA3-CA1 coherence, 3) the power decreases from the M_4 PAM, M_1 PAM and M_1 agonist could have driven the memory improvements found in previous studies, 4) the advantage or disadvantage of selectively increasing M_1 or M_4 activity may depend on the baseline state of the neural network. Throughout each theme, the drug effects on oscillatory synchrony and power in the hippocampus should be interpreted in light of the similar memory performance across drug conditions.

In line with previous studies (e.g. Trimper et al., 2014), we found that novel object exploration is associated with an increase in oscillatory synchrony between the CA3 and CA1 subregions of the hippocampus. Importantly, CA3-CA1 slow gamma coherence when rats were stationary and exploring novel objects was higher than when rats were stationary but not exploring novel objects. This supports the idea that transient increases in CA3-CA1 slow gamma coherence does not merely reflect changes in locomotive states, but reflects increased communication between CA1 and CA3 that is relevant to memory (Colgin et al., 2009; Trimper et al., 2014).

CA3-CA1 slow gamma coherence in each of the experimental drug conditions was not statistically different from control. This result was unexpected, as both the M_1 PAM and M_1 agonist increased CA3-CA1 slow gamma coherence above control in healthy young rats during a random foraging task (Lebois et al., 2016). Notably, in a previous study all of the experimental drugs improved memory performance only in otherwise healthy rats who were performing poorly at baseline (Galloway et al., 2014).

One possible explanation for the lower performance in the M_1 agonist condition in particular is that the dose used was higher than the dose that was previously associated with the most improvement in memory performance in rats (0.1 mg/kg in Galloway et al., 2014 vs. 1 mg/kg in the current study). In addition, rats in the current study showed above-chance performance on the object recognition memory task in the control condition, and the relatively low number of animals did not permit a parsing of the data by rat baseline performance. Thus, it is possible that selectively increasing the activity of M_4 or M_1 mAChRs would bias the network toward successful memory encoding that included changes in hippocampal oscillatory synchrony if the baseline network state was operating sub-optimally. It is also possible that changes in hippocampal oscillatory synchrony after acute, systemic administration of the M_1 PAM and the M_1 agonist depend on the background network state associated with different task demands (random foraging for food vs. voluntary exploration of objects).

The most robust finding in our analyses was that all of the experimental drugs decreased theta, slow gamma, or both theta and slow gamma power in the hippocampus when rats were not exploring novel objects. This may seem paradoxical, given the overall lack of drug effect on CA3-CA1 theta and slow gamma coherence. However, in addition to the consistency of the phase relationship between two signals, coherence values also reflect the degree of co-modulation of amplitude between the two signals, as opposed to the individual power of one signal or the other. Therefore, it seems that any given decrease in hippocampal theta or slow gamma power within CA1 or CA3 from any of the experimental drugs did not change the overall consistency in phase relationship or co-variation in theta or slow gamma power between CA1 and CA3 oscillations.

The lack of improvement in memory performance in any of the experimental drug conditions makes it difficult to know if the observed decreases in hippocampal theta or slow gamma power were meaningful for cognition, or considered a feature or drawback of the experimental drugs. Given that at least one dose of all three experimental drugs used in the current study improved memory in healthy young rats with poor baseline performance in a previous study (Galloway et al., 2014), it is possible that all of the patterns of change would benefit the overall network function in rats with poor baseline performance. For example, the M_4 PAM decreased CA3 slow gamma power when rats were exploring novel objects and when they were not. Perhaps the decreased power in CA3 resulted in less input from CA3 into CA1, which biased the hippocampal network to prioritize integrated sensory inputs from the entorhinal cortex into CA1 to facilitate encoding (Colgin et al., 2009). For the M_1 PAM and M_1 agonists, the reduction in hippocampal theta or slow gamma power that was prominent when rats were not exploring novel objects was not apparent when rats were exploring novel objects. Perhaps the decreased hippocampal theta or slow gamma power in the M_1 PAM and M_1 agonist conditions during non-exploration reduced coordinated activity that enables plasticity and encoding as means of reducing "noise" of irrelevant information and consequently increasing the "signal" of relevant object information when rats were actually exploring objects.

Interestingly, the decrease in hippocampal power in experimental drug conditions varied across CA regions, frequency ranges, and behaviors. Even the M_1 PAM and M_1 agonist, which both increase the activity of M_1, did not have affect hippocampal theta and slow gamma power in exactly the same way. Conversely, it is notable that the

M_4 PAM, which increases the action of the inhibitory $G_{i/o}$ protein and the M_1 PAM and M_1 agonist, which increase the activity of the excitatory G_q protein, would all impact hippocampal theta and slow gamma power in the same direction. Clearly, knowing how a drug will alter the excitability of cellular membranes that contain its target receptors does not necessarily predict the overall network response to systemic administration of experimental drugs. These results speak to the importance of using *in vivo* electrophysiology to measure the effects of experimental drug manipulations on dynamic neural networks.

Although we interpreted decreases in hippocampal theta or slow gamma power in experimental drug conditions in terms of benefiting overall hippocampal function and memory, decreases in theta or slow gamma power in CA1 and CA3 of the hippocampus might not have a simple, unidirectional relationship with brain networks and memory. Given that theta and gamma in the hippocampus coordinate memory-relevant network activity of cell assemblies and spiking activity of individual cells in the hippocampus, low to high hippocampal theta or slow gamma power might reflect worse to better coordination of network activity and by extension, memory performance. In line with this, the low average memory performance of rats in the M_1 agonist condition, although not statistically lower than the control condition, may mean that the decreased CA1 theta power and decreased CA1 slow gamma power in the M_1 agonist condition reflected dysfunction. In further support of decreased hippocampal power relating to network dysfunction and memory impairment, many animal models of aging, Alzheimer's disease, epilepsy, and TBI have shown reduced hippocampal theta and/or gamma power relative to controls (Arabadzisz et al., 2005; Jacobson et al., 2013; Pevzner et al., 2016;

Richard et al., 2013; Rubio et al., 2012; Scott et al., 2012). Yet other studies with animal models of Alzheimer's disease, schizophrenia, and Huntington's disease reported disease-related increases in hippocampal theta power (Cayzac et al., 2015; Jyoti et al., 2010; Lanre-Amos & Kocsis, 2010; Pignatelli et al., 2012; Siwek et al., 2015). Most relevant to the effects of selectively increasing mAChRs, one study found that blocking all mAChRs with scopolamine decreased memory performance and increased theta power (Masuoka et al., 2006). Notably, all of the studies mentioned above looked specifically at CA1 power and focused primarily on the theta frequency range, most were conducted with mice, and the behavioral state at the time of recording ranged from anesthetized to active engagement in a cognitive task. Given the prominence of positive and negative changes in hippocampal theta and slow gamma power observed across animal models of various brain dysfunctions and diseases, it seems likely that increases and decreases in theta and gamma power may not have straightforward consequences on cognition or brain health. Perhaps normal levels of hippocampal theta and slow gamma power reflect a healthy balance of network function, and significant alterations in either direction are pathological. In this view, selectively increasing M_1 or M_4 activity could be helpful for some diseases, or particular stages of the same disease, and harmful in others.

Alternatively, increasing M_1 or M_4 activity might have different effects on hippocampal theta or slow gamma power that depend on the baseline network state of the organism. The M_1 agonist is a partial agonist, and was demonstrated to independently activate the M_1 mAChRs with ~80% efficacy of ACh (Lebois et al., 2011). Therefore, the M_1 agonist might actually antagonize M_1 mAChRs in a brain with optimal ACh levels by locking the M_1 receptor into a submaximal state. In a diseased brain in which ACh and

other neurochemicals are not performing optimally, however, the ~80% activation could help restore M_1 activity to more closely resemble its level of activity in healthy brains. The M_1 and M_4 PAM can only increase the activity of ACh that is bound to the receptor, thereby circumventing problems that may arise from traditional agonist in disturbing the natural timing of ACh modulation (Bartfai & Wang, 2013). It is possible, however, that ACh function is most optimal for supporting cognition when it is neither too high or too low, and pushing it too high in a healthy animal will have neutral or negative outcomes. Indeed, several studies suggest that ACh levels have a range in which they are beneficial, and manipulations that push ACh levels too high or too low will impair memory performance (Balsters et al., 2011; Bentley et al., 2008; Chuah et al., 2009). Perhaps this is the case not just for ACh generally, but individual mAChR subtypes as well.

Future studies could investigate if increasing M_1 or M_4 activity in animals with brain dysfunction, such as transgenic rodent models of Alzheimer's disease, would improve cognition and how oscillatory synchrony and power within the hippocampus mediate the cognitive improvement. Perhaps doing so would allow us to use oscillations as a proxy for the state of neural networks and predict individual variation in response to therapies.

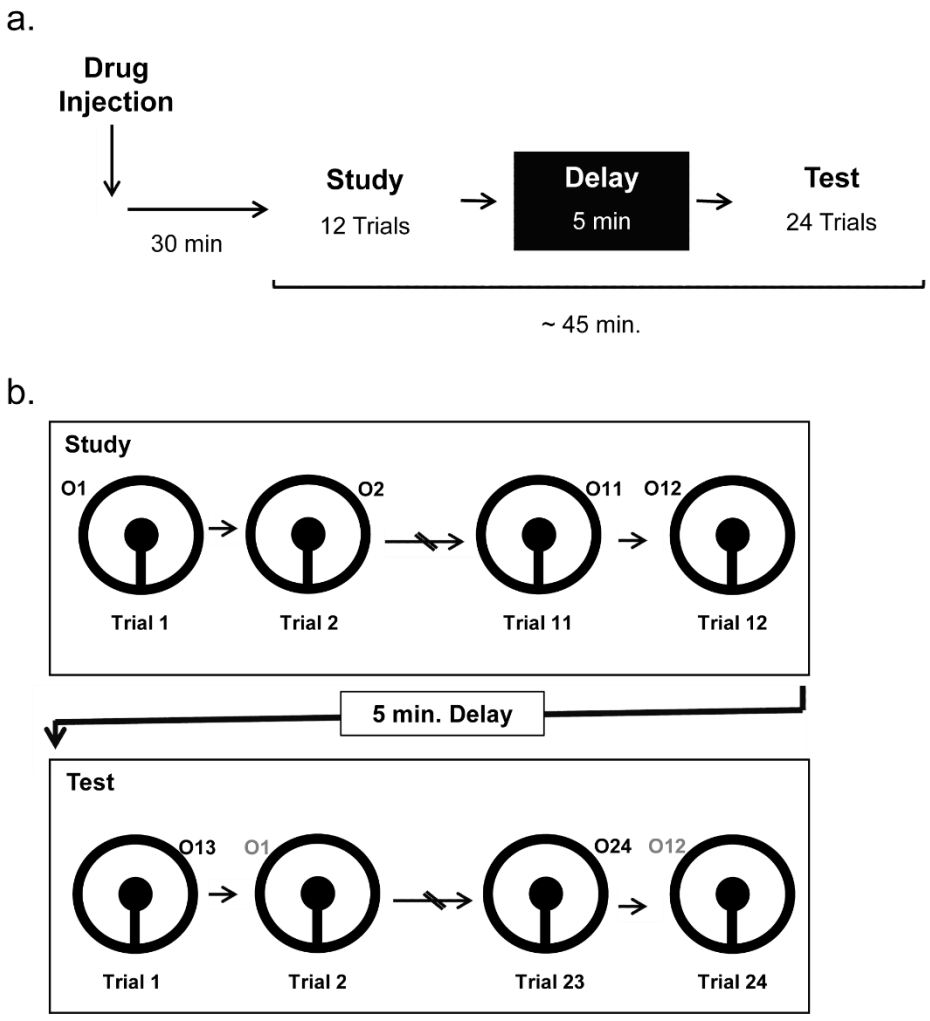

Figure 2.1 Schematic of the testing procedure. a. Each testing session began 30 min. after the drug injection, and consisted of a study phase, a 5 min delay, and a test phase. Each session lasted ~45 min. b. For each trial of both the study and test phase, rats encountered one object as they completed a clockwise lap around a circular track. During the study phase, rats encountered 12 different novel objects. During the test phase, rats encountered 12 duplicates of study phase objects (repeated objects) intermixed with an additional 12 novel objects. Individual objects are denoted by "O".

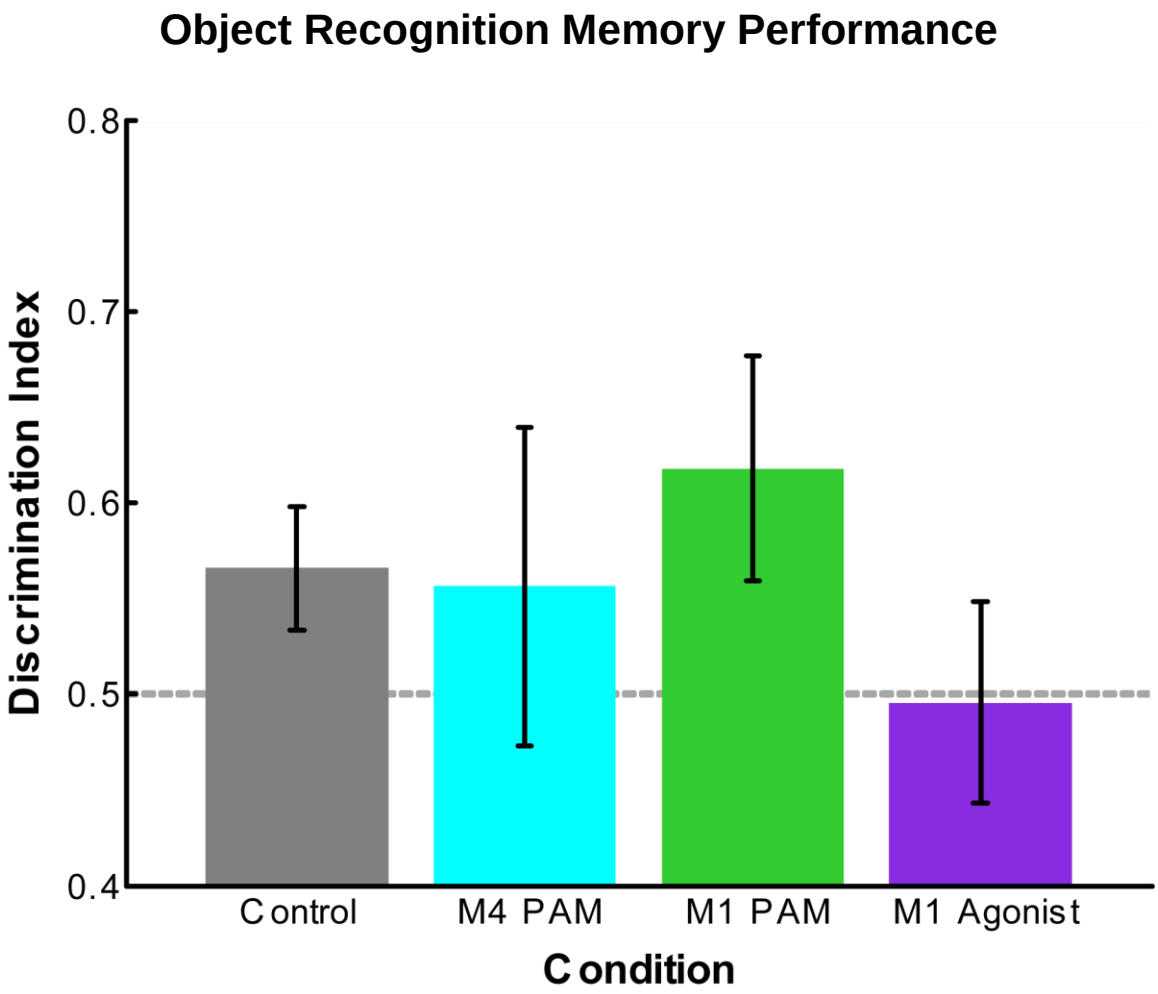

Figure 2.2. Memory performance for each drug condition. The results are shown as mean Discrimination Index (DI) across rats (N = 7). Error bars show *SEM*. The dashed line indicates chance performance. Memory performance of rats was similar across drug conditions.

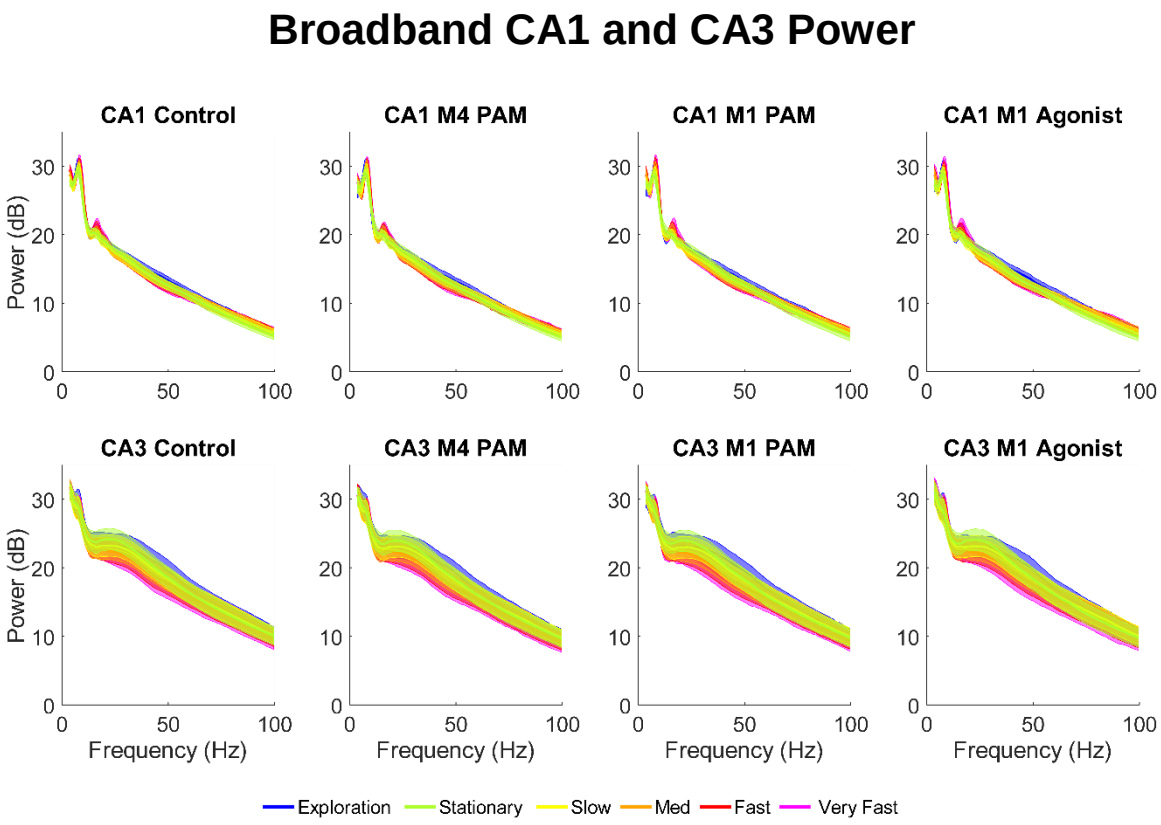

Figure 2.3. CA1 (top panel) and CA3 (bottom panel) broadband power (3-100 Hz). The means of power across rats (*N* = 7) are shown with thin dark lines and +/- *SEM* is shown in lighter shades. Different colors represent run speeds when rats were not exploring novel objects, from stationary (1-12 cm/sec) to very fast (45-56 cm/sec. Cool to warm colors reflect stationary to very fast run speeds, and novel object exploration is shown in blue. Across drug conditions, power was not increased when rats were exploring novel objects relative to when they were stationary but not exploring novel objects.

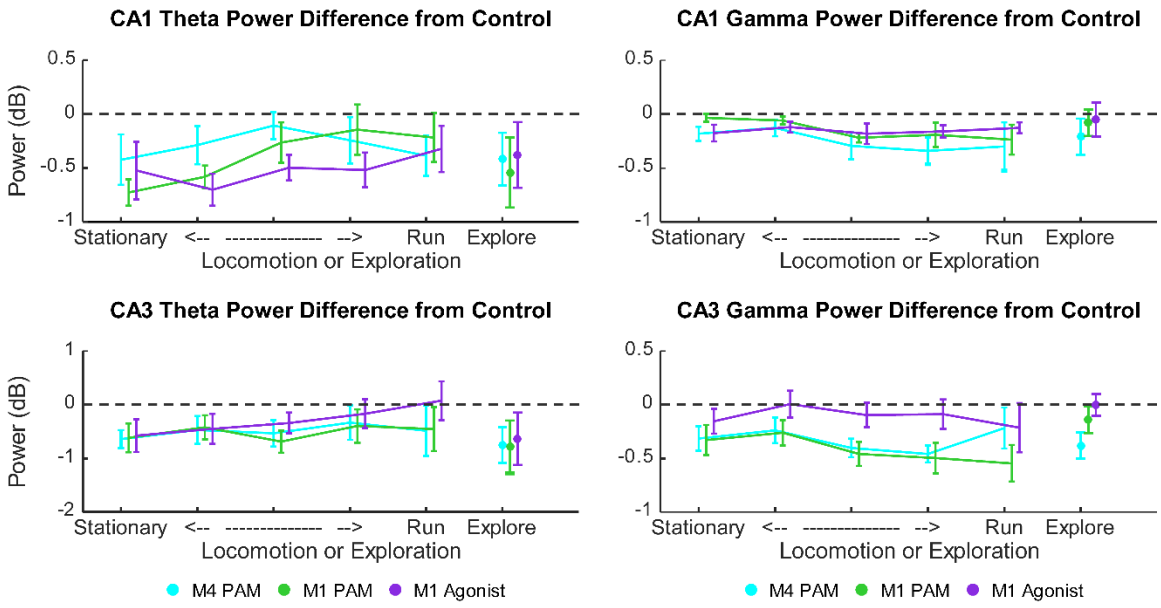

Figure 2.4. Mean difference of drugs from control in CA1 or CA3 power in theta or slow gamma frequency bands during different behavioral states. Results are shown as means across rats (*N* = 7). Error bars represent +/- *SEM*. Generally, the experimental drugs decreased hippocampal power.

