2013

The Effects of Acute Nicotine Administration on Memory Formation and Neural Activity in the Hippocampus, Perirhinal Cortex, and Medial Septum: Implications for Neurodegenerative Disorders

Matthew Wishnoff
Connecticut College, mattswishnoff@gmail.com

The Effects of Acute Nicotine Administration on Memory Formation and Neural Activity in the Hippocampus, Perirhinal Cortex, and Medial Septum: Implications for Neurodegenerative Disorders

A thesis presented by
Matthew S. Wishnoff
to the Department of Psychology
in partial fulfillment of the requirements
for the degree of
Bachelor of Arts

Connecticut College
New London, CT
5/1/2013

Abstract

Within the general public, nicotine is commonly thought of as a harmful molecule due to its role in tobacco addiction. However, nicotinic stimulation of the cholinergic system has also been shown to enhance cognitive functioning. This enhancement is thought to be caused by an increase in the release of the neurotransmitter, acetylcholine (ACh), which is responsible for mediating a variety of cognitive processes, such as REM sleep and memory formation. Recent research by Melichercik and colleagues shows that systemic nicotine administration enhances memory acquisition for both object location and object recognition memory in rats, as assessed by a modified version of the novel object recognition test (NOR). Using a standard NOR test we were able to reproduce their behavioral results: systemic nicotine administration enhances object recognition memory acquisition. Furthermore, we show for the first time that these behavioral results can be correlated with an increase in neuronal activation in the medial septum using immunohistochemical techniques. This research has implications for understanding the pathology that underlies neurodegenerative disorders with cholinergic involvement such as Alzheimer's Disease.

Dedication

I dedicate this thesis to my parents, without whom none of this would have been possible. Their constant advise and patience helped me over every stumbling block. To my dad who tirelessly read every page and provided me with invaluable feedback. Your comments and devotion helped me improve quality of my work tremendously. And to my mom, for always being there when I needed support. I am grateful for everything that you have done for me.

Acknowledgements

First, I would like to express my incredible gratitude for my advisor, Professor Grahn for the enormous amount of assistance that she have given me over the past three years. Writing this thesis has been a long and gratifying process that without her enthusiasm and expansive knowledge would have not been possible. I would also like to thank my other readers: Professor Schroeder and Professor Eastman for their valuable incite and critiques. And finally, to my friends who were there to encourage me along the way; their company during late nights in the lab and long writing sessions was truly extraordinary.

Table of Contents

List of Tables and Figures

An introduction to smoking, nicotine, acetylcholine, learning and memory

Smoking

People who start smoking during adolescence account for the largest proportion of people who struggle with addiction to cigarettes (Shram, 2007). Due to the copious amounts of carcinogenic substances found in tobacco, it is estimated that smoking-related deaths will be the leading cause of fatality worldwide by 2015. In addition to these carcinogenic substances, tobacco also contains nicotine. While the general public usually only hears about the addictive nature of smoking and the carcinogenic effects associated with tobacco use, neuroscientists believe that nicotine may actually facilitate memory formation through the activation of nicotinic acetylcholine receptors.

Acetylcholine, a Brief Overview

Acetylcholine (ACh) is responsible for all muscle movement, plays a role in REM sleep, and is essential for facilitating perpetual learning (Shiromani, 1987 and Yakel, 2012). Once released, ACh is able to activate nicotinic and muscarinic acetylcholine receptors (nAChR and mAChR). There are several subcategories of nAChRs and mAChRs spread throughout the brain (Howes, 2009). The main difference between the two categories is that nAChRs are ionotropic, whereas mAChR are metabotropic (Fisher, 2008). Both nAChRs and mAChR are commonly found on the surface of neuronal cells. One way that nAChRs are able to alter cellular activity is by modifying the electropotential gradient present between the inside environment of the neuron and the extracellular matrix. The binding of ACh to nAChRs causes ligand gated ion channels located in the cell membrane to open, allowing positively charged calcium ions to diffuse into the negatively charged environment of the

neuron. This influx of positive ions causes a brief depolarization across the cell membrane which is propagated and eventually causes the release of neurotransmitter into the synaptic cleft. ACh is degraded by the enzyme acetylcholinesterase shortly after activating the cholinergic receptor. Once ACh is no longer bound to the receptor, the calcium ion channels close and repolarization begins.

Nicotinic Acetylcholine Receptors (nAChRs)

All neuronal nAChRs found in the CNS are pentameric, meaning they are composed of 5 subunits (*Figure 1)*. These five subunits are arranged in a symmetric fashion around a central ion pore (Decker, 2000). According to genetic studies, there are at least 11 unique subunits that can combine to make a pentameric nAChR. Eight of these subunits have been designated as α-subunits (α2-α9), and three have been designated as β-subunits (β2-β4). Each subunit contains a N-terminal hydrophilic domain, a variable C-terminal domain located in the cytoplasm that is capable of being phosphorylated, and four transmembrane domains designated M1-M4 (Changeux, 1998).

Two main subtypes of neuronal nAChRs involved in cognitive processes are the heteropentameric α4β2, and homopentameric α7 receptors. The α4β2 receptor can either assume a $(\alpha4)_3(\beta2)_2$ or $(\alpha4)_2(\beta2)_3$ stoichiometry, while the α7 receptor exists only as the $(\alpha7)_5$ configuration. Both α7 and α4β2 subtypes are located pre and postsynaptically within hippocampal neurons, suggesting they play a critical role in modifying synaptic transmission and neurotransmitter release (Kenny, 2008). Severe spatial memory impairments can be seen upon using an antisense knockdown of the α7 nicotinic receptor in rats, indicating that the α7 receptor is crucial for spatial memory processes (Cruzon, 2006). Administration of the α4β2

receptor agonist, RJR 2403, has been shown to significantly improve working memory, indicating a role for the α4β2 receptor in working memory (Levin, 2002). Research also shows that activation of α7 and α4 nAChRs can help limit neuronal apoptosis by preventing glutamate-induced excitotoxicity, a common problem in several neurodegenerative disorders (Akaike, 2010).

Muscarinic Acetylcholine Receptors (mAChRs)

Acetylcholine is also able to activate muscarinic acetylcholine receptors (mAChRs) in the CNS. Like nAChRs, mAChRs are also composed of 5 subunits, M_1-M_5. All mAChRs are metabotropic, class I heptahelical G-protein-coupled receptors (*Figure 2)*. Upon activation, mAChRs regulate second messengers and ion channel activation through G-protein binding (Caulfield, 1998). In addition to the normal agonist-binding site, mAChRs activity can be modified through allosteric binding of other molecules (Cristopoulos, 1998).

M_1 mAChRs are located both pre and post synaptically in the PNS and CNS (Ito, 2009). They are present throughout the cerebral cortex and hippocampus, areas that are involved in learning and memory processes. Current research shows that selective M_1 receptor agonism may be a useful therapeutic approach for treating Alzheimer's disease (Fisher, 2008). M_2 mAChRs are also widely expressed in the PNS and CNS. M_2 receptors are the most prevalent mAChR subtype in the thalamus, hypothalamus, midbrain, caudate putamen, and cerebellum. Selective activation of M_2 receptors in the putamen inhibits the release of dopamine, a feature that may one day lead to a treatment option for schizophrenia (Oki, 2005). A large number of M_2 receptors are also found in myocardial and smooth muscle tissue. M_3 receptors are involved in respiratory and gastrointestinal functioning in the PNS.

While they are also located throughout the CNS, they are present in much lower concentrations than their M_1 and M_2 relatives. Due to their relatively low concentration, the role of these receptors in the CNS is still largely unknown (Eglen, 2006). The role of M_4 mAChRs in the CNS is somewhat unknown as well. The majority of M_4 receptors can be found in the corpus striatum where they co-localize with dopamine receptors. Recent research indicates that M_4 receptors help mediate the antipsychotic effects of xanomeline, a drug currently being developed to treat schizophrenia (Dencker, 2011). The M_5 receptor is the only muscarinic receptor to be expressed in the substantia nigra, the primary location of dopaminergic cells; and activation of the M_5 receptor has been shown to facilitate dopamine release (Eglen, 2006). M_5 receptor activation is also thought to facilitate cognitive processes due to its ability to dilate cerebral blood vessels. Genetically modified M_5 knockout mice exhibit a significant decrease in cerebral blood vessel dilation, a common occurrence in AD and focal ischemia. Therefore, selective activation of the M_5 receptor may represent an attractive target for novel drug therapies (Yamada, 2001).

Figure 1: Heteropentameric α4β2 and homopentameric α7 nicotinic acetylcholine receptors (nAChRs) and associated ACh binding site (adapted from Davis, 1999).

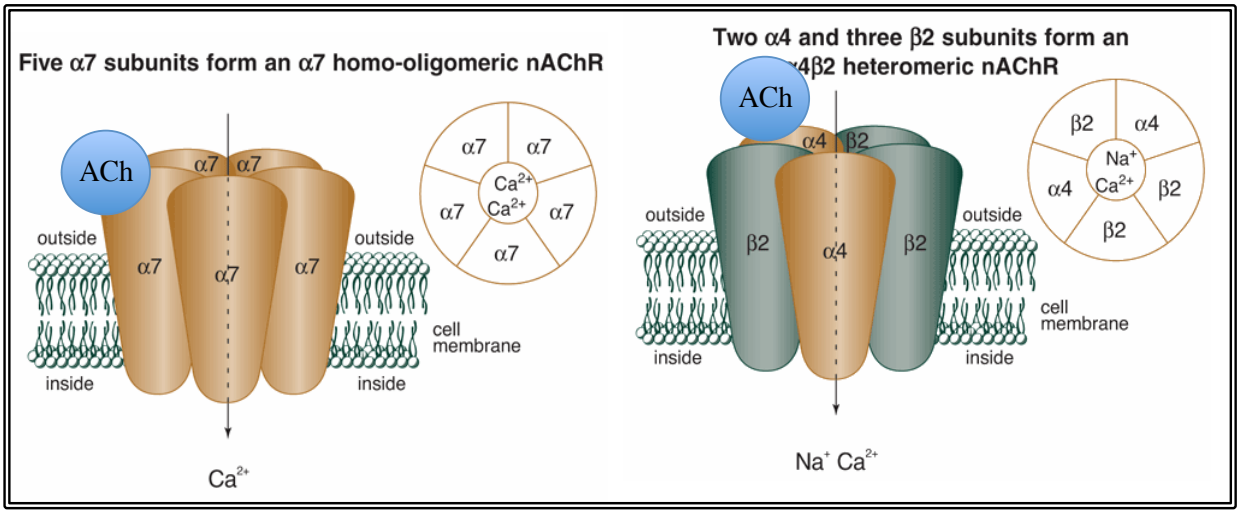
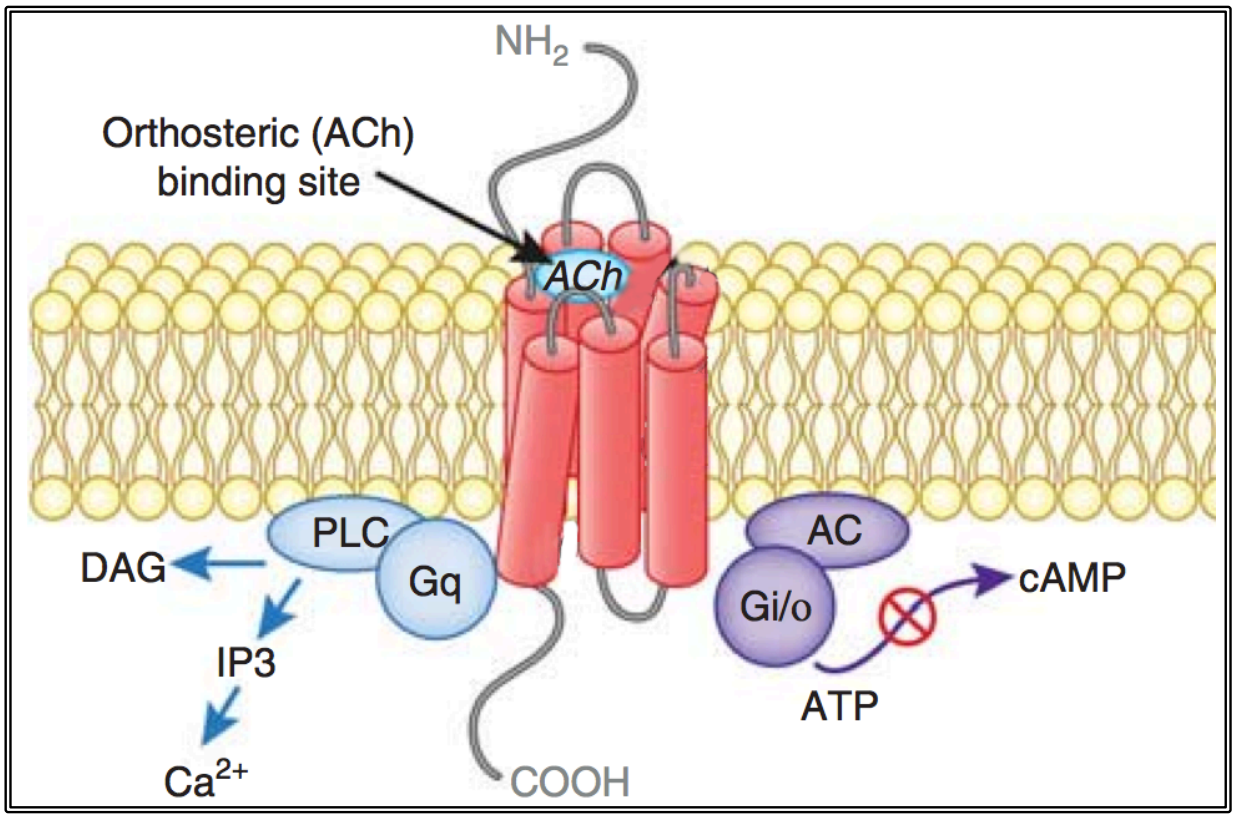


Figure 2: Representative muscarinic acetylcholine receptor (mAChR) and associated ACh binding site (adapted from Jones, 2012).



Nicotinic Activation of the Cholinergic System

Nicotinic activation of nAChRs results in the same mechanism of depolarization across the cell membrane as ACh. In addition to mimicking the effects of ACh at postsynaptic nAChRs, activation of presynaptic nAChRs by nicotine stimulates the release of ACh itself, resulting in an increase of neuronal activity. Unlike ACh, nicotine is not degraded by acetylcholinesterase. This means that when nicotine is bound to a nAChR, the ligand gated ion channel is able to remain open for a longer period of time, resulting in an extended period of depolarization (Carlson, 2010). Repeated administration of nicotine appears to cause a desensitization of nAChR functioning; however, this desensitization does not reduce nicotine's ability to facilitate memory formation (Gould, 2009). Therefore, other receptors must also be involved in nicotine's memory enhancing abilities. One hypothesis is that nicotine may also elicit a secondary effect through activation of mAChRs. A recent two-part study determined that one form of memory, spatial memory, improved with nicotine administration. However, when mAChR activity was blocked using chemical inhibitors, nicotine administration did not enhance spatial memory, implementing nicotinic activation of mAChRs in spatial memory processes (Liu, 2004).

Long Term Potentiation (LTP)

Synaptic plasticity is the constant rewiring of neuronal circuitry that serves as the basis for memory formation. The connections between neurons are strengthened, by making them more likely to be activated together in the future, when they are subject increased levels of stimulation, in a process known as long term potentiation (LTP). LTP was first observed in human hippocampal cells in 1973 (Bliss, 1993). Acetylcholine is known to be one of

many biological factors that affects LTP. Cholinergic receptors are commonly found on the surface of neuronal cells. One mechanism through which these receptors function is by modifying the electropotential gradient present between the inside environment of the neuron and the extracellular matrix. Upon stimulation from ACh, cholinergic receptors cause ligand gated ion channels located in the cell membrane to open, allowing for the diffusion of positively charged calcium ions into the negatively charged environment of the neuron, causing a brief depolarization across the cell membrane. Shortly after activating the cholinergic receptor, Ach is degraded by the enzyme acetylcholinesterase, causing ion channels to close and therefore cessation of the flow of calcium ions into the cell. Activation of nAChRs by nicotine causes the same mechanism of depolarization across the cell membrane. However, nicotine is not degraded by acetylcholinesterase, which allows the ligand gated ion channel to remain open for a longer period of time, resulting in an extended period of depolarization (Carlson, 2010). Repeated nAChR activation resulting in LTP is thought to be a major way in which high levels of ACh facilitate encoding (Radcliffe, 1999). Research has shown that the acquisition of new memories happens within 48 hours, making memory formation a relatively quick process (Tse 2007).

Types of Memory

Ever since researchers first attempted to propose a mechanism that would describe how memory formation occurs, no one has been able to create a unanimously agreed upon and complete model. Early hypotheses about how brain structure affects memory function were guided by the observations made by neurologists and neuropsychologists as they

worked with patients who had suffered various forms of brain damage. The most famous example of how brain structure is related to function comes from the case of Henry Gustav Molaison, long known as H.M. In the 1950's, H.M. underwent a bilateral medial temporal lobectomy, removing his hippocampus in an attempt to cure his epileptic seizures. As a result of the procedure, H.M. displayed severe anterograde amnesia, implicating hippocampal involvement in memory formation. Because some forms of his memory were left intact, such as information pertaining to his childhood, H.M.'s case also demonstrated that memory exists in multiple unique forms and that particular brain areas are responsible for certain forms of memory. For over 50 years, Wilder Penfield and Brenda Milner continued to study the effects of the lobectomy on H.M.'s behavior, making him one of the first cases used to create the field of cognitive neuropsychology. Currently, neuropsychology is one of the fastest growing fields as it attempts to understand how brain structure is related to function.

The Three Stage Model

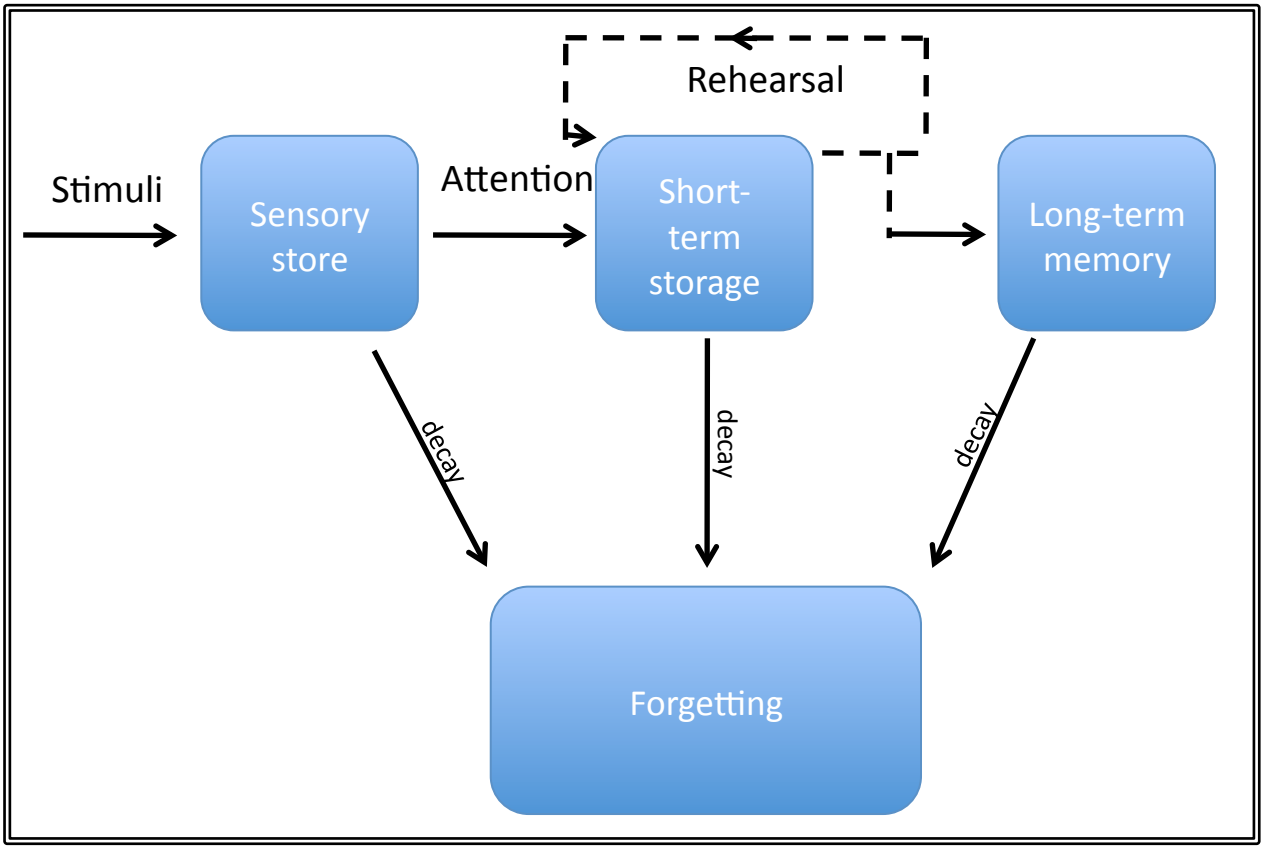
In 1962, psychologists Richard Atkinson and Richard Shiffrin proposed The Three Stage Model of Memory, which would eventually become the most commonly accepted psychological model used to describe the basic mechanism underlying memory formation (*Figure 3*). The first stage of the model describes how incoming information is dealt with. First, sensory information about the outside world is collected. After being collected, the information is encoded and sent to the sensory store. The sensory store is often referred to as a multimodal storage system because it deals with both visual and auditory information. The encoding of visual information results in iconic memory formation, while the encoding of

auditory information becomes echoic memory. The sensory memory store has a relatively small capacity and is only able to retain information for about half a second. If the brain actively pays attention to one of these incoming streams of information, the information is transferred to the second stage, short-term storage. However, if attention is not directed towards the incoming streams, the information decays and is forgotten.

Short-term storage has a smaller capacity than the sensory store and is limited to around 7+/- 2 chunks of information. Information can be stored in short-term storage for up to 18 seconds (Milner, 1956). Just as with the sensory store, if information contained in short-term storage is ignored, it will decay and be forgotten. If the information in the short-term store is further rehearsed through active thought processes, it has the opportunity to be transferred into the final compartment, long-term memory. Theoretically, information transferred to long-term memory should remain indefinitely. This being said, the Atkinson-Shiffrin model also states that information is susceptible to degradation over time, or corruption through interference, the process by which new information replaces existing information (Gazzaniga, 2009).

The Three Stage Model of Memory is often criticized for being too linear and simplistic because it does not take into account the various subdivisions of short-term memory and long-term memory, or incorporate working memory. While more recent models of memory formation still contain the basics of the three-stage model, they focus more on functioning of working memory. In current models of memory formation, short-term memory refers to small amounts information that are held passively and are later able to be reproduced without any manipulation, such as a repeating a phone number back to someone (Swanson, 2007).

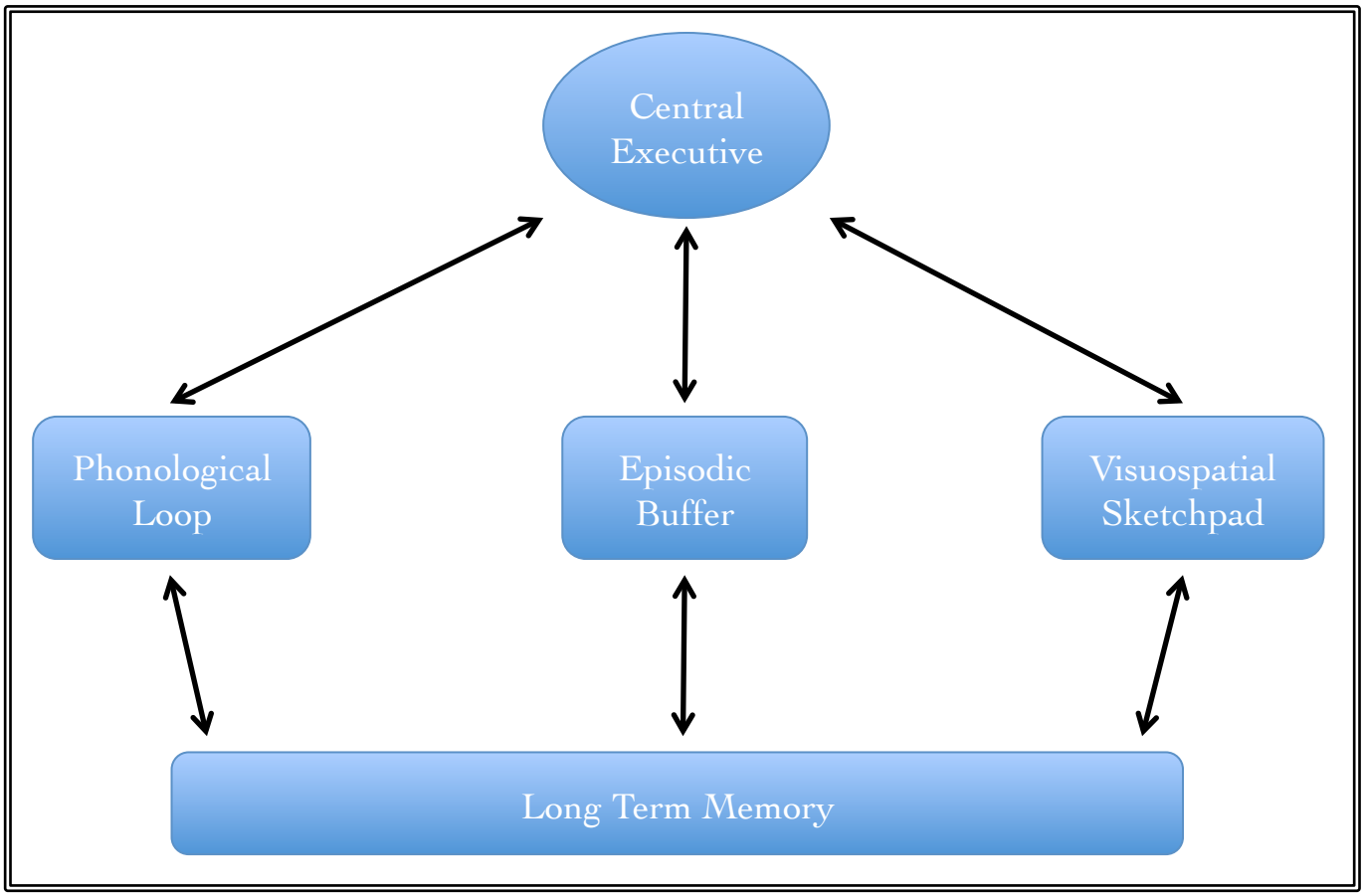
Figure 3: The Three Stage Model of Working Memory



Working Memory

Dissatisfied with the description of the short-term store in the Atkinson-Shiffrin three-stage model of memory, Alan Baddeley and Graham Hitch proposed a new model of working memory (WM) in 1974. Their model proposed that WM is responsible for not only the temporary storage, but also the manipulation of information needed for complex cognitive processes including both verbal and non-verbal tasks. Working memory receives input from both long-term memory and short-term stores. Their model initially divided WM into three individual components: the central executive, visuospatial sketchpad, and phonological loop (*Figure 4*) (Baddeley, 1992). Eventually, a fourth component known as the episodic buffer, was added to the model to account for phenomena that could not be represented within the initial model (Baddeley, 2000). Within this model, the central executive acts as a manager, controlling the flow of information to and from the visuospatial sketchpad and phonological loop. The visuospatial sketchpad is responsible for the storage and manipulation of visuospatial information, while the phonological loop is responsible for the storage and manipulation of auditory information. Because visual and verbal memory tasks can be performed simultaneously with minimal interference, the sketchpad and loop are believed to operate totally independently of each other (Cocchini, 2002). PET imaging studies provide further evidence for the physical separation of verbal and spatial processing as verbal tasks only produce increased neuronal activity in left hemispherical regions, while spatial tasks only produce increased neuronal activity in right-hemispherical regions (Smith, 1996).

