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Neurotransmitter Systems and Age Related Cognitive Decline: A Focus on Attention and Plasticity

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Neurotransmitter Systems and Age Related Cognitive Decline: A Focus on
Attention and Plasticity

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A thesis presented to the Graduate Faculty
of the College of William and Mary in Candidacy for the Degree of
Master of Arts

Department of Psychology

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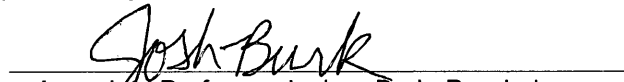
APPROVAL PAGE

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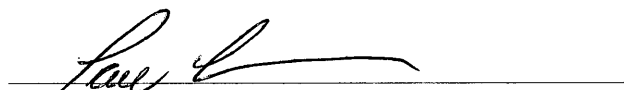
Master of Arts

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COMPLIANCE PAGE

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Research approved by

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ABSTRACT

Normal and pathological forms of aging, such as Alzheimer's Disease, are characterized by declines in attention and neuroplasticity. However, the underlying neurobiological changes associated with cognitive deterioration have not been fully characterized. This paper describes two experiments that strive to further the understanding of these two age-related cognitive declines. Experiment 1 investigated the interaction between GABAergic basal forebrain neurons and the cholinergic system and how these systems separately and in combination with one another affect performance on the two-choice sustained attention task for rats. The results from experiment 1 found that immunotoxic GABAergic basal forebrain neurons lesions left animals more susceptible to the attentional deficits due to the blocking of muscarinic receptors with scopolamine. Experiment 2 tested the effects of the addition of a flashing houselight distracter on neuroplasticity in aged rats. This was conducted through comparing performance in animals that had the addition of a flashing houselight distracter and those that performed with a stable houselight on a learned two-choice sustained attention task and a newly introduced light-location discrimination task. The results from experiment 2 indicated that once the rats were able to overcome the initial increase in attentional demand due the flashing houselight, the distracter-exposed animals showed an increase in attentional performance during the 25 ms signal duration trials during the sustained attention task beyond the non-distracter-exposed animals while maintaining performance on the other attention trial types. However, cholinergic manipulations due to injections of scopolamine and nicotine did not differentially alter performance for the distracter-exposed versus the non-distracter-exposed rats. These results indicate that the ability to overcome high attentional demands may increase neuroplasticity in aged rats.

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ACKNOWLEDGEMENTS

I would like to dedicate my masters thesis to my wonderful family, loving boyfriend, Nick, and patient cat, Dr. Tobias. Thank you for always having faith in me. I could not have done this without all of your unwavering support.

LIST OF FIGURES

Age-related cognitive decline can range from mild confusion and memory loss to severe dementia and Alzheimer's Disease (AD). Declines in aged individuals' cognitive function can include changes in attention, memory, learning, executive function, and language capabilities that can negatively affect quality of life, personal relationships, and the capacity for making informed decisions about health care and other matters (Wagster, King, Resnick, & Rapp, 2012). As of 2013, an estimated 16 million people are living with cognitive impairment due to aging. Another 5 million Americans 65 and older are thought to have AD (Hebert, Weuve, Scherr, & Evans, 2013).

Moreover, treating cognitive decline is extremely costly. Patients with cognitive impairment report more than three times as many hospital stays beyond those who are hospitalized for other conditions (Bynum, 2009). For people with conditions related to cognitive decline, aggregate payments for health care, long-term care, and hospice are projected to increase from $203 billion in 2013 to $1.2 trillion per year in 2050 (Alzheimer's Association, 2013). According the Centers for Disease Control, this not only makes cognitive decline and AD mental health problems that affect the elderly but also a public health issue ("Cognitive Impairment: A Call for Action, Now!", 2011).

Researching the neural circuitry that underlies the difficulties related to cognitive decline may lead to the development of more targeted pharmacological treatments and a better understanding about the effects that pharmacological treatments have within the brain. The experiments that are the focus of this paper were designed to examine two of the most common issues associated with age-related cognitive decline:

decreased attention and plasticity. The experiments will further investigate how changes in neurotransmitter systems may contribute to these two effects of aging.