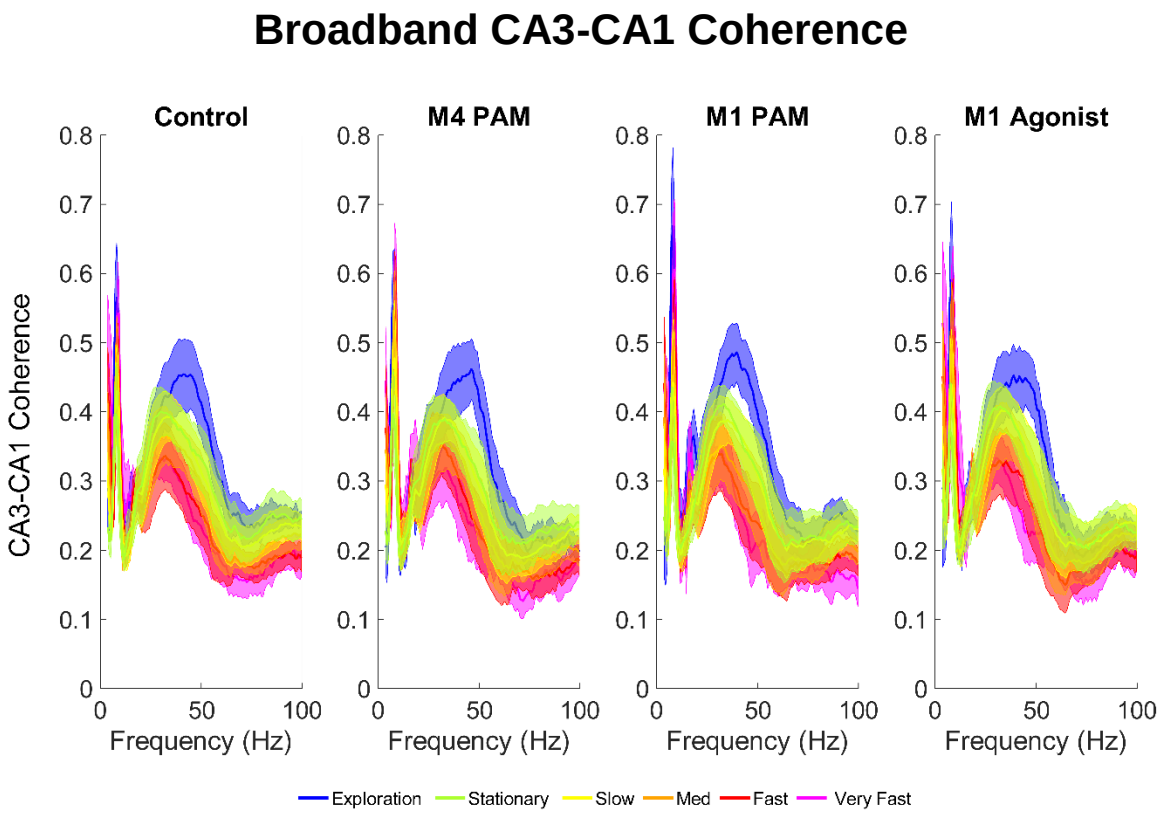

Figure 2.5. CA3-CA1 broadband (3-100 Hz) coherence. The means of coherence values across rats (N = 7) are shown with thin dark lines and +/- *SEM* is shown in lighter shades. Different colors represent run speeds when rats were not exploring novel objects, from stationary (1-12 cm/sec) to very fast (45-56 cm/sec. Cool to warm colors reflect stationary to very fast run speeds, and novel object exploration is shown in blue. For all drug conditions, CA3-CA1 slow gamma (30-55 Hz) coherence was higher during novel object exploration relative to when rats were stationary but not exploring novel objects.

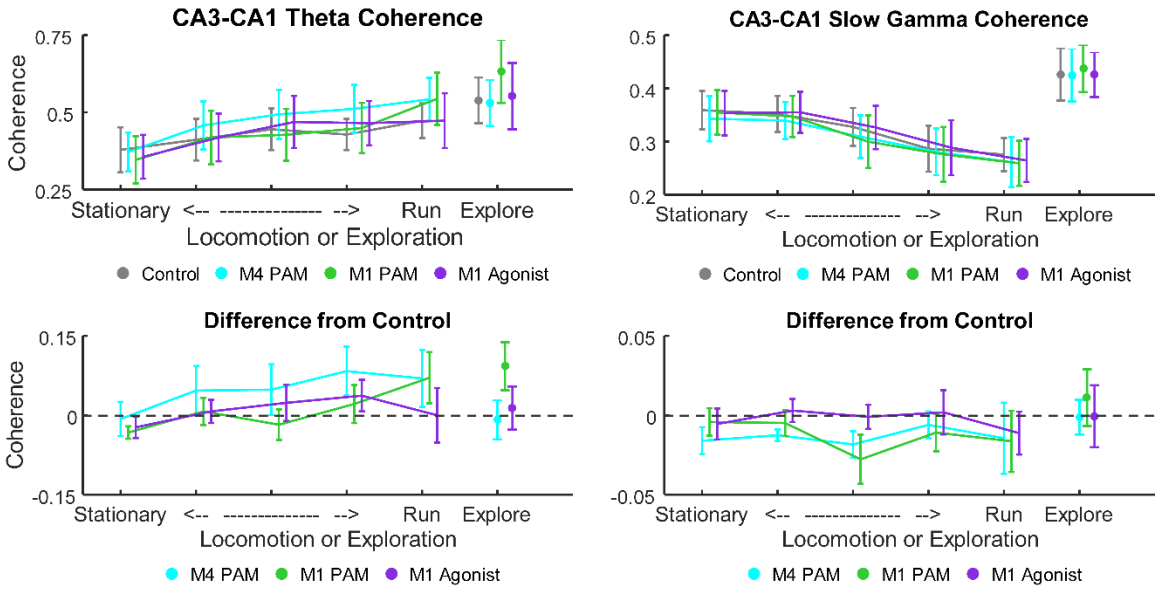

Figure 2.6. Mean CA3-CA1 coherence (top panels) and mean CA3-CA1 coherence minus control (bottom panels) in theta (left panels) or slow gamma (right panels) frequency ranges across different run speeds or during novel object exploration. Results are shown as means across rats (N = 7). Error bars represent +/- *SEM*. Generally, CA3-CA1 theta and slow gamma coherence was similar across drug conditions at different run speeds or during novel object exploration.

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Chapter 3

Longitudinal Assessment of Non-spatial and Spatial Memory in a Rat Model of Alzheimer's Disease

Abstract

Alzheimer's disease (AD) is a growing health problem that affects millions of people in the U.S., but current treatment options are limited. Many new candidate drug treatments have demonstrated efficacy in transgenic mouse models of AD, but ultimately fail in the clinic. One potential reason for the difficulty in translating preclinical findings into viable drug treatments for AD may be due to the differences between pathological features of human AD, such as tau pathology and cell death, and the pathology of transgenic AD mouse models that do not show these features. Recently, a transgenic rat model of Alzheimer's disease (AD rats) with human APP_{Swe} and PS1ΔE9 mutations was developed. AD rats age-dependently develop amyloid pathology, tau pathology and profound cell death, making them a good model to probe questions about AD that may be more readily translatable to clinical populations. AD rats were shown to have intact spatial memory performance at 6 months of age but performed poorly on a spatial memory task at 16 months. In order to further understand this rat model of AD, we asked at what age between 6 -16 months the AD rats would first show memory impairments, and whether spatial memory and non-spatial memory would be differentially impaired. Sixteen female AD rats were tested monthly from 5 months of age on a non-spatial and a spatial recognition memory task. We found that AD rats (n=8) had impaired spatial recognition memory performance by 9-12 months of age relative to wild-type controls (n=8). In contrast, AD rats performed similarly to WT controls on the spatial memory task from 5-8 months of age and on the non-spatial recognition memory task from 5-12 months. The selective memory impairment of AD rats from 9-12 months supports an important role of spatial memory dysfunction relatively early in the disease process.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by beta-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) of hyperphosphorylated tau (Querfurth & LaFerla, 2010). AD involves other signs of neural dysfunction, such as inflammation, oxidative stress, synaptic dysfunction, and cell death in later stages of AD (Querfurth & LaFerla, 2010). The degree of synaptic dysfunction and cellular loss correlate with the severity of dementia, perhaps the most well-known behavioral symptom of AD (Dekosky & Scheff, 1990; Dickson et al., 1995; Terry et al., 1991). The neuropathological changes in AD disproportionally target certain brain regions. For example, the hippocampus is an early target of NFTs, AD patients have reduced hippocampal volumes, and the hippocampi of AD patients show functional abnormalities (Braak & Braak, 2005; Chhatwal & Sperling, 2012; Vemuri & Jack, 2010). Although hippocampal dysfunction is widely understood to be an important part of AD, the nature of the dysfunction in the surviving hippocampal circuits is still not well understood, particularly on the level of single-cell activity *in vivo*.

Consistent with the hippocampal dysfunction that occurs in AD, tasks that depend on the integrity of the hippocampus are well-suited to detect cognitive impairments in AD. Several studies in human AD patients and transgenic rodent models have found that AD may particularly impair spatial associative memory, such as the ability to remember the location of a previously encountered object (Good et al., 2007; Hampstead et al., 2011; Hanaki et al., 2011; Kessels et al., 2010). This type of object-in-location spatial associative memory relates to everyday activities, such as remembering where one parked one's car in a large parking lot. Object-in-location recognition memory involves a network of brain regions that is disproportionately impacted by AD pathology, including

the hippocampus and surrounding medial temporal lobe structures (Barker & Warburton, 2011; Braak & Braak, 1995; Postma et al., 2008). Thus, object-in-location recognition memory task performance is likely to reflect hippocampal dysfunction that occurs in AD.

Animal models of AD give the opportunity to characterize changes from AD pathology at the single cell level, and use these changes as biomarkers for testing potential treatments of AD. Transgenic mouse models of AD typically express one or more familial AD mutations of one of the following three genes: APP, presenilin 1 (PS1), and presenilin 2 (PS2; Morrissette et al., 2009; Wu et al., 2012). All of these genetic mutations alter APP processing in AD mice, and give rise to many AD-like features (LaFerla & Green, 2012; Schaeffer et al., 2011, Wu et al., 2012). Although there is evidence that the characteristic NFTs of hyperphosphorylated tau are downstream consequences of Aβ plaques in humans (Gotz et al., 2001; Oddo et al., 2003), most mouse models of AD do not develop NFTs or show robust cell death unless additional genetic mutations are introduced that have not been linked to AD in humans (LaFerla & Green, 2012). However, a newly developed rat model of AD has potential to shed light on how AD progression normally unfolds in humans. Cohen and colleagues (2013) developed the TgF344 rat model of AD with the human genetic mutations APP_{Swe} and PS1ΔE9 which inevitably cause AD in humans. The AD rats age-dependently develop all of the major pathological hallmarks of the disease – Aβ plaques, NFTs of hyperphosphorylated tau, neuroinflammation, and eventually cell death (Cohen et al., 2013). AD rats also develop age-dependent cognitive impairments and show intact memory performance at 6 months, but were found to have impaired spatial memory performance at 16 months (Cohen et al., 2013). What remains to be determined is

whether spatial memory impairments are detectable in AD rats between the ages of 6 and 16 months, and if the memory impairments are specific to spatial (e.g. object-in-location) memory or extend to non-spatial (e.g. object-only) memory as well

The goal of this study was to determine if AD rats would develop age-dependent spatial memory impairments relative to their wild-type (WT) littermates at ages when AD rats still showed comparable non-spatial memory performance relative to WT rats. In order to test this prediction, we periodically tested non-spatial memory performance using an object-only recognition memory task and spatial memory performance using an object-in-location recognition memory task. Testing began when rats were young and continued until AD rats developed spatial memory impairments relative to WT rats. The object-in-location recognition memory task used in this study depends on the functional integrity of the hippocampal memory system, and is well-suited to a longitudinal design (Barker & Warburton, 2011). We found that AD rats developed selective spatial memory impairments by 9-12 months of age, when they still showed intact non-spatial memory performance.

Method

Subjects

Female rats (*n*=8 F344 [WT] and *n*=8 Tg-F344 [AD]) were tested each month from 5-12 months of age. The rats were kept on a 12-hour light/dark cycle (testing occurred during the light period) and were individually housed with free access to water and food. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

Procedure

Each month the memory performance of rats was tested with object-only and object-in-location recognition memory tasks. Object recognition memory tasks take advantage of the innate novelty preference of rats (Ennaceur & Delacour, 1988), so that memory for a repeated object can be inferred by the less time rats spontaneously explore repeated objects relative to novel objects. When rats were 4 months of age, they each were habituated for 10 minutes to the testing environment (a 91.5x91.5x61.0 cm. black box with four velcro patches, 2.5 $cm.^2$ in size, placed 30.5 cm. apart in the center) for 3 subsequent days. When rats were 5 months of age, they each were habituated for 10 minutes to the testing environment on one day that occurred 4-10 days prior to testing. For each subsequent month of testing, rats were re-habituated to the testing environment for 5 minutes, both 1 day before and immediately prior to testing.

For each month of testing, rats completed two sessions of an object-only recognition memory task within the same day, followed by two sessions of an object-in-location recognition memory task the next day. The objects used in the study were 78 – 5,000 cm^3 and made from ceramic, wood, plastic or metal material. Figure 3.1 shows the testing procedure of example trials for both tasks. Both tasks consisted of a 3 min study phase, a 2 min delay, and a 3 min test phase. During the study phase of the object-only recognition memory task, rats encountered two novel objects that were affixed in two of four possible locations within the box. During the test phase, one of the objects was replaced with a duplicate (the ‘repeated’ object) and the other was replaced with a novel object. During the study phase of the object-in-location recognition memory task, rats encountered three novel objects that were affixed in three of four possible locations within the box. All of the objects were replaced by duplicates during the test phase, but

one duplicate was placed in the same location that the object had appeared during the study phase (the ‘repeated location’ object) and two of the objects swapped locations (the ‘novel location’ objects) .

New sets of objects were used across sessions, tasks, and months. Which objects were repeated/repeated location and which were novel/novel location during the test phase was counterbalanced as much as possible across rats. In order to maintain task interest of rats throughout both sessions within a day, the location within the testing environment that objects were presented differed between the two sessions. For example, if session 1 objects in the object-only recognition memory task were in the SW and SE locations, session 2 objects were in the NW and NE locations. Every rat was tested by the same experimenter who was blind to the genotype of each rat.

Analyses

Each session was digitally recorded by a video camera mounted to the ceiling of the testing room, and object exploration for the study and test phase was scored separately by experimenters who were blind to both rat genotype and conditions of the objects (e.g. whether a given object was repeated or novel). For both tasks, a discrimination index (DI) was calculated from the raw exploration times of rats during the test phase as a measure of memory performance. DI = mean novel exploration / (mean novel exploration + mean repeated exploration). For the object-in-location task, the mean exploration of the two novel location objects was entered into the DI calculation for that session. If a rat did not explore the to-be-repeated object during the study phase of the object-only recognition memory task, the DI for the subsequent test phase was not included in the analysis. Similarly, if a rat did not explore the to-be-repeated-location

object and at least one of the to-be-novel-location objects during the study phase of the object-in-location memory task, the DI for the subsequent test phase was excluded from the analysis. For both tasks, only exploration times for objects that were explored for at least one second over the entire test phase for a given session were included in the analyses. For each month, the relative memory performance of WT and AD rats was determined by calculating the mean of each rat's DI for both sessions of each task for that month, and then calculating the mean DI across rats of each genotype. In order to reduce the month-to-month variability in mean DI scores for both genotypes, 4 month averages were also calculated to determine age-related differences between WT and AD rats. Analyses focused on genotype differences between tasks and age groups (5-8 months vs. 9-12 months).

Results

AD rats showed age-dependent spatial memory impairments relative to WT rats. Figure 3.2 shows DI scores for AD and WT rats on both the object-only and object-in-location recognition memory tasks. The results are shown for the mean DIs of each genotype across 5-8 months of age and 9-12 months of age. A three-way 2 (age: 5-8 or 9-12 months) x 2 (task: object-only or object-in-location recognition memory) x 2 (genotype: WT or AD) mixed ANOVA with repeated measures on DIs revealed that generally, older rats had impaired performance relative to youngers rats ($F[1,14]=5.779$, $p=0.031$). In addition, the effect of genotype on memory performance differed between tasks ($F[1,14]=9.067$, $p=0.009$). Two-way 2 (age: 5-8 or 9-12 months x 2 (genotype: WT or AD) mixed ANOVAs with repeated measures on DIs for each task revealed that for object-only recognition memory, older rats performed worse than younger rats

(F[1,14]=4.865, p=0.045) and AD rats performed similarly to WT rats. For the object-in-location task, memory performance was worse for AD than WT rats (F[1,14]=12.319, p=0.003), and there was a trend for the effect of genotype to differ between age groups (age x genotype interaction, F[1,14]=3.478, p=0.083). Indeed, preplanned comparisons confirmed that there was no difference in object-in-location memory performance between AD and WT rats ages 5-8 months (F[1,14]=0.247, p=0.627), but AD rats were impaired relative to WT rats ages 9-12 months (F[1,14]=8.969, p=0.010). Although study exploration times for both tasks declined over the months of testing for both AD and WT rats, the spatial memory impairment of AD rats at 9-12 months remained significant even when study exploration was included as a covariate (F[1]=7.513, p=0.017). The results indicate that AD rats have intact non-spatial and spatial memory when they are 5-8 months of age, but develop a selective spatial memory impairment relative to WT rats between 9-12 months of age.

Discussion

The current study investigated the age range at which AD rats show impaired memory relative to WT rats. The results indicated that AD rats developed a selective memory impairment when they were as young as 9-12 months of age. Specifically, AD rats performed similarly to WT rats in a non-spatial object recognition memory task at both 5-8 months of age and 9-12 months of age. In contrast, AD rats performed similarly to WT rats in a spatial object-in-location recognition memory task at 5-8 months of age, but showed impairments by 9-12 months of age.

The results are consistent with previous research that has shown a disproportional impairment of object-in-location memory in AD generally, especially in early stages of

the disease. This AD rat strain in particular was shown to have impaired spatial memory when tested at 16 months of age, and impaired non-spatial memory at 24 months of age (Cohen et al., 2013). The current study extended those results by longitudinally testing spatial and non-spatial memory of AD and WT rats at the same time, each month, beginning 1 month before the youngest age this this AD rat strain was previously shown to have intact memory. Notably, study exploration times in both tasks declined significantly with age, and were numerically lower in AD rats relative to WT rats at ages 9-12 months in the object-in-location recognition memory task. Analyses with study exploration times included as a covariate, however, showed that the poor spatial memory performance of AD rats was not the consequence of AD rats spending less time encoding the individual objects in their locations during the study phase of the object-in-location task.

It is noteworthy that although AD rats performed similarly to WT rats in the object-only task, both genotypes showed worse non-spatial memory at 9-12 months of age relative to 5-8 months of age. One interpretation may be that both genotypes have non-spatial memory impairments by 9-12 months. The mean DI for the non-spatial task relative to chance (0.5) did not quite reach statistical significance for AD rats ($p=.059$), therefore it is possible that AD rats also have non-spatial memory impairments at 9-12 months. However, the same WT rats were performing well on the more difficult spatial object-in-location task at the same time point, and good object-in-location performance requires memory for individual objects in addition to the location in which those objects had appeared. Thus, it seems unlikely that WT rats had impaired memory for individual objects during the object-only task but intact memory for individual objects during the

object-in-location task. Given that the AD rats performed numerically higher than WT rats on the object-only recognition memory task, it seems more likely that AD rats' non-spatial memory was intact at 9-12 months of age. Nevertheless, AD rats showed a clear spatial memory deficit relative to WT rats and relative to chance at 9-12 months of age.

The results shed light on the progression of memory impairments in AD rats, and provide clues to which brain region to target for research aimed at understanding brain network dysfunction *in vivo*, as well as which age-range to administer drug treatments to prevent or delay AD-related memory impairments. Future studies are needed to take advantage of the similarity in pathological progression between this AD rat model and AD in humans to better understand the relationship between different pathological changes (Aβ plaques, NFTs of hyperphosorylated tau, neuroinflammatory markers, cell death), and the progression of memory impairments within individual rats. Important questions could be answered, such as whether AD pathology and memory impairments follow a linear progression or if there is a certain threshold of pathology that, once reached, suddenly manifests behaviorally. In addition to these questions, future studies could examine the sort of network dysfunction (e.g. hippocampal hyperactivity) that occur in AD brains as different time points of the disease, and how neural network dysfunction relates to memory impairments and pathological progression.