Figure 4: The Baddely Model of Working Memory



The Phonological Loop

The phonological loop is responsible for the storage and manipulation of auditory information and can be further divided into of two parts: the phonological store and articulatory loop. The phonological store is responsible for temporarily holding information while the articulatory loop continuously rehearses it in order to prevent the inherently rapid decay of information from the store.

The phonological loop is composed of neuronal network located in the left lateral frontal and inferior parietal lobes. This is verified by several case studies in which participants who have sustained injury to the left inferior parietal lobe exhibit phonological working memory deficits as well as reduced capacity for auditory information (Gazzaniga, 2009). Likewise, damage to the left hemispherical portion of premotor cortex, supplementary motor cortex, or Broca's area results in impaired phonological rehearsal function (Gathercole, 1999) .

One of the more important roles that the phonological loop assumes is storing auditory information about unfamiliar words while they are being committed to memory (Baddeley, 1998a). By demonstrating that participants have a more difficult time remembering sequences of similar sounding letters such as B, C, D, G, T, than sequences of letters that had distinct sounds such as F, Y, W, R, T, it was determined that the phonological loop encodes incoming information acoustically as opposed to visually. Studies also show that working memory is encoded acoustically rather than semantically because participants have an easier time recalling a list of dissimilar sounding, unrelated words, than a list of related, similar sounding words (Gazzaniga, 2009).

The Visuospatial Sketchpad

Analogous to how the phonological loop is responsible for the temporary storage and manipulation of auditory information, the visuospatial sketchpad is responsible for the temporary storage and manipulation of visuospatial information. The sketchpad is responsible for visual orientated tasks such as creating a virtual map of one's environment, optical memory recall, and mental calculations. Unlike the phonological loop, the components of the sketchpad are not fully understood; however, it is thought to have separate visual, spatial/sequential, and kinesthetic components (Baddeley, 2003).

The Central Executive

The central executive acts as the primary control mechanism and relay center for working memory. It is often thought of as a homunculus, a term used in psychology to describe a "little man" who makes executive decisions. It is able to direct attention by selectively focusing on or ignoring incoming information. Unlike the phonological loop and visuospatial sketchpad, the central executive does not have a singular modality. This multimodality allows for the exchange of information between the sketchpad, loop, as and long term memory (Baddeley, 1998b).

The Episodic Buffer

Some of the initial inspiration to rethink the original model of working memory came from case studies of highly intelligent but densely amnesic patients. While these amnesic patients were unable to encode new information and transfer it to long-term memory, they still had the ability to recall recent stories. These stories were much too long to be held only in the phonological loop, therefore another storage system was needed. In order to account

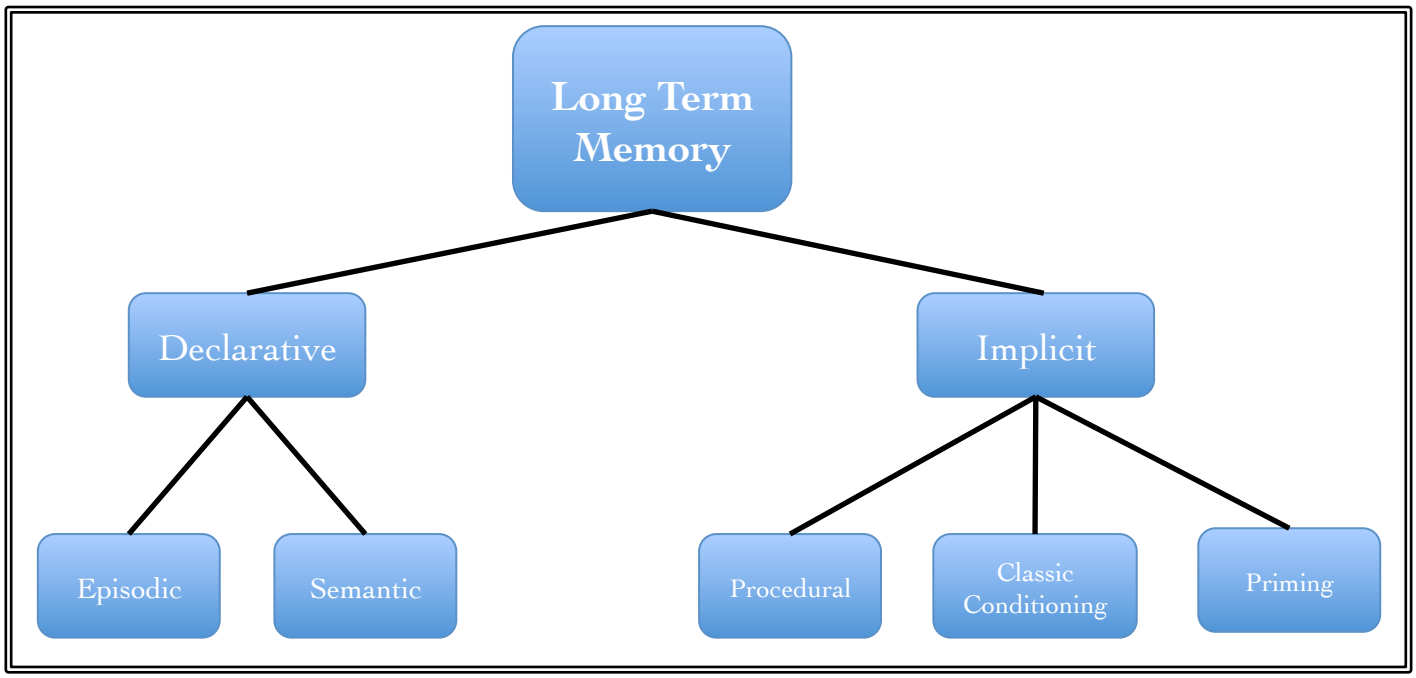
for phenomena like these that could not be represented in the original model of working memory, Baddeley introduced a new component, the episodic buffer. The episodic buffer was added in order to act as a temporary, multimodal storage space capable of chronologically integrating auditory and visual information with information stored in long term memory, in order to create a kind of cognitive movie (Baddeley, 2002).

The episodic buffer is also thought to be involved with chunking, the process by which prior knowledge is repackaged in a more efficient manor thereby increasing the capacity of working memory. Due to its wide range of abilities and functions, it is unlikely that the episodic buffer is contained in a single anatomical location (Baddeley, 2003).

Long-Term Memory

Long-term memory (LTM) has an exponentially larger capacity for information than both working and sensory memory, and is theoretically able to retain it indefinitely. Information stored in LTM can be further distinguished as either declarative or implicit memory. Declarative memory encompasses all conscious information that can be recalled such as general knowledge. Implicit memory, which is often referred to as procedural memory, includes all unconsciously recalled information such as learned skills like riding a bicycle (Gazzaniga, 2009)

Figure 5: Subdivisions of Long Term Memory



Declarative Memory

Declarative memory encompasses all the information we are consciously able to recall, such as events facts. FMRI studies show that prefrontal and medial temporal areas, especially the hippocampus and perirhinal cortex, are involved in declarative memory formation and retrieval processes. During the formation of declarative memories, the hippocampus receives information from multiple brain regions including the sensory and motor association cortex, the basal ganglia and the amygdala. The hippocampus is able to form associations between various information inputs during the consolidation process, allowing us to remember relationships such as how something tasted and where we were when we tasted it (Carlson, 2008). Studies show that information is constantly sent back and forth between working and declarative memory. Since the prefrontal cortex is partially responsible for the selection and organization of information entering working memory, it may be indirectly involved in declarative memory formation (Weis, 2004).

Declarative memory can be further distinguished as either episodic or semantic memory. Episodic memory pertains to the time and place where personal events occurred, such as remembering when and where your 16th birthday party was held. Conversely, semantic memories contain information unrelated to one's personal life such as facts learned in school. However, these two types of memory are not mutually exclusive. They are combined when forming autobiographical memories; complex combinations of episodic and semantic memories about the events of one's life (Purves, 2007). For example, a piece of semantic information may be that Connecticut College is located in New London, CT. A student who attended Conn will have his own episodic memories about his time there which will in turn help to reconstruct his semantic knowledge of the campus.

Implicit Memory

The other branch of LTM, implicit memory, accounts for learned information below the level of consciousness, and is able to be accessed without conscious control or attention. Implicit memory is named so because it is clinically assessed using implicit tests; tests in which memory is not directly assessed but rather inferred from behavior. Unlike declarative memory, acquiring implicit information does not require deliberate, active, memorization. The first evidence that there were multiple forms of LTM arose from the study of patients with damage to the medial temporal region. As demonstrated by H.M. and others, damage to the medial temporal region results in the inability to recall past events and information while leaving skill based learning tasks intact. Implicit memory can be further subdivided into three categories: procedural memory, classical conditioning, and priming (Carlson, 2008, Gray, 2006, Nelson, 2008).

Procedural Implicit Memory

Procedural memory, a branch of implicit memory, accounts for learned motor skills, habits, and tacit rules. The acquisition of procedural memories occurs below the level of conscious thought through repetitive actions. Some of the major brain areas involved in implicit memory include the basal ganglia, motor cortex, and cerebellum (Carlson, 2008). One example of implicit memory is knowing how to ride a bike. As everyone knows, the first attempt at riding usually results in failure. However, the skill can be mastered through successive attempts. While we are aware of our improvement, we are not conscious that sensorimotor learning is taking place that allows us to stay on the bike.

One subcategory of procedural memory, rule-based procedural memory, can be demonstrated through artificial grammar tests. Artificial grammar tests involve a set of rules that dictate which letters may be placed next to each other. For example, one rule may be that X's must be followed by either X's or Y's. During the test, subjects are not explicitly told the rules, but rather given examples of grammatical and non-grammatical strings of letters. Later, subjects are given new strings of letters and asked if they think the strings fulfill the requirements to be considered grammatical. While participants are typically unable to explicitly state the requirements for a string to be considered grammatical, they are able to correctly define them as such because the rules have been implicitly learned (Gray, 2006).

Classical Conditioning

Classical conditioning, another type of implicit memory, is primarily dependent on the cerebellar circuit (Lindquist, 2007). During classical conditioning, an association is learned between two stimuli; an unconditional stimulus (UC), something that elicits a natural response; and a conditional stimulus (CS), something that does not elicit a natural response. The UC and CS are paired together during a series of trials until the CS elicits the same response as the UC, at which point the CS is said to be a conditioned response (CR). The most well recognized example of classical conditioning is Pavlov's work with dogs, an experiment in which he was able to condition them to salivate at the sound of a bell. In this example, the presentation of food serves as the CS, with salvation as the natural response. The bell is the UC, as it does not normally elicit a salivatory response. Upon several weeks of pairing the ringing of the bell with the presentation of dinner, an association was formed between the two stimuli. Eventually the dogs began salivating at the sound of a bell even in

the absence of food (CR). This indicates that an implicit association between dinner and the bell had been learned.

Priming

The final kind of implicit memory, priming, refers to the process by which previous exposure to a stimulus elicits an exaggerated response upon later exposure to the same stimulus (Gazzaniga, 2009). The most common assessment of priming memory is the word-stem completion test. During the first stage of the test, a participant is asked to study a list of words. A short time later, anywhere from a few minutes to a few hours, the participant is presented with word-stems that have multiple possible completions and is asked to complete the stem with the first word that comes to mind. For example, the participant is presented with the stem "Mot" with the target word, motel, having appeared on a previously encountered word list. Studies show that the participant is more likely to complete the stem with a word on the previously provided list as opposed to previously unstudied words if priming has occurred (Schacter, 1998).

The most compelling evidence that priming occurs below the conscious level comes from the study of amnesic patients who have sustained brain damage to the hippocampus and or temporal lobes. While these patients are unable to recall recent experiences, a task that would involve normal brain function at the conscious level, they exhibit normal performance in priming tests (Schacter, 2001 & McDonald, 2010).

Summary of Different Forms of Memory

As described in the sections above, memory can be divided into several subcategories and involves a multitude of brain regions. Working memory has a relatively small capacity,

and is responsible for the short-term storage and manipulation of information that arrives from the sensory systems as well as LTM. If information contained within WM is rehearsed enough, it can be transferred to LTM, which has a nearly endless capacity. It should now be apparent that memory formation and storage is a very complex system that involves several cortical regions. The study of memory acquisition and consolidation of information within WM will be the main focus of this paper. How the cholinergic system facilitates hippocampal dependent memory formation will be addressed in the upcoming sections.

Types of Memory Tests

Several methods have been developed to test the effects of various drugs on memory through behavioral observations. Some of the most common tests for evaluating short-term memory and working memory are the Brown Peterson Test, the Novel Object Recognition Test, the Morris Water Maze, and several others. In order to gain a more complete understanding of how the brain works, these behavioral tests can be paired with tests showing biological mechanisms and cellular activity, such as RT-PCR and immunohistochemistry. The purpose of this section is to briefly introduce some behavioral tests that have become a mainstay of neuroscience research. They will frequently reappear in later sections as they provide excellent models for understanding how memory formation occurs.

The Brown Peterson Test

The Brown-Peterson Test is a commonly employed technique used to evaluate short-term memory function. In the first part of the test, subjects are given a string of short words

(three to four letters) to remember. After receiving the string of words, subjects are asked to perform a 20-30 second "distraction task". The distraction task is usually something simple, such as counting backwards by 3s. Once the distraction task has ended, the subjects are asked to recall the string of words. Numerous studies have proved that there is a negative correlation between the length of the distraction tasks and the number of words the subject is able to recall (Morrow, 2002). The Brown-Peterson task can be modified to test the effect of various drugs on short-term memory. For example, by administering nicotine before the word is presented, it is possible to increase the number of words a subject is able to recall after the distraction task. These results were also seen in a study using positron emission tomography (PET) in conjunction with the Brown Peterson test, which indicate that there is a positive correlation between Brown-Peterson test performance and hippocampal activity (Eustache, 1995).

The Nonrecurring-Items Delayed Nonmatching-to-Sample Test (DNMS)

The nonrecurring-items delayed nonmatching-to-sample (DNMS) task was created to test non-spatial working memory such as object recognition. The DNMS test was first used to test non-spatial working memory in monkeys and was eventually modified to test non-spatial working memory in rats as well (Mumby, 1990). Additionally, DNMS has been used to elucidate the role of various brain areas in object recognition. For example, one study shows that by lesioning the hippocampus before the trials began, rats exhibited severe deficits in object recognition (Mumby, 1992).

The T-Maze

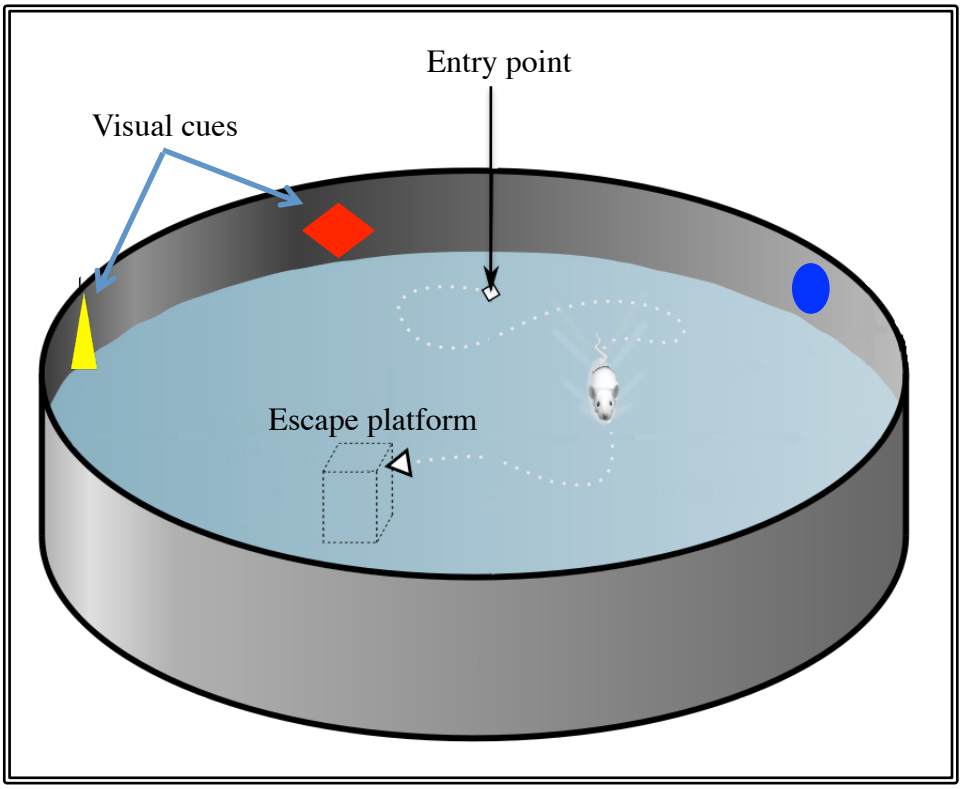
The T-maze is an apparatus commonly used to study spatial memory and other hippocampal-dependent processes. The maze, as the name suggests, is shaped like the letter T. During the first trial a subject, usually a rat, is placed at the base of the T and allowed to explore the maze. The trial is over once they reach the end of one of the arms at the top of the T. Countless studies show that if the subject is placed at the base of the maze for a second, consecutive trial, the natural tendency is to alternate which arm is explored. This tendency is known as spontaneous alternation. Researchers are able to reinforce spontaneous alternation by giving a reward if the animal alternates which side they choose between subsequent trials. Like the Brown-Peterson test, the T-Maze protocol can be modified to explore the effects of various drugs on behavior. Studies show that both surgical and neurotoxic induced lesioning of the hippocampus can disrupt spontaneous alternation behaviors, implicating a role for the hippocampus in spatial memory formation (Farr, 2000 & Volpe, 1988).

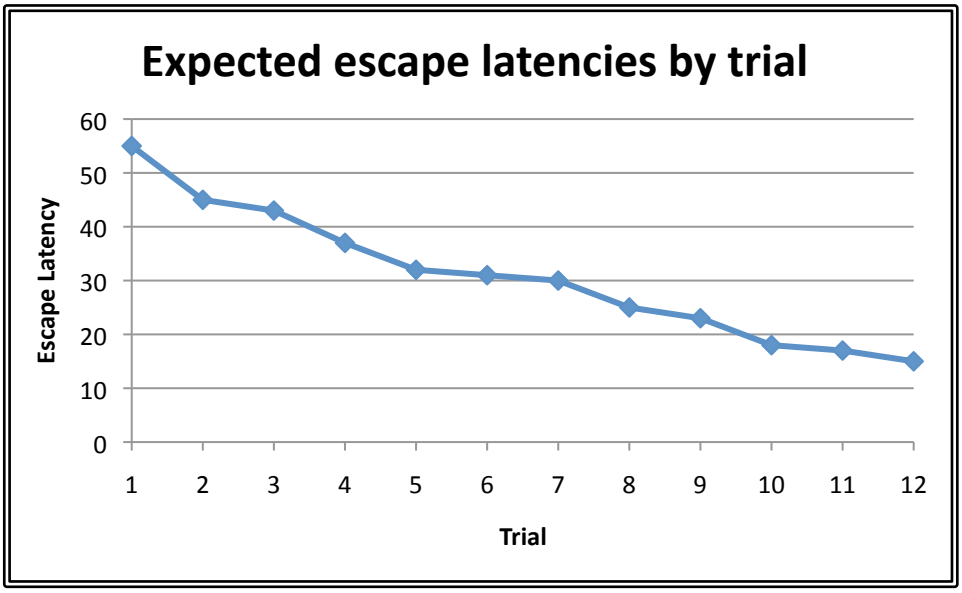
The Morris Water Maze

The Morris Water Maze (MWM) is another behavioral test that is frequently used to assess spatial memory formation and other forms of hippocampus-dependent learning. Its designer, Richard Morris, originally used the maze to demonstrate that hippocampal legions caused severe spatial learning impairments in rats (Figure 6). The maze itself consists of a round pool in a room with visual cues placed around the edges and an escape platform submerged slightly under the surface of the water. The rat is placed in the pool and the time it takes (latency) to reach the escape platform is recorded. As the rat is subject to more trials the

escape latency decreases, indicating that spatial memory formation has occurred. As in the previously mentioned behavioral tests, the MWM protocol can be modified in order to determine the effects of various drugs on learning and memory processes. The Morris water maze has a distinct advantage over the T-maze for studying spatial memory formation in that there are no fixed formals that the rat can memorize, such as learning that a reward is granted when the chosen side is alternated (Morris, 1984).

Figure 6: Morris Water Maze Diagram and Typical Escape Latency Graph



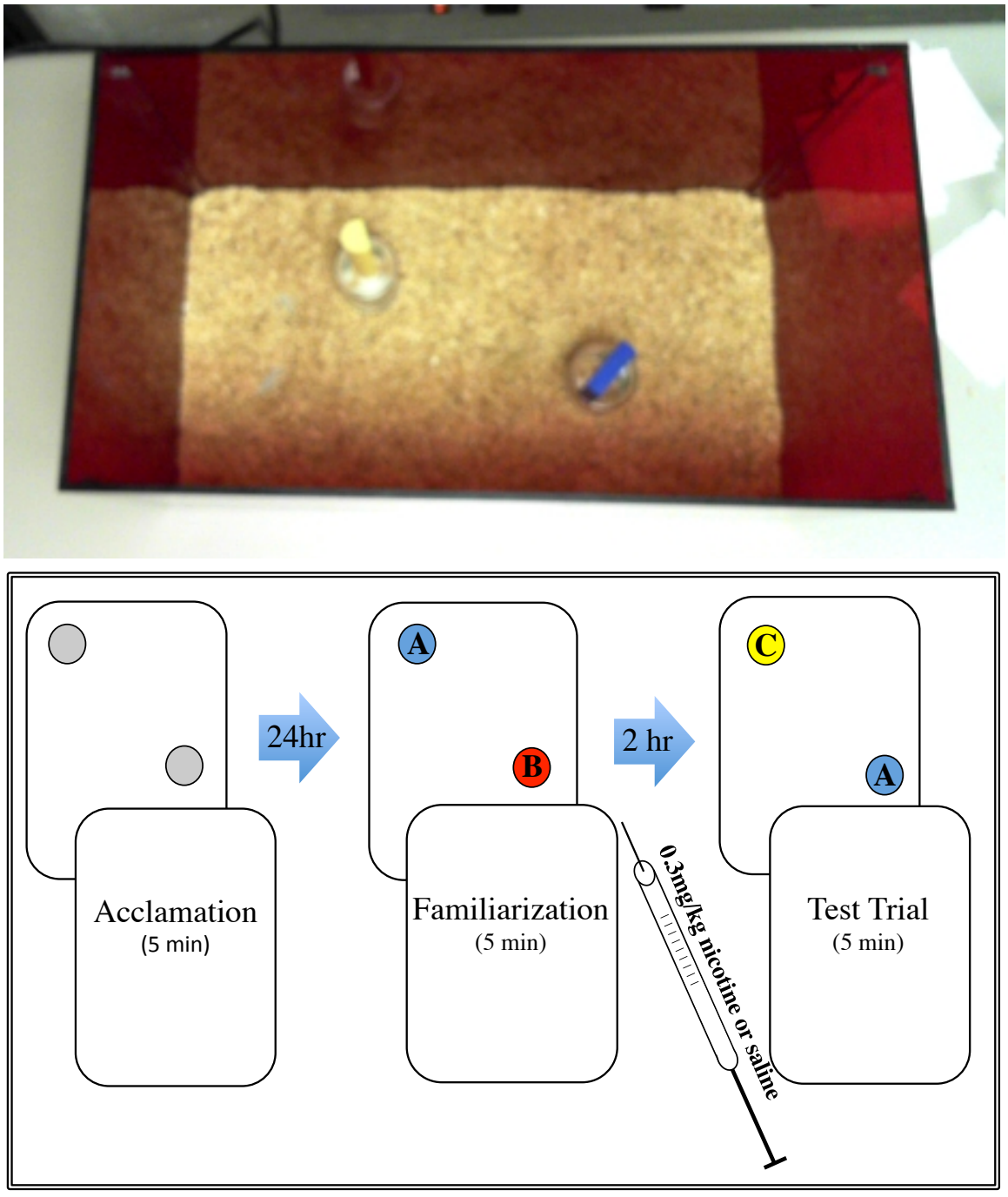


The Novel Object Recognition Test

The Novel Object Recognition (NOR) test is one of the most frequently used methods of studying hippocampus-dependent memory formation. The NOR tests is based on a rat's unconditional predisposition to explore novel objects significantly more than objects which they have previously encountered. A typical NOR protocol involves two trials: the familiarization trial and a test trial (Figure 7). The day before the familiarization trial, the rat is placed in the testing apparatus for a few minutes in order to acclimate with its surroundings.