Basal Forebrain and Attention

Cholinergic Neurons. The basal forebrain (BF) is comprised of a collection of subcortical structures located ventral and rostral to the striatum that include the medial septum, ventral pallidum, diagonal band nuclei, substantia innominata/extended amygdala, and peripallidal regions (Mesulam, Mufson, Wainer, & Levey, 1983). Neurons located within the BF essentially project to all cortical areas and layers (Similey, Subramanian, & Mesulam, 1999). Furthermore, cortically projecting acetylcholine (ACh)-releasing neurons, or cholinergic neurons, from the BF have been shown to impact a number of cognitive functions and behaviors such as attention, impulsivity, wakefulness, drug abuse, cognitive decline due to aging and a number of psychiatric disorders (Zaborszky, van den Pol, & Gyengesi, 2012).

A two-choice sustained attention task has been widely used in animal models to investigate the neural underpinnings, including the BF ACh projections, that contribute to attentional processing. This task requires the animal to respond to two different trial types. On signal trials, the light is illuminated for variable short durations and on non-signal trials the light is not illuminated. The subjects are trained to press one of two levers to receive a reward of water for signal trials and the other lever to receive a reward on the non-signal trials. Many studies have used this task to highlight the importance of the BF cholinergic system on performance in this task (i.e. McGaughy & Sarter, 1999; McGaughy & Sarter, 1995).

Previous studies provide evidence of the importance of BF ACh projections using a variety of methods to manipulate or measure the activity of this system during performance in this two-choice sustained attention task. For example, a number of experiments have used the immunotoxin 192 IgG-saporin to pharmacologically lesion ACh neurons within the BF through targeting and eliminating p75^{NTR}positive neurons and assess subsequent task performance. The 192 IgG-saproin complex is an antibody that binds with high specificity to the p75 nerve growth factor receptor and is then endocytosed which allows the saporin to inactivate the ribosomes within the cell leading to cell death. P75^{NTR} is only expressed in cholinergic neurons and not in neighboring non-cholinergic neurons within the area (Wrenn & Wiley, 1998; http://www.atsbio.com/catalog/toxins/it01.php). Therefore, the use of this immunotoxin leads to cholinergic cell death but the preservation of other non-cholinergic neurons. These studies then tested the effects of the cholinergic lesion using different attention tasks (i.e. Gibbs & Johnson, 2007; McGaughy, Kaiser, & Sarter, 1996). The results from both of these previous studies indicate that lesioning the cholinergic system within the BF leads to poorer attention performance especially when examining the trials when a visual signal needs to be detected.

Measurement of ACh release in rats using *in vivo* microdialysis during a visual sustained attention task found higher ACh levels within medial prefrontal cortex during this task compared with ACh release during performance of a task that controlled for lever pressing and reward access, but did not require explicit attention to visual cues' (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002). An additional study examined extracellular recordings of posterior parietal cortex (PPC) neurons after selectively

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removing ACh projections from the BF to the recording site. After the removal of ACh projections, fewer neurons within the PPC fired when a cue directed stimulus was presented but more PPC neurons fired when distracter stimuli were presented. These results imply an association between cholinergic activation and PPC neural response to stimuli that increase demands on attentional processing (Broussard, Karelina, Sarter, & Givens, 2009). All of these studies contribute evidence supporting the conclusion that ACh cortical projections from the BF are a critical aspect of the neural circuitry underlying attentional ability.

Several theories regarding the cholinergic modulation of attention have been suggested. One prominent theory proposes that activation of the cholinergic system optimizes cognitive and sensory components of attentional performance through contributing to both top-down and bottom-up processing (Sarter, Bruno, & Givens, 2003). As defined in Sarter, Givens, & Bruno (2001), top-down regulation indicates an internally motivated, knowledge driven guidance of attention whereas bottom-up regulation of attention indicates a sensory or environmental influence on attention. Top-down processing within the two-choice sustained attention task would indicate the subject's knowledge as to where to look for a signal, capacity to discern between a signal and a distracter, awareness of the general probability of a signal and ability to apply the rules of the task to properly respond to that signal. Evidence has also shown that the BF corticopetal cholinergic system is key in an organism's top-down control and ability to switch between rules for signal and non-signal trials (Howe et al., 2013). Bottom-up processing in the two-choice sustained attention task would be the animal's ability to distinguish sensory information. Theoretical models have proposed that the

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cholinergic system may be important for the integration of bottom-up and top-down aspects of attention (Yu & Dayan, 2002). This theory suggests that perception and attention involves inferring the most appropriate representation for sensory inputs. This inference is influenced by both top-down inputs, providing contextual and probability information, and bottom-up inputs from sensory processing. The authors propose that ACh reports on the uncertainty associated with top-down information, and has the effect of modulating the relative strengths of input sources associated with bottom-up information. In combination, the evidence to date suggests that ACh optimizes multiple components of attentional processing.