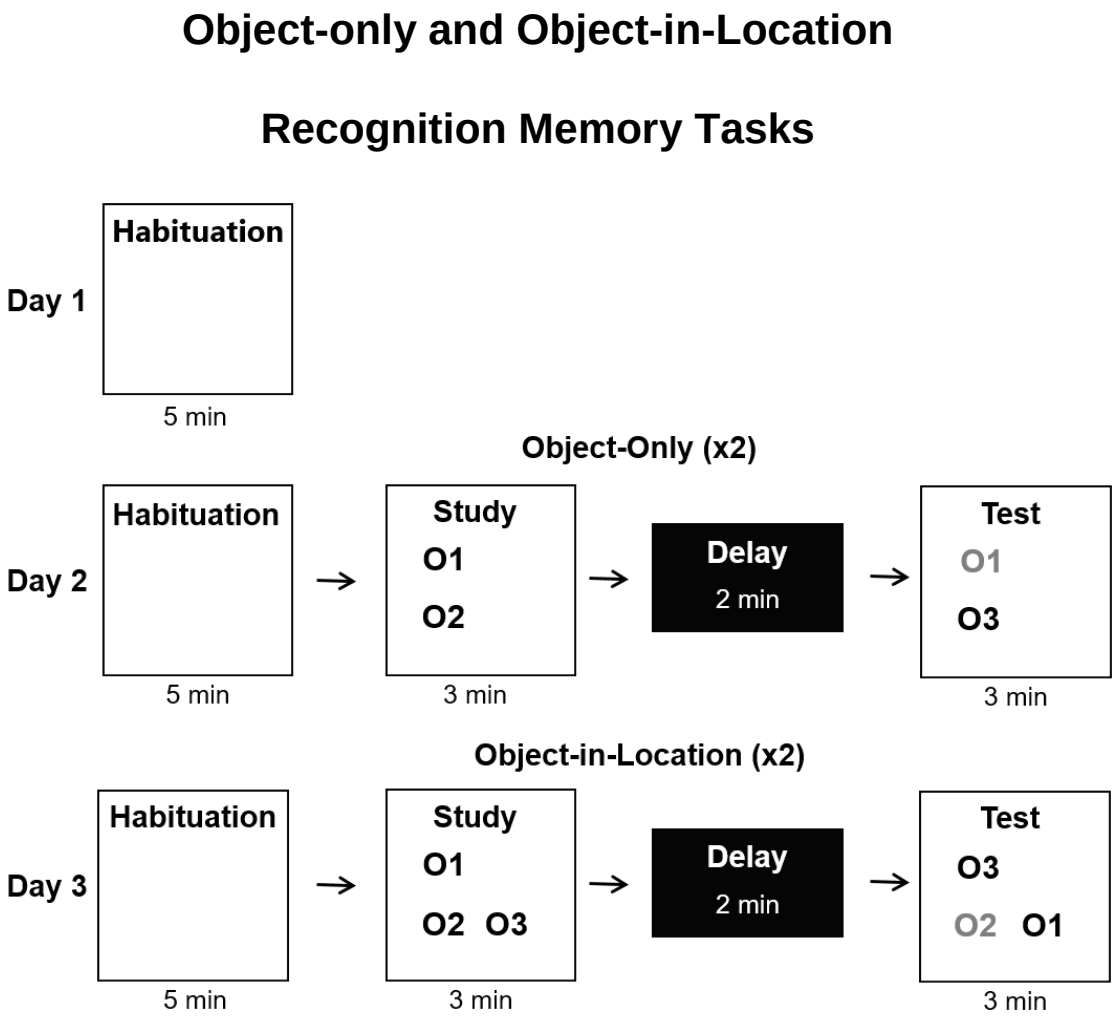

Figure 3.1. Schematic of the testing procedure. Each month of testing took place over 3 days. On day 1, rats were habituated to the testing environment with no objects for 5 min each. On day 2 rats completed two different object-only recognition memory sessions, and on day 3 rats completed two different object-in-location recognition memory sessions. Each test session began with a 5 min habituation, immediately followed by a 3 min study phase, a 2 min delay, and a 3 min test phase. For the object recognition memory task sessions, rats encountered two novel objects during the study phase and a duplicate of one of the study objects (repeated object) and a different novel object during the test phase. For the object-in-location memory task sessions, rats encountered three

novel objects during the study phase and three duplicates of the study phase objects during the test phase. One duplicate was placed in the same location as it appeared during the study phase (repeated location) and two duplicates swapped locations (novel location). Individual objects are denoted by “O”.

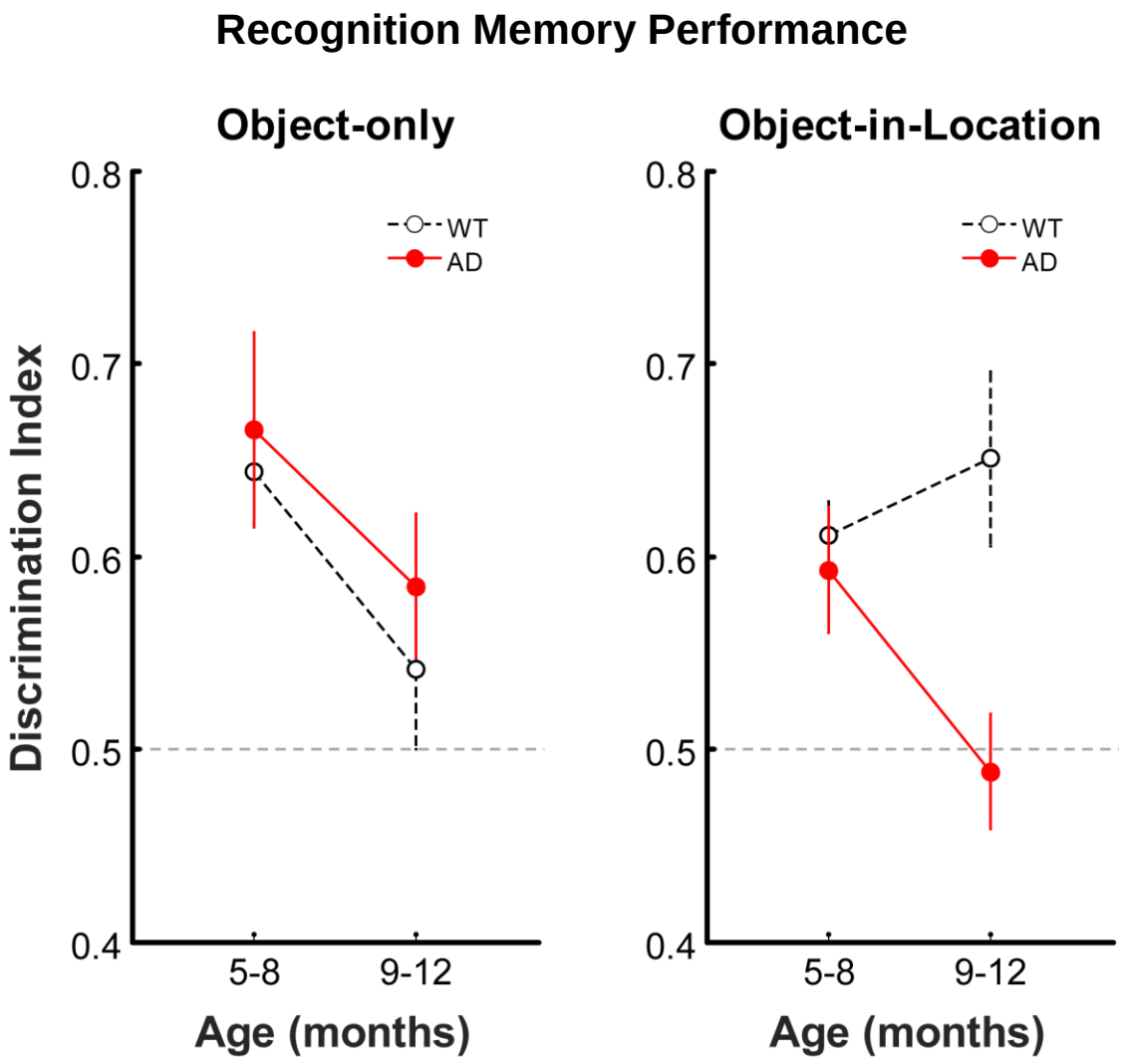

Figure 3.2. Recognition memory performance on object-only recognition memory (left panel) and object-in-location recognition memory (right panel) at different age ranges. The results are shown as mean Discrimination Index (DI) across rats (WT n = 8, AD n = 8). Error bars show ± *SEM*. The dashed line indicates chance performance. AD rats showed impaired object-in-location memory performance relative to WT rats by 9-12 months of age.

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Chapter 4

Hippocampal dysfunction and M_1 agonism in a Rat Model of Alzheimer's Disease

Abstract

Alzheimer's disease (AD) disproportionately impacts the hippocampus and memory function. Little is known about how hippocampal dysfunction *in vivo* contributes to memory symptoms in AD. Investigating hippocampal place cells, which fire preferentially within a specific location in a given environment, in a transgenic rat model of AD provides an opportunity to understand hippocampal and memory dysfunction in AD. The standard treatments for AD increase acetylcholine (ACh) levels but have limited efficacy that is related to dose-limiting side effects from activating peripheral ACh receptors. Selective activation of the muscarinic ACh receptor subtype M_1 may be able to maximize the memory-enhancement while minimizing the peripheral side effects from ACh treatments for AD. In order to test if acute, systemic administration of the M_1 agonist (VU0364572) can improve hippocampal dysfunction in AD, we administered the M_1 agonist to AD (n=7) and wild-type (n=4) rats prior to completing laps around an empty track while recording single cell activity from CA1, CA2, and CA3 of the hippocampus. We also administered the M_1 agonist to AD (n=6) and WT (n=4) rats prior to completing a recognition memory task that assessed both object-only and object-in-location memory. We found that AD rats had reduced spatial fidelity that was most apparent in CA2/3 place cells, and seemed to relate to a decreased signal-to-noise ratio in the firing rates. M_1 activation showed potential to increase the signal-to-noise ratio of AD CA2/3 place cells. AD rats also had impaired object recognition memory that was not improved by M_1 activation. The results suggested that the hippocampal dysfunction that underlies memory impairments in AD rats is primarily due to dysfunction in CA2/3 cells of the hippocampus and may benefit from selective M_1 activation.

Alzheimer's disease (AD) disproportionately impacts certain brain regions, including the hippocampus and surrounding structures in the medial temporal lobe (Braak & Braak, 1995). Hippocampal dysfunction can impair memory performance, one of the key cognitive symptoms in AD patients (Alzheimer's Association, 2014; Gallagher & Koh, 2011). The genetic and molecular pathology and clinical manifestations of AD have been studied extensively, and neuronal death resulting from AD pathology in brain regions relevant to memory (e.g. the basal forebrain and the hippocampus) clearly contributes to AD memory loss (Hardy & Selkoe, 2002; Simic et al., 2009; Whitehouse et al., 1982). Much less is known about how AD pathology influences the activity of surviving neurons to contribute to memory symptoms in AD.

One relatively well understood connection between neuronal activity and cognition is the activity of place cells, first discovered in the rodent hippocampus (O'Keefe & Dostrovsky, 1971). Place cells fire preferentially within a specific location of a given environment (the place field of the place cell). Place cells form distinct assemblies that allow the hippocampus to form cognitive maps of multiple environments (O'Keefe & Nadel, 1978). Hippocampal place cells have well-characterized relationships with prominent network rhythms (e.g. Foster & Wilson, 2007; O'Keefe & Recce, 1993) and upstream afferent structures in the medial temporal lobe (Brun et al., 2008; Jeffery, 2007), and also process temporal and stimuli-specific information that could support associative or episodic memory (Griffin & Hallock, 2013; Manns & Eichenbaum, 2009; Moser et al., 2008), all of which are altered in AD (Braak & Braak, 1995; Gallagher & Koh, 2011; Hanaki et al., 2011; Jackson & Snyder, 2008; Ulas & Cotman, 1997). Thus,

studying hippocampal place cells in rats provides an opportunity to evaluate how dysfunctional cell activity may underlie memory loss symptoms in AD.

Taking advantage of a newly developed rat model of AD (Tg-F344 AD rats with human genetic mutations APP_{Swe} and PS1ΔE9; Cohen et al., 2013) would allow for a better understanding of hippocampal place cell dysfunction in AD. Although hippocampal place cells have been studied in transgenic AD mice models (e.g. Cacucci et al., 2008; Cheng & Ji, 2013; Zhao et al., 2014), almost all studies that have characterized place cells in young and non-diseased aged animals have been in rats. In addition, relative to AD mice, AD rats more fully recapitulate the neuropathology seen in human AD including age-dependent tau pathology and cell death (Cohen et al., 2013). Therefore, investigating well-characterized place cell function and evaluating the efficacy of experimental AD treatments in AD rats opens up opportunities to shed light on human AD.

General acetylcholine (ACh) levels have been linked to memory symptoms in AD, and drugs that selectively target specific muscarinic ACh receptors (mAChRs) have potential to be effective treatments for AD. The observation that AD patients have profound and disproportional cell loss in the basal forebrain, which is the major supplier of ACh to the hippocampus and cortex, led to the long-standing hypothesis that ACh dysfunction plays a major role in AD cognitive dysfunction (Bartus et al., 1982). In line with this hypothesis, the most widely-prescribed FDA-approved drugs for treating AD memory loss are acetylcholinesterase inhibitors (AChEIs; Anand & Singh, 2013). AChEIs increase ACh levels by inhibiting the enzyme acetylcholinesterase, which normally functions within synapses to break down ACh (Anand & Singh, 2013).

Although AChEIs do provide temporary relief of memory loss symptoms in some AD patients, the nonspecific activation of both nicotinic and muscarinic acetylcholine receptors (mAChRs) by AChEIs can cause dose-limiting negative side effects (Levey, 1996). It is also possible that simultaneously activating mAChRs coupled to both G_q and G_i proteins may have antagonistic effects and blunt treatment efficacy (e.g. Davis et al., 2010). Thus, selective activation of particular mAChRs has the potential to be a more tolerable and effective treatment option than generally increasing ACh levels.

In AD, selective activation of the mAChR subtype M_1 may be able to maximize the memory-enhancement from AChEIs while minimizing the peripheral side effects. M_1, as well as M_4, is highly expressed in brain regions implicated in memory, such as the hippocampus, striatum, and amygdala (Levey et al., 1991), and is also less implicated in the dose-limiting peripheral side-effects caused by global ACh activation mediated by other mAChRs (M_2 and M_3; Bymaster et al., 2003). Both M_1 and M_4 have the potential to be viable treatment options for memory disorders, but the M_1 agonist VU0364572 used in the current study has been shown to improve performance on a hippocampal-dependent test of spatial memory and increase spatial correlations of hippocampal place cells across similar environments in healthy young rats after acute administration (Digby et al., 2012; Lebois et al., 2016), and reduce amyloid pathology in a mouse model of AD after chronic administration (Lebois, 2014). Investigating if and how the M_1 agonist will affect memory performance and hippocampal place cell fidelity in AD rats may contribute to developing more effective AD therapies.

Consistent with the hippocampal dysfunction that occurs early in the disease, memory tasks that depend on the integrity of the hippocampal memory system are well-

suited to detect early cognitive impairments in AD. Several studies in humans and transgenic animal models of AD suggest that the ability of individuals to remember the location in which a previously presented object initially appeared (object-in-location recognition memory) may be particularly impaired in AD (Good et al., 2007; Hampstead et al., 2011; Hanaki et al., 2011; Kessels et al., 2010). We also found that AD rats in particular developed age-dependent object-in-location recognition memory impairments at 9-12 months of age, when their object-only recognition memory was still similar to WT rats in that age range (Chapter 2). Importantly for the objectives of the current study, the hippocampus is part of a network of brain regions that is crucial for the successful performance of object-in-location recognition memory (Barker & Warburton, 2011; Braak & Braak, 1995; Postma et al., 2008). Thus, an object-in-location recognition memory task is likely to show memory impairments in AD rats, and may reflect improvements in hippocampal function from increasing M_1 activity.

The goals of this study were to investigate if AD rats would have impaired spatial fidelity of hippocampal place cells and to determine if acute oral administration of the M_1 agonist would reverse impairments in hippocampal place cell fidelity and improve performance on a recognition memory task in AD rats. Hippocampal spatial fidelity was assessed by the activity of hippocampal place cells as rats completed laps around a circular track after receiving an oral dose (either 10 mg/kg or 30 mg/kg) of the M_1 agonist or the vehicle control. Memory performance was assessed with a modified version of the tasks previously used to assess object-only and object-in-location recognition memory performance in AD rats. For the task used in this study, rats spontaneously explored novel and repeated objects, some of which appeared in different locations as their initial

presentation, as they completed laps around a circular track after receiving an oral dose (10 mg/kg) of the M_1 agonist of the vehicle control. We found that impaired hippocampal spatial fidelity was most apparent in the CA2/3 region of the hippocampus in AD rats, and that the M_1 agonist may improve the spatial fidelity of the hippocampal place cells of AD rats. Moreover, AD rats have impaired recognition memory performance that was not improved by M_1 agonism.

Method

Subjects

Adult female rats (*N*=11; F344 [WT] *n*=4 and Tg-F344 [AD] expressing the human genetic mutations APP_{Swe} and PS1ΔE9 *n*=7; Cohen et al., 2013) were used to evaluate the effects of genotype and M_1 activation on the spatial fidelity of hippocampal place cells, and 10 of those rats (WT *n*=4 AD *n*=6) were used to evaluate the effects of genotype and M_1 activation on recognition memory performance. Rats were implanted with a micro recording assembly when they were between 11 and 20 months of age (WT *M*=15, AD *M*=16), and testing occurred when rats were 12-21 months of age (WT *M*=16, AD *M*=17.5). One WT and 4 AD rats had previously performed several sessions of object-only and object-in-location recognition memory tasks from 5-12 months of age that involved voluntary exploration of various objects in a box for a longitudinal study (see Chapter 2) that ended several months before any of these rats were tested in the current study. The rats were kept on a 12-hour light/dark cycle (testing occurred during the light period), individually housed with free access to water, and placed on a restricted diet such that they maintained at least 90% of their free-feeding weight. All experimental

procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

Drugs

The M_1 agonist used was VU0364572, a bitopic agonist highly selective for M_1 (Lebois et al., 2011). Before each place cell and recognition memory test session rats were orally administered either 10 mg/kg M_1 agonist, 30 mg/kg M_1 agonist, or vehicle control. The M_1 agonist was formulated as an HCl salt in nuclease free H_2O, and then mixed into strawberry-flavored gelatin for a total volume of 1.2 mL. This particular M_1 agonist was chosen for its selectivity for M_1 versus the other mAChR subtypes, lack of activity at other off-target proteins (e.g. ion channels, G-protein coupled receptors, etc.), brain penetration, and evidence of a known range of doses having *in vivo* efficacy of altering memory performance and hippocampal function in mice and rats (Digby et al., 2012; Galloway et al., 2014; Lebois et al., 2011). The specific 10 mg/kg and 30mg/kg doses were selected for their demonstrated efficacy in altering hippocampal function in healthy young rats (Lebois et al., 2016).

Surgery

Sterile tip surgery was performed on each rat to implant a chronic recording assembly with up to 8 tetrodes to record cellular activity. The recording assembly was affixed to the skull above a craniotomy centered approximately 3.7 mm posterior to bregma and 2.6 mm lateral to the central suture so that tetrodes could be independently lowered through a hole in the skull into the CA1, CA2 and CA3 pyramidal layers of the hippocampus. Each tetrode consisted of four 12.5 µm nichrome wires. The tips of each

tetrode were plated in gold to reduce impedance to 100-200 kΩ at 1 kHz to facilitate the detection of individual units in the hippocampus.

Prior to surgery, rats were anesthetized with isoflurane in 1-3% oxygen and subcutaneously (s.c.) injected with 2 mg/kg injectable meloxicam and 5mL lactated ringers (2.5 mL for each haunch). Just prior to the incision, up to 0.1 mL of 0.25% bupivacaine with 1:200,000 epinephrine was administered over 2-3 s.c. injections along the sagittal suture as a local anesthetic. Nine stainless-steel screws were secured along the ridges of the skull to serve as an anchor for the recording assembly. One additional stainless steel screw, soldered to a wire attached to the recording assembly, served as the ground and was secured to the posterior portion of the skull above the cerebellum. The base of the chronic recording assembly was secured to the anchor screws with dental acrylic. During surgery, tetrodes were slowly lowered into the brain so that they were roughly 1mm above the target regions. Topical antibiotics were applied to the incision site. Before rats were removed from anesthesia, they were given an additional 5mL of lactated ringers (2.5 mL in each haunch). For the first two days after surgery, rats were given s.c. injections of 1 mg/kg of injectable meloxicam. For the first day after surgery, rats were also given an additional s.c. injection of 5 mL of lactated ringers. Four AD rats, instead of injectable meloxicam and local bupivacaine, received s.c. injections of 0.05 mg/kg buprenorphine prior to surgery followed by an oral dose of 0.75mL meloxicam immediately after the rats began to ambulate after surgery. These same four rats, instead of receiving injectable meloxicam the following days after surgery, received s.c. injections of 0.05 mg/kg buprenorphine and an oral dose of .75mL meloxicam one day after surgery, and received an oral dose of 0.75mL meloxicam two days after surgery. All

rats were given 1 week to recover, and then tetrodes were slowly lowered 20-80 µm at a time into the target regions of the hippocampus using hallmark electrophysiological cues. In order to minimize movement between sessions, tetrodes were not raised or lowered from 24 hours prior to the first testing session until the rat was euthanized. After testing was completed and just prior to euthanasia, small lesions were made at the tips of recording electrodes by passing 20-40 µA of current for 20 seconds each. After euthanasia, brains were sectioned into 40 µm-thick coronal slices and stained with cresyl violet stain to confirm tetrode locations based off of lesion location.