During the familiarization trial the rat is placed in a box with two different objects that are affixed to the box for a short period of time. The amount of time rat spends exploring both objects is recorded. Once familiarization is complete, the rat is removed from the box and placed back in its cage. At this point, one of the original objects is replaced with a new (novel) object, while the other (familiar) object remains untouched. After a period of time has passed, the rat is returned to box for the test trial and the amount of time rat spends exploring both objects is recorded. Countless studies show that the rat will spend significantly more time exploring the novel object than the familiar object during the test trial due to their natural predisposition to investigate novel objects (Broadbent, 2009). This type of NOR test is considered a one-trial object recognition tasks, and has been implemented in trying to understand many different aspects of memory formation.

Figure 7: Standard NOR Apparatus and Protocol



Note: memory acquisition version of protocol shown

The basic NOR procedure can be modified in a variety of ways to test different aspects of memory formation. For example, one study was able to prove that the hippocampus is involved in memory formation. This was done by adding a step to the previously mentioned NOR procedure between the familiarization and the test trial test trial. Between the two trials, half of the rats in the study underwent a procedure in which their hippocampal tissue was lesioned. The other half of the rats in the study remained untouched, serving as a control group. During the subsequent test trial, the rats that had received the hippocampal lesions exhibited moderate to severe memory impairment indicating that hippocampus played a role in object recognition (Gaskin, 2010).

Considerations for Experimental Design

There are a variety of testing condition issues that need to be taken into account when performing a NOR study. One of the most important issues is understanding to what degree the animals relate to the intrinsic properties of the objects used in the study. For example, it was found that rats show preference for objects that they are able to "interact" with. In other words, rats prefer objects that they are able to climb on or crawl into verses those that they cannot, regardless of how many times they have seen the objects in the past (Chemero, 2005). One way to make sure that object preference does not confound the results is to make sure that all objects are roughly the same height. Another way to control for differences in object sizes is to elevate the objects above the rat's normal visual zone. When an object is on ground level, the top of the object is the primary focus as the rat has the most interaction with the top through climbing activities. By placing objects on clear glass jars approximately 6 cm

high, the rat gains a more complete perspective of the object as a whole that results in a more complete memory formation (Mumby, 1990).

Rats are unable to see as broad of a color spectrum as humans. Recent studies have shown that rats are more attuned to differences in brightness rather than colors, meaning that two different colors of similar brightness may look very different to humans but identical to rats. For this reason it is important to choose objects that not only vary in color but also brightness (Jacobs, 2001).

Odor also plays an important role in memory and recognition processes. Using the same objects in multiple studies and with different animals may create an unintentional bias. If during a trial, a subject explores and touches one object preferentially over another, they may impart an odor on that object, which could affect how the next subject interacts with it. Therefore, the objects should be washed between animals (Ennaceur, 2010).

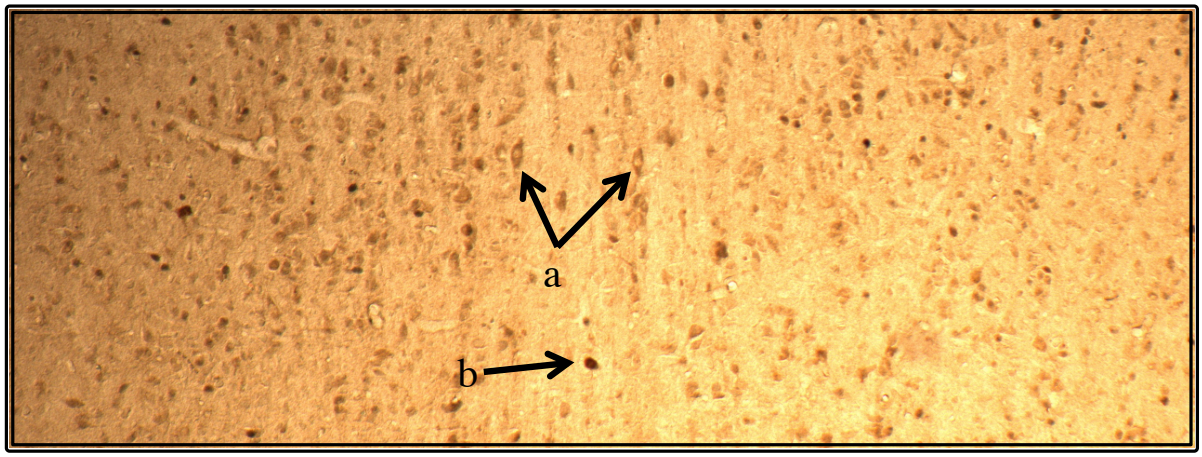
Immunohistochemistry

While the previously described behavioral tests examine learning and memory through behavioral observations, they do not provide an understanding of the changes in neural activity that are responsible for the behavioral changes. One of the most common ways to visualize changes in neuronal activation is to use immediate-early genes (IGE) immunohistochemistry, as the induction of IGEs is commonly associated with increases in neuronal activity. One IGE, c-Fos, encodes for a transcriptional factor. Because basal levels of c-Fos are low, an increase in c-Fos is representative of an increase in gene activity (Kovacs, 1998 & Worley, 1991). Because the c-Fos gene codes for a transcription factor, only nucleus of the cell is labeled during immunohistochemistry staining.

Immunohistochemistry can also be used to visualize the presence of various neurotransmitters within the cell. This technique was first used to visualize the anatomical location of cholinergic neurons in the rat brain. As mentioned on page 9, acetylcholine is assembled by the biosynthetic enzyme, choline acetyltransferase (ChAT). As demonstrated by Armstrong and colleagues, it is possible to determine the neuroanatomical location of cholinergic neurons within the rat brain using anti ChAT primary antibodies. Using this technique, a large concentration of reactive cells was found within the medial septum as expected (Armstrong, 1983). Due to the fact that acetylcholine is assembled within the cytoplasm of the cell, immunohistochemistry results in staining of the whole cell.

By using c-Fos and ChAT antibodies in a dual staining immunohistochemistry procedure, it is possible to visualize cholinergic neurons in the medial septum that have recently undergone increased levels of activation (Modirrousta, 2004).

Figure 8: Example of c-Fos and ChAT Dual Staining in the Medial Septum



20x magnification (a) ChAT only, (b) dual ChAT/ c-Fos labeled cells

The link between smoking and working memory

Although nicotine has been shown to have cognitive effects in humans, the mechanisms by which it causes these effects are still unclear. In a study to determine the effects of nicotine on life-long non-smokers, it was determined that subjects given nicotine instead of a placebo performed better in a variety of working memory orientated tasks: producing faster, more accurate responses (Kumari, 2003). Several studies have also reported that nicotine withdrawal has a negative effect on cognitive tasks involving working memory. Understanding how chronic nicotine administration via smoking cigarettes affects neurophysiology is a rapidly growing field of research.

Human Studies

Numerous human studies have shown that the effects of chronic smoking followed by abrupt withdrawal can have a negative effect on performances in working memory tasks, especially those that involve recognition memory. It is possible that these deleterious effects contribute to the social and physiological factors associated with nicotine dependence, and are therefore associated with the difficult task of quitting smoking (Mendrek, 2006). Furthermore, studies show nicotine deprivation causes a significant decline in mood, which may contribute to the psychological aspect of nicotine dependence (Heffernan, 2005).

Due to the all of the negative health effects associated with smoking cigarettes, the primary method of nicotine delivery into the body, some studies set out to determine the effects of nicotine in humans when delivered via battery operated electronic cigarettes (e-cigarettes). The use of e-cigarettes provides a potentially safer nicotine delivery system because no tobacco is burned in the process, eliminating the normal co-inhalation of tar and

carbon monoxide. Replacing normal cigarettes with e-cigarettes has been shown to help curb withdrawal symptoms during the quitting process. One study found that some brands of e-cigarettes cause both a reduction in the desire to smoke as well as a reduction in the severity of withdrawal symptoms without actually raising blood nicotine levels (Vansickel, 2010). Further studies confirmed this notion by using cigarettes that contain denicotinized tobacco. These findings show that simply performing the physical processes associated with smoking help to curb withdrawal symptoms, and that nicotine addiction has both a biological and psychological component (Rose, 2010).

Using the Brown-Peterson task it was determined that nicotine inhalation via e-cigarettes was able to improve attention to and speed of visual-spatial processing in humans. Likewise, a Brown-Peterson Memory test, commonly used to evaluate working memory, showed that e-cigarettes also improve recognition memory performance (Dawkins, 2012).

Nicotine and Cognition: the Hippocampus and Perirhinal Cortex.

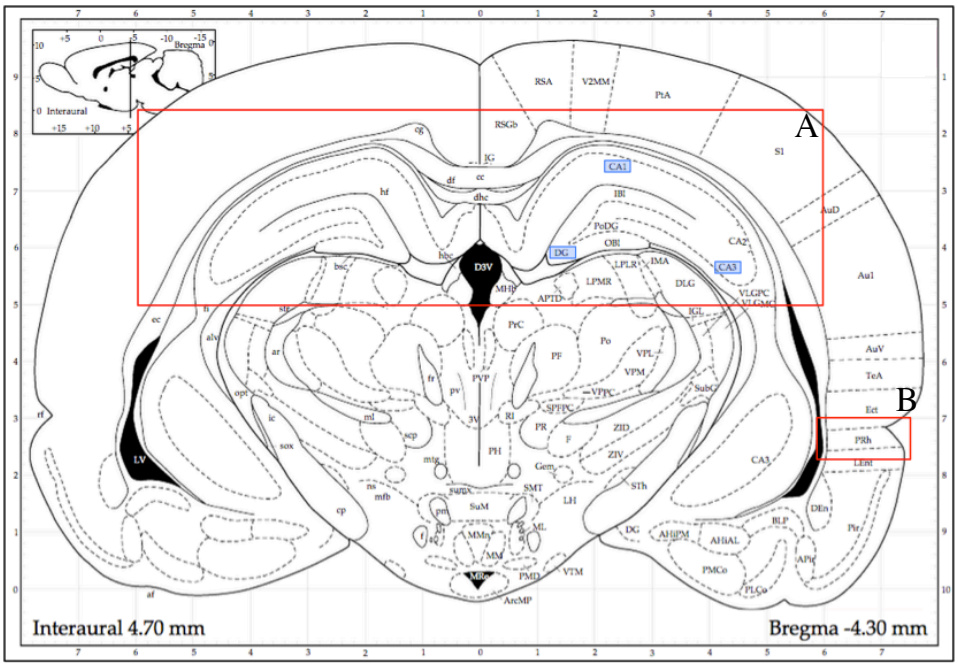
It has been suggested that many of nicotine's effects on cognitive behavior involve areas of the brain that have projections into the hippocampus, a structure largely involved in spatial recognition memory (Gray, 1994). There are several subtypes of nAChRs located within the hippocampus. Two subtypes, $\alpha 7$ and $\alpha 4\beta 2$, are thought to contribute to synaptic plasticity due to their location on both pre- and postsynaptic membranes. This dual location is thought to increase their ability to modify synaptic transmission and neurotransmitter release, consequently increasing synaptic plasticity (Kenney, 2008). The $\alpha 7$ and $\alpha 4\beta 2$ nAChRs have also been found in other brain areas involved in recognition memory, such as the perirhinal cortex (Melichercik, 2012). The location and subtypes of nAChRs within the

perirhinal cortex and hippocampus suggest a role for cholinergic signaling within these cortical structures.

The Hippocampus

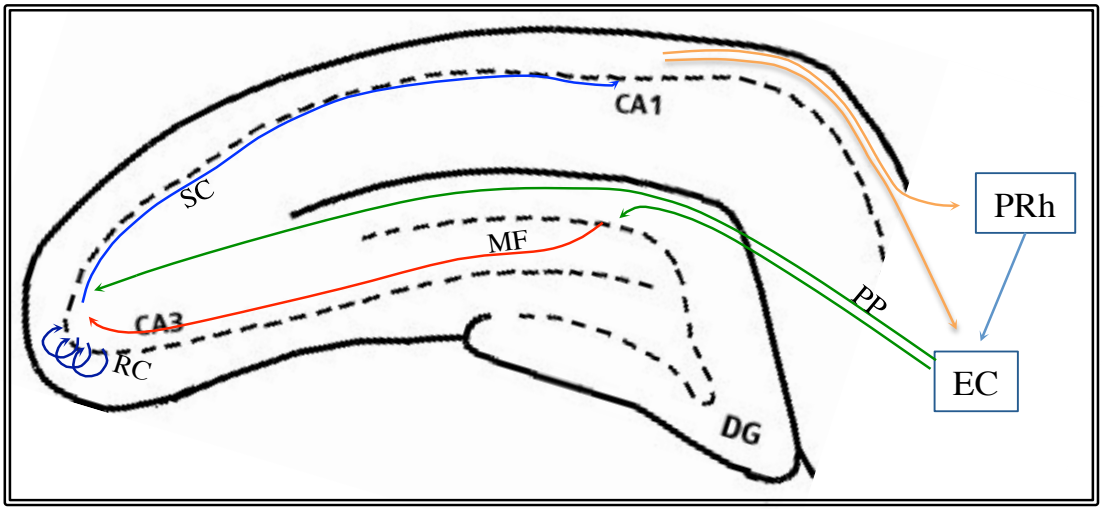
The following information about the organization of the hippocampus is summary adapted from The Hippocampus Book (Anderson, 2007). The hippocampus has a unique, unidirectional circuitry, which allows it to effectively process information from a variety of cortical regions (*Figure 9)*. Its unique circuitry enables it to integrate incoming, afferent sensory information with previously made associations stored in long term memory. There are three main divisions in the hippocampus: The dentate gyrus, CA1, and CA3/CA2. The main source of hippocampal input comes from the entorhinal cortex and arrives in the granular cells of the dentate gyrus (DG). Information is then relayed via mossy fiber synapses to the pyramidal cells of CA3 where it is processed through a variety of recurrent connections. After processing, information is sent both to the pyramidal cells of the CA1 and to the contralateral hippocampus via Schaffer collateral synapses. After processing, information exits the hippocampus through the subiculum and terminates in various parts of the cortex (*Figure 10*).

Figure 9: Atlas cross-section of a rat brain



(**A**) The Hippocampus with CA1, CA3, and the DG highlighted, (**B**) the Perirhinal cortex (adapted from Paxinos, 2007)

Figure 10: A Simplified Schematic of Informational Flow in the Hippocampus



The entorhinal cortex (EC) provides the main source input to the hippocampus via the perforant pathway (PP). The cells of the PP terminate on the molecular layer of the dentate gyrus (DG) as well as the stratum lacunosum-molecular (SL-M) layer of the pyramidal cells of CA3. Mossy fiber projections (MF), which also originate in the DG, terminate just above the pyramidal layer of CA3. There are a large amount of recurrent collaterals (RC) pathways within CA3. Schaffer collateral connections (SC) connect CA3 to CA1. From CA1 information travels out of the hippocampus to the Perirhinal cortex (PRh) and back to the EC.

The Dentate Gyrus

The dentate gyrus (DG) is a "V" shaped formation of cells that is the first stop for information coming into the hippocampus. There are three distinct cell layers present in the DG: the molecular layer, the principle layer, and the polymorphic layer.

The molecular layer of the DG is superficially located closest to the hippocampal fissure. For the most part, it is composed of granular and basket cell dendrites originating from bodies located within the principle layer. While the molecular layer contains a few multipolar cells, it is relatively free of cell bodies as compared to the other two layers. The few cell bodies that it does contain are GABAergic and provide inhibition to the granular cells of the principle layer.

The principle layer of the DG, often referred to as the granular cell layer, is located just under the molecular layer. The principle layer is densely packed, ranging anywhere from 4-8 granule cells thick and often times there is no glial sheath between adjacent cells. Dentate granule cells found in the principle layer are primarily glutamatergic. Their dendrites form tree or cone shaped projections that point towards the superficial plane of the molecular layer and are responsible for sending projections to other hippocampal region, primarily CA3. It has been estimated that there are approximately 1.2 million granular cells in the human hippocampus.

Pyramidal basket cells, a type of GABAergic interneuron, are the main inhibitory cells within the principle layer. As their name suggests, they have a pyramidal cell body. The *basket* nomenclature denotes the shape of their dendrites, which surround and form

synapses with the synapses of the granule cells. Although fewer in number and less well defined, other types of GABAergic interneuron can be found in the principle layer.

The outermost layer of cells that encloses the DG is known as the polymorphic layer. The polymorphic is composed of a very diverse cell population whose function remains largely unknown. Mossy fiber cells are the most prevalent cell type found in the polymorphic layer. These cells have large bodies that are triangular or multipolar. They usually have at least three long dendrites that travel great distances within, but virtually never leave, the polymorphic layer.

Afferent Projections Arriving at the DG

The main source of input into the hippocampus comes from the entorhinal cortex via the perforant pathway, which terminates at the molecular layer of the DG as well as on the pyramidal cells of CA3. The medial septum nucleus (MS) and the diagonal band of Broca are also responsible for sending information to the hippocampus by way of the entorhinal cortex. While the majority of these afferent projections are cholinergic, GABAergic projections are present as well. Interestingly, there is a large amount of specificity where GABAergic axons usually terminate only at GABAergic nonpyramidal cells, such as basket cells; while cholinergic projections usually terminate only at the excitatory granule cells of the DG.

Efferent Projections Out of the DG

CA3 is the only region within the hippocampus to be innervated by the DG. These connections consist of mossy fiber projections from granule cells in the principle layer of the DG and terminate just above the pyramidal layer of the CA3 in the stratum lucidum. Mossy fibers projections have many unique features: they have unusually large, highly complex and

irregular terminals that form connections with proximal CA3 pyramidal dendrites; they have a large number of active zones; and they can make as many as 37 connections with a single CA3 pyramidal cell. Mossy fiber active zones usually synapse with a single pyramidal cell. Individual mossy fibers can have up to 15 active zones, making it able to connect with 15 pyramidal cells. Due to the disproportionate number of granule cells to pyramidal cells, each pyramidal cell is able to receive information from 72 different granule cells.

CA1, CA2 & CA3

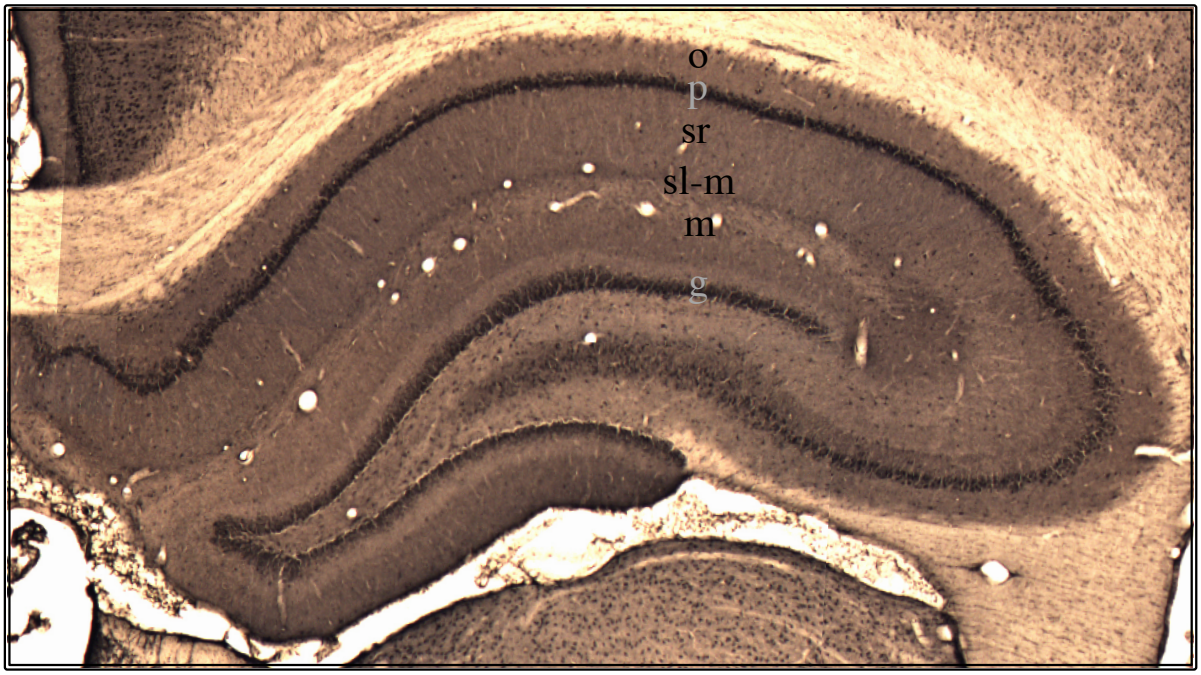
The laminar organization is well conserved throughout the hippocampus. The pyramidal cell layer (PCL) is the principle cell layer. It consists of tightly packed cells in CA1 and slightly more loosely packed cells in CA2/3. The stratum oreins (SO) is located just under the PCL and consists mainly of pyramidal cell dendrites and various interneurons. The Stratum radiatum (SR) is often referred to as the suprapyramidal region, and is located on the other side of the PCL. This region is home to both CA3 - CA3 recurrent collaterals and CA3 - CA1 Schaffer collateral connections. The most superficial layer of the hippocampus, called the stratum lacunosum-molecular (SL-M), is located just above the SR (*Figure 11)*.

Pyramidal cells are the most prevalent neurons found in the hippocampus. Their cell body is located within the PCL, with a basal dendritic tree that extends into the SO, and an apical dendritic tree with projections terminating in both SR and SL-M. There are also several groups of GABAergic interneurons found throughout all layers of the hippocampus. The most prevalent of these cells, the basket pyramidal cell, has a cell body located either in the PLC or the SR and dendritic extensions into the SO, SR, and SL-M (*Figure 12*). A typical basket cell is able to innervate as many as 1000 pyramidal cells. Another class of

interneuron, the interneuron-selective (IS) neuron, has axons that connect exclusively to other interneurons. Furthermore, there are several other interneurons with similar morphology to the basket cell, such as the bistratified cell and chandelier cell. The main source of output from the hippocampus originates at CA1 which sends the majority of outgoing information the entorhinal cortex and perirhinal cortex.

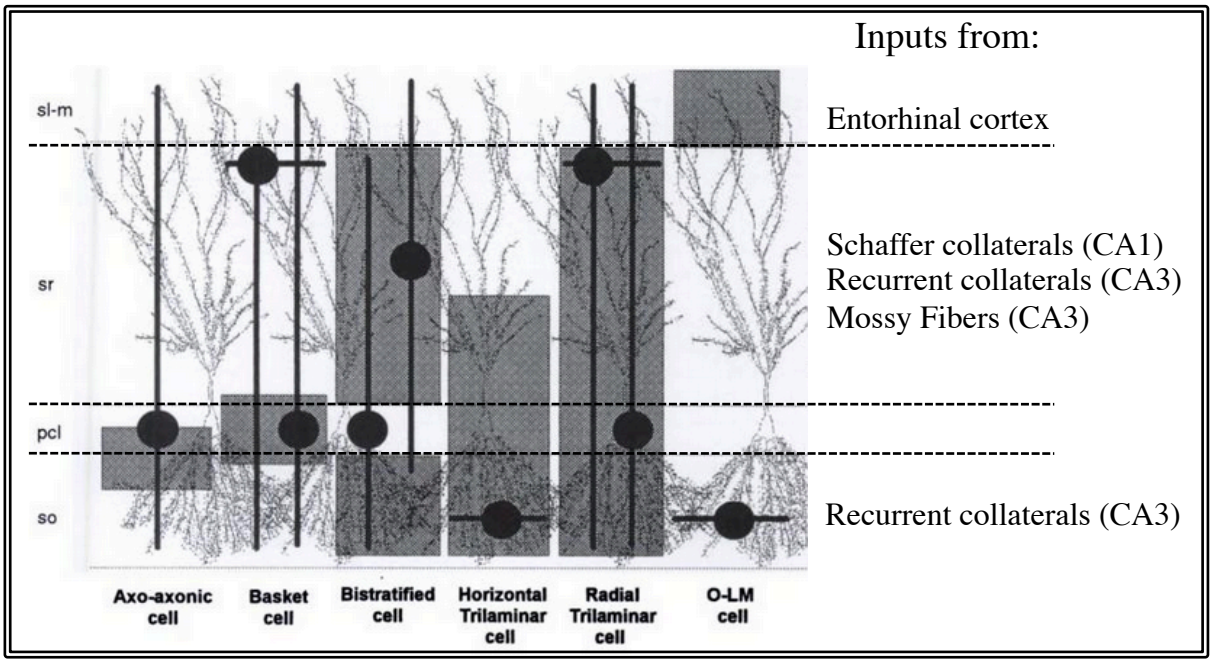
The previous sections provide a simplified anatomical description of the laminar organization of the hippocampus. While the information presented above will serve as a basis for understanding one way in which the cholinergic system influences hippocampal memory formation, research into this complex circuitry is still underway.

Figure 11: Laminar organization of CA1-3



Hippocampus dual stained with c-Fos and ChAT. **(o)** stratum oriens, **(p)** stratum pyramidale, **(sr)** stratum radiatum, **(sl-m)** stratum lacunosum-molecular, **(m)** dentate molecular layer, **(g)** granule cell layer.

Figure 12: Laminar organization of CA1-3 by cell type (Adapted from Grey, 1994)



Learning and Memory: a General Role for the Hippocampus

The CA1 and CA3 regions of the hippocampus have repeatedly been associated with memory formation. The rapid influx of information to CA3 causes a change in synaptic plasticity that leads to the formation of associations between previously unrelated stimuli (Do, 2000). Since CA3 receives information from a variety of sources and is involved in physically making new associations, it is thought to play a role in the formation of working memory, spatial memory, and shock avoidance behaviors. CA1 also receives information from a variety of different cortical regions implicated in spatial and temporal pathways. For example, mice that have undergone surgical procedures disrupting CA1 were unable to form cognitive spatial maps and retrieve spatial information (Daumas, 2005). In addition to their roles in spatial memory function, CA1 and CA3 have also been implicated in the formation and consolidation of long-term memories (Kenney, 2008). It is hard to consistently delineate the boarders of CA2 between studies, therefore less research into its functioning has been performed. However, due to its location between CA1 and CA3, CA2 is also thought to be involved in these memory processes.