Procedure

Prior to surgery, rats were trained to complete laps around a circular track for a small food reward (~0.5 g of sprinkles, chocolate flavor). After they reached a pre-surgery criteria of completing 40 laps around the circular track (track outside diameter = 91.5 cm; track width = 7 cm) in 40 minutes, they were implanted with an 8-tetrode chronic recording assembly. Testing typically occurred 3-7 weeks after surgery. After recovering from surgery, rats were retrained to the pre-test criteria to complete 40 laps in 40 minutes. Rats began testing once the minimum laps/min criteria was reached and as many tetrodes as possible reached their target region in the hippocampus. Only data collected from tetrodes with confirmed positions in CA1, CA2, or CA3 during testing sessions were included in the present analysis.

Place Cell Recording. Thirty minutes prior to each session, rats were given an oral dose of 10 mg/kg or 30 mg/kg of the M_1 agonist, or vehicle control only. Session 1 and session 6 were control days in which rats were given the vehicle. In sessions 2-5, rats received either 10 mg/kg or 30 mg/kg of the M_1 agonist. The order of administration of

the 10 mg/kg and 30 mg/kg doses of the M_1 agonist alternated, and which dose was administered first was counterbalanced between rats. Each session, rats completed laps around a circular track for 35-45 minutes. For each lap, rats exited a center stem, completed a counterclockwise circle, and returned to the center stem for the small food reward.

Recognition Memory Testing. After each rat completed the six sessions to evaluate place cell fidelity, they completed three sessions to evaluate the effect of M_1 agonism on recognition memory. Figure 4.1 shows a schematic of each session to test the effects of M_1 agonism on recognition memory performance. Object recognition memory tasks take advantage of the innate novelty preference of rats (Ennaceur & Delacour, 1988), so that memory for a repeated object can be inferred by the less time rats spontaneously explore repeated objects relative to novel objects. We used a modified version of a recognition memory task used previously in our laboratory (Trimper, 2016). Objects ranged from 10 – 2,000 cm^3 in size and were made of wood, ceramic, metal, or plastic material. Each day of testing, rats were orally administered 1.2 mL of 10 mg/kg M_1 agonist or vehicle control 30 minutes prior to performing the recognition memory task. Each rat completed three testing sessions on three separate days. Session 1 and 3 were control days, and in session 2 rats received 10 mg/kg M_1 agonist. Each session consisted of 6-12 (WT M=11, range 6-12; AD M=10, range 8-12) blocks of 4 laps around a circular track. For each lap, rats exited a center stem and completed a counterclockwise lap around a circular track with adjustable flaps on which different objects can be presented, and returned to the center stem for a small food reward. Lap 1 of each block was always an empty lap in which no objects were presented. On lap 2 (novel object),

two novel objects were presented at the 10 o'clock and 2 o'clock locations on the track. These same two items were repeated on laps 3 and 4, but on lap 3 (repeated object, repeated location) the objects were presented in the same locations as they appeared on the previous lap, and on lap 4 (repeated object, novel location) the locations that they appeared were swapped. Repeated items were always duplicates. New sets of objects were used across blocks and across sessions for each rat. For both place cell and recognition memory testing, every rat was tested by the same experimenter who was blind to the genotype of each rat.

Analyses

The position of rats during place cell testing and exploration of objects during recognition memory testing was recorded by a digital video camera mounted to the ceiling of the testing environment at a 30 Hz sampling rate (30 frames per second). After testing, a rat's location on each frame was calculated using custom MATLAB software, or manually by a trained experimenter, using information emitted from red and green light emitting diodes that were attached to the recording assembly during testing. Object exploration was scored by an experimenter who was blind to the genotype of each rat.

Place Cell Recording. Spiking activity of single units was recorded at a sampling rate of 30,000 Hz and filtered at 600-6,000 Hz with the software NSpike data acquisition system (nspike.sourceforge.net). After testing, spiking data was manually sorted using software that gave a 3D view of spike waveform characteristics across wires of a given tetrode (Offline Sorter, Plexon, Dallas, Texas). Spiking data was analyzed with custom MATLAB scripts.

A total of 1,800 pyramidal cells were recorded from 4 WT and 7 AD rats. This total reflects the sum of pyramidal cells from each rat across all 6 days of testing, and there was likely a large degree of overlap between cell populations between sessions for any given rat. 787 were recorded from tetrodes placed in CA1, and 1,013 were recorded from tetrodes placed in CA2, on the CA2/3 border, or in CA3. In order to compare potential differences in hippocampal subregions for all of the place field analyses, only units from tetrodes that were unambiguously CA1 were used as CA1 units and compared to units from tetrodes that were in CA2, the CA2/3 border, or CA3. Units in CA2, CA2/3 border, and CA3 were combined into one group and will subsequently be referred to as CA2/3. This grouping of CA2 and CA3 neurons was done because it was not always possible to definitively distinguish the two regions during histological inspection. Of the 1,800 pyramidal units, 825 (445 CA1 and 380 CA2/3) were further characterized as place cells if they had at least one area of the environment (place field) in which the firing rate of the unit preferentially increased when the rat was within that place field relative to other locations in the environment. 56.5% of CA1 pyramidal units were place cells, whereas only 37.5% of CA2/3 units were place cells. Only units that were identified as place cells were used in the present analyses. Of the 445 CA1 place cells, 94 were recorded from AD rats and 351 were recorded from WT rats. Of the 380 CA2/3 place cells, 236 were recorded from AD rats and 144 were recorded from WT rats.

In order to reduce variations in firing rates due to stationary/feeding activity versus ambulating on the track, data (spikes and rat's position on the track) that occurred in the very center of the stem where rats consumed the small chocolate reward after the

completion of each lap were not considered. Furthermore, only spikes that occurred when the rat was ambulating above 5 cm/sec were included in the analyses.

Spatial fidelity of hippocampal place cells was assessed in several different ways, but the primary metrics reported here are spatial information scores, sparsity, firing rates, and firing rate signal-to-noise ratios. Spatial information scores reflect the amount of spatial information emitted by each spike in terms of bits per spike, using the formula $\sum p_i(R_i/R)\log2(R_i/R)$ if p_i = probability the rat was at spatial bin *i*, R_i = firing rate at spatial bin *i*, and R = overall firing rate of the place cell (Skaggs et al., 1996). Sparsity is a measure of the proportion of space within the environment that the unit fired in, using the formula $\sum(p_i*R_i^2)/R^2$ if p_i = probability the rat was at spatial bin *i*, R_i = firing rate at spatial bin *i*, and R = overall firing rate of the place cell (Skaggs et al., 1996). Firing rates were calculated as the number of spikes per second, and the firing rate signal-to-noise ratio was calculated for each unit with the following formula: mean in-field firing rate / (mean in-field firing rate + mean out-of-field firing rate).

In order to calculate the spatial fidelity of each place cell, a firing rate grid was calculated for each session by dividing the environment into spatial bins of 4 squared pixels. The firing rate for each bin was determined as the number of spikes in that spatial bin divided by the total time spent in that particular bin. Place field boundaries were determined by the bins in which the firing rates were at least 20% of the maximum in-field firing rate. Place fields less than 15 bins were likely due to spurious activity and excluded from the analyses. Note that this calculation does not exclude the possibility that a given unit could have multiple place fields.

If a unit preferentially increased its firing rate in the very center of the stem, where the rat consumed its chocolate reward after each lap, it would be unclear if the activity of a unit with a "place field" close to the center stem was representing spatial location, or if the spiking activity correlated with eating and/or grooming. In order to eliminate the possibility of including spurious place fields that may reflect behavioral state instead of spatial location, only place fields in which less than 10% of the in-field spikes occurred within the center stem between laps were included in the analyses. In addition, only place cells in which the rat passed through the place field in at least 50% of the laps of any given session were included in the analyses.

Spatial information scores, sparsity scores, and firing rates for each unit were calculated separately for all spikes that occurred while rats were running laps, spikes that occurred outside of the place field(s) (out-of-field) of units while rats were running laps, and spikes that occurred within the place field (in-field) of units. Note that lap spikes included in-field, out of field, and any spikes that may have been part of an invalid place field near the center of the stem or in a location that the rat occupied on less than 50% of laps in a given session.

Recognition Memory Testing. Object-only memory performance was measured as a discrimination index (DI) calculated from the mean raw exploration of rats during lap 2 and lap 3: DI = mean novel object / (mean novel object + mean repeated object, repeated location). Object-in-location memory performance was measured as a DI calculated from the mean raw exploration of rats during lap 3 and lap 4: DI = mean repeated object, novel location / (mean repeated object, novel location + mean repeated object, repeated location). Data processing and statistical analyses for place cell and

recognition memory data were conducted with SPSS 19 (IBM) and MATLAB R2011 (Mathworks).

Results

Place Cell Testing

This strain of AD rats showed impaired recognition memory performance relative to WT rats at 9-12 months of age (Chapter 2). Although all rats were at least 12 months of age at the time of testing, the age of rats at testing varied considerably across rats (Range: 12- 21 months). In order to account for the possibility that hippocampal place cell function was influenced by age in both AD and WT rats, the age of rats was included as a covariate for all analyses. Accordingly, all metrics used to evaluate place cell fidelity were first analyzed with a three-way 2 (CA region: CA1 or CA2/3) x 2 (genotype: WT or AD) x 3 (drug: control, M_1 low, or M_1 high) mixed ANCOVA with repeated measures controlling for rat age. Many studies have also found differences between CA1 and CA3 place cell characteristics (Leutgeb & Leutgeb, 2007; Mizuseki et al., 2012), and subregional difference in hippocampal function in aging and AD (e.g. Bakker et al., 2012; Thome et al., 2015). Thus, the initial three way ANCOVA for each metric of spatial fidelity was always followed by separate analyses for CA1 and CA2/3 place cells using two-way 2 (genotype: WT or AD) x 3 (drug: control, M_1 low, or M_1 high) mixed ANCOVAs with repeated measures and controlling for rat age. Unless otherwise noted, there was a main effect of rat age for each three way and two way ANCOVA. Finally, preplanned within-subjects contrasts controlling for rat age were used to compare means within each region between genotypes of each drug condition. In an effort to show the potential effect of M_1 agonism, all p values reported from preplanned contrasts were

calculated with Tukey's LSD, and alpha levels were not adjusted for possible inflation from multiple comparisons. However, the consistent pattern of which contrasts were significant across metrics of spatial fidelity suggests that the significant contrasts were not spurious, but revealed meaningful differences between AD and WT rats and effects of the M_1 agonist on hippocampal place cell function.

AD and WT rats had similar run speeds across all drug conditions. Firing rates of units can be influenced by the run speed of rats (e.g. Huxter et al., 2003). In order to address the possibility that differences between AD and WT place cells across drug conditions were the consequence of differing run speeds of rats during laps, we calculated the average run speed for every session for each rat. A two way (2 (genotype: WT or AD) x 3 (drug: control, M_1 low, or M_1 high) mixed ANCOVA with repeated measures controlling for rat age on rat run speeds was conducted, and found no significant differences in run speed between genotype or across drug conditions. One way 3 (drug: control, M_1 low, or M_1 high) ANCOVAs with repeated measures controlling for rat age on rat run speeds were then conducted separately for AD and WT rats, followed by preplanned within subjects contrasts comparing means of each drug condition. Even when AD and WT rats were analyzed separately, there were no significant differences in mean run speeds in any drug condition (*M* ± *SEM*: WT control=16.472 ± 2.493; WT M_1 low=16.301 ± 1.600; WT M_1 high=16.139 ± 1.573; AD control=15.764 ± 1.864, AD M_1 low=16.234 ± 1.196; AD M_1 high=15.893 ± 1.176). Thus it is unlikely that any differences between AD and WT rats across drug conditions, or drug effects within either genotype, are the indirect result of differences in locomotion-related changes in firing rate of any given unit.

Data related to place cell spatial fidelity are reported next. Overall, the results suggest that place cells of AD rats have impaired spatial fidelity relative to WT place cells. The impairments in spatial fidelity of hippocampal place cells of AD rats seemed to relate to higher and more spread out-of-field firing of CA2/3 place cells in particular. There was some evidence that M_1 agonism may ameliorate the excessive out-of-field firing of CA2/3 place cells of AD rats.

AD CA2/3 place cells had reduced spatial fidelity when all spikes during laps were considered. Figure 4.2 shows spatial information scores calculated from all spikes that occurred when rats were running laps for WT and AD rats. The results for spatial information scores and the remaining metrics to evaluate spatial fidelity of place cells (Figures 4.2 – 4.11) are shown for the means across units for each drug condition, calculated separately for CA1 and CA2/3. AD place cells had lower spatial information scores relative to WT place cells (F[1,812]=5.785, p=0.016), spatial information scores of CA1 place cells were higher than CA2/3 place cells (F[1,812]=15.470, p=0.000), and the difference between spatial information scores for AD and WT place cells differed between CA1 and CA2/3 place cells (F[1,812]=16.092, p=0.000). There was also a trend for spatial information scores to differ across drug conditions (F[2,812]=2.508, p=0.082). Within CA1, there was no main effect of drug on spatial information scores in CA1 place cells, but preplanned within-subjects contrasts showed a trend for spatial information scores of AD CA1 place cells to improve in the M_1 high condition relative to control (p=0.064). There was also no difference between drug conditions in spatial information scores of CA2/3 place cells, but AD CA2/3 place cells had significantly lower spatial information scores of WT CA2/3 place cells (F[1,373]=35.016, p=0.000), and preplanned

within-subjects contrasts showed that spatial information scores for AD CA2/3 place cells were significantly lower than WT CA2/3 place cells in each drug condition (control: p=0.005; M_1 low: p=0.003; M_1 high: p=0.007). The results indicate that the activity of AD CA2/3 place cells provide less information about the spatial location of rats relative to WT CA2/3 place cells, and that M_1 agonism may improve spatial information scores of AD CA1 place cells.

Sparsity scores showed a similar, but not identical pattern to spatial information. Figure 4.3 shows that just as with spatial information, AD place cells had worse (higher) sparsity scores relative to WT place cells (F[1,812]=4.362, p=0.037), CA1 place cells had better (lower) sparsity scores than CA2/3 place cells (F[1,812]=18.001, p=0.000), and the difference between AD and WT place cells differed between CA1 and CA2/3 (F[1,812]=7.275, p=0.007). Unlike spatial information scores, there was no trend for sparsity scores to differ across drug conditions, nor was there a main effect of rat age on sparsity scores in CA1 place cells. In contrast, AD CA3/CA3 place cells had significantly worse sparsity scores relative to WT CA2/3 place cells (F[1,373]=12.805, p=0.000), and there was at least a trend for AD CA2/3 place cells to have worse sparsity scores across all drug conditions (control: p=0.006; M_1 low : p=0.048; M_1 high: p=0.094). Although there was no main effect of drug, the contrasts also showed a trend for the high dose of the M_1 agonist to improve sparsity scores of AD CA2/3 place cells relative to control (p=0.065). The results indicate that AD CA2/3 place cells had higher sparsity scores than WT CA2/3, and thus the activity of these cells was distributed over a larger area of the environment in the control condition. There was some evidence that M_1 agonism may improve sparsity scores of AD CA2/3 place cells.

Differences in firing rates that were calculated from all lap spikes corroborated the spatial information and sparsity findings that the difference between AD and WT place cells are more pronounced in CA2/3. Figure 4.4 shows that similarly to spatial information and sparsity scores, there was a trend for CA2/3 place cells to have higher firing rates than CA1 place cells (F[1,812]=3.615, p=0.058), and the difference between AD and WT place cells differed between CA1 and CA2/3 (F[1,812]=5.285, p=0.022). In contrast to spatial information and sparsity scores, overall firing rates were not different between AD and WT place cells, and there was a trend for the difference between AD and WT place cells to differ across drug conditions (F[2,812]=2.492, p=0.083). For CA1 place cells, there was a significant interaction between genotype and drug condition (F[2,438]=3.272, p=0.039), yet there were no significant differences between the mean firing rates calculated for each drug condition for either AD or WT CA1 place cell firing rates. There was also not a main effect of rat age on firing rates for CA1 place cells. Similar to spatial information and sparsity scores, firing rates of CA2/3 differed between AD and WT, with AD CA2/3 place cells firing at a higher rate relative to WT CA2/3 place cells (F[1,373]=6.193, p=0.013). There was no main effect of drug, but preplanned within subjects contrasts provided limited evidence that M_1 agonism may suppress abnormally high firing rates of AD CA2/3 place cells. AD CA2/3 place cell firing rates were significantly higher than WT in the control condition only (p=0.002), but differences between AD and WT CA2/3 place cell firing rates were not significantly different in either dose of the M_1 agonist (low: p=0.402; high: p=0.199). The results suggest that AD CA2/3 place cells are hyperactive, and M_1 agonism may have a general effect to decrease firing rates of AD place cells to similar levels of WT place cells.

Next, we addressed the possibility that the reduced spatial fidelity of AD CA2/3 place cells was the consequence of AD CA2/3 place cells having larger place fields or more place fields per unit relative to WT. That is, activity of place cells with larger place fields and/or multiple place fields would spread over a larger proportion of the environment and perhaps convey less useful information about where the rat is in space, thus driving the lower spatial information per spike and higher sparsity in AD place cells. Figure 4.5 shows the mean number of place fields per unit and Figure 4.6 mean place field area for WT and AD rats.

The difference between mean number of place fields per unit in CA1 and CA2/3 differed between AD and WT place cells (F[1,812]=4.294, p=0.039), and the difference across drug conditions differed between AD and WT place cells (F[2,812]=4.963, p=0.007). There was not a main effect of rat age on mean number of place fields per unit. Within CA1, there was a trend for the difference between AD and WT to differ across drug conditions (F[1,438]=2.988, p=0.051), and preplanned within-subjects contrasts revealed that this was due to WT CA1 place cells the in M_1 high condition having more place fields relative to the WT M_1 low condition (p=0.044). In the M_1 high condition only, WT CA1 place cells also had significantly higher mean number of place fields relative to the AD CA1 mean number of place fields (p=0.043). AD CA2/3 cells generally had more place fields relative to WT CA2/3 place cells (F[1,373]=4.258, p=0.040), but preplanned within subjects contrasts revealed that this difference was driven solely by a significant increase of mean place fields per unit of AD CA2/3 place cells in the M_1 low condition (control vs. low: p=0.033; low vs. high: p=0.030). The difference between AD CA2/3 and WT CA3/CA3 was significant only in the M_1 low

condition (p=0.002), but there was no evidence that AD CA2/3 place cells had more place fields per unit relative to WT in the control or M_1 high condition,. Overall, the results suggested that AD place cell dysfunction in control conditions was not driven by AD place cells having more place fields relative to WT.

Similar to mean number of place fields per unit, there were trends for the difference in place field area between CA1 and CA2/3 place cells to differ between AD and WT (F[1,812]=3.025, p=0.082), and for the difference in place field areas between drug conditions to differ between AD and WT (F[2,812]=2.365, p=0.095). WT CA1 place cells had generally larger place fields than AD CA1 place cells (F[1,438]=5.276, p=0.044), and preplanned within subjects contrasts suggested that the genotype difference between place field area in CA1 place cells was driven by an increase in WT CA1 place field areas in the M_1 high condition (control vs. high: p=0.025). There were no main effects of rat age, genotype, drug, interactions between drug and genotype, or significant differences in individual comparisons in place field areas of CA2/3 place cells. Thus, the results suggested that AD place cell dysfunction was not driven by AD place cells having larger or more place fields relative to WT place cells.

The reduced spatial fidelity of AD CA2/3 place cells is specific to out-of-field activity. In order to gain further insight into how AD place cells firing patterns result in poor spatial fidelity, we also analyzed spatial information, sparsity, and firing rates calculated separately from spikes occurring within and outside place field(s) for a given unit. Figures 4.7, 4.8, and 4.9 show spatial information, sparsity scores, and firing rates that were calculated from spikes that occurred only outside of place fields for WT and AD rats, and Figure 4.10 shows firing rates that were calculated from spikes that occurred

only inside place fields for WT and AD rats. In short, the results showed that the poor spatial fidelity in AD place cells, particularly in CA2/3, are not the result of abnormal activity of place cells within their place fields. Instead, poor spatial fidelity of AD place cells seems to be driven by AD CA2/3 place cells firing more often and in a larger proportion of the environment outside of the place fields. Because it is not clear how spatial information and sparsity scores calculated from in-field spikes would be meaningful, only the spatial information and sparsity scores calculated from out-of-field spikes will be reported here.

Just as with spatial information and sparsity scores calculated with all spikes within and outside of place fields, the spatial fidelity calculated from out-of-field spikes of AD place cells was impaired (lower for spatial information, higher for sparsity) relative to WT place cells (spatial information: $F[1,812]=5.493$, $p=0.019$; sparsity: $F[1,812]=7.186$, $p=0.007$), CA2/3 place cells were impaired relative to CA1 place cells (spatial information: $F[1,812]=10.523$, $p=0.001$; sparsity: $F[1,812]=13.220$, $p=0.000$), and the difference between AD and WT differed between CA1 and CA2/3 (spatial information: $F[1,812]=19.481$, $p=0.000$); sparsity: $F[1,812]=6.575$, $p=0.011$). There was no main effect of rat age on out-of-field sparsity scores in CA1 place cells. In CA1 place cells, there was no overall effect of genotype, drug, or genotype by drug interaction on spatial information scores, but there was a trend for the high dose of the M_1 agonist to improve spatial information, but not sparsity, scores in AD CA1 place cells ($p=0.093$). AD CA2/3 place cells had significantly worse spatial fidelity as measured by both metrics (spatial information: $F[1,373]=37.641$, $p=0.000$; sparsity: $F[1,373]=14.408$, $p=0.000$), and preplanned comparisons confirmed that AD CA2/3 place cells were

significantly impaired relative to WT for spatial information (control: p=0.003, M_1 low: p=0.002; M_1 high: p=0.003) and sparsity (control: p=0.004; M_1 low: p=0.051; M_1 high: p=0.033). Thus, the pattern of results of spatial metrics restricted to spikes that occurred outside of place fields was very similar to the pattern of results from spatial metrics calculated from all spikes when rats were running laps.

Analyses for in-field firing rates or out-of-field firing rates revealed an interesting divergence in firing rate patterns between AD and WT place cells. For out-of-field firing rates, there was no main effect of genotype, CA region, or drug, but there was an interaction between genotype and CA region (F[1,812]=7.643, p=0.006). Within CA1, there were no significant main effects, interactions, or contrasts on out-of-field firing rates. AD CA2/3 place cells had higher out-of-field firing rates relative to WT CA2/3 place cells (F[1,373]=5.751, p=0.017). The out-of-field firing rates of AD CA2/3 place cells were only significantly higher than WT CA2/3 place cells in the control condition (p=0.007), but were not different in the M_1 agonist conditions.

The results from in-field firing rates analyses showed a different pattern. WT place cells had generally higher firing rates inside place fields (F[1,812]=5.383, p=0.021), but this genotype difference interacted with region (F[1,812]=4.140, p=0.041) and drug (F[2,812]=3.848, p=0.017). There was only a trend for in-field firing rates to differ by age (F[1,812]=3.437, p=0.064). Within CA1, there were no main effects of genotype or drug, or interaction between the two, but the preplanned within-subjects contrasts did provide evidence that M_1 agonism increased in-field firing of WT CA1 place cells relative to control (control vs. M_1 low: p=0.077; control vs. M_1 high: p=0.004). The effect of M_1 agonism on out-of-field firing rates of AD and WT CA2/3

place cells showed the inverse pattern that was found with in-field firing rates. Within CA2/3, in-field firing rates for WT place cells were generally higher than AD place cells, and the difference between WT and AD CA2/3 in-field firing rates was not uniform across drug conditions. Pre-planned within-subject contrasts confirmed that AD CA2/3 and WT CA2/3 in field firing rates were not different in the control condition, but in-field firing rates for AD and WT CA2/3 place cells were significantly different in both the M_1 low ($p=0.006$) and M_1 high ($p=0.005$) conditions. Together, M_1 agonism seems to decrease in-field firing rates of AD place cells but increase in-field firing rates in WT place cells. Taken together with the out-of-field firing rates of place cells, it is possible that M_1 agonism attenuates AD CA2/3 hyperactivity. This pattern of results pointed to the possibility for M_1 agonism to increase the signal-to-noise ratio of hippocampal place cells, particularly by reducing the noise in AD CA2/3 hippocampal place cells and perhaps increasing the signal in WT CA1 hippocampal place cells as well.

AD CA2/3 place cells decreased spatial fidelity seems to reflect a decreased signal-to-noise ratio that is driven by increased noise, not decreased signal. In order to directly investigate if hippocampal place cell dysfunction AD rats, and the effects of M_1 agonism, were best described in terms of firing rate signal(in-field)-to-noise(out-of-field) ratios, we calculated a signal-to-noise ratio for each unit with the formula (mean in-field firing rate) / (mean in-field firing rate + mean out-of-field firing rate). Figure 4.11 shows the signal-to-noise ratios for WT and AD rats.

CA1 units had higher signal-to-noise ratios than CA2/3 units ($F[1,812]=15.780$, $p=0.000$), and the difference between CA1 and CA2/3 signal-to-noise ratios differed between AD and WT ($F[1,812]=6.204$, $p=0.013$). There was a trend for signal-to-noise to

differ across drug conditions (F[2,812]=2.669, p=0.070). When CA1 and CA2/3 were analyzed separately, there were no main effects, interactions, or direct contrasts that significantly impacted the signal-to-noise ratios in CA1 place cells, with the exception of rat age. This indicates that although in-field firing rates of WT CA1 place cells increased with M_1 agonism, this increase in signal was perhaps not sufficient to cause an overall increase in the signal-to-noise ratio. The signal-to-noise ratios in CA2/3 place cells were significantly lower in AD CA2/3 relative to WT CA2/3 place cells (F[1,373]=7.836, p=0.005). Although there was no main effect of drug, preplanned within subjects contrasts provided evidence that M_1 agonism decreased signal-to-noise ratio in AD CA2/3 place cells. The signal-to-noise ratios of AD CA2/3 place cells were only significantly lower than WT CA2/3 place cells in the control condition (p=0.008), but not in the M_1 low or M_1 high conditions. In addition, there was a trend for signal-to-noise ratios of AD CA2/3 place cells to be higher in the M_1 low condition relative to control (p=0.089), and this difference reached significance in the M_1 high condition (p=0.019). These results indicated that the dysfunction in AD CA2/3 cells was driven by a decreased signal-to-noise ratio, and the individual effects of the M_1 agonist on in-field and out-of-field firing rates of AD CA2/3 place cells may have a combined effect to improve the signal-to-noise ratio.

Altogether, the results indicated that the dysfunction of AD hippocampal place cells was best characterized by AD CA2/3 place cells having a decreased signal-to-noise ratio resulting from an increase in noise, rather than a decrease in signal. Moreover, M_1 agonism may be able to improve signal-to-noise ratios of AD CA2/3 place cells to comparable signal-to-noise ratios of WT CA2/3 place cells. Initially, the higher in-field

firing rates of WT CA1 place cells supported the role of M_1 agonism to increase the signal of CA1 WT place cells. The results from the analyses on signal-to-noise ratios, however, suggests that the increased in-field firing rates of WT CA1 place cells in the M_1 agonist conditions did not correspond with an overall improvement in the signal-to-noise ratio.

Recognition Memory Testing

AD rats have impaired recognition memory performance. AD rats generally had much higher exploration times on lap 2 relative to WT rats in the control condition (*M* ± *SEM*: WT=4.024 ± 0.737; AD=8.310 ± 1.846), which made comparing exploration times on subsequent laps problematic. Therefore, only data from blocks in which the average exploration on lap 2 (novel object, novel location) was higher than 0.69 seconds (1 *SD* below the *M* exploration times across genotypes on lap 2 in the control condition) for WT rats and below 14.42 seconds (1 *SD* above the *M* exploration times across genotypes on lap 2 in the control condition) for AD rats were included in the analyses. This exclusion criteria brought the mean lap 2 exploration in the control condition for AD rats within one second of the mean lap 2 exploration in the control condition for WT rats (*M* ± *SEM*: WT=4.634 ± 0.378; AD=5.589 ± 0.9.55). Recognition memory performance was analyzed with two-way 2 (genotype: WT or AD) x 2 (drug: control or M_1) mixed ANCOVA with repeated measures controlling for rat age for both object-only DIs and object-in-location DIs. Figure 4.12 shows the object-only recognition memory performance for WT and AD rats, and Figure 4.13 shows the object-in-location recognition memory performance for WT and AD rats. For both Figures 4.12 and 4.13, the results are shown for the means across rats for the control and M_1 agonist condition.

AD rats had generally lower object-only DIs relative to WT (F[1,7]=12.180, p=0.010), although preplanned contrasts controlling for age showed that AD rats were only significantly impaired relative to WT rats in the M_1 agonist condition (F[1,7]=23.665, p=0.002). There was no difference between AD and WT object-in-location DIs across or within drug conditions. The results confirmed that AD rats between 12-19 months of age have recognition memory impairments relative to WT rats, although this impairment only reached statistical significance in the M_1 agonist condition.

Discussion

The current study investigated how hippocampal place cell function differed between AD and WT rats at an age at which AD rats have known spatial memory impairments. The study also asked whether systemic administration of an M_1 agonist could improve hippocampal dysfunction in the AD rats as measured by spatial fidelity of hippocampal place cells and recognition memory performance. Our results indicated that spatial fidelity of hippocampal place cells is impaired in AD rats, and an M_1 agonist may help alleviate some aspects of AD hippocampal place cell dysfunction albeit without apparent effects on recognition memory performance. The poor spatial fidelity and decreased firing rate signal-to-noise ratios of AD place cells was most apparent in CA2/3. There was some evidence that M_1 agonism may improve the signal-to-noise ratio in AD CA2/3 place cells by decreasing the rate and spread of out-of-field firing. However, there was less indication that M_1 agonism improved spatial information or sparsity scores, when either all spikes or only out-of-field spikes were considered.

Consistent with a previous study in our laboratory (see Chapter 2) and the impaired hippocampal place cell function of AD rats in the current study, AD rats

showed memory impairments relative to WT. In this particular task, AD rats were impaired in object-only recognition memory performance relative to WT rats, and there was no evidence that M_1 agonism improved object-only recognition memory performance of AD rats. The recognition memory task was designed so that the same objects appeared three times in a given block, but the objects swapped location on the last lap. The results showed that AD rats did not show memory for repeated objects when they were repeated in the same location as they had first appeared. Put together with the results from Chapter 2, which revealed AD rats at 9-12 months of age had impaired object-in-location memory performance but intact object-only recognition memory performance, it may be that the memory impairments of AD rats considerably older than 12 months begin to extend to the object-only domain as well. This object-only memory impairment in AD rats at older ages is consistent with Cohen and colleagues (2013), who found object-only recognition memory impairments in AD rats of 24 months of age. Due to the task design, it seems unlikely that the comparable exploration between AD and WT rats of repeated objects in swapped locations is due to the AD rats showing good memory for the location in which the repeated objects were first presented. Instead, it seems more likely that AD rats continued to indiscriminately explore repeated objects across different laps, regardless of location, because of an impairment to encode the objects themselves. Thus, the poor object-only recognition memory performance prevented meaningful interpretation of genotype or drug effects on object-in-location recognition memory performance. It should also be noted that the group size for each genotype was sufficient for recoding large numbers of hippocampal pyramidal neurons (N=1,800) but was nevertheless underpowered for detecting behavior effects (WT n=4; AD n=6). Future

studies investigating the effects of M_1 agonism on memory performance should use a higher number of AD and WT rats and employ tasks that would separate non-spatial and spatial memory performance to better understand how M_1 agonism impacts memory performance of AD rats.

The results of the current study are consistent with a large body of literature showing impairments on hippocampal-dependent memory tasks and synaptic plasticity abnormalities in transgenic animal models of AD (Morissette et al., 2009). Moreover, the few studies that have investigated hippocampal place cells in mouse models of AD and AD-relevant pathology have also found impairments in AD animals relative to WT (Cacucci et al., 2008; Cheng & Ji, 2013; Zhao et al., 2014). Yet there are notable dissimilarities between the results of previous studies and the current study. Cacucci et al. (2008) investigated hippocampal place cell function in the Tg2576 (with Swedish APP mutations K670N and M671L) mouse model of AD. Similar to our results, Tg2576 mice had age-dependent impairments in spatial information scores relative to AD, but there was no indication if this finding was more pronounced in a specific subregion as it was in the current study. The place cells of Tg2576 mice also had larger place field sizes relative to WT, which was not the case in AD CA1 or CA2/3 place cells in the current study. Zhao and colleagues (2014) looked at hippocampal place cells in an APP (with Swedish and Indiana APP mutations MMRRC #34845) mouse of model of AD, targeting CA1 hippocampal place cells only. Similar to the findings from Cacucci and colleagues (2008), APP mice had impaired spatial information scores and larger place field sizes in a familiar environment relative to controls. This is in contrast to the current results, as AD CA1 place cells had similar spatial information and place field sizes relative to WT CA1

place cells. Although Cheng and Ji (2013) also investigated hippocampal function in the rTg4510 model of tauopathy, hippocampal pyramidal cells in 7-9 month old rTg4510 mice did not show location-specific firing at all (although this seems to be age-dependent, see Ciupek et al., 2015). It seems that the selectivity for CA2/3 place cells and nature (increased noise) of impairments in AD rats is a new finding. More studies are needed with animal models and humans to determine if the specific impairments found in the current study are a reliable consequence of AD pathology and will extend to human AD patients.

Although there are very few studies that have examined hippocampal place cell activity in animal models of AD, hippocampal place cells in aged rats and fMRI activity in aged humans have been studied extensively. Many studies have also reported age-related to hyperactivity in CA3 place cells in rats and primates (Rosenzweig & Barnes, 2003; Thome et al., 2015). However, one study found CA3 hyperactivity of aged rats was particularly pronounced in novel environments (Wilson et al., 2005), but the recording environment for the current study was highly familiar to AD and WT rats. Moreover, Wilson and colleagues (2005) also found that aged CA3 place cells had larger place fields, but the current results did not find a difference between the place field sizes of AD and WT CA2/3 place cells in the control condition. One interesting possibility is that CA3 is disproportionally targeted by normal aging and AD relative to CA1, but the nature of the CA3 dysfunction in normal aging and AD is different. For example, hippocampal place cells in aged animals seem to have particular impairments in "remapping" by changing their collective pattern of activity between dissimilar environments to form distinct cognitive maps of the environment (Rosenzweig & Barnes,

2003), whereas the current study suggest hippocampal place cells in AD seem to have particular impairments in constraining CA2/3 place cell firing within their place fields. More research is needed to systemically compare AD place cell function with known aged-related place cell dysfunction using task paradigms that allow comparison of activity between novel and familiar environments. Relatedly, it is also possible that the current paradigm of evaluating AD hippocampal place cell activity in a high familiar environment did not properly tax CA1 place cells. Although the current results suggest that AD CA1 place cells may have similar spatial fidelity as WT CA1 place cells, a different paradigm that compared activity in a novel versus familiar environment, or the ability to distinguish between two familiar environments, may reveal impairments in AD CA1 place cells relative to WT.

There are few studies on the effects of manipulating mAChRs on hippocampal place cell function. Brazhnik and colleagues (2003) found that acute administration of scopolamine, the non-specific mAChR antagonist that has been used as a pharmacological model of dementia (Drachman & Leavitt, 1974; Ebert & Kirch, 1998), in healthy young rats disrupted place cell firing by decreasing the ratio of in-field vs. out-of-field firing. Interestingly, scopolamine decreased in-field firing rates and increased out-of-field firing rates in healthy young rats. It is interesting that the overall impairment in hippocampal place cell function from acute administration of scopolamine is similar to the differences between AD and WT CA2/3 place cells, and selectively increasing the activity of M_1 may have the opposite effect on signal-to-noise ratios as scopolamine by decreasing noise in AD CA2/3 place cells. M_1 activation may have excited hippocampal interneurons which in turn inhibited pyramidal cells in the hippocampus (Yi et al., 2014),

to dampen the location-irrelevant activity of hippocampal place cells to reduce out-of-field firing. The results of the current study together with the findings from Brazhnik et al. (2003) indicate that the activity of mAChRs, especially M_1, influence signal-to-noise ratios in the hippocampus. This may be helpful for AD and other diseases marked by an imbalance in signal-to-noise ratios, such as schizophrenia (Winterer & Weinberger, 2004).

Notably, the current study was conducted entirely in female AD and WT rats. Thus, we cannot dismiss the possibility that AD pathology or M_1 agonism may differ by sex. It is also possible that hippocampal place cells characteristics differ across estrous cycle in female AD rats, as synaptic plasticity and dendritic density in the hippocampus change throughout the estrous cycle (Woolley, 1998). There were no sex differences in pathological markers or memory performance in the initial characterization of AD rats, however (Cohen, R., personal communication). Moreover, Tropp and colleagues (2005) did not find any differences across the estrous cycle of female Sprague-Dawley rats on spatial information scores, in-field firing rates, the number of place fields per place cells, the number of place cells, field size, or burst properties of CA1 or CA3 pyramidal cells. The only difference they reported was a decrease in firing rate of CA1 pyramidal cells when rats were in proestrus, but the low number of cells in CA3 prevented them from investigating CA3 firing rates (Tropp et al., 2005). It seems unlikely that estrous cycle would play a major role in the current results, although it cannot be ruled out completely. Future studies could systematically examine hippocampal place cell activity in female rats in AD and WT rats to determine if there is an interaction between genotype and

estrous cycle on hippocampal place cell properties, as well as how estrous cycle interacts with potential treatments.

It was beyond the scope of the current study to investigate the relationship between place cell dysfunction in AD rats relative to hallmark AD pathology, but other studies have found interesting results in relation to pathology and hippocampal function. Hippocampal place cell function in Tg2576 mice correlated with Aβ plaque burden in the hippocampus, and TgCRND8 mice (with Swedish KM670/671NL and Indiana V717F APP mutations) show signs of hippocampal network dysfunction prior to the detection of Aβ pathology (Cacucci et al., 2008; Goutagny et al., 2013). One promising avenue of research would be to determine the timing of hippocampal place dysfunction relative to different AD pathological hallmarks, and whether the dysfunction occurs after a certain threshold is reached or slowly degrades in correspondence with the increase of pathology.

Finally, it should be noted that the current study only examined the acute effects of M_1 agonism on hippocampal place cell activity and recognition memory in AD rats. The same M_1 agonist used in this study was shown to reduce Aβ pathology and improve performance on a hippocampal-dependent spatial memory task in 5xFAD mice (expressing 5 early onset human AD mutations of APP and PS1 genes) following chronic administration (Lebois, 2014). Future studies should determine if chronic administration of the M_1 agonist in AD rats before they show impairments in pathology, network function, or behavior, could prevent or slow hippocampal dysfunction.

Overall, this is the first study to find a specific pattern of dysfunction in the CA2/3 region of the AD hippocampus that relates to a decreased signal-to-noise ratio through an increased firing rate and spread of out-of-field firing. The subtle effects of M_1

agonism on hippocampal place function demonstrate how using *in vivo* electrophysiology to investigate AD therapeutics can provide rich information that may help translate therapies to humans and understand differences between responders and non-responders to experimental therapies.

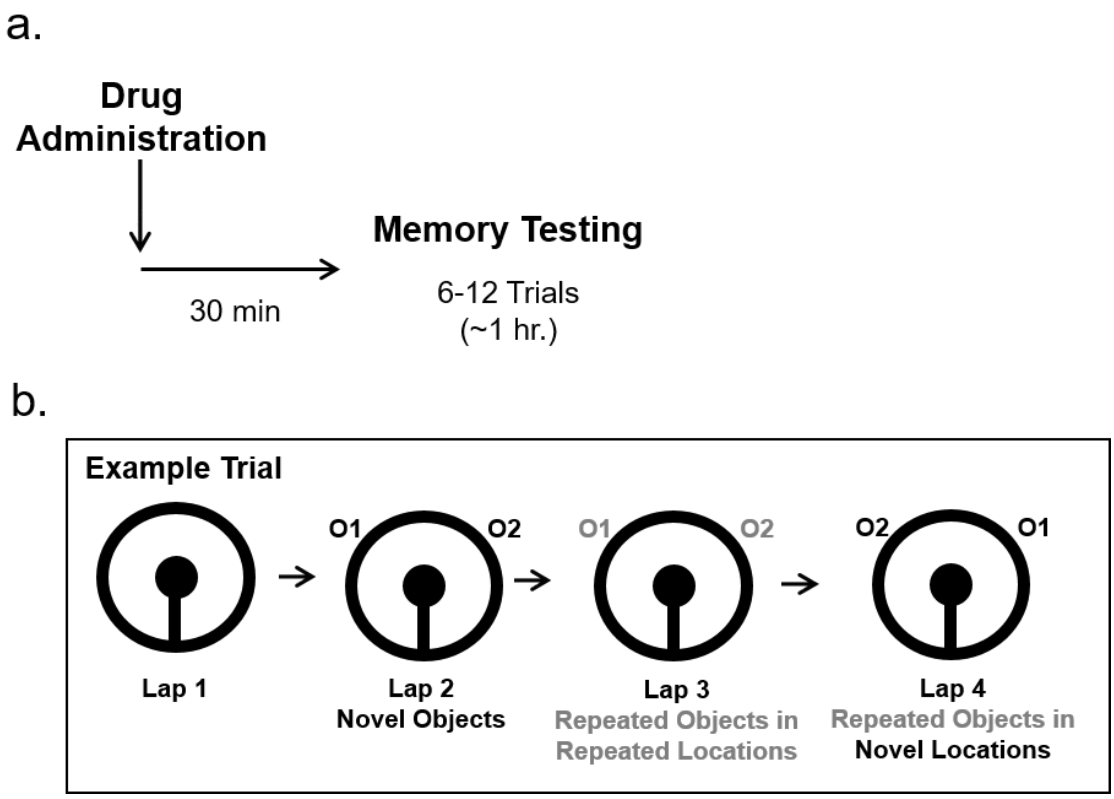

Figure 4.1. Schematic of the testing procedure. a. Each testing session began 30 min after drug administration, and consisted of a memory testing session of 6-12 trials that lasted ~1 hour. b. Each trial, rats completed 4 clockwise laps around a circular track. Each trial began with an empty lap in which rats did not encounter any objects. On lap 2, rats encountered two novel objects in two different locations. On lap 3, rats encountered duplicates of the lap 2 objects in the same locations they had appeared on lap 2 (repeated objects, repeated locations). On lap 4, rats encountered duplicates of the lap 2 objects, but the locations in which they appeared were swapped (repeated objects, novel locations). Individual objects are denoted by "O".