6. The Perirhinal Cortex

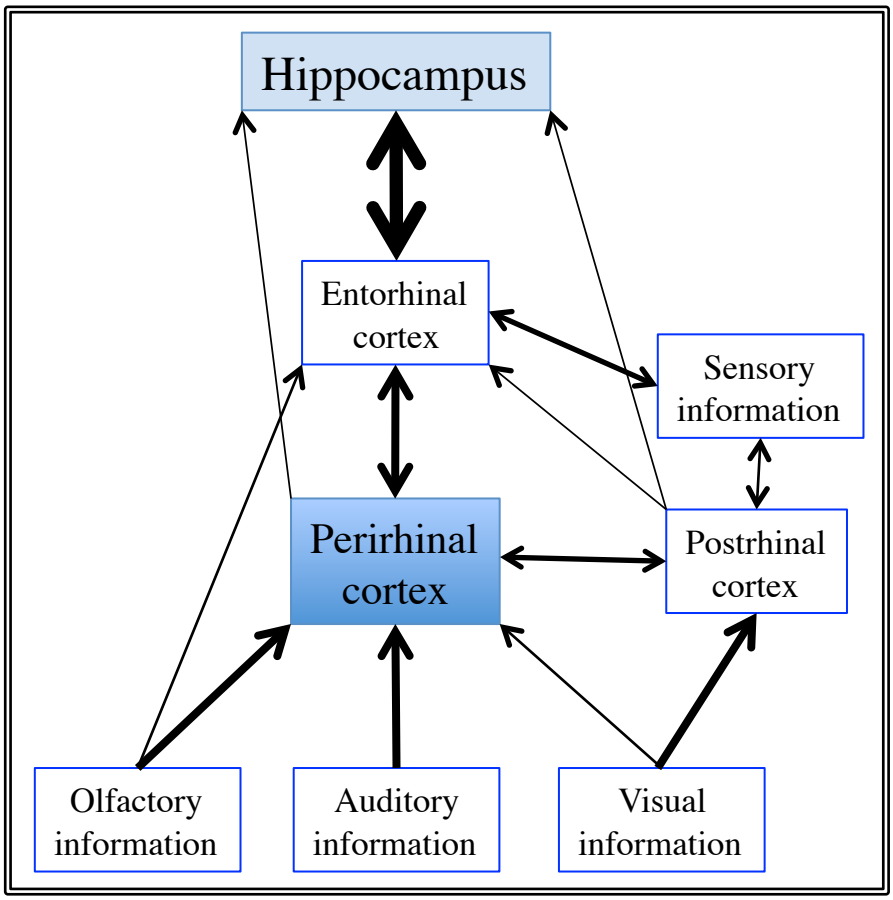
6.1 General Structure

The perirhinal cortex (PRh) is located on the surface of the temporal lobe near the visual cortical area. The exact boundaries of the PRh are not universally agreed upon. In fact, a large degree of variation can be found when trying to delineate its boundaries from various scientific papers. Like the hippocampus, the PRh contains nAChRs, however, it is not

thought to be significantly involved in spatial memory recognition tasks, but rather it object recognition tasks (Parker and Gaffan, 1998). The perirhinal cortex receives information from a variety of cortical regions involved in making associations as well as the visual, auditory, olfactory, and somatosensory cortexes. Some of the most well studied afferent projections terminate at the caudate nucleus, putamen and the nucleus accumbens.

The PRh also has reciprocal connections, meaning it both sends and receives information, with several brain areas such as the entorhinal cortex; the main source of input to the DG and therefore hippocampus (Canning, 1997). Due to the close proximity of the PRh and entorhinal cortex, they are frequently grouped together and referred to as the rhinal cortex (Brown, 1997). Reciprocal connections can also be found between the PRh and amygdala, as well as the PRh and thalamus. Several of these reciprocal pathways are capable of undergoing LTP, providing a possible mechanism for memory formation (Liu, 1996).

Figure 13: Inputs and Putputs of the Perirhinal Cortex



Arrow thickness indicates relative amount of information flow between brain regions

Differences Between the Perirhinal and Hippocampal Dependent Memory

While the perirhinal cortex and hippocampus are both involved in recognition memory they serve unique, distinct, functions. It is thought that the PRh is primarily responsible for recognizing that an object has previously been encountered, while the hippocampus is responsible for knowing the circumstances surrounding the last time the object was encountered (Brown, 1997).

Lesioning Studies

The distinctive roles of the hippocampus and PRh have been demonstrated using lesioning studies. For example, spatial memory in rats, as assessed by the Morris Water Maze, was impaired upon receiving hippocampal lesions (Broadbent, 2006). Severe memory deficits caused by hippocampal lesioning were also demonstrated using the Novel Object Recognition Test (Gaskin, 2010). Interestingly, no memory deficits were observed in rats that had received hippocampal lesions as assessed by the nonrecurring-items delayed nonmatching-to-sample (DNMS) test (Mumby, 1992). Since the DNMS is used to test non-spatial object recognition, while the WMW and NOR are used to test spatial object recognition, these results suggest that the hippocampus is more involved in object location rather than object recognition memory processes.

An inverse pattern of memory disruption is present upon examining the effects of perirhinal lesions on object recognition and object location memory. In a equivalent DNMS experiment to that of Mumby and colleagues described above, rats that received neurotoxic lesions to the perirhinal cortex showed severe deficits in object recognition memory (Ennaceur, 1996). Severe impairments in object recognition caused by perirhinal lesioning

has also been observed in monkeys using the DNMS test (Meunier, 1993, Parker and Gaffan, 1998). Unlike with hippocampal lesions, lesions to the perirhinal cortex to not appear to disrupt spatial memory processes. Wiig and colleagues demonstrated that rats who received perirhinal cortex lesions did not display deficits in spatial memory as measured by the MWM (Wiig, 1940). These experiments suggest that unlike the hippocampus, the perirhinal cortex is more involved in object recognition rather than object location memory processes. Intriguingly, studies show that surgically disconnecting the hippocampus from the perirhinal cortex causes severe memory impairments for both object recognition and object location tasks (Warburton, 2003). Therefore, communication between the hippocampus and PRh must be necessary for both object recognition and object location tasks.

Immunohistochemistry Studies

A distinct functional separation between the hippocampus and PRh can be visualized using immediate early gene (IEG) activation patterns. Immunohistochemistry c-fos studies show that PRh neurons undergo a higher level of activation following exposure to novel visual stimuli than familiar visual stimuli, a pattern not seen in the hippocampus (Zhu, 1995). An inverse pattern of activation can be seen in the hippocampus when using c-fos immunohistochemistry to determine neuronal levels of activation in response to novel versus familiar environments. Activation due to changes in environment are more closely related to location memory while activation due to changes in visual stimuli are related to object recognition memory (Wan, 1999). Taken together, the lesioning and immunohistochemistry studies show that while the PRh and hippocampus play distinct roles recognition memory, communication between the two regions is crucial for memory formation.

Learning and Memory: A General Role for the Perirhinal Cortex

The commonly accepted mechanistic theory for how the perirhinal cortex is able to make distinctions between novel and familiar objects is based on synaptic plasticity. Similar to the CA3's aforementioned ability of to make spatial connections, the simultaneous influx of information from several cortical regions pertaining to an objects physical attribute, such as color and size, causes in changes in neuronal wiring in the PRh. These new connections create a consolidated representation of an object's physical properties which is the basis of object recognition memory (Murray and Richmond, 2001).

Slice studies performed on the rat perirhinal cortex show that as the PRh is repeatedly exposed to a stimulus, the neuronal response to that stimulus declines. This reduced response does not fade or diminish over time. It provides a mechanistic explanation for how the PRh is able to facilitate object discrimination and is commonly referred to as long-term depression (LTD) (Warburton, 2003). Just as in the hippocampus, pre and post synaptic nAChR activity may help modulate LTD facilitated synaptic plasticity in the PRh.

By using scopolamine to block mAChRs during acquisition, retrieval, and consolidation, Winters and colleagues tested how ACh levels in the PRh are involved different stages of the novel object recognition test. In order to determine the role of ACh in memory acquisition, a cannula was used to deliver an intra-PRh injection of scopolamine prior to the familiarization trial. Rats treated with scopolamine performed significantly worse during the test trial than those treated with saline, indicating that ACh in the PRh is necessary for memory acquisition. Scopolamine administration directly after the familiarization trial did not produce any negative effects during the test trial, indicating that ACh may not play a role in PRh consolidation. Interestingly, rats given the intra-PRh scopolamine infusions three hours prior

to the test trial (after the consolidation period) performed significantly better during the test trial than those given saline (Winters, 2006). In a subsequent set of experiments it was determined that these enhancing effects were due to interference prevention caused by low ACh levels. Essentially, when a novel object is presented between the familiarization and test trials a new memory gets formed. This new memory can interfere with the memory formed during the familiarization trial, in a process known as retroactive interference. Studies show that retroactive interference is able to be avoided by administering an intra-PRh infusion of scopolamine prior to the presentation of the extraneous object. Scopolamine is able to enhance memory formation in this test by blocking the acquisition of the interfering object, allowing for the previously encoded memory to remain unadulterated (Winters, 2007). These experiments provide strong evidence that recognition memory processes in the PRh are mediated by ACh. While there has been some research into the workings of the perirhinal cortex, further research is needed to solidify its exact role in memory formation.

Modification to the Cholinergic System Disrupts Memory Processes

There is overwhelming amount of evidence implicating the cholinergic system in a variety of learning and memory processes. It is largely accepted that the cholinergic system able to mediate these processes via the release of the neurotransmitter, acetylcholine (ACh).

The first line of evidence indicating that ACh helps facilitate memory formation comes from research showing that cholinergic projections from basal forebrain to the hippocampus and frontal cortex release ACh upon exposure to a novel stimulus. In primates, activation of basal forebrain neurons upon exposure to a novel stimulus occurs at a higher

level when the stimulus is associated with something that is advantageous; such as a reward or punishment (Wilson, 1990). In other words, the degree to which a novel stimulus directly affects an animals life is correlated with how well the animal remembers that stimulus.

Microdialysis: Real-Time Measurements of Neurotransmitter Release

ACh is Released Upon Exposure to Novel Stimuli, a Microdialysis Study

By implanting a microdialysis probe in the brain of a living animal, researchers are able to measure fluctuations in the release of neurotransmitters in real time. For example, exposure to a novel environment has been shown to cause elevated levels of ACh in the hippocampus using microdialysis technique. Microdialysis has also been used to measure the amount of ACh released during different stages of a behavioral test by placing a probe in the basal forebrain. One study set out to determine the extent to which ACh was released in response to both conditioned and unconditioned stimuli in rats using three experimental groups (habituation, novel stimuli, and conditioned fear). During the first phase of the experiment, training, rats were placed in the same apparatus for twelve, 20 minute sessions. During training, the habituation group received constant exposure to both light and sound stimuli, while the novel stimuli group were placed in the apparatus without receiving any stimuli. The conditioned fear group was exposed to the same stimuli as the habituation group, with the caveat that every time they were exposed to the light or sound stimuli they received a footshock.

Microdialysis probes were placed in the frontal cortex and hippocampus after completion of the training trials. After recovering from surgery, the rats were returned to the apparatus and once again exposed to their respective conditions, except for rats in the novel

condition group which were exposed to the light and sound stimuli for the first time. Both the novel stimuli group (unconditioned stimuli) and conditioned fear group (conditioned stimuli) had significantly elevated levels of ACh in both the frontal cortex and hippocampus. The rats in the habituation group showed no changes in ACh levels between the two trials. These results indicate that ACh is released upon encountering novel stimuli, as in the unconditioned stimuli group, and behaviorally relevant, as in the conditioned fear group, but not upon experiencing familiar, non relevant stimuli as in the habilitation group (Acquas, 1996). Furthermore, an increase in exploratory behavior is seen upon exposure to a novel environment, suggesting a role for ACh in instigating investigatory behaviors (Thiel, 1998).

ACh is Released in Learned Anticipation Tasks

Another study using *in vivo* microdialysis determined that ACh is involved in learned anticipation tasks. In this study, rats were placed in a testing apparatus and given either water or liquid chocolate before being returned to their home cage. Once a day for 14 days the rats were given their respective treatment with the idea being that rats that received chocolate would learn to anticipate this treat, while the rats given water served as a control group. On the 15th day, the same procedure was followed while monitoring hippocampal and frontal cortex levels of ACh. The rats used to receiving the liquid chocolate had significantly higher levels of frontal cortex and hippocampal ACh as compared to those given water, indicating a role for ACh in learned anticipation (Inglis, 1994).

Morris Water Maze: Spatial Memory

Studies using the Morris Water Maze (MWM) have provided substantial evidence for understanding how ACh is involved in modulating learning and memory processes. The MWM consists of a round pool of water with various visual cues located around the edges and a hidden escape platform submerged a few millimeters below the surface. In the beginning of a trial, a rat is placed in the pool and the time it takes (latency) to reach the escape platform is recorded. As an animal undergoes several subsequent trials, their escape latency decreases, indicating that spatial memory formation has taken place and that the rodent has successfully completed the acquisition stage of training. In some studies, the rodent is examined further to determine the extent to which acquisition has led to a stable memory of the platform location. It is possible to study the effects of various drugs on spatial memory processes by administering treatments during different stages of the MWM procedure and recording changes in escape latencies. For example, selectively destroying cholinergic neurons has been shown to cause deficits in memory acquisition using the MWM (Myhrer, 2003).

IgG-192-Saporin Injections in the Hippocampus Disrupt Memory Acquisition, but not Consolidation

The immunotoxin, IgG-192-saporin, selectively binds to and kills cholinergic cells in the basal forebrain and hippocampus, while sparing cholinergic cells in the brainstem. This same pattern of cell death is common in Alzheimer's disease, making IgG-192-saporin a useful tool for creating an animal model of the disease (Nillson, 1992). By injecting IgG-192-saporin at different stages of the MWM, Nillson and colleagues were able to show that cholinergic neurons play a role in spatial memory acquisition. In their study, a cohort of rats was divided into three groups (control, acquisition, and consolidation) before receiving

bilateral hippocampal cannulas. After recovering from surgery, the rats underwent several training sessions to familiarize them with the MWM. The rats in the acquisition group received 4ug/kg IgG-192-saporin before the training sessions, while the rats in the consolidation group were injected after the training sessions. The rats in the control group did not receive any drug infusions. After the training sessions, the rats underwent a series of six trials to calculate their escape efficiency. The rats in the acquisition group had significantly longer escape latencies than those in the consolidation or control group. Additionally, there was no significant difference in escape latencies between the consolidation or control group. Taken together, these results indicate that cholinergic neurons are involved in memory acquisition, but not consolidation processes (Walsh, 1995).

Scopolamine Injections in the Hippocampus Disrupt Memory Acquisition, but not Consolidation

Another common way of studying what a particular neurotransmitter does is by understanding how it affects different receptor systems. For example, studying what happens upon activation or inhibition of mAChRs and nAChRs has helped scientists piece together the role of the cholinergic system in memory formation. Researchers have numerous drugs at their disposal that are selective for specific mAChR and nAChR subtypes. Administering receptor agonists allow researchers to examine behavioral changes as a result of augmented receptor functioning, while receptor antagonists can be used to temporally interrupt receptor function.

The role of muscarinic acetylcholine receptors (mAChRs) in memory processes has been extensively researched. In one set of experiments, hippocampal cannulas were used to deliver bilateral infusions of scopolamine, a potent mAChR antagonist, at different stages of testing in the MWM. As in the IgG-192-saporin study mentioned above, a cohort of rats was

divided into three different groups: control, acquisition, and consolidation. The rats in the acquisition group received scopolamine infusions before the training sessions, while the consolidation group received infusions after the training sessions. The rats in the control group did not receive any drugs, and as expected, they escaped progressively faster during the training and trial sessions indicating that spatial learning had taken place. There were significantly longer escape latencies for the rats in the acquisition group than in both the consolidation and control groups, implying mAChRs help to facilitate the acquisition of spatial memories. However, there was no significant difference in escape latency between the consolidation and control groups, indicating that mAChRs do not influence the consolidation of spatial memories (Riekkinen, 1997).

Pirenzepine Disrupts Memory Acquisition

Some research has attempted to examine the role of specific mAChR subtypes on memory acquisition using the M1 muscarinic receptor antagonist, pirenzepine, with similar testing protocols as those described above. Although not conclusive, it is thought that M1 receptor inhibition leads to impaired memory acquisition. Due to pirenzepine's low level of selectivity for the M1 receptor, these effects may have actually been mediated by M2 receptor inhibition as well (Hunter, 1988). More research needs to be performed in order to elucidate the exact roles of the various mAChR subtypes.

Mecamylamine and Hexamethonium Disrupt Memory Acquisition, but not Consolidation

The role of nicotinic acetylcholine receptors (nAChRs) in memory formation has also been expansively researched using the MWM. In this version of the MWM test, the rats were divided into four groups, mecamylamine i.p. 3mg/kg, mecamylamine i.p. 10mg/kg,

hexamethonium i.c.v., and saline. Mecamylamine is a nicotinic antagonist active in the CNS, while hexamethonium is a peripherally acting nicotinic antagonist. The effects of the nicotinic antagonists were tested for both acquisition and consolidation processes.

The escape latencies revealed a significant drug effect at both mecamylamine doses when given before the acquisition trials. While still significant, the escape latencies of the rats given hexamethonium injections were comparable to those that received the lower dose of mecamylamine. These findings demonstrate that nicotinic antagonists are able to disrupt acquisition, implicating nAChR activity in the memory acquisition process. These effects were not observed when the drugs were administered between training and trial sessions, suggesting that memory consolidation processes are independent of nAChR function (Decker, 1992).

Phenserine, an AChE inhibitor, Restores Scopolamine - Induced Memory Deficits

Administration of acetylcholinesterase (AChE) inhibitors allow ACh to elicit a greater synaptic response by delaying the enzymatic degradation of ACh by acetylcholinesterase. AChE inhibitors have been shown to have a restorative effect on memory deficits induced by scopolamine.

Administration of the AChE inhibitor, phenserine (PHEN), has been shown to have a restorative effect on memory deficits induced by scopolamine. In a recent study rats were acclimated to the MWM before receiving one of the following i.p. treatments: saline (CON), 1.0mg/kg scopolamine (SCOP), 1.0mg/kg PHEN (PHEN1), 2.0mg/kg PHEN + 1.0mg/kg scopolamine (PHEN2), or 4.0mg/kg PHEN + 1.0mg/kg scopolamine (PHEN4). After receiving their respective treatments, the rats underwent 4 more trials, during with their

escape latencies were measured. As expected, the rats in the SCOP group had significantly longer escape latencies than those in the CON group. The escape latencies of rats in the PHEN4 group were significantly shorter than those in the SCOP group, showing that by using an AChE inhibitor it is possible to reverse spatial learning deficits caused by attenuated cholinergic function (Janas, 2005).

Novel Object Recognition

The Novel object recognition (NOR) tests is based on rat's natural tendency to spend a greater time exploring novel objects than objects which they have previously encountered, making it a valuable tool to examine object memory processes. A standard NOR test is comprised of two of trials, the familiarization trial and the test trial. During the familiarization trial, the rat is placed in the testing apparatus (NORbox) with two different objects to investigate for a short period of time, before being returned to its cage. After a predetermined period of time, the rat is returned to the NORbox for the test trial. During the test trial, the NORbox contains one of the original objects along with one never before seen, novel object. The amount of time the rat spends exploring both objects is recorded with the expectation being that the rat will spend a greater amount of time exploring the novel object. By administering drug treatments at various stages of the experiment it is possible to determine the effects of these treatments on object memory processes (Broadbent, 2009).

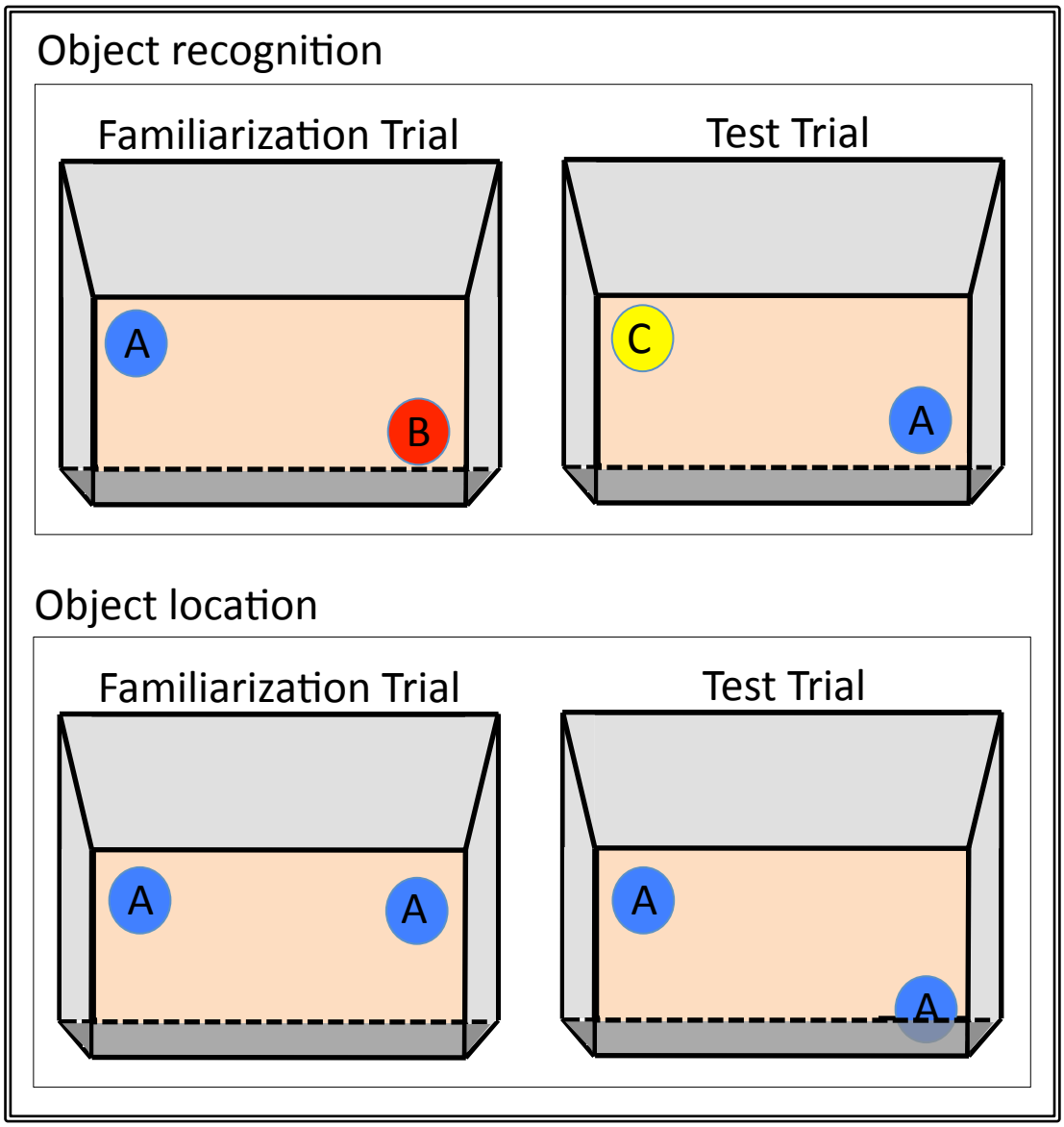
Nicotine Enhances Memory Both Object Recognition and Location Memory Formation

In a series of experiments, Melichercik and colleagues demonstrated that pre-familiarization, systemic, intra-perirhinal and intra-hippocampal nicotine administration is able to enhance both object recognition and location memory formation. A slightly modified

version of the novel recognition test was used to assess object recognition memory; A Y-shaped apparatus was used instead of the standard NOR box. The Y-shaped apparatus contained two exploratory arms and one arm with a starting area separated by a guillotine door. The experiment started with the rat located in the starting area with the guillotine door lowered. To start the trial the door was raised allowing the animal free exploration of both arms. Once the animal exited the starting area the door was closed. During familiarization, identical objects (A and A1) were located in each arm, and the time the animal spent exploring each object was recorded. The test trial was conducted 72 hours after familiarization. During the test trial one arm contained a familiar object (A or A1), while the other contained a novel object (B), and the time the animal spent exploring each object was recorded. From this experiment it was determined that systemic, intra-perirhinal and intra-hippocampal nicotine administration enhanced object recognition memory.

A standard NOR apparatus test was used to examine object location memory by slightly modifying the protocol; during familiarization two identical objects were located in adjacent corners of the apparatus, and during the test trial the location of one object was moved (*Figure 14*). In order to form spatial associations for where the objects were located, several visual cues were present in the testing room. By changing the position of one object during the test trial, the translocated object gains novelty and therefore should be more thoroughly investigated during the test trial (Melichercik, 2012). These experiments demonstrate nicotine's ability to enhance multiple forms of memory formation in various brain regions.

Figure 14: Object recognition vs. object location NOR tests



Metrifonate and Donepezil (AChE inhibitors) Enhance Memory Acquisition for Object Recognition

The NOR test has also been used to determine the effects of two AChE inhibitors, metrifonate and donepezil, on memory acquisition and consolidation. Prickaerts and colleagues administered oral does of the AChE inhibitors prior to familiarization in order to determine if AChE inhibitors can enhance memory acquisition. They discovered that the rats treated with either metrifonate and donepezil spent significantly more time examining the novel object during the test trial, as compared to those treated with saline in the control group. These results indicate that increased ACh levels are able to enhance memory acquisition.

When metrifonate or donepezil were administered post familiarization, there was no change in exploration times during the test trial, suggesting that ACh may not be involved in consolidation (Prickaerts, 2005). Due to the improvement in memory acquisition facilitated by metrifonate and donepezil further signifies a role for acetylcholine in object recognition memory acquisition.

IgG-192-Saporin Injections in the MS do not Disrupt Memory Acquisition for Object Recognition

As previously mentioned, IgG-192-saporin induced lesioning of hippocampal cholinergic neurons has been shown to cause spatial memory acquisition impairments in the MWM. Furthermore, IgG-192-saporin induced lesions have been shown cause memory impairments using spontaneous alternation tasks such the T-Maze (Chang, 2004).

Since the medial septum (MS) is the seat for cholinergic projections into the hippocampus, it was hypothesized that IgG-192-saporin induced lesioning of the MS may also produce memory deficits. In order to test this hypothesis, injections of IgG-192-saporin

into the MS were given between the familiarization and test trials of a standard NOR test. Amusingly, rats with MS lesions spent more time examining the novel object during the test trial, indicating that MS ACh projections to the hippocampus are not involved in the novel object acquisition process. Although these projections are not involved in novel object acquisition processes, they are thought to be involved in object location memory (Cai, 2012).