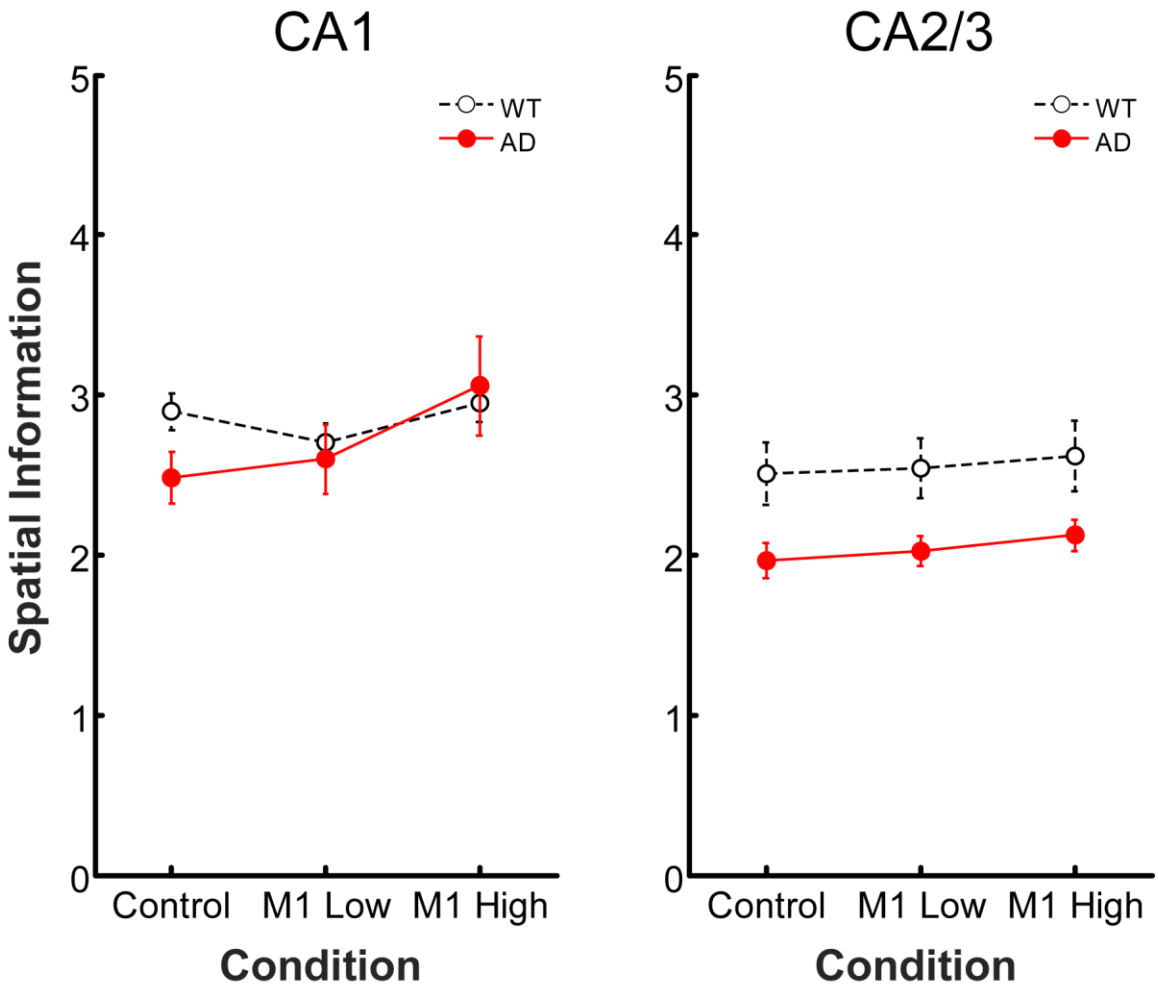

Figure 4.2. Spatial information scores of CA1 and CA2/3 units of all spikes that occurred while rats ran laps, by genotype and drug condition. The results are shown as mean spatial information scores (bits/spike) across units. Error bars show *SEM*. CA2/3 place cells of AD rats had lower spatial information scores relative to CA2/3 place cells of WT rats.

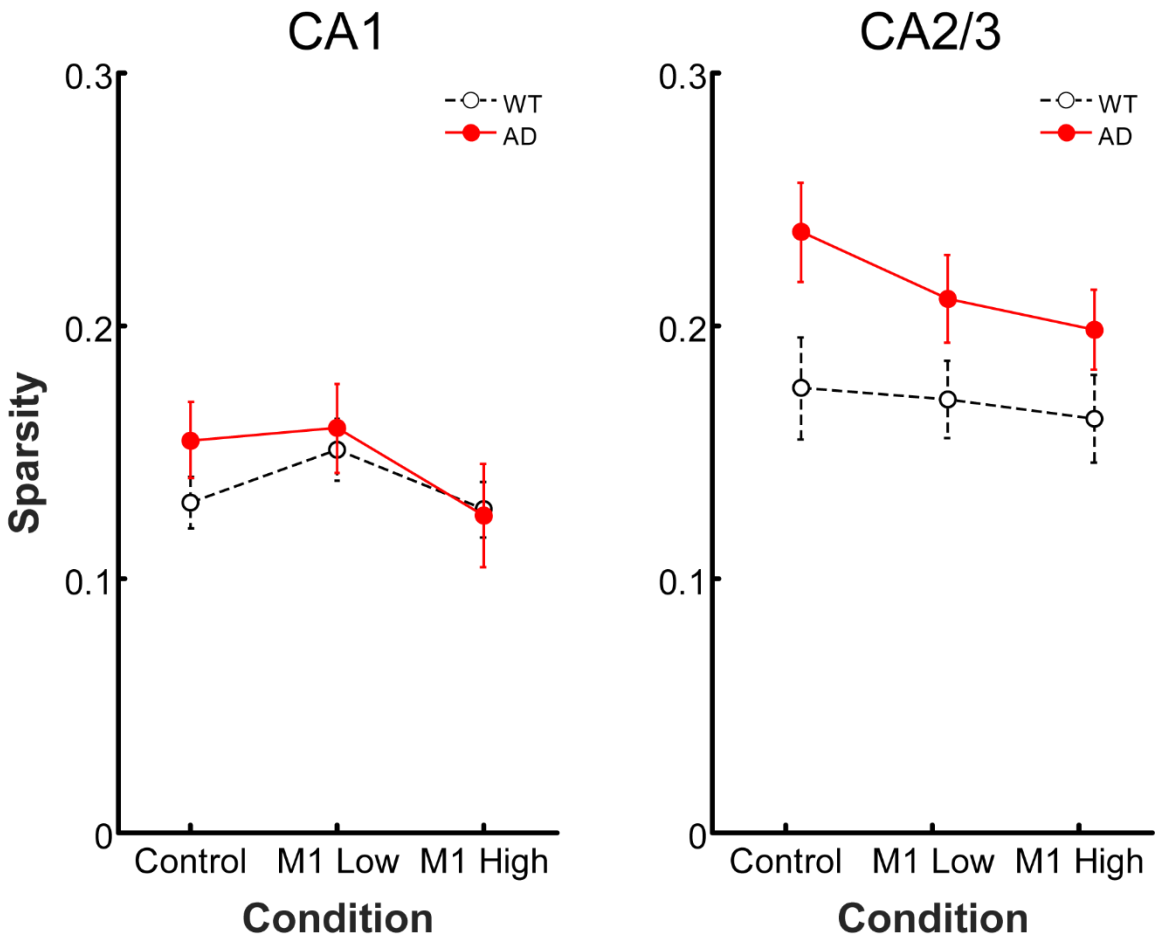

Figure 4.3. Sparsity scores of CA1 and CA2/3 units of all spikes that occurred while rats ran laps, by genotype and drug condition. The results are shown as mean sparsity scores across units. Error bars show *SEM*. CA2/3 place cells of AD rats had increased sparsity scores relative to CA2/3 place cells of WT rats, particularly in the control condition.

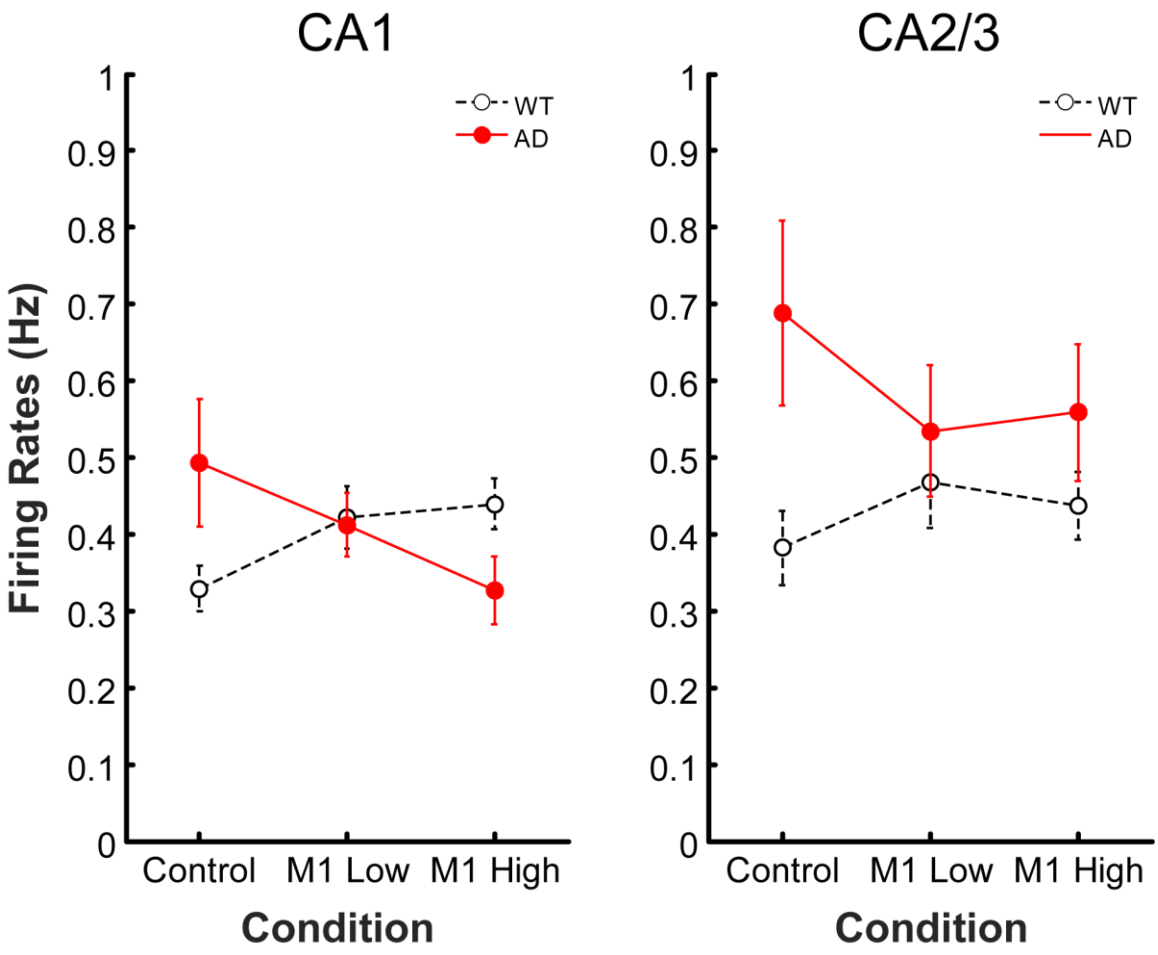

Figure 4.4. Firing rates of CA1 and CA2/C3 units of all spikes that occurred while rats ran laps, by genotype and drug condition. The results are shown as mean firing rates across units. Error bars show *SEM*. CA2/3 place cell firing rates of AD rats are higher than CA2/3 place cell firing rates of WT rats, particularly in the control condition.

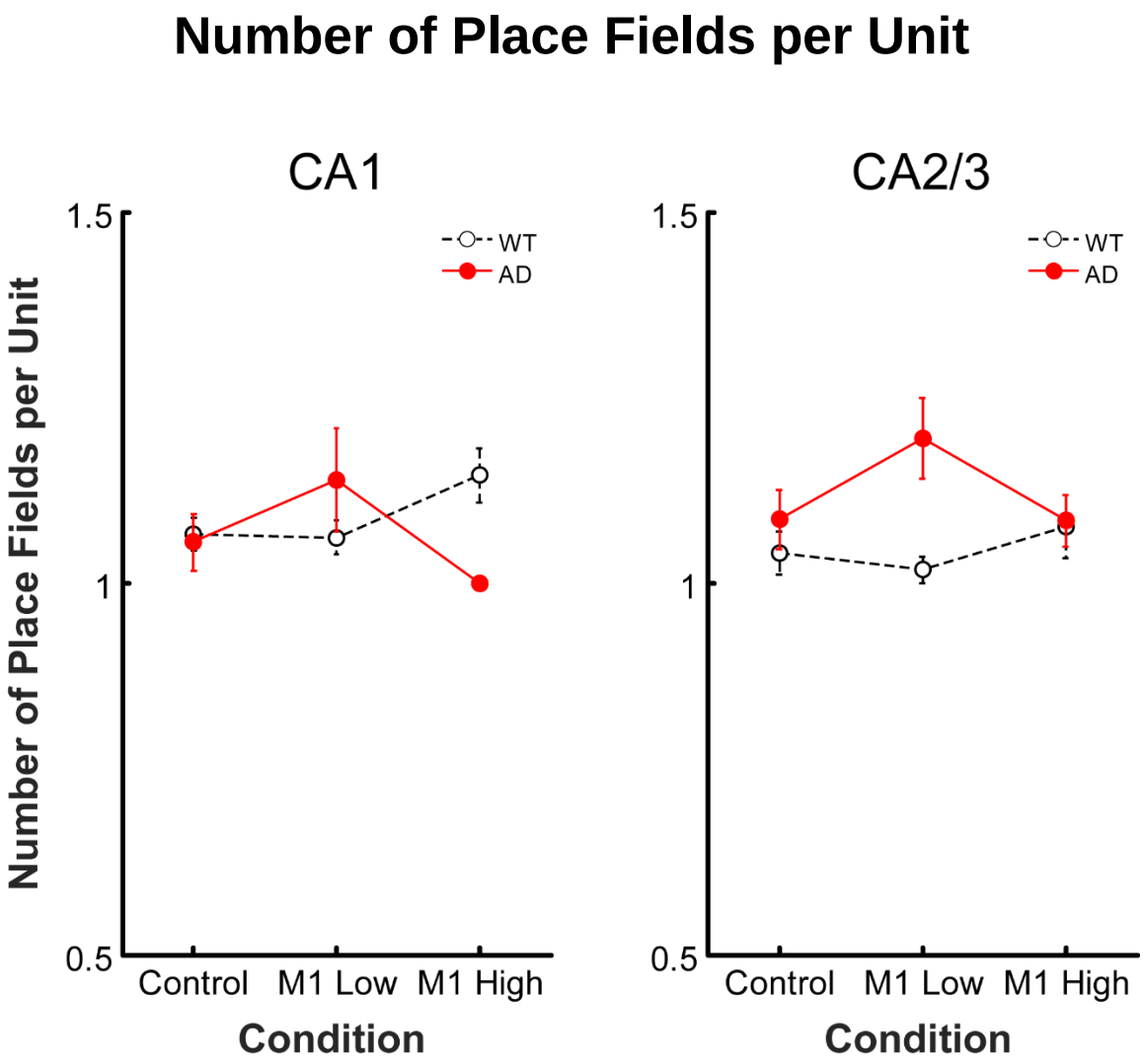

Figure 4.5. Number of place fields of CA1 and CA2/3 units, by genotype and drug condition. The results are shown as mean number of place fields across units. Error bars show *SEM*. CA1 and CA2/3 place cells of WT and AD rats had a similar number of place fields per unit.

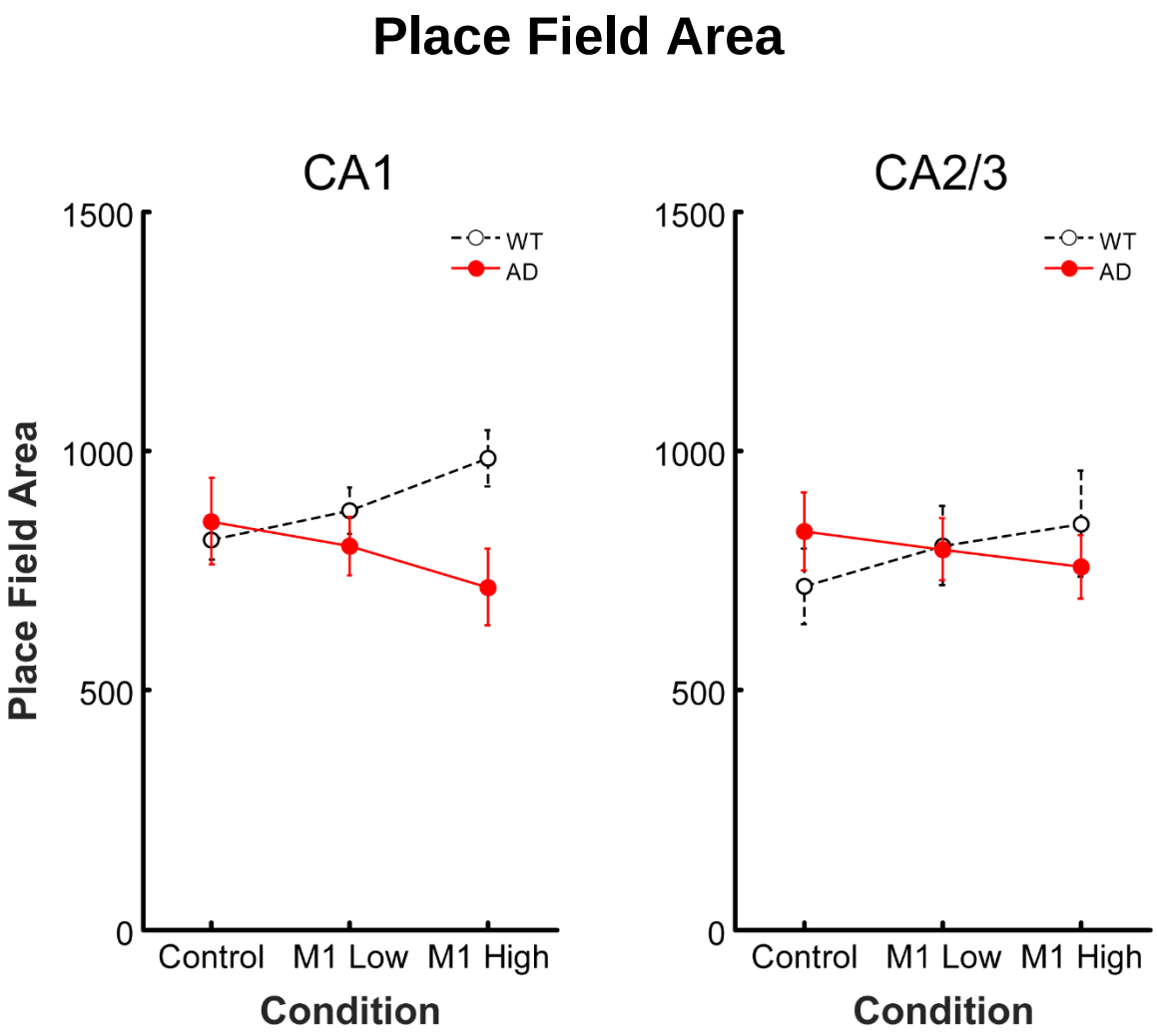

Figure 4.6. Place field area of CA1 and CA2/CA3 units, by genotype and drug condition. The results are shown as mean place field area across units. Error bars show *SEM*. CA1 and CA/3 place cells of WT and AD rats had similar place field sizes.

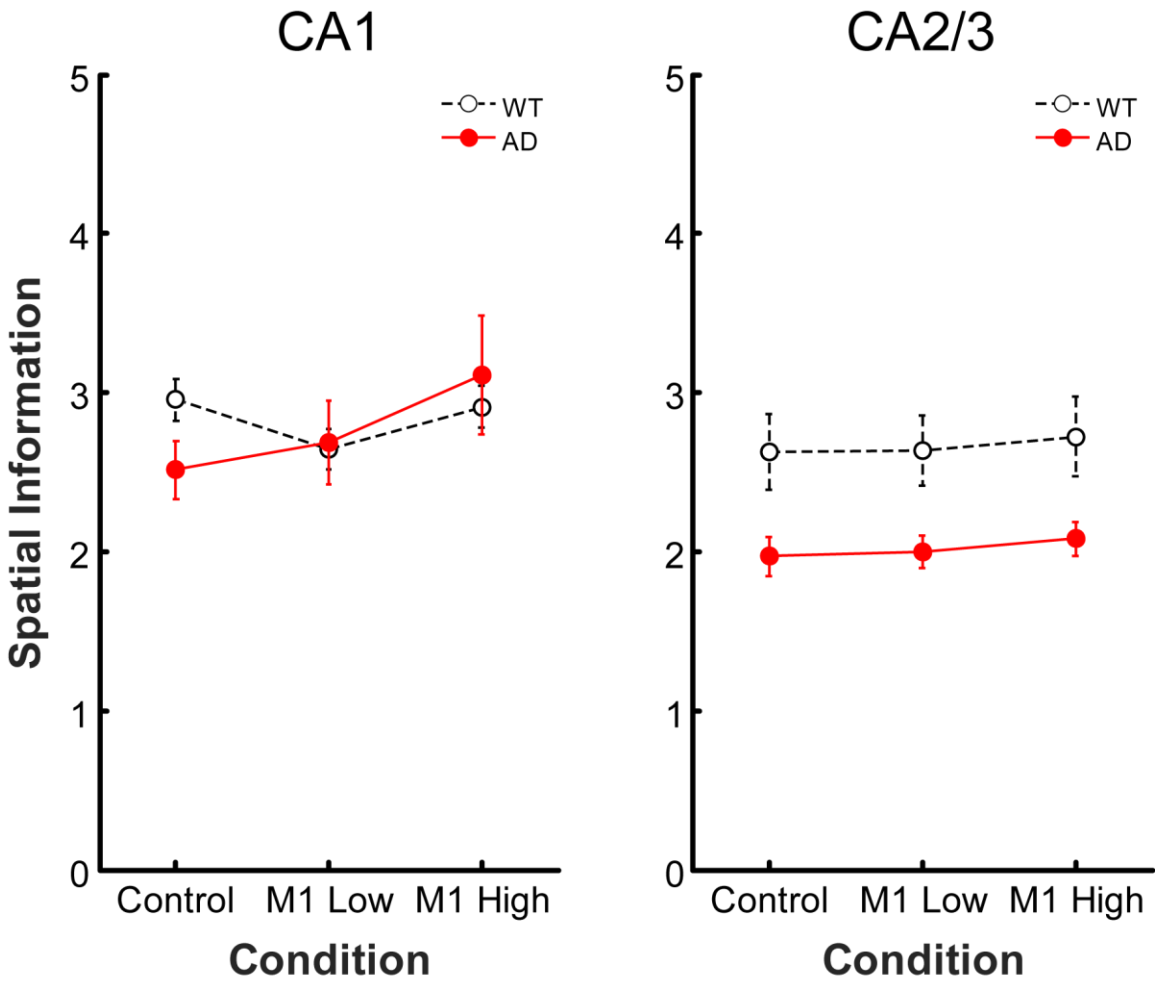

Figure 4.7. Spatial information scores of CA1 and CA2/3 units of spikes that occurred outside of the place field(s) of units while rats ran laps, by genotype and drug condition. The results are shown as mean out-of-field spatial information scores across units. Error bars show *SEM*. The out-of-field spatial information of CA2/3 place cells of AD rats had lower out-of-field spatial information relative to CA2/3 place cells of WT rats.