IgG-192-Saporin Injections into the MS Disrupts Memory Acquisition in a Modified Object Location NOR Test

In a subsequent study, rats received IgG-192-saporin induced lesions ACh neurons in the MS between the familiarization and test trials. This time they were tested using the previously mentioned object location version of the NOR test [sec 7.3.1] (Cai 2012). The rats given MS ACh lesions spent approximately the same about of time examining both objects during the test trial, indicating that ACh lesioning of the MS disrupts spatial memory formation. Taken together, the results of these two lesioning studies show that ACh projections from the MS to the hippocampus are involved in object location, but not object recognition memory formation (Cai, 2012).

IgG-192-Saporin Injections into the PRH Disrupt Object Location Memory

Bilateral injections of IgG-192-saporin into the perirhinal cortex (PRh) have also been shown to cause object recognition memory acquisition deficits in a slightly modified NOR test. In this study rats received bilateral surgical implantation of cannulas into the PRH before familiarization. After recovering from surgery, one group of animals received IgG-192-saporin infusions, while the other group received saline. The two groups then participated in a familiarization trial in which the NORbox contained two identical objects. During the test trial, one of the objects was replaced with a novel object. As expected, rats

that received only saline spent a greater proportion of time exploring the novel object during the test trial. However, those with PRh lesions spent the same proportion of time exploring the novel and familiar objects during the test trial, indicating that the cholinergic neurons in the PRh facilitate memory acquisition for object recognition (Winters, 2005).

Both Systemic and PRh Specific Scopolamine Injections Disrupt Novel Object Recognition Memory Acquisition

Just as in the MWM experiments, the role of mAChRs have been evaluated using the NOR test. By administering i.p. injections of the mAChR antagonist, scopolamine, before the familiarization trial it was determined that mAChRs help facilitate memory acquisition. In this experiment, rats were either treated with i.p. injections of saline or scopolamine, 0.05mg/kg, prior to the familiarization trial. As expected, rats treated with saline spent significantly more time investigating the novel object during the test trial. The rats treated with scopolamine showed no preference for the novel object during the test trial. The lack of discrimination between the novel and familiar object exhibited by the rats treated with scopolamine indicates that mAChRs help facilitate memory acquisition. These results are consistent with several NOR studies examining the effects of s.c. injections of scopolamine on memory formation in which scopolamine disrupts memory acquisition (Dodart, 1997 & Besheer, 2001). Furthermore, bilateral infusions of scopolamine into the perirhinal cortex produced the same lack of object discrimination, specifying mAChR activity in the perirhinal cortex is involved in the acquisition process (Warburton, 2003).

Systemic Scopolamine Injections Disrupt Object Recognition Memory Acquisition in a Modified NOR Test

Other versions of the NOR test have been used to examine the effects of nAChR and mAChR antagonism on memory acquisition processes. One set of experiments conducted

Besheer and colleagues (2001) used a three chambered NOR apparatus instead of the standard one chamber NORbox. The modified NOR apparatus consisted of a small, central chamber flanked by two doors, each leading to a larger chamber. During the familiarization trial the doors remained closed and a rat was placed in one of the side chambers with a single object for 5 minutes. During the test trial, the previously encountered object remained in place while a novel object was placed in the other side chamber. To start the trial both doors were opened and a rat was placed in the central compartment. Just as in a typical NOR test, the assumption was that the rats would better remember the object encountered in the familiarization trial and therefore spend more time exploring the novel one during the test trial.

Scopolamine was delivered s.c. (0.3, 1.0, and 3.0 mg/kg) twenty minutes prior to the familiarization trial in order to disrupt systemic mAChR functioning, while s.c injections saline served as a control. The rats given scopolamine spent approximately the same amount of time investigating both objects during the test trial for all doses, indicating that proper mAChR functioning is imperative for memory acquisition process; these results confirm the findings of the previously mentioned study [page 70].

Systemic Mecamylamine Injections Disrupt Object Recognition Memory Acquisition in a Modified NOR Test

Besheer and colleagues carried out the same experiment as in the previous section using the nAChR antagonist mecamylamine (0.3, 1.0, and 3.0 mg/kg, s.c.), and saline. All rats, regardless of treatment, spent a significantly greater amount of time exploring the novel object during the test trial. These results call into question the role of nAChR activity novel object recognition acquisition processes. As previously mentioned, several studies show

discrepancies between the effects of nicotinic agonists and antagonists in memory formation. This topic will be further examined in the discussion section (Besheer, 2001).

Summary of Modification to the Cholinergic System that Disrupt Memory Processes

Blocking ACh receptors and obliteration of cholinergic cells have been shown to produce deficits in memory acquisition processes, while augmenting the cholinergic system with agonists has been shown to facilitate these processes (*Table 1)*. While these studies provide a definitive role for ACh in memory formation, the underlying cellular mechanism by which ACh exerts its effect remains unclear.

Table 1: Summary of the effects of cholinergic modifications on memory processes

Behavioral test	Type of memory tested	Modification	Drug	Results
MWM	Spatial Memory	Cholinergic lesioning	IgG -192-saporin Hp-c	acquisition disrupted, consolidation intact
MWM	Spatial Memory	mAChR antagonist	Scopolamine i.c.v.	acquisition disrupted, consolidation intact
MWM	Spatial Memory	M1 mAChR antagonist	Pirenzepine	acquisition disrupted
MWM	Spatial Memory	CNS nAChR antagonist	Mecamylamine i.p.	acquisition disrupted, retrieval intact
MWM	Spatial Memory	peripheral nAChR antagonist	Hexamethonium i.c.v	acquisition disrupted, retrieval intact
MWM	Spatial Memory	AChEi + mAChR antagonist	Phenserine + Scopolamine	no memory disruption
NOR	Recognition + Location	nAChR agonist	Nicotine	enhanced recognition and location memory
NOR	Object Recognition	AChEi	Metrifonate	Enhanced recognition memory
NOR	Object Recognition	AChEi	Donepezil	Enhanced recognition memory
NOR	Object Recognition	Cholinergic lesioning	IgG -192-saporin PRh-c	no memory disruption
NOR	Object Location	Cholinergic lesioning	IgG -192-saporin MS-c	acquisition disrupted
NOR	Object Recognition	Cholinergic lesioning	IgG -192-saporin PRh-c	acquisition disrupted
NOR	Object Recognition	mAChR antagonist	Scopolamine i.p.	acquisition disrupted
NOR	Object Recognition	mAChR antagonist	Scopolamine PRh-c	acquisition disrupted
NOR	Object Recognition	mAChR antagonist	Scopolamine s.c.	acquisition disrupted
NOR	Object Recognition	nAChR antagonist	mecamylamine	acquisition disrupted

Hp-c., hippocampal cannula; PRh-c, perirhinal cannula; MS-c, Medial Septum cannula i.p., intraperitoneal; i.c.v., intracerebroventricular; s.c., subcutaneous

The Mechanisms by Which Acetylcholine Modulates Memory Formation

The exact mechanism by which the cholinergic system modulates hippocampus dependent memory acquisition is extremely complex. So far two approaches have been employed in order to understand how this system functions. The older, more common approach, relies on data obtained from patch-clamp studies investigating the effects of ACh agonists and antagonists on various hippocampal neurons. In recent years, the data obtained from these patch-clamp studies has been compiled and analyzed using computational models in order to create a more complete schematic of the cholinergic systems. While a conclusive mechanism remains to be seen, the general mechanism is thought to be as follows:

During the learning process, previous associations need to be curtailed so that new information can be successfully be processed. For example, take a version of the paired association task in which a participant is first asked to remember an association between the words "boot" and "leather". As the words are repeated together several times Hebbian consolidation occurs, meaning that if participant is presented with one of the words, their neural network will now automatically recall the other word as well. If the participant is then asked to remember an association between the words "leather" and "holster" they are presented with a problem, as the previous association has been engrained in their memory. In order to combat this problem, the brain is able to temporarily inhibit the retrieval of the previously made association using proactive interference, as to more efficiently learn the new association (Easton, 2012).

The "Encoding Versus Retrieval Scheduling" (ERS) Framework Model

The "encoding versus retrieval scheduling" (ERS) framework is a model that was created to ascertain how the hippocampus switches between modes of information encoding and retrieving. The idea that ACh levels play a crucial role in determining which mode dominates is central to the ERS model. During low levels of cholinergic activation, projections from areas of long term memory provide the main source of input to the hippocampus. Conversely, high levels of cholinergic activation allow for new associations to be formed. Elevated levels of acetylcholine are thought to facilitate the formation of novel associations by both reducing interference from cortical regions associated with long term memories, and enhancing signal transmission from afferent pathways (Hasselmo,1995 & Hasselmo, 2006).

Low Levels of ACh Set the Stage for Consolidation and Retrieval

According to the ERS model, consolidation and retrieval processes dominate during periods of low ACh levels. Hippocampal consolidation is particularly well studied during sleep. Several studies have implicated sharp wave / ripple (SWR) events as playing an important role in these consolidation process. SWRs are the most synchronous pattern of neuronal discharge observed in the hippocampus. They appear in EEG recordings as high frequency oscillations that last for only a few milliseconds (Ylinen, 1995). SWRs most frequently occur during slow-wave sleep (SWS) when ACh levels are low. While SWRs also occur during learning tasks, they are generally less frequent, and are seldom observed during REM sleep (Jadhav, 2012).

There are two stages of hippocampus-dependent memory formation. First, the hippocampus rapidly encodes information as SWR patterns during alert periods where learning is taking place. EEG recordings of CA1 and CA3 in rats show that clusters of neurons display SWR patterns during exploration of a novel environment. These patterns of activation could form the basis of memory encoding. Reactivation of the previously encoded patterns occur during sleep, actively strengthening the newly encoded memories (O'Neill, 2010 & Sutherland 2000).

EEG studies indicate that there is an increase in SWR activity during SWS following learning events. In one experiment, rats participated in a training session during which they learned to associate a odor with a food reward. EEG recordings were taken during night following training. As expected, the rats that learned to associate the odor with the reward showed significant amounts of SWR activity during SWS, while those who did not form the association did not show elevated levels of SWR activity (Eschenko, 2008). The same pattern of neuronal firing has also been shown for associative learning of spatial discrimination (Ramadan, 2009).

Using the drug physostigmine, an acetylcholinesterase inhibitor, it has been proven that low levels of ACh are crucial for sleep enabled memory consolidation. During SWS, excitatory feedback synapses in CA3 are suppressed by low levels of ACh activity. Normally, recurrent connections between CA1 and CA3, as well as between the hippocampus and entorhinal cortex, allow for reactivation of SWR patterns of recently learned associations. Reactivation of these patterns allow for consolidation and therefore LTP to take place (Power, 2004). In an experiment performed by Gais and colleagues in 2003, human participants showed enhanced memory formation for both declarative and procedural

memory tasks following a full night's sleep. However, upon receiving a dose of physostigmine before going to sleep, the participants did not benefit from sleep induced declarative memory consolidation, suggesting that low levels of ACh are critical for memory consolidation processes (Gais, 2003). Administering physostigamine before sleep effectively raises ACh levels, therefore suppressing activation of SWS recurrent connections in the hippocampus which prevents consolidation facilitated LTP from occurring.

Recurrent Connections Regulate Hippocampal Activity

As previously mentioned, the ERS model dictates that low ACh levels direct the hippocampus towards consolidation and retrieval processes while high ACh levels prompt it to encode new memories. As seen on page 59, scopolamine impairs acquisition but leaves retrieval / consolidation intact, indicating that high levels of ACh are required for acquisition and not consolidation (Riekkinen, 1997 & Dodart, 1997).

One way in which elevated levels of ACh are able gear the hippocampus towards encoding new memories is through the selective activation of recurrent connections. Recurrent connections are found in several brain areas including the olfactory cortex, neocortex, and hippocampus. For the most part, it is thought that these recurrent connections consist of interneuron whose primary function is to modulate activation levels within the areas that they innervate (Douglas, 1990). Depending on the location and neurotransmitter profile of these interneurons, they are able perform a variety of tasks such as limiting feedback within the circuit or strengthen previously learned associations through the enhancement of previously formed synapses (Hasselmo, 1994).

There are several mechanisms by which ACh is able to suppress feedback within the hippocampus. Isolated slice preparations have been used to demonstrate the effects of ACh on various layers of the hippocampus. For example, ACh agonists have very little effect on excitatory transmission from the entorhinal inputs to the molecular layer of the dentate gyrus, while simultaneously suppressing excitatory transmission on the Schaffer collateral inputs in CA1. These agonists also suppress excitatory recurrent connections in the stratum radiatum of CA3, further limiting internal feedback (Hasselmo, 2004).

Using a patch-clamp technique it has been shown that nAChR activation by both non-selective ACh, and selective nAChR agonists results in the depolarization of GABAergic interneurons in CA1 (Albuquerque, 2001). Due to the large amount of recurrent connections within the interneuron circuitry, this increased GABA release is thought to reduce feedback within the hippocampus, allowing it to focus on novel information arriving at the pyramidal cells. (Hasselmo, 2004). Similar patterns of suppression have been demonstrated in intracortical synaptic transmission in the primary visual cortex as well as the frontal cortex (Vidal, 1993). Furthermore, ACh is able to suppress excitatory feedback but not afferent olfactory input to the piriform cortex (Hasselmo, 1992)

Enhancement of Excitatory Transmission From Afferent Inputs

As previously mentioned, behavioral data shows that cholinergic agonists, nicotine in particular, are able to enhance hippocampal-dependent learning. Giocomo and colleagues have shown that nicotine is able to selectively enhance afferent input to the hippocampus. In their experiments, unipolar stimulating and recording electrodes were placed in several areas of hippocampal brain slices extracted from male Sprague-Dawley rats. Electrodes were

placed in the Stratum radiatum (SR) to record the evoked synaptic potentials of recurrent connections; interneurons of the SR are primarily GABAergic and express α4, α5, α7, and β2 nAChR subunits (Liu, 1996). Electrodes were also placed in the striatum lacunosum-molecular (SLM) to record the evoked postsynaptic potentials of afferent connections. Since projections from entorhinal cortex, thalamus, amygdala, and inferotemporal cortex converge in the SLM, it is thought to be an important center of integration. Afferent projections from the entorhinal cortex terminate in the SLM of CA3 where they synapse primarily with pyramidal cells (Capogna, 2011).

In order to visualize the role of nicotine on these connections, the difference in excitatory postsynaptic potential (EPSP) measurements were taken before and after the slices were bathed in nicotine. If increasing the level of cholinergic activity resulted in an increase in SLM activity, it could be inferred that nicotine helps facilitate memory formation by enhancing the input of sensory information to the hippocampus. Their results show that nicotine administration did not cause any change in ESPS measurements for the SR, while causing SLM transmission to undergo a brief suppression followed by a long-lasting enhancement of synaptic transmission. These results suggest that one way in which nicotine is able to facilitate learning is by selectively enhancing afferent synaptic transmission, therefore allowing the individual to focus on outside stimuli (Giocomo, 2005). As mentioned on page 15, periods of enhanced synaptic transmission make neurons more likely to be activated together in the future, in a process known as long term potentiation.

Alzheimer's Disease

As illustrated in the sections above, the cholinergic system plays a complex role in learning and memory. That being said, it should come as no surprise that cholinergic dysfunction causes a wide range of adverse effects. In fact, cholinergic dysfunction is a hallmark of Alzheimer's Disease, one of the most prevalent neurodegenerative disorders.

Alzheimer's Disease (AD) is the most common form of dementia, accounting for 50-80% of all cases, and is the 6th leading cause of death in the United States. The Alzheimer's Association uses seven "stages" to describe the decline in cognitive function a patient experiences during disease progression. During the early stages of AD, the patient may start to notice a decline cognitive functions, such as forgetting where they placed their keys. These changes, however, may simply be a function of the normal aging process rather than the onset of AD and often do not cause suspicion during routine medical examinations. As disease progression continues, changes in cognitive function become more apparent. Common symptoms include difficulty recalling the name of ordinary objects, committing the names of new people to memory, and misplacing objects become common place in everyday life. In addition, patients start to have difficulty with arithmetic, such as figuring out tip on dinner bills and balancing a check book. As the disease progresses, lapses in memory are no longer limited to recent events as memories of earlier life start to fade away. Changes in mood are common as a patient may become agitated or withdrawn upon forgetting common events in social situations. During moderate or mid-stage AD, patients may start to require assistance for everyday life. They may have trouble remembering their own phone number or address, become easily confused as to what day of the week it is or where they are, and exhibit changes in sleeping patterns. Increased supervision is needed as daily tasks, such as

operating the toilet and preparing meals, become more difficult. Eventually larger deficits in memory such as forget the names of family members and large portions of their personal histories become common. Often times these changes are accompanied delusions and obsessive compulsive behaviors. As they progress into severe or late-stage AD, patients are unable to carry on a conversation and control muscle movements, making it increasingly difficult to sit up write, smiling, or swallowing, and simple reflexes are no longer functional (Alzheimer's Association, 2012).

Diagnosing Alzheimer's Disease

A definitive diagnosis of AD relies on the presence of amyloid plaques and neurofibrillary tangles during a postmortem examination (Shen, 2004). The highest concentration of plaques and tangles are usually observed in brain areas that rely on neurotransmitter acetylcholine (ACh) for communication, such as the hippocampus and medial septum (Marco-Contelles, 2006). Deficits in ACh are seen in all patents with AD and are often times the first manifestations to appear. Many researchers believe that this cholinergic dysfunction, dubbed "the cholinergic hypothesis" may instigate the degradation of others (Zamani, 2001).

The Cholinergic System in Alzheimer's Disease

Cholinergic dysfunction may account for many of the cognitive deficits observed in patients suffering from AD due to its involvement in learning and memory processes. While there are several treatment options aimed at ameliorating the symptoms, there is no known cure for AD. All of the treatment options currently approved by the FDA belong to a class of drug known as acetylcholinesterase inhibitors (AChEIs). AChEIs impede the natural

enzymatic degradation of ACh by acetylcholinesterase in the synaptic cleft, allowing ACh to elicit a greater response (Mattson, 2004).

Although the majority of AD cases are sporadic, studies suggest genetic predispositions may play a role in instigating cholinergic dysfunction. For example, individuals with certain polymorphisms of the apolipoprotein E (ApoE) allele may be at higher risk for developing AD (Kita, 2012).

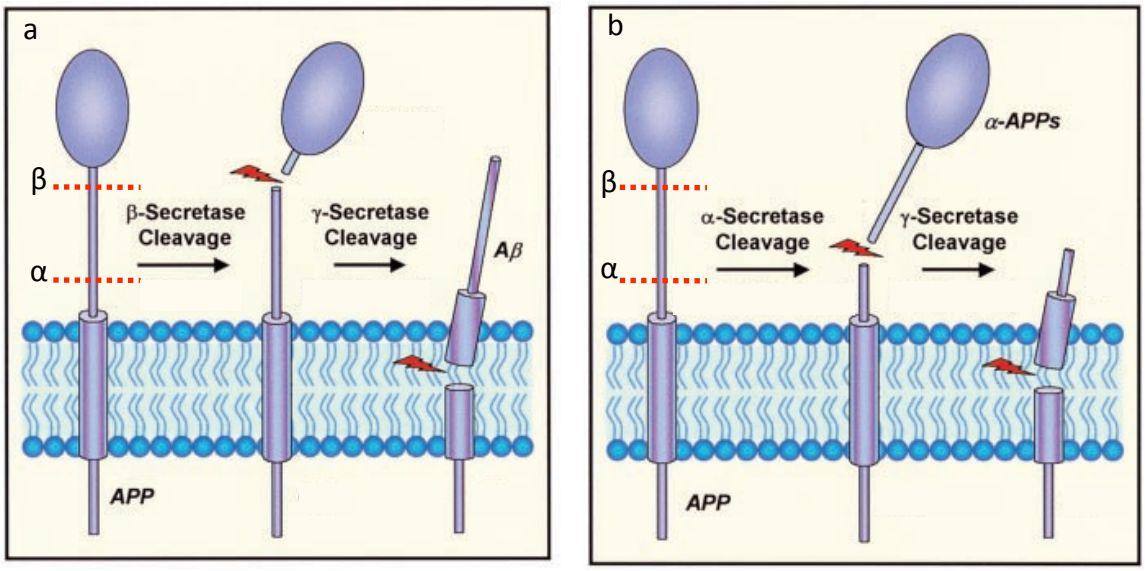
The Amyloid Hypothesis

As previously mentioned, one of the main hallmarks of AD is the appearance of amyloid plaques. These plaques are primarily composed of aggregated 40-42 amino acid polypeptide chains, Amyloid-β (Aβ). Aβ polypeptides are formed as a result of proteolytic cleavage of a type 1 transmembrane protein, Amoloid-β Precursor Protein (APP). In normal cellular function, APP is thought to act as a trophic factor as well as play a role in cell recognition and adhesion. Once synthesized, APP is transported to the cell membrane where it is subject to proteolytic processing by α, β, and γ-secretases, resulting in the formation of several Aβ isoforms (Schroder, 1994). In addition the APP isoforms there are also several APP-like proteins which are capable of activating the same biochemical pathways resulting in similar cellular activities (Heber, 2000).

The APP molecule itself has extracellular cleavage sites for α-secretases and β-secretases (BASE), and transmembrane cleavage sites for γ-secretases. If APP is first cleaved by BASE, the remaining transmembrane portion is able to undergo cleavage by γ-secretase resulting in the formation of Aβ. However, if APP is first processed by α-secretase, BASE

cleavage is inhibited, and subsequent cleavage by γ-secretase will not produce Aβ and will therefore not generate amyloid plaques (Esler, 2001).

Figure 15*:* APP processing and the formation of Aβ



Full length APP processing by α, β, and γ-secretases **(a)** initial processing by β-secretase allows for subsequent processing by γ-secretase, resulting in the formation of Aβ. **(b)** initial processing by α-secretase inhibits β-secretase cleavage. No Aβ is produced upon further processing by γ-secretase, precluding the formation of Aβ. (Adapted from Esler, 2001)

In healthy individuals the majority of the Aβ peptides produced are 40 amino acids in length (Aβ40). The Aβ40 are readily digested within the cellular environment and therefore are not thought to cause any negative effects. While infrequent, the synthesis of a more hydrophobic, 42 amino acid polypeptide (Aβ42) is also possible in healthy individuals. In AD, alternative processing of APP results in the formation of large amounts of (Aβ42) (Kamenetz, 2003 & Jackish, 2009). Once synthesized, the Aβ42 peptide exists in an equilibrium of conformational states. Aβ42 is able to fold into α-helical monomers or β-sheet structures. The α-helical monomers are soluble and therefore pose very little threat to the human brain, while the β-sheets can be more problematic. As the β-sheets are formed, they aggregate, becoming oligomeric. When enough of the aggregate form is present, it precipitates out as neurotoxic amyloid plaques and is unable to reassume the soluble α-helical form (Zeng, 2001). Additionally there are several complex molecules which coordinate the binding of Cu2+, Fe2+, and Zn2+ to Aβ, inducing it to assume the β-sheet conformation (Bossy-Wetzel, 2004). Recent research has shown that nicotine may regulate metal ion homeostasis thereby attenuating Aβ neurotoxicity, making nicotine administration a possible treatment strategy for AD (Zhang, 2006).

Determination as to which Aβ is produced seems to be largely controlled by a complex consisting of several proteins, γ-secretase. One sub-complex, the presenilin (PS) family of proteins, is responsible for providing catalytic energy necessary for cleavage of APP. Included in the PS family is presenilin 1 (PS1), located on chromosome 14, and presenilin 2 (PS2), located on chromosome 1(Van Broeckhoven, 1995). Mutations in PS1 and PS2, as well as in the APP gene itself, change the way that APP is processed and cause increased $A\beta_{42}$ metabolism. Neuronal activity itself is also able to regulate both the

production and secretion of Aβ through γ-secretase mediated processing of APP. Research shows that an increase neuronal activity causes an increase in Aβ formation. Consequently, this increase in Aβ formation causes a depression in synaptic activity suggesting that Aβ generation may be implicated in regulating neuronal excitability (Kamenetz, 2003).

The exact mechanism by which Aβ plaque deposition leads to neural toxicity is not fully understood, however several explanations have been proposed. First, it has been shown that soluble Aβ is able insert itself into cell membranes, increasing their fluidity. Many membrane bound proteins rely on specific membrane fluidities to function properly, especially in synaptic membranes, therefore a disruption in membrane fluidity caused by Aβ may result in neural dysfunction (Avdulov, 1997). Additionally, the formation of Aβ plaques generates free radicals (Hensley, 1994). These free radicals then interact with membrane bound fatty acids, producing a variety of aldehydes such as 4-hydroxynoneal (HNE). Upon exposure to HNE, cultured hippocampal cells undergo neurite fragmentation and cell body vacuolation, common in neuron degeneration (Mark, 1997).

Treatment Options

Early pharmacological treatments of AD included administration of acetylcholine precursor molecules, muscarinic and nicotinic agonists. The administration choline in hopes of stimulating ACh production proved ineffective and caused several adverse side effects. The use of mAChR agonists was also ineffective due to their low bioavailability and receptor subtype specificity, as well as the high rate of side effects. Conversely, stimulating nAChRs resulted in improved cognitive function and was fairly well tolerated. There are inherent problems associated with the used of nAChR agonists such as difficulty targeting CNS while

leaving the periphery unaffected, and selectively activating specific receptor subtypes. Furthermore, nAChRs become desensitized upon constant stimulation, making nAChR agonists ineffective after a short period of time (Farlow, 2001).

All of the drug treatments currently approved by the FDA to treat AD – Cognex, Donepezil, Rivastigmine, and Galantamine – are acetylcholinesterase inhibitors (AChEIs.) Shortly after ACh is released into the synaptic cleft it is captured by the protease, acetylcholinesterase (AChE), which hydrolyzes its ester bond liberating choline and acetate. Although it is less abundant, the protease, butyrylcholinesterase (BuChE), is also able to degrade ACh by hydrolyzing its ester bond (Darvesh, 2002). By inhibiting the natural degradation of ACh by AChE they are able to help alleviate some of the cognitive deficits caused by cholinergic neurodegeneration. These drugs only offer symptomatic relief, as they are unable to stop, slow, or reverse the degradation of cholinergic neurons. Furthermore, the use of AChEIs usually becomes ineffective within a year due to the progressive nature of neurodegeneration found in AD (Michaelis, 2003). Acquiring a more complete view of how the cholinergic system influences memory is the first step in creating drugs that will, some day, more effectively treat or even cure AD.

Summary

Based on previous research and the information provided above, it is thought that nicotinic stimulation of the cholinergic system may facilitate memory acquisition processes related to object recognition. In order to test this hypothesis, we employed a NOR test during which rats received an injection of nicotine after the familiarization trial. We expect that rats treated with nicotine will spend a greater proportion of time investigating the novel object

during the test trial than those treated with saline. Immunohistochemistry will then be used to analyze the effects of nicotine on a cellular level. We expect that there will be an increase in c-Fos expression, the gold standard for measuring neuronal activation, in the hippocampus and PRh, two areas commonly associated with object recognition. Furthermore, since ACh is thought to play an important role in memory formation we expect to see a high level of ChAT expression in the medial septum, the main source of ACh to the hippocampus, in rats treated with nicotine.

Methods

Subjects and Drug Administration

The subjects for this experiment were twelve male rats, of the genus Rattus and species norwegicus, from the Sprague-Dawley strain. They were provided from Charles River Labs, and had no prior experience with experiments of this kind. At the time of testing the rats were approximately four months old and weighed between 368g and 471g. They were housed in same sex pairs, in a temperature controlled room kept on a 12 hour light cycle, and given free access to food and water. The subjects were randomly divided into either the control or treatment group, and received an i.p. injection of saline or 0.3mg/kg nicotine tartrate, respectively. The researchers were kept blinded to prevent any bias. Approximately two hours after injection, carbon dioxide was used to sacrifice the animals. The procedures used in this project were approved by the Connecticut College Animal Care and Use Committee, in accordance with guidelines provided by the Guide for the Care and Use of Laboratory Animals.

Materials

Nicotine tartrate was obtained from Sigma-Aldrich, and administered as a 0.3 mg/ml solution in saline. The objects used in the test were attached to the top of small glass jars, which could be secured to the floor of the novel object recognition box. Three objects of similar dimensions were used: a yellow cylindrical block, a blue triangular wooden block, and a red wooden block. Which objects were used for the familiarization and test trials were systematically alternated to prevent any object bias. A video camera with and ANY-maze software was used to record the videos for later analysis.

The subjects were sacrificed using carbon dioxide gas, and immediately perfuse using a 0.1M phosphate buffer solution (PB) along with an injection of heparin, then with a 4% paraformaldehyde solution. The brains were then extracted and stored in a 30% solution of sucrose containing sodium azide and stored in a refrigerator until being sliced with a cryostat. Immunohistochemistry was performed using Santacruz Biotech and Jackson ImmunoResearch reagents. The samples were individually stained using c-Fos and ChAT primary antibodies, then goat anti-rabbit polyclonal biotinylated secondary antibodies, followed by ABC/DAB detection kit. The results were visualized using an Olympus BX41 microscope and quantified using IPLab software.

Procedure

All twelve rats were handled several times in the weeks prior to testing in order to familiarize them with human interaction. Twenty-four hours prior to testing, the rats were placed in the novel object recognition box (NORbox) for five minutes without any objects as to prevent any novelty bias on test day. On the morning of test day, the 0.3mg/kg nicotine in saline solution test solution and the control saline solution were prepared by a third party, placed in vials labeled “A” and “B”, and given to the researcher administering the test, as to not introduce any biases. The rats were then randomly assigned to a treatment group. During the familiarization trial (FT) each rat spent five minutes in the NORbox with two objects located in opposite corners. The locations and the identities of the objects were systematically arranged so that every combination of object and location was possible during the FT and the test trial (TT). Immediately after completion of the FT, the rats were given an 1ml/kg intraperitoneal injection of the appropriate solution, and placed back in their original cages for eighty-five minutes.

During the test trial one of the familiar objects was replaced with the other novel object according to the systematic plan. The rats were then placed back in the NORbox for the TT, which also lasted 5 minutes. Both the FT and TT trial were recorded using a video camera and recorded for later analysis.

The rats were sacrificed immediately after the TT using a carbon dioxide chamber. They then received an injection of heparin directly into their hearts to thin their blood for perfusion. In order to clear all blood from the tissue, the rats were then perfused using a 0.1M solution of PB. Next, the rats were perfused with a 4% paraformaldehyde solution in order to prevent tissue degradation. The brains were extracted and stored in a 4% paraformaldehyde solution for twelve hours before being transferred into a 30% sucrose solution containing sodium azide and stored in a refrigerator until slicing.

Prior to slicing, the brains were rinsed in phosphate buffer solution to remove any paraformaldehyde present and a block was used to isolate a segment of the brain located between the optic chiasm and the end of the hippocampus. Approximately 200, 40 micron segments were taken from each brain using a cryostat. The samples were stored in cryoprotectant and refrigerated until staining.

A set of three slices were selected from approximately the same region of both the hippocampus and medial septum (MS) for staining. All tissue samples were first stained for c-Fos expression using a rabbit anti-Fos polyclonal IgG primary antibody from Santacruz Biotech. The primary c-Fos antibody was prepared in a 1:8000 ratio with 50ml blocking solution (0.01MPBS, normal goat serum, fetal bovine serum, and 30% 100x Triton). All tissue was first washed three times in 0.01M PBS for ten minutes, and then placed in the c-

Fos primary antibody overnight. Twenty-four hours later, both sets of tissue were washed for ten minutes in 0.01M PBS, three times to remove the primary antibody. Both sets of tissue were placed in the same, 1:200 solution of biotinylated goat anti-rabbit polyclonal IgG secondary antibody from Jackson Laboratories in 50ml blocking solution, for two hours. The tissue was washed for ten minutes in 0.01M PBS, three times to remove the secondary antibody and placed in Avidin-Biotin complexed solution with horseradish peroxidase from an ABC detection kit by Vectastain, for one hour. The tissue was then washed for ten minutes in 0.01M phosphate buffer, three times to remove the ABC solution. The tissue was then placed in an enhanced diaminobenzidine (DAB) solution for eight minutes (25μL glucose oxidase, 800μL nickel ammonium sulfate soln., 500μL cobalt chloride soln., 20μL ammonium chloride, 10mg DAB, 20mg beta-D-glucose, in 50mL 0.1M PB). Both sets of tissue were washed for ten minutes in 0.01M PBS, three times to remove the DAB solution. The hippocampal slices were mounted onto slides and left to dry for two days, while the MS slices were placed in ChAT primary antibody over night. The primary ChAT rabbit anti-Fos polyclonal IgG primary antibody was obtained from Millipore and prepared in a 1:5000 ratio with 50ml blocking solution. The next day, the tissue was washed for ten minutes in 0.01M PBS, three times to remove the primary antibody. Next the tissue was placed in a 1:200 solution of biotinylated goat anti-rabbit polyclonal IgG secondary antibody for two hours. The secondary antibody was removed by washing the tissue three times for ten minutes in 0.01M PBS. The tissue was then placed in Avidin-Biotin complexed solution with horseradish peroxidase from an ABC detection kit by Vectastain, for one hour. The ABC solution was removed by washing the tissue for ten minutes in 0.01M phosphate buffer, three times. In order to create a discernable difference between the c-Fos and ChAT staining, the

tissue was incubated in a non-enhanced diaminobenzidine (DAB) solution for six minutes (25υL glucose oxidase, 10mg DAB, 20mg beta-D-glucose, in 50mL 01M PB). The tissue was then washed three times, for ten minutes, in 0.01M PBS, and then mounted onto slides. The slides were then placed with their hippocampal counterparts and left to dry overnight. The following day all of the slides were rinsed in a graded ethanol wash (50%, 75%, 90%, 95%, and 100%) followed by a clearing solution before being cover-slipped using Permount.

The activated c-Fos particles in the DG, CA1, CA3, and PRh regions were examined using an Olympus BX41 microscope at 10x magnification. A template for each brain region was made using a stereotaxic atlas to delineate the boarders and kept constant across animals. The hippocampus and perirhinal cortex were -3.30mm from bregma, and the medial septum was 1.00mm from bregma (Paxinos, 2007). Once a template had been obtained the number of cells expressing activated c-Fos particles were counted by hand by an observer blinded to treatment. Only the darkest stained cells were counted as expressing c-Fos particles. The cells dual labeled for c-Fos / ChAT in the medial septum were quantified using the same technique. Only cells stained for both c-Fos and ChAT were counted. Two of the most evenly stained tissue samples from each animal were selected for data analysis. The number of cells expressing the various immunohistochemical markers were averaged for each animal.

Analysis

The videos created during the FT and the TT were analyzed; the average distance traveled during each trial was quantified using the ANY-maze software while the number of visits to the novel object and the familiar object were recorded by a blinded observer. A visit was defined as anytime a rat had his nose touching, or within 0.5 cm of an object. In addition to the number of visits to each object, a difference score was created in order to account for

individual variations in exploration during the test trial: Difference score = (number of visits to the novel object) – (number of visits to the familiar object). The amount of c-Fos and ChAT expression was counted by a blinded researcher. Three tissue samples were analyzed per brain area. The hippocampal and perirhinal cortex tissue samples each contained two brain regions, one left and one right, allowing for two data points per tissue sample. Due to the central location of the medial septum, only one data point could be collected per tissue sample. Once the samples had been collected the two that exhibited the most even staining were analyzed. The results of the immunohistochemistry experiments as well as the behavioral results were analyzed using a students t- test and a Pearson correlation test in SPSS. In both cases analysis was done using each individual animal's behavioral and immunohistochemical data.

Results

Novel Object Recognition: Behavioral Results

To assess the effects of nicotine on memory formation from a behavioral perspective, the number of visits to both objects during familiarization and the test trial were recorded both for the nicotine and saline treated rats. This data was considered in multiple forms. First, the average number of visits, to both objects, during familiarization and the test trial was calculated for each treatment and represented graphically (Figure 16).

The average number of visits, for both treatment groups combined, to the familiar object during familiarization and the test trial was also examined and displayed graphically (Figure 17). A students t-test measures revealed that rats explored the familiar object significantly fewer times during the test trial (M= 3.75) than the familiarization trial (M=6.330) $T(22) = 2.1295$, $P = 0.0450$. There was no significant difference present when examining the effects of drug treatment on exploration of the familiar object between trials.

The average number of visits, for both treatment groups combined, to the temporary object (the object which was later replaced by the novel object) during familiarization was compared to the number of visits to the novel object during the test trial and displayed graphically (Figure 18). A students t-test revealed that there was no significant difference between the number of visits for each object.

The effects of drug treatment on average number of visits to the novel and familiar objects during the test trial was calculated (Table 2) and represented graphically (Figure 19). Upon analysis with a students t-test, it was determined that the effect of drug was not significant.

In order to account for individual differences in exploration during the test trial we computed a difference score for each animal: (number of visits to the novel object) – (number of visits to the familiar object), (Figure 20). Using a students t-test, we determined that there was a significant effect of drug treatment on the difference score; rats treated with nicotine had a significantly larger difference score (*M*=2.5) than those treated with saline (M=-.33) T(5) = -2.996, P=0.030. Additionally, the average distance traveled by the saline and nicotine treatment groups was examined. It was determined that there was no significant difference in the average distance that the nicotine and saline treated animals traveled during the test trial (Figure 21).

Figure 16: Average number of visits, to both objects, during the familiarization and test trials

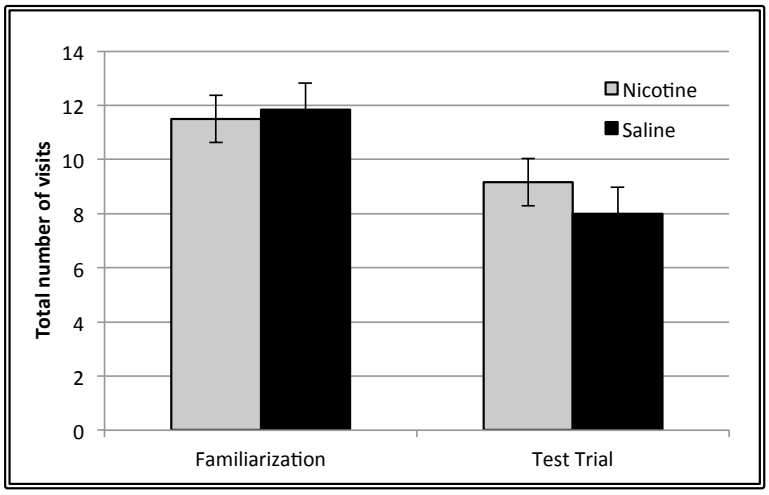
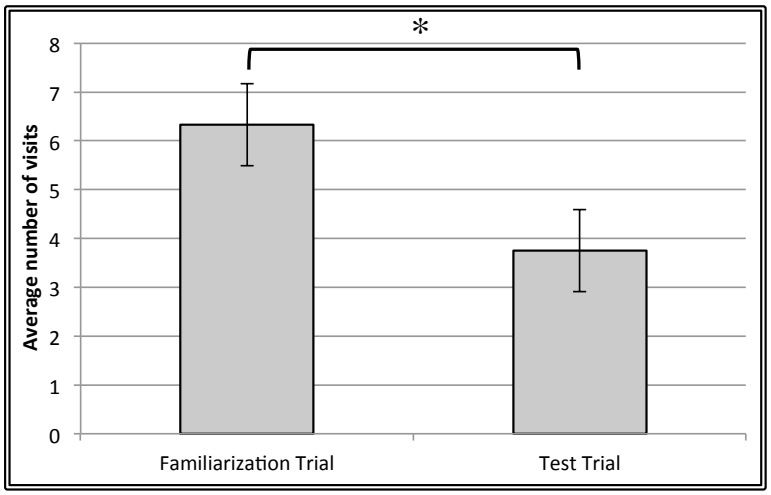


Figure 17: Average number of visits, for both treatment groups combined, to the familiar object during familiarization and test trials



*indicates significance at the α=.05 level

Figure 18: Average number of visits, for both treatment groups combined, to the temporary and novel objects

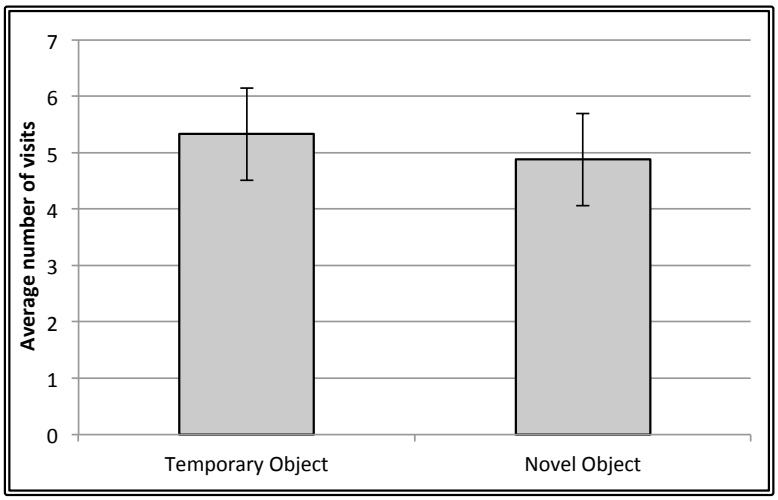


Table 2: Average number of visits to each object during the test trial
Means and Standard Deviations (N = 12)

	Familiar Object		Novel Object	
Variable	Mean	SD	Mean	SD
Nicotine	3.33	2.50	5.83	3.25
Saline	4.17	3.54	3.83	2.79

Figure 19: Average number of visits to each object during the test trial

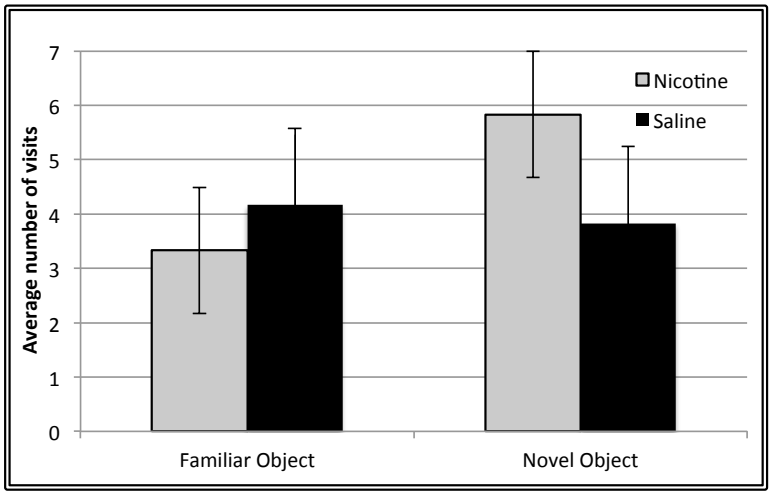
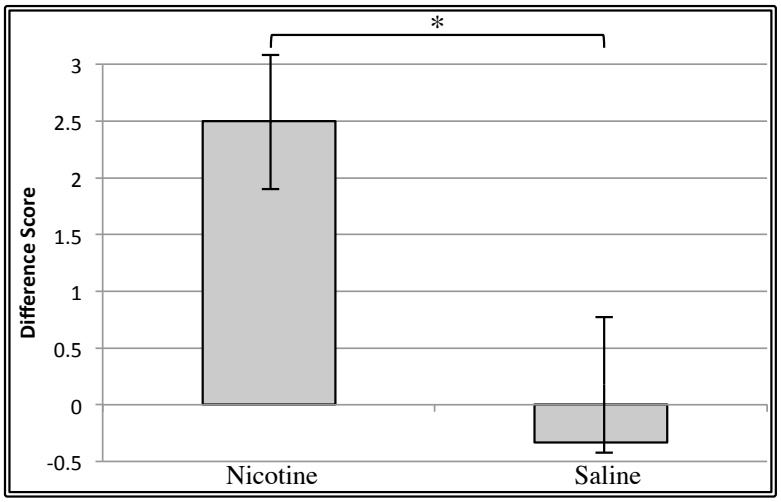
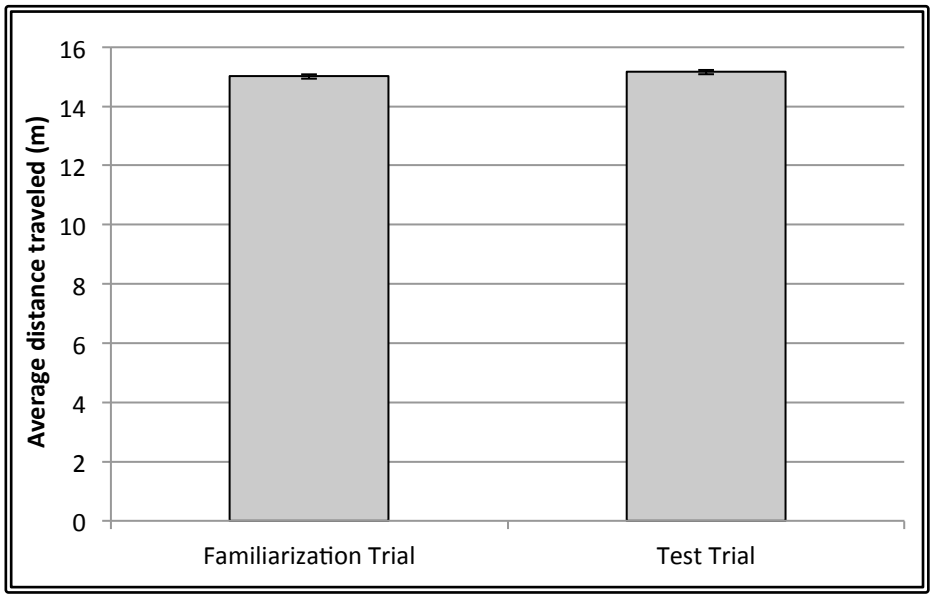


Figure 20: Average difference in exploration during the test trial



*indicates significance at the α=.05 level

Figure 21: Average distance traveled during the test trial



Immunohistochemistry:

The average number of cells expressing c-Fos within the DG, CA1, and CA3 of the hippocampus, as well as the in the PRh, were counted in order to determine the effects of nicotine on neuronal activation (Table 3). Analysis using a students t-test revealed that the rats treated with nicotine displayed significantly more c-Fos expression in the hippocampus and perirhinal cortex combined than those treated with saline, $T(86)=1.9965$, $P=0.0490$ (Figure 22). Nicotine administration resulted in a significant increase of c-Fos expression in the hippocampus, $T(64)=2.0219$, $P=0.0474$, but not in the perirhinal cortex (Figure 23). Additionally, nicotine administration resulted in elevated c-Fos expression within the DG: $T(20)=3.1943$, $P=0.005$, and CA3: $T(20)= -1.525$, $P=0.005$, but not CA1 of the hippocampus (Figure 24).

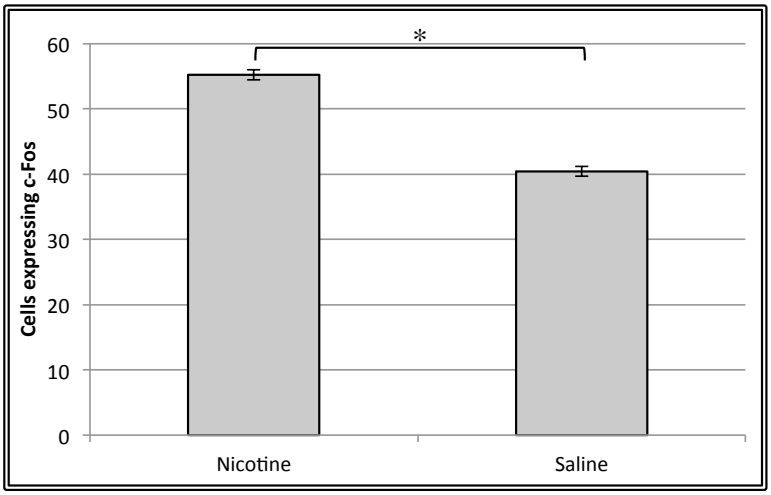
The average number of cells in the medial septum (MS) labeled for both c-Fos and ChAT was calculated in order to determine the effect of nicotine on the activation of cholinergic cells. It was determined using a students t-test that drug treatment did not significantly influence the number of cells expressing both c-Fos and ChAT in the MS (Figure25).

A Pearson correlation was used in order to determine any correlations between the behavioral and immunohistological data. The increased the number of cells expressing both c-Fos and ChAT in the MS is strongly and positively correlated to the difference in exploration during the test trail ($r=.840$, $p=.01$). There was no significant correlation between the difference score and the c-Fos expression for both treatments in the hippocampus and perirhinal cortex.

Table 3: c-Fos expression by brain region
Means and Standard Deviations (N = 12)

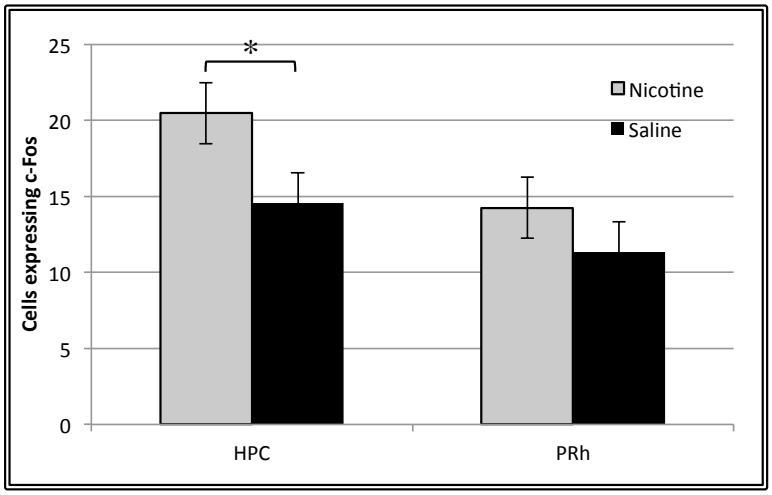
	DG		CA1		CA3		HPC		PRh	
	Mean	SD	Mean	SD	Mean	SD	Total	SD	Mean	SD
Nicotine	13.08	2.85	3.25	1.27	4.13	1.00	20.46	4.87	14.25	3.49
Saline	9.45	2.40	2.50	1.25	2.60	1.31	14.55	3.70	11.35	3.90

Figure 22: Graphical representation of c-Fos expression for the HPC and PRh combined



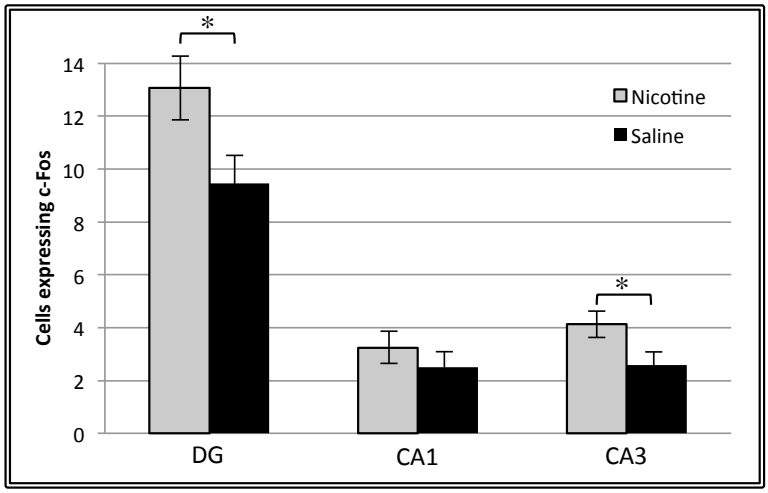
*indicates significance at the α=.05 level

Figure 23: Graphical representation of c-Fos expression in the hippocampus and PRh



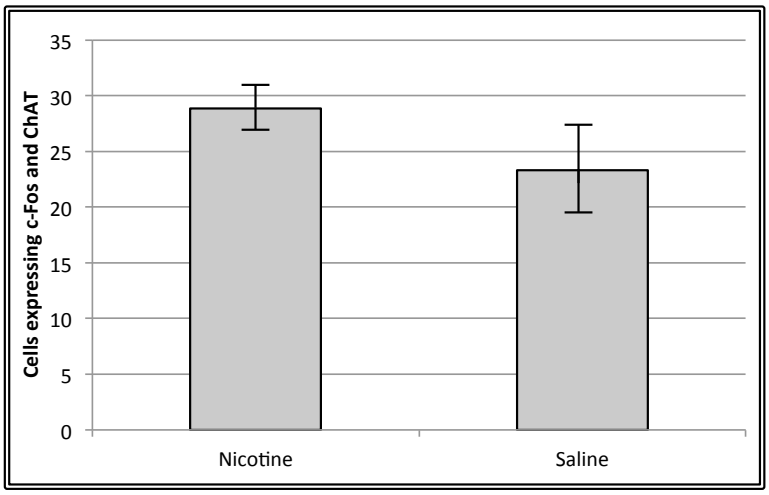
*indicates significance at the α=.05 level

Figure 24: Graphical representation of c-Fos expression in the hippocampus by region



*indicates significance at the α=.05 level

Figure 25: Graphical representation of c-Fos and ChAT co-expression in the MS



Discussion

Behavior

The hypothesis for this experiment was that nicotinic stimulation of the cholinergic system facilitates memory acquisition as related to object recognition. The results of the novel object recognition test, as well as the immunohistochemistry study, appear to support this hypothesis. Furthermore, these results are consistent with those of other researchers demonstrating nicotine facilitated enhancements of memory acquisition processes in NOR (Melichercik, 2012).