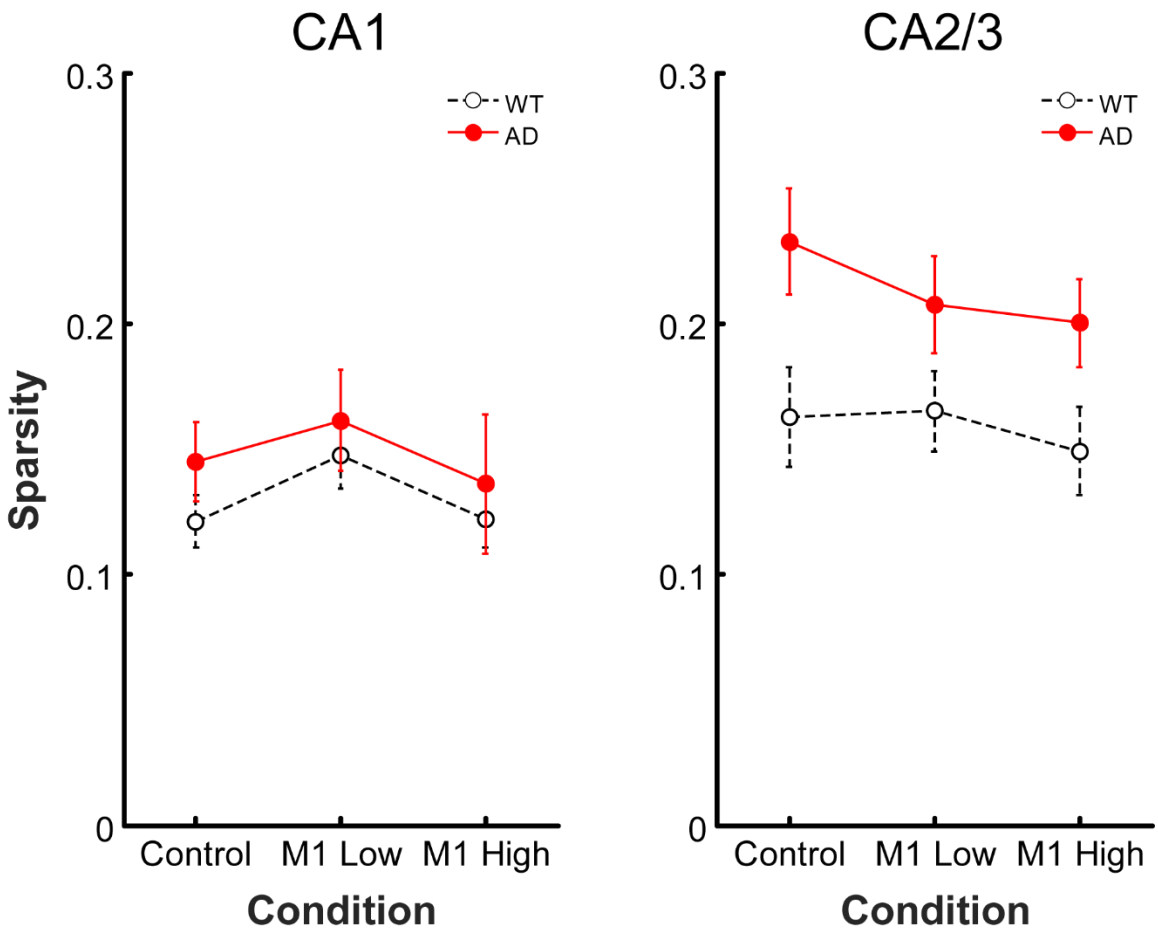

Figure 4.8. Sparsity scores of CA1 and CA2/3 units of spikes that occurred outside of the place field(s) of the units while rats ran laps, by genotype and drug condition. The results are shown as mean out-of-field sparsity scores across units. Error bars show *SEM*. CA2/3 place cells of AD rats had higher sparsity scores relative to CA2/3 place cells of WT rats, particularly in the control condition.

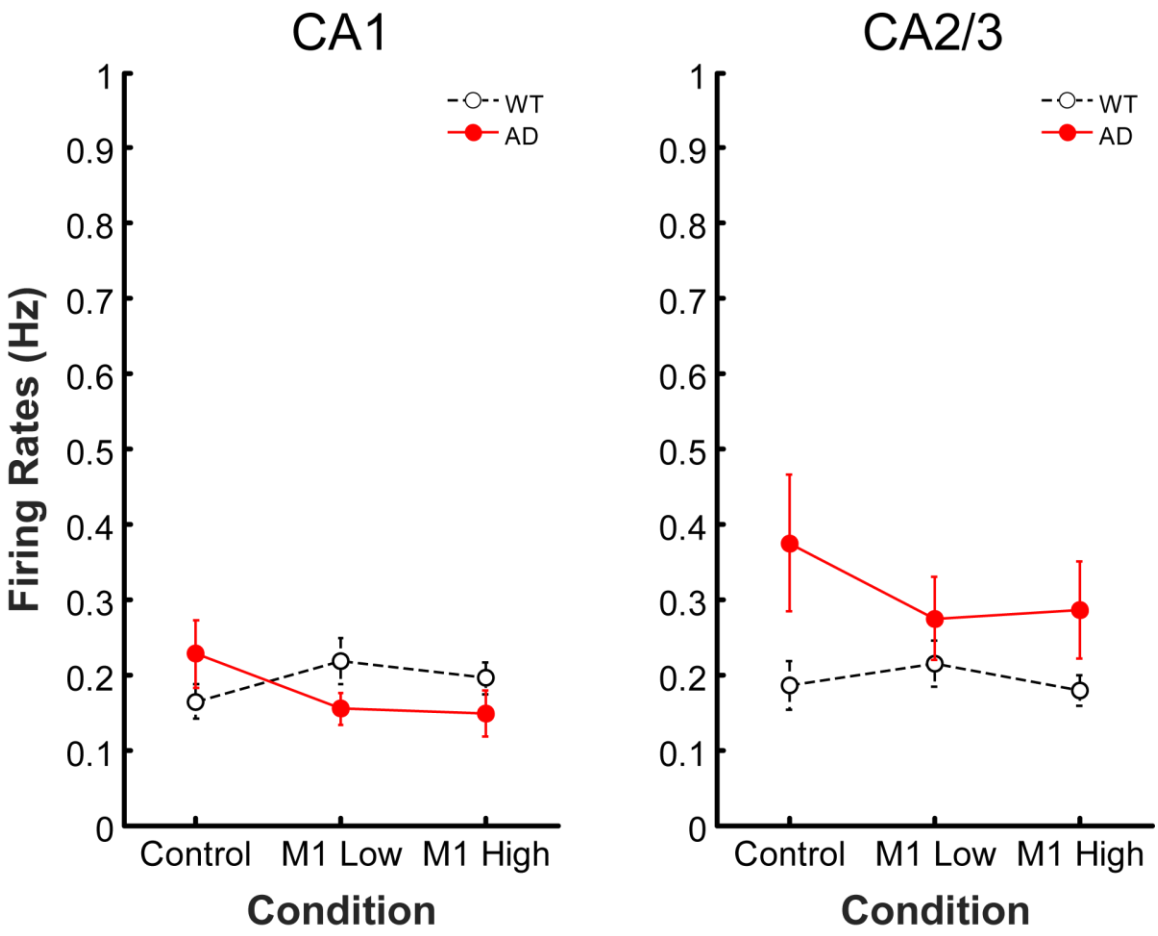

Figure 4.9. Firing rates of CA1 and CA2/C3 units of spikes that occurred outside of the place field(s) while rats ran laps, by genotype and drug condition. The results are shown as mean firing rates across units. Error bars show *SEM*. CA2/3 place cell firing rates of AD rats are higher than CA2/3 place cell firing rates of WT rats, particularly in the control condition.

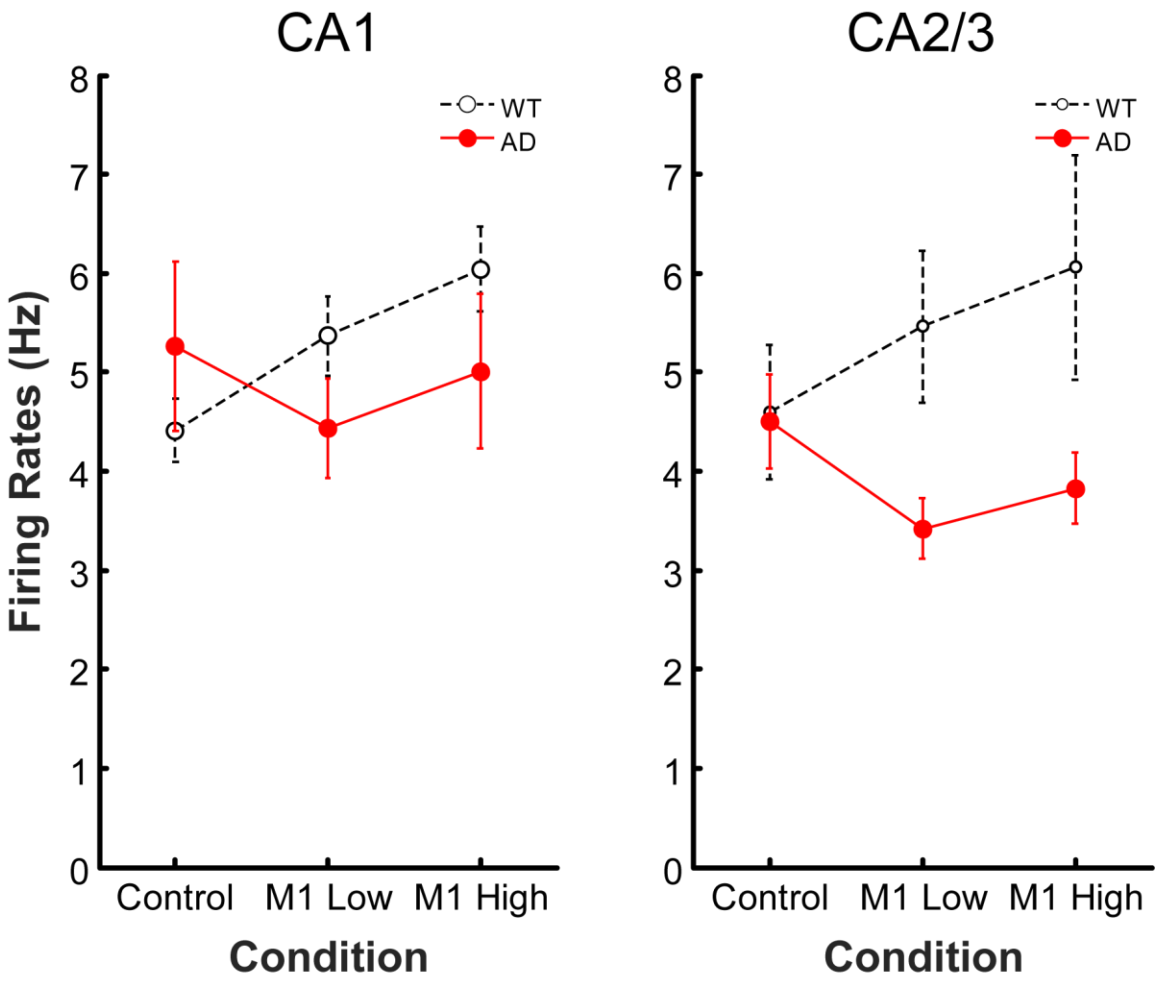

Figure 4.10. Firing rates of CA1 and CA2/C3 units of spikes that occurred inside of the place field(s) while rats ran laps, by genotype and drug condition. The results are shown as mean firing rates across units. Error bars show *SEM*. CA2/3 place cell firing rates of WT rats are higher than CA2/3 place cell firing rates of AD rats, particularly in the M_1 low and M_1 high conditions.

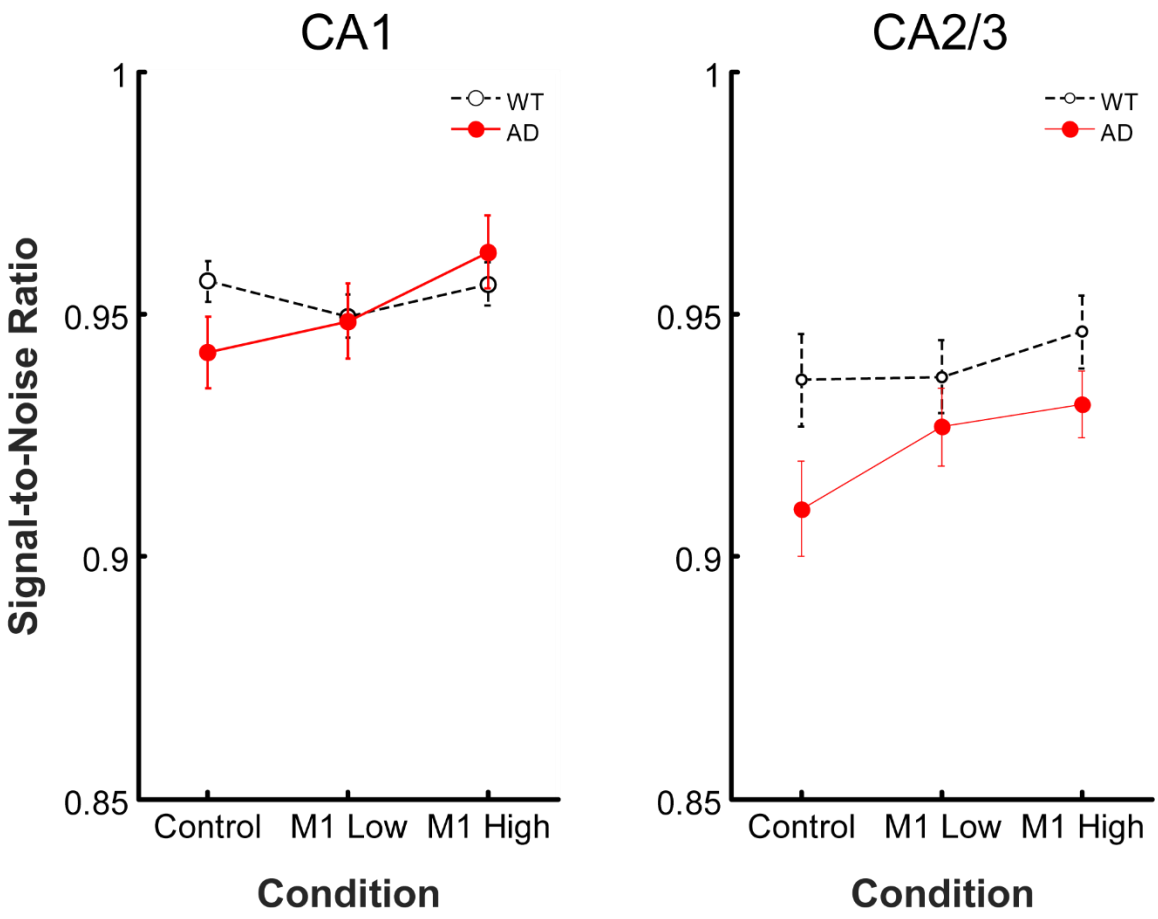

Figure 4.11. Signal-to-noise ratio of CA1 and CA2/C3 calculated from the average firing rates within the place field(s) divided by the sum of the average firing rates within and outside the place field(s) of units by genotype and drug condition. The results are shown as mean signal-to-noise ratio across units. Error bars show *SEM*. CA2/3 place cell of AD rats have decreased signal-to-noise ratios relative to CA2/3 place cells of WT rats, particularly in the control condition.

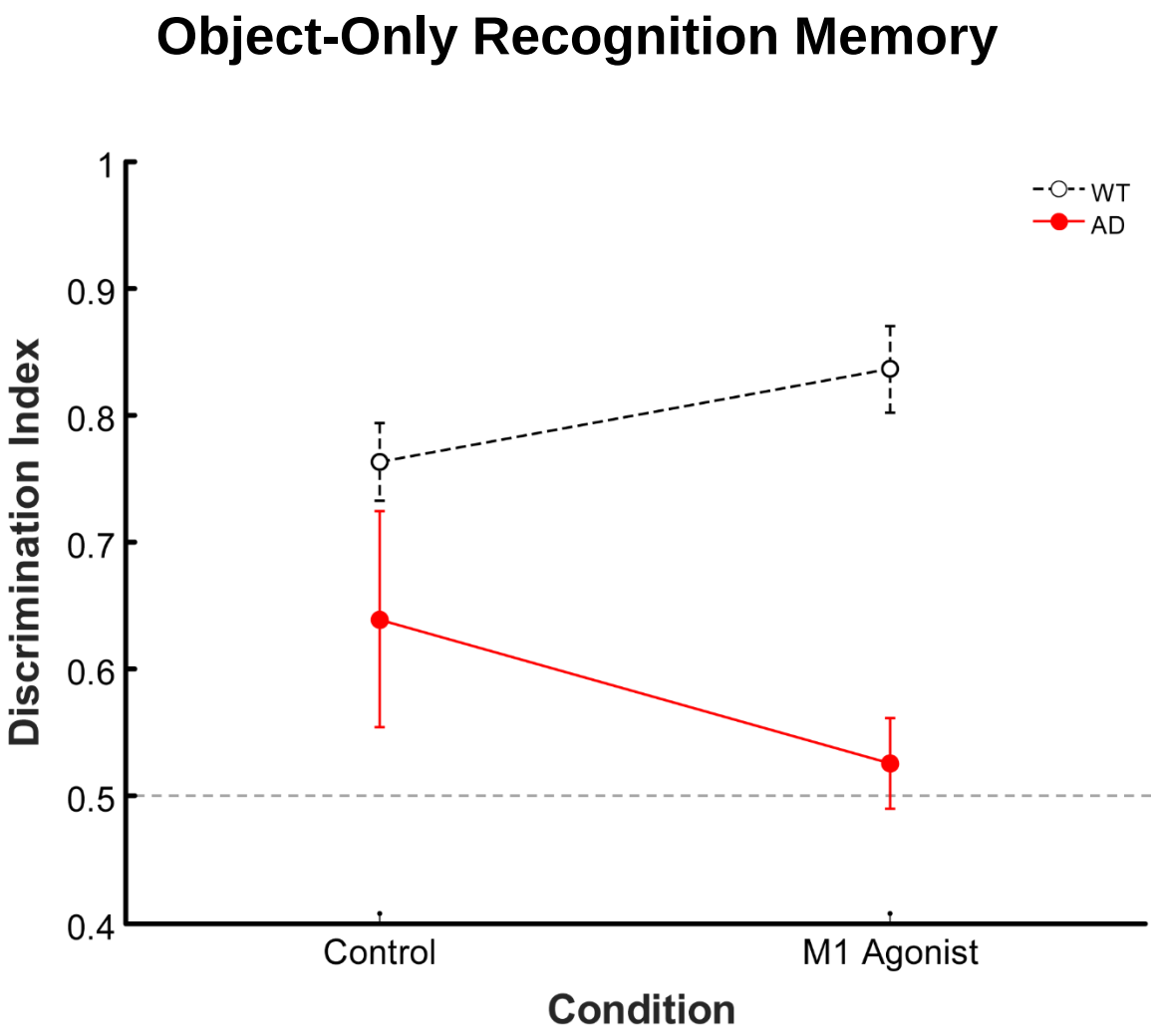

Figure 4.12. Object-only recognition memory performance by genotype and condition. The results are shown as mean Discrimination Index (DI) across rats (WT n = 4, AD n = 6). Error bars show ± *SEM*. The dashed line indicates chance performance. AD rats showed impaired object-only memory performance relative to WT rats, especially in the M_1 agonist condition.