The NOR test is a frequently used method of studying hippocampal dependent learning based upon the tendency of rats to explore novel objects more than previously encountered objects (Broadbent, 2009). A typical NOR test consists of two phases: familiarization and test trial. During familiarization, the rats are placed in the NOR apparatus for five minutes with two never before encountered objects designated the "temporary object" and the "familiar object". After a predetermined amount of time, the rats are returned to the NOR apparatus for a five-minute test trial. During the test trial, the NOR apparatus contains the "familiar object" and the "novel object" (replacing the temporary object). Since nicotine is thought to facilitate memory acquisition, giving the rats a dose of nicotine shortly after the familiarization trial should create a stronger memory as to which object the rats previously encountered. Therefore, the rats treated with nicotine should investigate the novel object a significantly greater proportion of times than the familiar object.

The ANY-maze tracking software used to quantify the total distance traveled seemed to have some difficulty differentiating between the front and back of the animal. While this

problem most likely did not interfere with the validity of the mobility results, it made it impossible to quantify the number of visits made to each object using the software provided. Consequently, the data for number of visits was calculated manually by a blinded observer watching recordings of each familiarization and test session.

The results of the NOR test help support the hypothesis that acute nicotine administration enhances the acquisition of object recognition memory. The drug treatment did not produce a significant difference in the total number of visits during a given trial (Figure 16). This is a good indication that any novelty result is due to better remembering the familiar object and not just a result of increased exploration. In addition, nicotine administration did not affect the average distance traveled during the test trial, indicating that any nicotine facilitated memory enhancements observed during the test trial were actually caused by improved memory formation, and not simply a byproduct of nicotine-induced locomotion. The number of total visits to the familiar object decreased significantly between the familiarization trial and the test trial. This shows that the rats remembered encountering the familiar object from the familiarization trial, and as a result, spent less time investigating it during the test trial (Figure 17). While this was expected, it helps confirm the validity of our results. There was no significant difference between the number of visits to the temporary object during the familiarization trial, and the number of visits to the novel object during the test trial (Figure 18). Since the object location, as well as the particular object used for each trial, were rotated between subjects, this is good proof that none of the objects or object locations were favored and therefore caused the rats to investigate them more thoroughly.

Although not statistically significant, the rats in the nicotine group tended to visit the novel object more than any other object during the test trial (Figure 19). In order to investigate this trend further, a difference score was calculated for each animal: (number of visits to the novel object) – (number of visits to the familiar object). The use of a difference score has been validated by several researchers (Besheer, 2000 & Hannesson, 2004). It allows for a more accurate representation of the drug effect by accounting for individual differences in exploration during the test trial (Figure 20). Using the difference score, it was determined that the drug treatment had a significant effect; rats treated with nicotine visited the novel object significantly more during the test trial than those treated with saline. On average, the rats in the nicotine group visited the novel object three more times than the familiar object during the test trial, while rats treated with saline visited both objects roughly the same.

Our results, which show that acute nicotine administration facilitates object recognition memory acquisition, are consistent with the literature data. Furthermore our difference score is comparable to that obtained by Melicherick and colleagues (Melichercik, 2012). Most, if not all of the behavioral studies mentioned in the introduction, focus only on the behavioral changes facilitated by the administration of various drugs without attempting to elucidate the underlying neuronal mechanism responsible for these changes. In addition to supporting the hypothesis that nicotine stimulation of the cholinergic system facilitates memory acquisition, the results of our immunohistochemical study provide insight into the neuronal mechanisms responsible for these behavioral changes.

Immunohistochemistry

The intermediate-early gene, c-Fos, is maximally expressed ninety minutes after periods of intense neuronal activation, making it the gold standard for measuring neuronal activation (Kovacs, 1998 & Worley, 1991). The number of cells expressing c-Fos in the hippocampus and perirhinal cortex was combined in order to determine the effect of drug on neuronal activation. As expected, nicotine administration caused an overall increase in neuronal activation (Figure 22). When the hippocampus and perirhinal cortex were analyzed individually, nicotine caused a highly significant increase in c-Fos expression in the hippocampus, while only producing a nearly significant increase in the perirhinal cortex. The individual regions of the hippocampus were also examined and it was determined that nicotine administration caused a significant increase in neuronal activation in dentate gyrus (DG) and CA3, but not CA1. These results confirm the literature findings that a nicotine-facilitated enhancement of acquisition of object recognition memory is a hippocampal dependent process. Contrary to these results, some studies show that the hippocampus is more involved in object location rather than object recognition processes, while the opposite is true for the perirhinal cortex (Mumby, 1992 & Ennaceur, 1996). However, surgically disconnecting the hippocampus from the perirhinal cortex causes severe deficits in both object location and object recognition processes, demonstrating that communication between these two areas is necessary for both forms of memory formation (Warburton, 2003).

The discrepancy between the literature data and our data may be a result of the NOR protocol used in our study. As demonstrated in *figure 7* on page 35, three objects were used in our NOR protocol. During familiarization, objects "A" and "B" were placed kitty-corner to each other. During the test trial the locations of objects "A" and "B" were swapped and

object “B” was replaced by object “C”, thereby making “C” the novel object and “A” the familiar object. It is possible that changing the location of object “A” between the familiarization trial the test trial could have imparted object location novelty aspects to the familiar object, activating the hippocampal dependent spatial memory processes. Furthermore, the increased difference score caused by nicotine administration could be attributed to a combination of object location and object recognition memory formation. This means that object location memory processes could have been activated by both objects “A” and “C”, while object recognition memory processes were only activated by object “C” during the test trial. This could account for the significant increase in hippocampal activation, which is heavily involved in spatial recognition, and the almost significant PRh activation that is primarily involved in object recognition memory. It would be beneficial to control for object location if this experiment were to be repeated.

Timing is an important aspect of immunohistochemistry protocol design when measuring immediate early gene product expression in order to characterize neuronal activation. Several studies indicate that the maximum level of c-Fos expression occurs between one and three hours after periods of extended neuronal activation (Kovacs, 1998). Further research indicates that c-Fos levels may fluctuate within this two-hour window, with absolute maximum expression occurring at ninety minutes (Meyza, 2007). For this reason, we attempted to collect tissue samples from each animal approximately ninety minutes after drug administration.

Because the familiarization and test trials were only spaced two hours apart, we believe that we are measuring nicotine-facilitated changes in acquisition, rather than consolidation memory. This rational is due mainly to the fact that, according to the ERS

framework model of hippocampal activity, low levels of cholinergic activity during slow wave sleep are thought to set the stage for consolidation (Hasselmo, 2006). However, due to a lack of research investigating hippocampal dependent memory acquisition on c-Fos expression levels, we are unable to conclusively say that ninety minutes after injection was the ideal time to collect tissue. In future studies it would be beneficial to collect tissue at fifteen-minute intervals and determine the point at which c-Fos expression is at a maximum. This would allow for a more accurate representation of memory acquisition facilitated changes in c-Fos expression.

One of the highest concentrations of cholinergic cells is located in the medial septum (MS). As described in the introduction, the medial septum is responsible for sending a large amount of information to the DG. It was also hypothesized that nicotine administration would selectively increase the level of cholinergic activation in the medial septum (MS), since nicotine is an agonist for the cholinergic system and is capable of stimulating the release of acetylcholine. Previous research shows that it is possible to label cholinergic cells by probing with an antibody specific for choline acetyltransferase (ChAT), the enzyme responsible for the biosynthesis of acetylcholine (Armstrong, 1983). It is also possible to specifically label cholinergic neurons that have recently undergone a period of increased activation by dual staining with both c-Fos and ChAT antibodies (Modirrousta, 2004).

While the results of the c-Fos / ChAT dual staining show a trend that nicotine increases cholinergic activation in the MS, they are not statistically significant. This is most likely due to the small sample size. Since the medial septum is located directly in the center of the brain, each slide only yields one data set. The hippocampus and perirhinal cortex, on the other hand, yields two data sets per slice, one on each side of the brain. While the results

of the dual staining were insignificant when viewed alone, it was determined that the trend of nicotine facilitated increase in cholinergic activation was strongly correlated to the nicotine facilitated increase difference score using a Pearson correlation. This is the first study to physically link cholinergic activation in the MS with behavioral data showing an increase in hippocampal dependent processes, providing further evidence for a relationship between the cholinergic system and hippocampus. Since nAChRs are located both pre- and postsynaptically in the hippocampus and perirhinal cortex, they are thought to play a significant role in modifying synaptic plasticity, making it possible that these nicotine facilitated increases in neuronal activation and novel object recognition processes were a result of nAChR facilitated LTP (Gould, 2008).

Research Implications

The study of nicotine-facilitated memory enhancements extends far beyond the realm of behavioral studies in rats. For example, there is a large amount of research regarding the role of cholinergic system in Alzheimer's disease. As previously mentioned, cholinergic dysfunction is one of the hallmarks of AD.

Several transgenic animal models have been created to mimic the amyloid-driven neurodegeneration seen in humans. The Tg2576 mouse was one of the first transgenic mice models to express human APP (hAPP) and develop amyloid plaques. It has been repeatedly shown that Tg2576 mice have severe deficits in spatial memory as assessed by the MWM (Arendash, 2002). APP23 mice, like Tg2476 mice, express hAPP and display the histopathological and behavioral hallmarks of AD. Furthermore, APP23 mice display neuronal loss in the cholinergic system, making them useful for understanding the behavioral

manifestations of cholinergic dysfunction in AD. Like the Tg2476 mice, APP23 mice show deficits in spatial memory as assessed by the MWM (Kobayashi, 2005). These strains have also been examined using NOR tests. Studies reveal that both APP23 and Tg2476 transgenic mice display deficits in object recognition as assessed by the NOR test (Bruin, 2006 & Taglialatela, 2009)

While the first drugs approved to treat AD acted by inhibiting the degradation of ACh, the most recently approved therapeutic drug for the treatment of AD, galantamine (marketed as Nivalin), is an allosteric modulator of the α-subunit of nAChRs. By allosterically binding to the α-subunit, galantamine is able to enhance nAChR activity by increasing their sensitivity to endogenous ligands (Schroeder, 1994). Activation of α7-nAChRs has been shown to protect against glutamate-induced apoptosis, a common cause of neurodegeneration in AD (Takada, 2003). Furthermore, acute administration of galantamine causes an increase in neurotrophic growth factors such as insulin-like growth factor 2 in the mouse hippocampus. These effects were not seen when co-administered with methyllycaconitine, a selective α7-nAChR antagonist, suggesting the increase insulin-like growth factor 2 was caused by α7-nAChR activation (Kita, 2012).

Behavioral techniques have also been used to demonstrate the effects of galantamine on spatial memory formation in rats. For example, galantamine has been shown to attenuate scopolamine-induced deficits of spatial memory acquisition in rats, as assessed by both the MWM and T-Maze (Fishkin, 1993). Bruin and colleagues also demonstrated that 10mg/kg of galantamine reversed the effects of scopolamine induced cognitive deficits in a modified version of the NOR test (Bruin, 2006). Furthermore, galantamine administration has been show to improve MWM performance in APP23 mice (Van Dam, 2003 & Van Dam 2007).

The use of muscarinic agonists in AD treatment has also been extensively studied. As previously mentioned, one of the hallmarks of AD is the formation of amyloid plaques, which are composed of insoluble Aβ aggregates. These Aβ proteins are a produced by the proteolytic processing of the amyloid precursor protein (APP) by α, β, and γ-secretases. There are two possible outcomes of APP processing: if APP is first cleaved by β-secretase and then by γ-secretase, Aβ is produced. However, if APP is first cleaved by α-secretas, β-secretase is unable to cleave and no Aβ is produced. Therefore, inducing proteolytic processing by α-secretase provides a possible mechanism for inhibiting amyloid plaque formation. Several studies have shown that administration of M1 and M3 muscarinic agonists can decrease Aβ production (Esler, 2001). These effects are thought to be mediated by a variety of cell signaling processes such as activation of the protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) dependent pathways. Activation of these pathways ultimately results in increased α-secretase production, thereby causing a decrease in Aβ production (Haring, 1998). Interestingly, M2 and M4 receptor activation does not cause an increase in α-secretase production. M2 activation may even decrease α-secretase release. nAChR activation may also stimulate the production of α-secretase, making both nAChR and mAChR stimulation potentially useful for AD treatment (Fisher, 2008).

There are several studies that investigate nicotine's potential to act as a therapeutic AD drug. The two forms of Aβ, Aβ40 and Aβ42, are synthesized in healthy individuals. In AD there is a marketed increase in Aβ42 production that presents a problem; while Aβ40 is easily digested within the cell, the more hydrophobic Aβ42 is not, allowing it to be present for longer periods of time and aggregate into amyloid plaques. Aβ42 exist in an equilibrium of conformational states, folding into either α-helical monomers or β-sheet structures. The β-

sheet structures have a high propensity to aggregate into amyloid plaques, while the α-helical monomers are not thought to cause neurotoxic effects. Nicotine administration has been shown to inhibit the formation of amyloid plaques from β-sheet structures in vitro. While the exact mechanism by which this occurs is not fully understood, it is thought to be a result of nicotine binding to β-sheet structures and inhibiting aggregation (Zeng, 2001). There is also substantial evidence that several complex molecules help bind metals such as Cu2+, Fe2+, and Zn2+ to Aβ42. This metal binding induces it to assume the β-sheet conformation. Research shows that nicotine administration is able to stabilize metal ion homeostasis, decreasing the amount of ions available for Aβ42 binding, effectively lowering the amount of Aβ42 in the β-sheet conformation (Zhang, 2006).

The cholinergic system is an unbelievably complex system, and the brain areas that it influences are quite widespread. There is still a large amount of research that needs to be conducted in order to fully understand all of its responsibilities. As I have shown in the pages above, nicotine is able to stimulate the cholinergic system and enhance memory acquisition processes. So while nicotine often gets a bad reputation due to its addictive qualities, it may one day offer significant therapeutic benefits.

References

Acquas, E., Wilson, C., Fibiger, H. C. (1996). Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: Effects of novelty, habituation, and fear. *The Journal of Neuroscience*, 76(9), 3089-309.

Akaike, A., Takada, Y., Kume, T., Izumi, Y. (2010). Mechanisms of neuroprotective effects of nicotine and acetylcholinesterase inhibitors: Role of a4 and a7 receptors in neuroprotection. *Journal of Molecular Neuroscience*, 40(1-2), 211-216.

Albuquerque, E. X., Pereira, E. F., Mike, A., Eisenberg, H. M., Maelicke, A., Alkondon, M. (2000). Neuronal nicotinic receptors in synaptic functions in humans and rats: physiological and clinical relevance. *Behavioral Brain Research*, 113(1-2):131-41.

Alkondon, M., Albuquerque, E. X. (2001). Nicotinic acetylcholine receptor alpha7 and alpha4beta2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *Journal of Neurophysiology*, 86(6), 3043-3055.

Allen, T. G. J., Abogadie, F. C., and Brown, D. A. (2006). Simultaneous release of glutamate and acetylcholine from single magnocellular "cholinergic" basal forebrain neurons, *The Journal of Neuroscience,* 26(5), 1588 –1595.

Anderson, P., Morris, R., Amaral, D., Bliss, T., O'Keefe, J. (2007). *The hippocampus book*. Oxford, UK: Oxford Neuroscience Series.

Arendash, G. W., King, D. L. (2002). Intra- and intertask relationships in behavioral test battery given to Tg2576 transgenic mice and controls. *Physiological Behavior,* 75(5), 643-652.

Armstrong, D. M., Saper, C. B., Levey, A. I., Wainer, B. H., Terry, R. D. (1983). Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. *The Journal of Comparative Neurology,* 216(1), 53-68.

Attwell, D., Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism*, 21(10), 1133-1145

Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., MacVicar, B. A., Newman, E. A. (2010). Glial and neuronal control of brain blood flow. *Nature,* 468(7321), 232-243.

Avdulov, N. A., Chochina, S. V., Igbavboa, U., O'Hare, E. U., Schroeder, F., Cleary, J. P., Wood, W. G. (1997). Amyloid beta-peptides increase annular and bulk fluidity and induce lipid peroxidation in brain synaptic plasma membranes. *Journal of Neurochemistry*, 68(5), 2068-2091.

Baddeley, A. (1992). Working memory. *Science*, 255(5044), 556-559.

Baddeley, A., Gathercole, S. (1998a). The phonological loop as a language learning device. *Psychological review*, 105(1), 158-173.

Baddeley, A. (1998b). The central executive: A concept and some misconceptions. *Journal of the International Neuropsychological Society*, 4(5), 523-526.

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends in cognitive Science,*

Baddeley, A., Wilson, B. A. (2002). Prose recall and amnesia: implications for the structure of working memory. *Neuropsychologia,* 40(10), 1737 – 1743.

Baddeley, A. (2003). Working memory: looking back and looking forward. *Nature*, 4, 829-893.

Baddeley, A. D., Kopelman, M. D., Wilson, B. A., (2004). Essential handbook of memory disorders for clinicians. Hoboken, NJ: Wiley.

Barker, G. R. I., Warburton, E. C. (2011). When is the hippocampus involved in recognition memory? *Neuroscience*, 31(29), 10721-10731.

Besheer, J., Bevins, R. A. (2000). The role of environmental familirization in novel-object preference. *Behavioral Processes*, 50, 19-29.

Besheer, J., Short, K. R., Bevins, R. A. (2001). Dopaminergic and cholinergic antagonism in a novel-object detection task with rats. *Behavioral Brain Research*, 126(1-2), 211-217.

Bliss, T. V., Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, 361(6407), 31-39.

Bossy-Wetzel, E., Schwarenzbacher, R., Lipton, S. A. (2004). Molecular pathways to neurodegeneration. *Nature,* S2-S8.

Bozon, B., Kelly, A., Josselyn, S. A., Silva, A. J., Davis, S., & Laroche, S. (2003). MAPK, CREB and zif268 are all required for the consolidation of recognition memory. *Philosophical Transactions of the Royal Society,* 358(1432), 805-814.

Broadbent, N. J., Gaskin, S., Squire, L. R, & Clark, R. E. (2009). Object recognition memory ad the rodent hippocampus. *Learning & Memory*, 17(1), 5-11.

Broadbent, N. J., Squire, L. R., Clark, R. E. (2006). Reversible hippocampal lesions disrupt water maze performance during both recent and remote memory tests. *Learning and Memory*, 13(2), 187-191.

Brown, M. W., Xiang, J. Z. (1997). Recognition memory: neuronal substrates of the judgment of prior occurrence. *Progress in Neurobiology*, 55, 149-189.

Brown, M. W., Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and the hippocampus? *Nature Reviews*, 2, 51-61.

Bruin, D. N., Pouzet, B. (2006). Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: Deficits induced by scopolamine and by

prolonging the retention interval. *Pharmacology, Biochemistry and Behavior,* 85(1), 253-260.

Cai, L., Gibbs, R. B., Johnson, D. A. (2012). Recognition of novel objects and their locations in rats with selective cholinergic lesions of the medial septum. *Neuroscience Letters*, 506, 261-265.

Canning, K. J., Leung, L. S. (1997). Lateral entorhinal, perirhinal, and amygdala-entorhinal transition projections to hippocampal CA1 and dentate gyrus in the rat: a current source density study. *Hippocampus*, 7(6), 643-655.

Caponga, M. (2011). Neurogliaform cells and other interneurons of stratum lacunosum-molecular gate entorhinal-hippocampal dialogue. *Journal of Physiology,* 589(8), 1875-1883.

Carlson, N. R. (2010). Physiology of Behavior, Tenth Edition. Boston, MA: Allyn & Bacon.

Caulfield, M. P., Birdsall, N. J. M. (1998). International union of pharmacology, xvii. Classification of muscarinic acetylcholine receptors. *Pharmacological Review*, 50(2), 279-290.

Chang, Q. & Gold, P. E. (2004). Impaired and spared cholinergic functions in the hippocampus after lesions of the medial septum/vertical limb of the diagonal band with 192 IgG-saporin. *Hippocampus*, 14(2), 170-179.

Changeux, J. P., Bertrand, D., Corringer, P. J., Dehaene, S., Edelstein, S., Lena, C., Novere, N. L., Marubio, L., Picciotto, M., Zoli, M. (1998). Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. *Brain Research Reviews*, 26(2-3), 198-216.

Chemero, A., & Heyser, C. (2005). Object exploration and a problem with reductionism, *Syntheses*,147, 403–23.

Chiu, R., Boyle, W., Meek, J., Smeal, T., Hunter, T., & Karin, M. (1988). The c-Fos protein interacts with c-Jun / AP – 1 to stimulate transcription of AP – 1 responsive genes. *Cells,* 54(4), 541 – 552.

Christopoulos, L., Lanzafame, A., Mitchelson, F. (1998). Allosteric interactions at muscarinic choline receptors. *Clinical Experiments in Pharmacological Physiology*, 25, 185-194.

Clarke, P. B. S., Schwartz, R. D., Paul, S. M., Pert, C. B., Pert, A. (1985). Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H] nicotine, and [^{125}I]-a-bungarotoxin. *The Journal of Neuroscience*, 5(5), 1307-1315.

Cocchini, G., Logie, R. H., Della Sala, S., Macpherson, S. E., Baddeley (2002). Concurrent performance of two memory tasks: evidence for domain- specific working memory systems. *Memory & Cognition*, 30(7), 1087-1095.

Curzon, P., Anderson, D. J., Nikkel, A. L. Fox, G. B., Gopalakrishnan, M., Decker, M. W., Bitner, R. S. (2006). Antisense knockdown of the rat a7 nicotinic acetylcholine receptor produces spatial memory impairment. *Neuroscience Letters*, 410(1), 15-19.

Darvesh, S., Hompkins, D. A., Geula, C. (2002). Neurobiology of butyrylcholinesterase. *Nature Neuroscience,* 4,131,138.

Daumas, S., et al. (2005). Encoding, consolidation, and retrieval of contextual memory: differential involvement of dorsal CA3 and CA1 hippocampal subregions, *Learning and Memory*, 12(4), 375-382.

Davis, J. A., Gould, T. J. (2009). Hippocampal nAChRs mediate nicotine withdrawal related learning deficits, *European Neuropsychopharmacology*, 19(8), 551–561.

Davis, T. J., Fiebre, C. M. (1999). Alcohol's actions on neuronal nicotinic acetylcholine receptors. *Expert Reviews in Molecular Medicine,* 1-12.

Dawkins, L., Turner, J., Hasna, S. and Soar, K. (2012). The electronic- cigarette: Effects on desire to smoke, withdrawal symptoms and cognition, *Addictive Behaviors,* doi: 10.1016/j.addbeh.2012.03.004

Decker, M. W. & Majchrzak, M. J. (2000). Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotinic cholinergic antagonist, on spatial memory in rats. *Psychopharmacology*, 107(4), 530-534.

Decker, M. W., Rueter, L. E., Bitner, R. S. (2004). Nicotinic acetylcholine receptor agonists: a potential new class of analgesics. *Current Topics in Medicinal Chemistry,* 4(3), 369-384

Delacourte, A. & Buea, L. (2000). Tau Pathology: A marker of neurodegenerative disorders. *Current Opinions in Neurology*, 13, 371-376.

Dencker, D., Wortwein, G., Weikop, P., Jeon, J., Thomsen, M., Sager, T. N., Mork, A., Woldbye, D. P. D., Wess, J., Jensen, A. F. (2011). Involvement of a subpopulation of neuronal M4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M1/M4 preferring muscarinic receptors agonist xanomelin. *The Journal of Neuroscience*, 31(16), 5905 – 5908.

Diniz, L. S., & Oliveria, P. M. A. (1999). Clinical problems of sloths, *bradypus* sp. and *choloepus* sp. in captivity. *Journal of Zoo and Wildlife Medicine*, 30,76-80.

Do, V. H., Martinez, C. O., Martinez, J. L., Derrick, B. E. (2002). Long-term Potentiation in direct perforant path projections to the hippocampal CA3 region in vivo. *Journal of Neuropsychology,* 87(2), 669-678.

Dodart, J. C., Mathis, C., Ungerer, A. (1997). Scopolamine-induced deficits in a two-trial object recognition task in mice. *NeuroReport*, 8(5), 1173-1178.

Douglas, R. J., Martin, K. A. C. (1990), Synaptic organization of the brain. *Neocortex,* 389-399.

Easton, A., Webster, L. A., Eacott, M. J. (2012). A specific role for septohippocampal acetylcholine in memory? *Neuropsychologia,* 50(13) 3156-3168.

Eglen, R. M. (2006). Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. *Auton Autacoid Pharmacology,* 26(3), 219-233.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behavioral Brain Research,* 215(2), 244-254.

Ennaceur, A., Aggleton, J. P. (1996). The effects of neurotoxin lesions of the perirhinal cortex combined to fornix transaction on object recognition memory in the rat. *Behaviroal Brain Research*, 88, 181-193.

Eschenko, O., Ramadan, W., Mölle, M., Born, J., Sara, S. J. (2008). Sustained increase in hippocampal sharp-wave ripple activity during slow-wave sleep after learning. *Learning and Memory*, 15(4), 222-228.

Esler, W. P., Wolfe, M. S. (2001). A portrait of Alzheimer secretases – new features and familiar faces, *Science*, 293(5534), 1449-1454.

Eustache, F., Rioux, P., Desgrages, B., Baron., J. C. (1995). Healthy aging memory subsystems and regional cerebral oxygen consumption. *Neurophychologia,* 33(7), 867-887.

Farlow, M. R. (2001). Pharmacokinetic profiles of current therapies for Alzheimer's disease: implications for switching to Galantamine, *Clinical Therapies,* 23, A13-A24.

Farr, S. A., Banks, W. A., La Scola, M. E., Flood, J. F., & Morley, J. E. (2000). Permanent and temporary inactivation of the hippocampus impairs T-maze foot shock avoidance acquisition and retention. *Brain Research*, 872(1-2), 242-249.

Fisher, A. (2008). Cholinergic treatments with emphasis on M1 muscarinic agonists as potential disease-modifying agents for Alzheimer's disease. *Journal of the American Society for Experimental NeuroTherapeutics*, 5(3), 433 - 442.

Fishkin, R. J., Ince, E. S., Carlezon, W. A., Dunn, R. W. (1993). D-cycloserine attenuates scopolamine-induced learning and memory deficits in rats. *Behavioral and Neural Biology*, 59(2), 150-157.

Freund, T. F., Buzsaki, G. (1996) Interneurons of the hippocampus. *Hippocampus,* 6(4)*, 347-470.*

Gabrieli, J. D. E. (1998). Cognitive neuroscience of human memory. *Annual Review of Psychology,* 49, 87-115.

Gais, S., Born, J. (2003). Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation *Proceedings of the National Academy of Sciences,* 101(7), 2140-2144.

Gaskin, S., Tardif, M., Cole, E., Piterkin, P., Kayello, L., & Mumby, D. G. (2010). Object familiarization and novel-object preference in rats. *Behavioral Processes,* 83, 61-71.

Gathercole, S. E. (1999). Cognitive approaches to the development of short-term memory. *Trends in Cognitive Sciences*, 3(11), 410-419.

Gazzaniga, M. S., Ivry, R. B., Mangun, G. R., Steven, M. S. (2009). Cognitive neuroscience: The biology of the mind, 3rd edition. New York City, NY: Norton & Company.

Gray, J. A, Mitchell, S. N., Joseph, M. H., Grigoryan, G. A., Dawe, S., & Hodges, H., (1994). Neurochemical mechanisms mediating the behavioral and coactive effects of nicotine. *Drug Development*, 31, 3-17.

Gray, P. O. (2006). Psychology, 5th edition. Boston, MA: Worth Publishers.

Gray, R., Rajan, A. S., Radcliffe, K. A., Yakehiro, M. & Dani, J. A. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature*, 383, 713–716.

George, T. P., Vessicchio, G. C., Termine, A., Sahady, D. M., Head, C. A., Pepper, W. T., Kosten, T. R., Wexler, B. E. (2002). Effects of smoking abstinence on visuospatial working memory function in Schizophrenia. *Neuropsychopharmacology,* 26(1), 75-86.

Gould, T. J., Wehner, J. M. (2008). Nicotine enhancement of contextual fear conditioning, *Behavioral Brain Research*, 102(1), 31-39.

Gould, T. J., Davis, J. A. (2009). Hippocampal nAChRs mediate nicotine withdrawal related learning deficits, *European Neuropsychopharmacology*, 19(8), 551-561.

Hannesson, D. K., Howland, J. G., Phillips, A. G. (2004). Interactions between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *The Journal of Neuroscience*, 24(19), 4596-4604.

Harring R., Fisher, A., Marciano, D., Pittle, Z., Kloog, Y., Zuckerman, A., Eshhar, N., Heldman, E. (1998). Mitogen-activated protein kinase-dependent and protein kinase C-dependent pathways link the m1 muscarinic receptor to beta-amyloid precursor protein synthesis. *Journal of Neurochemistry*, 71(5), 2094-2103.

Hasselmo, M. E., Anderson, B. P., Bower, J. M. (1992). Cholinergic modulation of cortical associative memory function. *Journal of Neurophysiology*, 67(5), 1230-1246.

Hasselmo, M. E. (2004). Runaway synaptic modification in models of cortex: implications for Alzheimer's disease. *Neural Networks,* 7(1), 13-40.

Hasselmo, M. E., Schnell, S., Barkai, E. (2005). Dynamics of learning and recall at excitatory recurrent synapses and cholinergic modulation in rat hippocampal region ca3. *Journal of Neuroscience*, 15(7), 5249-5262.

Hasselmo, M. E., McGaughy, J. (2006). High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. *Progress in Brain Research*, 145, 207–231.

Heber, S., Herms, J., Gajic, V., Hainfellner, J., Aguzzi, A., Rulicke, T., von Kretzschmar, H., von Koch, C., Sisodia, S., Tremml, P., Lipp, H. P., Wolfer, D. P., & Muller, U., (2000). Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. *Journal of Neuroscience*, 20(21), 7951–7963.

Heffernan, T. M., Ling, J., Parrott, A. C., Buchanan, T., Scholey, A. B., Rodgers, J. (2005). Self-rated everyday and prospective memory abilities of cigarette smokers and non smokers: a web-based study. *Drug and Alcohol Dependence*, 78(3), 235-241.

Hensely, K., Carney, J. M., Mattson, M. P., Aksenova, M., Harris, M., Wu, J. F., Floyd, R. A., Butterfield, D. A. (1994). A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. *Proceedings of the National Academy of Science,* 91(8), 3270-3274.

Hollis, F., Cruz, M. G., Carrier, N., Dietz, D. M., & Kabbaj, M. (In Press) Juvenile and adult rats differ in cocaine reward and expression of ZIF268 in the forebrain, *Neuroscience*, doi: 10.1016.

Hossmann, K. A. (1994). Viability thresholds and the penumbra of focal ischemia. *Annals of Neurology,* 36(4), 557-565.

Howes, M. J., Houghton, P. J. (2009). Traditional medicine for memory enhancement. *Ethnomedicine,* 239-291.

Hunter, A. J. & Roberts, F. F. (1988). The effects of pirenzepine on spatial learning in the Morris water maze. *Pharmacology Biochemistry and Behavior*, 30(2), 519-523.

Inglis F. M., Day, J. C., Fibiger, H. C. (1994). Enhanced acetylcholine release in hippocampus and cortex during the anticipation and consumption of a palatable meal. *Neuroscience*, 62(4), 1049-1056.

Ito, Y., Oyunzul, L., Seki, M., Fujino, O. T., Matsui, M., Yamada, S. (2009). Quantitative analysis of the loss of muscarinic receptors in various peripheral tissue in M1-M5 receptor single knockout mice. *British Journal of Pharmacology*, 156(7), 1147-1153.

Jacobs, G. H., Fenwick, J. A., Williams, G. A. (2001). Cone-based vision of rats for ultraviolet and visible lights. *Journal of Experimental Biology*, 204(14), 2439–2446.

Jackish, R., Forster, S., Kammerer, M., Rothmaier, A. K., Ehret, A., Zentner, J., Feuestein, T. J. (2009). Inhibitory potency of cholinesterase inhibitors on acetylcholine release and cholinesterase activity in fresh specimens of human and rat neocortex. *Journal of Alzheimer's Disease*, 16(3), 635-647.

Jadhav, S. Banerjee, A. Bhawalker, J. (2012). Limitations of activation in patients with musculoskeletal disorders. *Anneals in Medical Health Science Research,* 2(1), 5-9.

Janas, A. M., Sunninghan, S. C., Duffy, K. B., Bevan, B. D., Grieg, N. H., Spangler, E. L. (2005). The cholinesterase inhibitor, phenserine, improves Morris water maze performance of scopolamine-treated rats. *Life Sciences*, 76(10), 1073-1081.

Jellinger, K. A. (2007). Morphologic diagnosis of "vascular dementia" – A critical update. *Journal of the Neurological Sciences*. 270(1-2), 1-12

Jones, C. K., Byun, N., Bubser, M. (2012). Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of Schizophrenia. *Neurophsychopharmacology Review,* 37(1) 16-42.

Kamenetz, F. Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S., Malinow, R. (2003). APP processing and synaptic function, *Neuron,* 37(6), 925-297.

Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K., Muller-Hill, B. & (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell–surface receptor. *Nature*, 325(1606), 733–736.

Kenney, J. W., Gould T. J. (2008). Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Molecular Neurobiology*, 38(1), 101-121.

Kim, K., Swanson, L. (2007). Working memory, short-term memory, and naming speed as predictors of children's mathematical performance. *Intelligence*, 35(2), 151-168.

Kita, Y., Ago, Y., Takano, E., Fukada, A., Takuma, K., Matsuda, T. (2012). Galantamine increases hippocampal insulin-like growth factor 2 expression via α7 nicotinic acetylcholine receptors in mice. Psychopharmacology, online.

Kobayashi, D. T., Chen, K. S. (2005). Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes, Brain, and Behavior*, 4(3), 173-196.

Kovacs, K. J. (1998). c-Fos as a transcription factor: A stressful review from a functional map. *Neurochemistry International,* 33(4), 287-297.

Kumari, V., Gray, J. A., Ffytche D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C. R, Simons, A., & Sharma, T. (2003). Cognitive effects of nicotine in humans: an fMRI study. *Neuroimage*, 19(3), 1002-1013.

Levin, E. D. (2002). Nicotinic receptor subtypes and cognitive function. *Journal of neurobiology*, 53, 633-640.

Lindquist, D. H., Sokoloff, G., & Steinmetz, J. E. (2007). Ethanol-exposed neonatal rats are impaired as adults in classical eye-blink conditioning at multiple unconditioned stimulus intensities. *Brain Research*, 1150, 155-166.

Liu, P., Bilkey, D. K. (1996). Direct connection between perirhinal cortex and hippocampus is a major constituent of the lateral perforant path. *Hippocampus*, 6(2), 125-135.

Liu, Y., Wang, Y., Sun, X., & Wang, H. (2004). Evidence that muscarinic receptors are involved in nicotine-facilitated spatial memory. *Pharmacology, Biochemistry and Behavior,* 78(4), 775-779.

Marco-Contelles, J., Carreiras, M. C., Rodriguez, C. R., Vellarroya, M., Garcia, A. G. (2006). Synthesis and pharmacology of Galantamine. *Chemical Reviews*, 106(1), 116-133.

Mattson, M. P. (2004). Pathways towards and away from Alzheimer's disease. *Nature*, 430, 631-639.

Mark, R. J., Lovell, M. A., Markesbery, W. R., Uchida, K., Mattson, M. P. (1997). A role for 4-hydroxylnonenal, and aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. *International Society for Neurochemistry*, 68(1), 225-264.

McDonald, C. R., Thesen, T., Carlson, C., et al. (2010). Multimodal imaging of repetition priming: using fMRI, MEG, and intracranial EEG to reveal spatiotemporal profiles of word processing. *Neuroimage*, 53(2), 707-717.

Mendrek, A., Monterosso, J. (2006). Working memory in cigarette smokers: Comparison to non-smokers and effects of abstinence, *Addictive Behaviors,* 31(3), 833–844.

Melichercik, A. M., Elliott, K. S., Bianchi, C., Ernst, S. M., Winters, B. D (2012). Nicotinic receptor activation in perirhinal cortex and hippocampus enhances object memory in rats. *Neuropharmacology,* 62(5-6), 2096-2105.

Meunier, M., Bachevalier, J., Mishkin, M., Murry, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *The Journal of Neuroscience*, 13(12), 5418-5432.

Meyza, K. Z., Boguszewski, P. M., Nikolaev, E., Zagrodzka, J. (2007). The effects of age on the dynamics and the lefel of c-Fos activation in response to acute restraint in Lewis rats. *Behavioral Brain Research*, 180(2), 183-189.

Michaelis, M. L. (2003). Drugs targeting Alzheimer's disease: some things old and some things new. *Journal of Pharmacology Experimental Therapy*, 304(3), 897-904.

Milner, G. A. (1956). The magic number seven. *The Psychological Review*, 63(2), 81-97.

Modirrousta, M., Mainville, L., Jones, B. E. (2004). GABAergic neurons with a2-Adrenergic receptors in basal forebrain and pre-optic area express c-Fos during sleep. *Neuroscience*, 129(3), 803-810.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods,* 11, 47-60.

Morrow, L. A & Ryan, C. (2002). Normative data for a working memory test: the four word short-term memory test. *The Clinical Neuropsychologist,* 16(3), 373-380.

Mumby, D. G., Pinel, J. P., Wood, E. R. (1990). Nonrecurring-items delayed non matching to sample in rats: a new paradigm for testing non-spatial working memory. *Psychobiology*, 18(3), 321-326.

Mumby, D. G., Pinel, J. P., Wood, E. R. (1992). Object-recognition memory is only mildly impaired in rats with lesions of the hippocampus and amygdala. *Psychobiology,* 20(1), 18-27.

Murray, E. A., Richmond, B. J. (2001). Role of perirhinal cortex in object perception, memory, and associations. *Current Opinions in Neurobiology*, 11, 188-193.

Myhrer, T. (2003). Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis based on studies of four behavioral tasks. *Brain Research Reviews*, 41(2-3), 268-287.

Nelson, C. A. & Luciana, M. (2008). Handbook of developmental cognitive neuroscience. Cambridge, MA: MIT Press.

Nilsson, O. G., Leanza, G., Rosenblad, C., Lappi, D. A., Wiley R. G., Bjorklund, A. (1992). Spatial learning impairment in rats with selective immunolesion of the forebrain cholinergic system. *Neuroreport,* 3(11), 1005-1008.

Nuutinen, S., Barik, J., Jones I. W., Wonnacott, S. (2007). Differential effects of acute and chronic nicotine on EIK-1 in rat hippocampus. *Molecular Neuroscience,* 18(2), 121-126.

O'Neill, J., Pleydell-Bouverie, B., Dupret, D., Csicsvari, J. (2010). Play it again: reactivation of waking experience and memory. *Trends in Neurosciences*, 33(5), 220-229.

Oki, T., Takagi, Y., Inagaki, S., Taketo, M. M., Manabe, T., Matsui, M., Yamada, S. (2005). Quantitative analysis of binding parameters of [^{3}H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptors in knockout mice. *Molecular Brain Research*, 133(1), 6-11.

Parker A., Gaffan D. (1998). Interactions of frontal and perirhinal cortices in visual object recognition memory in monkeys. *European Journal of Neuroscience*, 10(10), 3022-3057.

Paxinos, G. & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates*, 4th ed. New York, NY. Reed Elsevier.

Pidoplichko, V. I., DeBiasi, M., Wiliams, J. T., Dani, J. A. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature*, 390(6658), 401-404.

Penfield, W., Milner, B. (1958). Memory deficit produced by bilateral lesions in the hippocampal zone. *AMA Architectural Neurology and Psychiatry*, 79(5), 475-497.

Picciotto, M. R., Zoli, M. (2008). Neuroprotection via nAChRs: the role of nAChRs I neurodegenerative disorders such as Alzheimer's and Parkinson's disease. *Frontiers in Bioscience* 13(1), 492–504.

Power, A. E. (2004). Slow-wave sleep, acetylcholine, and memory consolidation. *Proceedings of the National Academy of Sciences,* 101(7), 1795-1796.

Prickaerts, J., Van Straveren, W. C. G., Sik, M., Markerink-Van Ittersum, M., Niewohner U., Van Der Staay, F. J., Blokland, A., De Vente, J. (2002). Effects of two selective phosphodiesterase type 5 inhibitors, sildenafil and vardenafil, on object recognition memory and hippocampal cyclic GMP levels in the rat. *Neuroscience*, 113(2), 351-361.

Prickaerts, J., Sik, M., Van Der Staay, F. J., De Vente, J., Blokland, A. (2005). Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. *Psychopharmacology,* 177(4), 381-390.

Priller, C., Bauer, T., Mitteregger, G., Bjarne, K., Kretzschmar, H. A., Herms, J. (2006). Synapse formation and function is modulated by the amyloid precursor protein. *The Journal of Neuroscience*. 26(27), 7212-7221.

Puryes, D. (2007). Principles of cognitive neuroscience. Sunderland, MA: Sinauer Associates Inc.

Quik, M., Huang, L. Z., Parameswaran, N., Bordia, T., Campos, C., & Perez, X. A. (2009). Multiple roles for nicotine in Parkinson's disease. *Biochemical Pharmacology*, 78(77), 677-685.

Radcliffe, K. A., Fisher, J. L., Gray, R., Dani, J. A. (1999). Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurons. *Annals of the New York Academy of Sciences*, 868, 591-610

Ramadan, W., Eschenko, O., Sara, S. J. (2009). Hippocampal sharp wave/ripples during sleep for consolidation of associative memory, *PLoS ONE* 4(8).

Riekkinen, M. & Riekkinen, P., Jr. (1997). Dorsal hippocampal muscarinic acetylcholine and NMDA receptors disrupt water maze navigation. *NeuroReport*, 8(3), 645-648.

Rose, J. E., Behm, F. M., Westman, E. C., & Johnson, M. (2000). Dissociating nicotine and non-nicotine components of cigarette smoking. *Pharmacology, Biochemistry and Behavior*, 67(1), 71- 81.

Schacter, D. L., Buckner, R. L. (1998). Priming and the Brain. *Neuron*, 20, 185-195.

Schacter, D. L., Badgaiyan, R. D. (2001). Neuroimaging of priming: New perspectives on implicit and explicit memory. *Current Directions in Psychological Science*, 10(1), 1-4.

Schroder, B., Reinhardt-Maelicke, S., Schrattenholz, A. (1994). Monoclonal antibodies FK1 and WF6 define two neighboring ligand binding sites on torpedo acetylcholine receptor α-polypeptide. *Journal of Biochemistry*, 269(14), 10407-10416.

Selkoe, D. J., Podlisny, M. B., Joachim, C. L., Vickers, E. A., Lee, G., Fritz, L. C., Oltersdorf, T. (1988). Beta-amyloid precursor protein of Alzheimer disease occurs as 110–135-kilodalton membrane-associated proteins in neural and non-neural tissues. *Proceedings of the National Academy of Sciences*. 85(19), 7341– 7345.

Seppa, T., Salminen, O., Moed, M., & Ahtee, L. (2001). Induction of Fos immunostaining by nicotine and nicotinic receptor antagonists in rat brain. *Neuropharmacology*, 41(4), 486-495.

Shantanu, P., Jadhav, L. Kemere, C., German, P. W., Frank, L. M. (2012). Awake hippocampal sharp-wave ripples support spatial memory. *Science*, 336(6087), 1454-1458.

Shen, Z. X. (2004). Brain cholinesterases: II. The molecular and cellular basis of Alzheimer's disease. *Medical Hypotheses*, 63(2), 308-321.

Shiromani, P. J., & Gillin, C. (1998). Acetylcholine and the regulation of REM sleep: Basic mechanisms and clinical implications for affective illness and narcolepsy. *Annual Reviews in Pharmacological Toxicology. 27, 137-156.*

Shram, M. J., Funk, D., Li, Z., & Le, A. D. (2007). Acute nicotine enhances c-Fos mRNA expression differentially in reward-related substrates of adolescent and adult rat brain. *Neuroscience Letters,* 418(3), 286-291.

Smith, E., Jonides, J., & Koeppe, R. A., (1996). Dissociating verbal and spatial working memory using PET. *Cerebral Cortex*, 6(11-20), 1047-3211.

Snowdon, D. A., Greiner, L. H., Mortimer, J. A., Riley, K. P, Greiner, P. A., Markesbery, W. R. (1997). Brain Infarction and the clinical Expression of Alzheimer Disease. *Journal of the American Medical Association*. 227(10), 813-817.

Sutherland, G. R., McNaughton, B. (2000). Memory trace reactivation in hippocampal and neocortical neuronal ensembles. *Current Opinion in Neurobiology*, 10, 180–186.

Swanson, L., Kim, K. (2007). Working memory, short-term memory, and naming speed as predictors of children's mathematical performances. *Intelligence*, 35(2), 151-168.

Sweet, L. H., Mulligan, R. C., Finnerty, C. E., Jerskey, B. A., David, S. P., Cohen, R. A., Niaura, R. S. (2010). Effects of nicotine withdrawal on verbal working memory and associated brain response. *Psychiatry Research,* 183(1), 69-74.

Taglialatela, G., Hogan, D., Z, W. R., Dinely, K.T. (2009). Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. *Behavioral Brain Research,* 200(1), 95-99.

Takada, Y., Yonezawa, A., Kume, T., Katsuki, H., Sugimoto, H., Akaike. A. (2003). Nicotinic acetylcholine receptor-mediated Neuroprotection by donepezil against glutamate neurotixicity in rat cortical neurons. *Journal of Pharmacological Experimental Therapy,* 306(2), 772-777.

Teather, L. A., Packard, M. G., Smite, D. E., Ellis-Behnke, R. G., Bazan, N. G. (2005). Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks. *Neurobiology of Learning and Memory,* 84(4), 75-84.

Theil, C. M., Huston, J. P., Schwarting, R. K. W. (1998). Hippocampal acetylcholine and habituation learning. *Neuroscience*, 85(4), 1253-1262.

Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P. A., Wood, E. R., Witter, M. P. & Morris, R. G. M. (2007). Schemas and memory consolidation. *Science,* 316(5821), 76-82.

Van Broeckhoven, C . (1995). Presenilins and Alzheimer disease. *Nature*, 11, 230 – 232.

Van Dam, D., D'Hooge, R. Staufenbiel, M., Van Ginneken, C., Van Meir, F., De Deyn, P. P. (2003). Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. *European Journal of Neuroscience*, 17(2), 388-396.

Van Dam, D., Abramowski, D., Staufenbiel, M., De Deyn, P. P. (2007). Symptomatic effect of donepezil, Rivastigmine, galantamine, and memantine on cognitive deficits in the APP23 model. *Psychopharmacology Berlin,* 180(1), 177-190.

Van Lieshout, J. J., Wieling, W., Karemaker, J. M., & Secher, N. H. (2003). Syncope, cerebral perfusion, and oxygenation. *Journal of Applied Physiology,* 94(3), 833-848.

Vansickel, A. R., Cobb, C. O., Weaver, M. F., & Eissenberg, T. E. (2010). A clinical laboratory model for evaluating the acute effects of electronic 'cigarettes': Nicotine delivery profile and cardiovascular and subjective effects. *Cancer Epidemiology*, Biomarkers & Prevention, 19(8), 1945-1953.

Vidal, C. & Changeux, J. P. (1993). Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. *Neuroscience*, 56(1), 23-32.

Volpe, B. T., Waczek, B., and Davis, H. P. (1998). Modified T-maze training demonstrates dissociated memory loss in rats with ischemic hippocampal injury. *Behavioral Brain Research*, 27(3), 259-268.

Walsh, T. J., Kelly, R. M., Dougherty, K. D., Stackman, R. W., Wiley, R. G., Kutscher, C. L. (1995). Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: cholinergic and non-cholinergic effects following i.c.v. injection. *Brain Research,* 702(1-2), 233-245.

Wan, H., Aggleton, J. P. & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *Journal of Neuroscience*, 19(3), 1142–1148.

Warburton, E. C., Koder, T., Cho, K., Massey, P. V., Duguid, G., Barker G. R. I., Aggleton, J. P., Bashir, Z. I., Brown, M. W. (2003). Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron*, 38(6), 987-996.

Weis, S., Klaver, P., Reul, J., Elger, C. E., Fernandez, G., (2004). Temporal and cerebellar brain regions that support both declarative memory formation and retrieval. *Cerebral Cortex*, 14, 256-267.

Wiig, K. A., Bilkey, D. K. (1994). The effects of perirhinal cortical lesions on spatial reference memory in the rat. *Behavioral Brain Research,* 63(1), 101-109.

Wilson, F. A. W., Rolls, E. T. (1990). Neuronal responses related to reinforcement in the primate basal forebrain. *Brain Research*, 509(2), 312-231.

Winters, B. D. & Bussey, T. J. (2005). Removal of cholinergic input to perirhinal cortex disrupts object recognition, but not spatial working memory in the rat. *European Journal of Neuroscience*, 21(8), 2263-2270.

Winters, B. D., Saksida, L. M., Bussey, T. J. (2006). Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: implications for cholinergic system function. *Journal of Neuroscience*, 26(37), 9520-9529.

Winters, B. D., Bartko, S. J., Saksida, L. M., Bussey, T. J. (2007). Scopolamine infused into perirhinal cortex improves object recognition memory by blocking the acquisition of interfering object information. *Learning and Memory*, 14(9), 590–596.

Worley, P. F., Christy, B. A., Nakabeppu, Y., Bhat, R. V., Cole, A. J., & Baraban, J. M. (1991). Constitutive expression of ZIF268 in neocortex is regulated by synaptic activity. *Neurobiology*, 88(12), 5106-5110.

Yamada, M., Lamping, K. G., Duttary, A., Zhang, W., Cui, Y., Bymaster, F. P., Faraci, F. M., Wess, J. (2001). Cholinergic dilation of cerebral blood vessels is abolished in M5 muscarinic acetylcholine receptor knockout mice. *Proclamation of the National Academy of Science,* 98(24), 14096 – 14101.

Yakel, J. L. (2012). Nicotinic ACh receptors in the hippocampus: role in excitability and plasticity. *Nicotine & Tobacco Research,* 1-8.

Ylinen, A., Bragin, A., Nádasdy, Z., Jandó, G., Szabó, I., Sik, A., Buzsáki, G. (1995). Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *Journal of Neuroscience*, 15(1), 30-46.

Zamani, M. R., Allen, Y. S. (2001). Nicotine and its interaction with beta amyloid protein: a short review. *Society of Biological Psychiatry*, 49(3), 221-232.

Zeng, H., Zhang, Y., Peng, L., Shao, H., Menon, N. K., Yang, J., Salomon, A. R., Freidland, R. P., & Zagorski, M. G. (2001). Nicotine and amyloid formation. *Society of Biological Psychiatry,* 49, 248-275.

Zhang, J., Liu, Q., Chen, Q., Liu, N. Q., Li, F. L., Lu, Z. B., Qin C., Zhu, H., Huang,Y. Y., He, W., Zhao, B. L., (2006). Nicotine attenuates the b-amyloid neurotoxicity through regulating metal homeostasis. *The FASEB Journal*, 20(8), 1210-1215.

Zhu, X. O., Brown, M. W., McCabe, B. J. & Aggleton, J. P. (1995). Effects of novelty or familiarity of visual stimuli on the expression of the immediate early gene c-Fos in rat brain. *Neuroscience*, 69(3), 821–829.