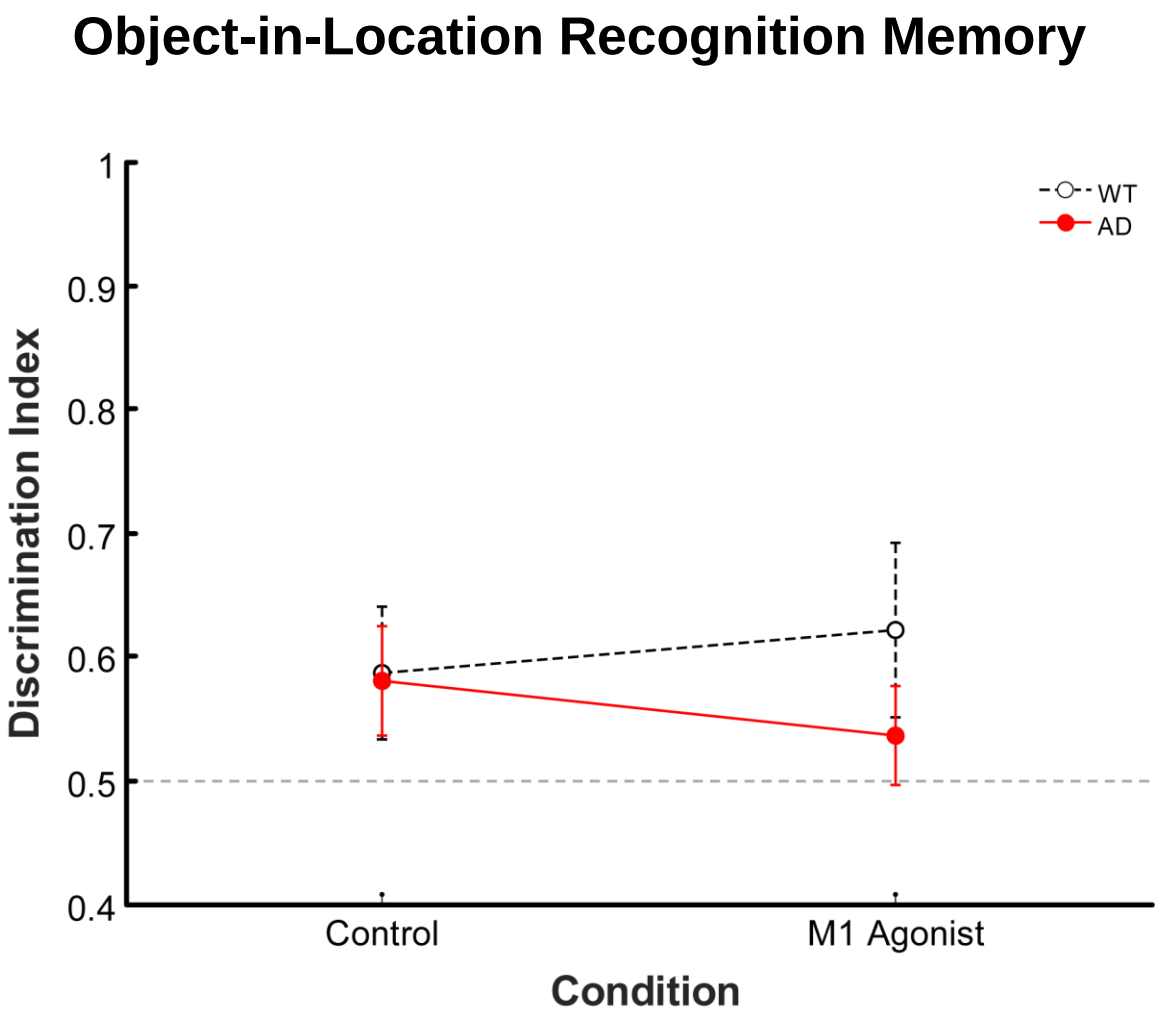

Figure 4.13. Object-in-location recognition memory performance by genotype and drug condition. The results are shown as mean Discrimination Index (DI) across rats (WT *n* = 4, AD *n* = 6). Error bars show ± *SEM*. The dashed line indicates chance performance. AD rats showed similar object-in-location memory performance relative to WT rats.

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Chapter 5

General Discussion

The experiments presented in Chapters 2-4 took advantage of *in vivo* electrophysiology techniques and behavioral tasks to investigate the effects of drugs that selectively increase the activity of M_1 or M_4 muscarinic acetylcholine receptors (mAChRs) on hippocampal function in healthy rats, and the efficacy of an M_1 agonist to improve hippocampal dysfunction in a rat model of Alzheimer's disease (AD) that closely resembles the pathological progression of human AD. Together, the studies in the previous chapters found that acute administration of drugs that selectively increase the activity of M_1 or M_4 (the M_4 Positive Allosteric modulator [PAM] VU0152100, the M_1 PAM BQCA, and the M_1 agonist VU0364572) may not improve hippocampal function in healthy rats performing well at baseline, but the M_1 agonist may improve hippocampal function in AD rats. The potential improvement in hippocampal function of AD rats from the M_1 agonist was detectable at the level of single cell activity, but not in memory performance. The findings of these studies point to the usefulness of *in vivo* electrophysiology to understand how single cells and cell assemblies in the hippocampus support memory at the network level, how potential drug therapies alter hippocampal network function, and how hippocampal network dysfunction contributes to AD-related memory impairment.

A summary of the findings from Chapters 2-4 is given below, followed by a discussion of the implications of the current studies in the context of hippocampal dysfunction in AD at the network level, muscarinic drug therapies for AD, and how pathological changes apart from the hallmark Aβ and tau pathology may interact with muscarinic drug therapies for AD.

Summary of Chapter 2

In the first study, we investigated if selectively increasing the activity of M_1 or M_4 would improve memory performance of rats on an object recognition memory task and increase measures of oscillatory synchrony between CA1 and CA3 of the hippocampus. We recorded local field potentials (LFPs) from CA1 and CA3 in healthy young rats as they performed an object recognition memory task. In all drug conditions, novel object exploration coincided with an increase in CA3-CA1 slow gamma coherence, a measure of oscillatory synchrony that reflects communication between CA1 and CA3. The increase in CA3-CA1 slow gamma coherence was higher during novel object exploration relative to when rats were stationary and not exploring novel objects, indicating that the increase in CA3-CA1 slow gamma coherence was not a movement-related phenomenon. All experimental drugs decreased CA1 or CA3 power in theta, slow gamma, or both frequency ranges, yet CA3-CA1 coherence during novel object exploration was similar across drug conditions. In addition, rats performed similarly well on the object recognition memory task across drug conditions. The results implicated that acute, systemic administration of drugs that selectively increase the activity of M_1 or M_4 may not provide an additional benefit to memory performance or oscillatory synchrony between CA1 and CA3 in healthy young rats.

Summary of Chapter 3

In the second study, we longitudinally assessed the performance of AD rats and WT rats on object-only and object-in-location recognition memory tasks from an age at which AD rats were previously shown to have intact memory performance relative to WT (5 months) until they developed spatial (object-in-location) memory impairments relative to WT rats. The goal was to determine a more precise time point at which AD rats

developed memory impairments to open up opportunities to investigate neural network activity underlying memory impairments and test potential drug therapies in AD rats at a relatively early stage in the pathological process. We found that by 9-12 months of age, AD rats had selective spatial memory impairments relative to WT rats.

Summary of Chapter 4

In the third study, we investigated if selectively activating M_1 would improve hippocampal function in AD rats at an age at which they had confirmed memory impairments. Hippocampal function was measured by hippocampal place cell fidelity and performance on a recognition memory task that was designed to assess both object-only and object-in-location memory. We found that AD rats had reduced hippocampal place cell fidelity relative to WT rats, most apparently in CA2/3 of the hippocampus. The dysfunction of AD CA2/3 hippocampal place cells seemed to reflect a decreased signal-to-noise ratio of firing rates that was specifically driven by the increased noise of spiking activity outside of place fields. There was some evidence that M_1 activation may reduce the noise of AD CA2/3 place cells to increase the signal-to-noise ratio. AD rats also had impaired object recognition memory relative WT rats, but M_1 activation did not improve memory performance of AD rats. The results suggested that hippocampal dysfunction that underlies memory impairments in AD rats is more apparent in CA2/3 cells of the hippocampus and may benefit from selective M_1 activation. The results also suggested that electrophysiological measures of hippocampal function complement behavioral tasks in their potential to uncover sensitive, precise mechanisms underlying the nature of hippocampal dysfunction in AD and potential benefit of drug therapies.

The current studies have several implications for the nature of hippocampal dysfunction in AD and the future of muscarinic drug therapies for AD. The following implications will be discussed along with their associated future directions: 1) place cell dysfunction in AD may share similarities with place cell dysfunction in normal aging, 2) hyperactivity in CA3 could contribute to AD pathology and cell death in CA1, 3) hyperactivity in CA3 could disrupt information coding in CA1 and relate to memory impairments in AD, 4) the cognitive benefits from selectively increasing the activity of M_1 and M_4 may be apparent in individuals who are performing poorly at baseline, 5) chronic administration of allosteric drugs that target specific mAChR subtypes may be needed to understand the therapeutic potential of these drugs, 6) using *in vivo* electrophysiology techniques to evaluate the impact of experimental drug therapies on network function may accelerate drug development, 7) how neuroinflammation interacts with the cholinergic system, contributes to AD pathology, and relates to hippocampal dysfunction at the network level should be better understood, 8) testing drug effects in animals that model the hormonal status in post-menopausal women may accelerate more effective treatments for the majority of AD patients.

Implications for network level mechanisms underlying AD

The results in Chapter 4 pointed to the possibility that place cell dysfunction in AD rats impacted CA2/3 more severely, which has interesting implications for how network activity and AD pathology may interact throughout the disease process. Many studies in aged rats have found selective impairments in CA3 place cells (Oh et al., 2016). CA3 place cells of aged rats have been reported to have larger place field sizes, higher firing rates in novel environments, and are more likely to represent two different

environments similarly (Wilson et al., 2005). CA3 hyperactivity has also been observed in aged monkeys and humans (Thome et al., 2015; Yassa et al., 2010). Yet in Chapter 4, we found the nature of CA2/3 place cell dysfunction was best understood in terms of hyperactivity outside of place fields that decreased the signal-to-noise ratio in a highly familiar environment, and was not related to place field size. One possibility is that CA3 is disproportionately impacted by aging and AD, but the nature of the dysfunction differs. In order to gain a better understanding of how AD memory impairments compare to age-related memory impairments, future studies should evaluate hippocampal place cell function of AD rats across multiple environments to determine if AD place cells follow the same pattern of age-related, subregion specific impairments in forming distinct representations of distinct environments.

Several studies in humans also indicate that AD does not impact all subregions of the hippocampus to the same degree or in the same way. Aβ pathology, tau pathology, and cell death of AD patients is disproportionally seen in CA1 (Braak et al., 2006; Thal et al., 2002; West et al., 1994), while fMRI studies found that CA3 and the DG was hyperactive early in the AD process and drug treatment that reduced the CA3/DG hyperactivity improved cognition (Bakker et al., 2012; Yassa et al., 2010). The subregional differences in vulnerability to the functional and pathological consequences of AD in the hippocampus may not be entirely incongruent. Both Aβ and tau pathology can contribute to excitotoxicity in the hippocampus (Minkeviciene et al., 2009; Palop et al., 2007; Siskova et al., 2014), but hippocampal hyperexcitability may also propagate AD pathology (Noebels, 2011). One possibility is that increased firing rates of CA3 pyramidal cells early in AD contribute to the pathological progression and cell death in

CA1. Future studies are needed to determine if drug therapies which decrease CA3 excitability early in the disease process could blunt the disease progression.

Hyperactive inputs from CA3 to CA1 may also drive cognitive dysfunction in AD by interrupting cortical input to CA1 from the entorhinal cortex. Hasselmo (1997) proposed that memory impairments in AD could reflect "runaway synaptic modification" due to insufficient cholinergic inhibition of previously stored synapses during new encoding. This runaway synaptic modification could cause interference from older memories so that newly encoded information would be inappropriately linked to older, previously stored information (Hasselmo, 1997). Given the role of M_4 to pre-synaptically inhibit glutamatergic inputs from CA3 to CA1 (Dasari & Gulledge, 2011), perhaps drug therapies that selectively increase the activity of M_4 would sufficiently inhibit inputs from CA3 to CA1 to allow new information from the entorhinal cortex to be encoded without interference from previously stored information coming from CA3. Theta oscillations in the hippocampus also play a role in separating new and old information coming into CA1, so that inputs from the entorhinal cortex occur at the peak of theta when CA1 is most depolarized and responsive to inputs, while inputs from CA3 occur at the trough of theta when CA1 is hyperpolarized (Hasselmo et al., 2002). One possibility is that CA3 spikes are more distributed across the theta phase of CA1 in AD rats, and M_4 activation helps restrict CA3 input to the trough of theta so that new information from the entorhinal cortex could be properly encoded and stored separately from old information. Drug therapies that selectively increase M_4 function in the hippocampus should be tested to directly assess whether increasing M_4 activity could rebalance hippocampal network activity to allow for encoding of new information in AD.

Implications for muscarinic therapies for AD

The findings from Chapter 2 suggested that selectively increasing the activity of M_1 or M_4 may not further improve hippocampal function in individuals who were already performing well in the control condition. This importance of baseline performance is in line with a previous study that tested memory performance of rats who were systemically administered the M_4 PAM, M_1 PAM, and M_1 agonist used in Chapter 2 (Galloway et al., 2014). In support of the potential for M_1 and M_4 to improve hippocampal function in impaired individuals, the M_1/M_4-preferring agonist Xanomeline effectively improved cognition and reduced the neuropsychiatric symptoms (hallucinations, delusions, violent outbursts) in AD patients (Bodick et al., 1997). However, Xanomeline was discontinued in phase II clinical trials due to intolerable side-effects, presumably mediated primarily through offsite activation of M_2 and M_3 (Bodick et al., 1997; Bymaster et al., 2003). Perhaps combining drugs with greater subtype selectivity like the M_4 PAM and M_1 PAM or M_1 agonist would be able to elicit the combined benefits of M_1/M_4 activation without the negative side effects. Although selective M_1 and M_4 activation may not push normal hippocampal function to superphysiological levels, activating M_1 and M_4 alone or together may be able to rebalance dysfunctional hippocampal network states. More studies are needed to investigate if the combination of the M_1 PAM or M_1 agonist with the M_4 PAM would have synergistic effects on hippocampal function in healthy young rats or AD rats. Moreover, experimental designs should include more difficult cognitive tasks or test more subjects in order to parse data by baseline performance.

One important factor to consider when evaluating the efficacy of the M_4 PAM, M_1 PAM, and M_1 agonist used in Chapter 2 and Chapter 4 is that all of the drugs were

administered acutely. For the M_1 agonist in particular, it is possible that the benefit for hippocampal function, especially in AD rats, would be most apparent after chronic dosing that began prior to detectable memory impairments and markers of AD pathology. For example, Lebois (2014) found that chronic (administered systemically ages 1.5 – 6 months), systemic administration of the M_1 agonist reduced Aβ pathology and improved memory performance in a mouse model of AD after a 24 hour wash-out period. The potential disease-modifying capability and memory improvement after chronic administration of the M_1 agonist is particularly promising in light of the temporary benefit of acetylcholinesterase inhibitor (AChEI) treatments for AD, which are due in part to the down-regulation of mAChRs (Anand & Singh, 2013; Volpicelli & Levey, 1993). There is some evidence that M_1 selective drugs that target allosteric sites may not activate the same regulatory pathways that mediate mAChR down-regulation, and so may be more suitable for chronic treatment (Davis et al., 2010; but see Yeatman et al., 2014). Perhaps the advantage of drugs that target allosteric mAChRs for the treatment of AD would be most apparent after chronic administration, especially if the drug therapy was initiated in early stages of the disease. Future studies should test the chronic effects of the M_4 PAM, M_1 PAM, and M_1 agonist in healthy young rats and AD rats to discover if prolonged treatment, particularly prior to memory impairments and build-up of AD pathology in AD rats, could prevent AD-related hippocampal dysfunction and remain effective over time.

Together, the studies also point to the important of using *in vivo* electrophysiology and other direct measures of *in vivo* brain function to evaluate the effects of potential drug therapies. Gaining a better understanding of how drugs influence

neural network activity both in healthy and diseased animals has the potential to accelerate treatments across diseases that may share some of the same underlying dysfunction. For example, the current study found that CA2/3 place cells of AD rats had a decreased signal-to-noise ratio. Perhaps the effect of the M_1 agonist to increase the signal-to-noise ratio through decreasing noise of CA2/3 place cells may benefit patients with schizophrenia or other diseases marked by decreased signal-to-noise ratios (Winterer & Weinberger, 2004). In addition, combining behavioral evaluations with measures of neural network activity holds great potential to better understand how experimental therapies are actually altering brain function "online," and inform downstream clinical trials about the potential limitations and appropriate application of a given drug therapy. For example, a drug which effectively reduced CA2/3 hyperactivity may be appropriate to administer to patients in the very early stages of AD when the hippocampus is known to be hyperactive, but may be ineffective or even harmful to patients in later stages of AD marked by hippocampal hypoactivity (Johnson et al., 2012). More studies are needed to understand which therapeutic interventions would be most effective at different stages of AD.

Implications for muscarinic therapies in relation to other pathological factors in AD

Although the disease-modifying potential of M_1 agonism in AD has mainly been investigated in terms of reducing Aβ and tau pathology, neuroinflammation may be another important metric for assessing the efficacy of AD treatments. For example, polymorphisms in genes involved in the regulation of inflammatory processes in the brain have been linked to AD (Wes et al., 2016), and chronic use of non-steroidal anti-inflammatory drugs (e.g. ibuprofen) has also been associated with decreased risk for AD

(Vlad et al., 2008). Neuroinflammation also plays a role in mediating the toxic effects of Aβ and tau pathology (Metcalfe & Figueiredo, 2010; Wilock, 2012), and therapies that target amyloid or tau pathology via microglia-mediated immune responses have disease-modifying potential (Lambracht-Washington & Rosenberg, 2013). The cholinergic system may also interact with inflammatory responses in the brain, as pro-inflammatory states were shown to decrease overall ACh levels by increasing acetylcholinesterase (Li et al., 2000), and acetylcholine was shown to reduce inflammatory markers (Pavlov & Tracey, 2005). Although M_1 and M_4 do not seem to be the primary receptors mediating ACh inflammatory responses (Pannell et al., 2016; Pavlov & Tracey, 2005), the M_1/M_4 preferring agonist Xanomeline blunted inflammatory responses in mice (Rosas-Bellina et al., 2015). In relation to hippocampal network dysfunction, neuroinflammation is related to neurotoxicity (Wang et al., 2005). An interesting possibility is that chronic administration of M_1 and M_4 together or alone could reduce CA3 hyperactivity and neuroinflammatory markers that would serve to improve cognitive dysfunction and alter the disease process that is exacerbated by neuroinflammation. Future studies should take advantage of *in vivo* electrophysiology techniques to investigate if and how markers of neuroinflammation relate to hyperactivity in the hippocampus and memory impairment in AD.

The efficacy of AD therapies, especially those that manipulate the cholinergic system, should be tested in females with compromised estrogen function to be most relevant to women with AD. Women are more at risk for AD and have more severe Aβ pathology relative to men (Warring et al., 1999; Zandi et al., 2002). The increased susceptibility to AD is likely mediated by decreased estrogen levels after menopause

(Waring et al., 1999; Zandi et al., 2002), as hormone replacement therapy in women soon after menopause has been correlated with a decreased risk for AD (Gibbs, 2010). In relation to hippocampal function, estrogen has been shown to increase ACh and other markers of ACh synthesis and release in the hippocampus (Spencer et al., 2008), and blocking mAChRs prevented estrogen-induced plasticity in the hippocampus (Stelly et al., 2012). One study found that combined administration of estradiol and the AChEI Donepezil improved memory performance in aged ovariectomized rats when neither estradiol nor Donepezil alone improved cognition in these animals (Gibbs, 2010). Thus, it seems likely that the severe decrease in estrogen could have important implications for the efficacy of AD therapies in post-menopausal women, especially therapies that involve the cholinergic system. Preclinical trials that more systematically test drug effects in ovariectomized female rats with or without estrogen replacement may be better able to predict positive or negative outcomes in AD women. Future studies should systematically investigate the progression of AD and response to potential drug therapies in both male and female AD rats that model post-menopausal hormonal states. It would be especially interesting to investigate if and how sex differences are reflected at the level of hippocampal place cell activity or CA3-CA1 oscillatory synchrony, and if estrogen treatment in combination with drugs such as the M_1 agonist VU0364572 would have synergistic effects in female AD rats.

Conclusion

In Chapter 2, we found that CA3-CA1 slow gamma coherence during novel object exploration was higher than CA3-CA1 slow gamma coherence during stationary periods when rats were not exploring novel objects, and drugs that selectively target M_1

and M_4 may not offer further improvement in memory or CA3-CA1 slow gamma coherence in young healthy subjects. In Chapter 3, we found that selective spatial memory impairments in AD rats were evident by 9-12 months of age. In Chapter 4, we found that hippocampal dysfunction in AD related to decreased signal-to-noise ratios of firing rates in CA2/3 place cells, and drugs that selectively target M_1 showed potential to increase the signal-to-noise ratio of CA2/3 place cells in the hippocampus. The studies in the previous chapters point to the opportunity to use *in vivo* electrophysiology to directly determine how diseases that involve brain dysfunction and potential drug therapies influence dynamic, complex neural networks. Moreover, the results advance our understanding of how muscarinic drug therapies affect hippocampal function in healthy and AD rats and the nature of hippocampal dysfunction that underlies memory impairments in Alzheimer's disease.

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