Non-Cholinergic Neurons. Although the cholinergic system within the BF has been the focus of much of the previous research on the area and attention, there are other populations of neurons within the BF, namely gamma-aminobutyric acid (GABA) transmission. In fact, GABAergic neurons outnumber ACh neurons within the globus pallidus/substantia innominata region of the BF (9,600 vs. 5,100 cells/ hemisphere; Gritti, Mainville, & Jones,1993). Given the relatively large number of non-cholinergic neurons intermingled with cholinergic neurons within the BF, any satisfactory theory of the role of the BF in attention needs to include an understanding of the contributions of non-cholinergic neurons along with their interactions with the cholinergic neurons (Sarter, Lustig, Howe, Gritton, & Berry, 2014; Baxter & Bucci, 2013). In order to investigate this relationship, Burk and Sarter (2001) administered either 192 IgG-saporin (which damages only cholinergic neurons) or ibotenic acid (which preferentially, but not selectively, damages non-cholinergic neurons) to the BF of rats. The animals were then tested on a two-choice sustained attention task

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previously described by McGaughy & Sarter (1995). The results from this experiment showed that attentional performance in the ibotenic acid-induced lesioned animals had a different pattern of deficits when compared to the 192 IgG-saporin lesioned animals. Ibotenic acid-induced lesions increased false alarms (incorrect responses on trials when no visual signal was provided). This result contrasts with the typical effect of BF cholinergic lesions, a decrease in the hit rate (more errors on trials when a visual signal is presented). This indicates that there is a key difference in the attentional effects of selective cholinergic lesions as compared with lesions that preferentially, but not selectively, destroy non-cholinergic neurons in the BF.

Recent studies have built upon these findings to provide further evidence of the role of non-cholinergic neurons in attention. For example, Lin and Nicolelis (2008) used a Go/NoGo task that linked auditory and visual cues to reward and aversive stimuli to provide neurophysiological evidence of the role of non-cholinergic BF neurons on attention in rats. The results showed phasic bursting of non-cholinergic BF neurons during the encoding of both reward and adverse cues implicating these neurons in the encoding attention to cues along with subsequent action toward motivationally salient stimuli. These results implicate non-cholinergic BF neurons in the attentional encoding of both positive and negative motivational stimuli.

Furthermore, Yi and colleagues (2014) demonstrated the interaction between cholinergic muscarinic-1 (M_1) receptors and GABA transmission. This study, using a mouse model, provided evidence that excitation of M_1 cholinergic receptors on parvalbumin containing neurons (putative GABA neurons) increased the GABAergic activity within hippocampal cells. However, because of a lack of available agents to

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selectively lesion non-cholinergic neurons, previous research has mostly ignored the function of non-cholinergic neurons within the BF (Baxter & Bucci, 2013).

Aging and Attention: Underlying Neural Circuitry

      Cholinergic Neurons. The cholinergic hypothesis of age related cognitive decline indicates that the cognitive impairment seen in AD patients and potentially normally aging elderly people is due primarily to loss of ACh neurons (Bartus, Dean, Beer, & Lippa, 1982). Since its original proposal in 1982, research on both normal and AD populations has supported and elaborated upon this theory.

      Research with animal models has confirmed that BF lesions to cholinergic neurons render animals more vulnerable to attentional decline due to aging (Burk, Herzog, Porter, & Sarter, 2002). This longitudinal study found that partial 192 IgG-saporin-induced lesions of BF ACh neurons early in life decrease sustained attention performance later in life (31 months) when compared to animals who received sham lesions. Additional studies have found BF cholinergic lesions produced by 192 IgG-saporin dampened the cognition-enhancing effects of a complex environmental stimulus in combination with the administration of a benzodiazepine receptor weak inverse agonist (Fadel, Sarter, & Bruno, 1999). This study also showed that aged lesioned rats had significantly lower cortical ACh efflux in this complex environment when compared to sham and lesion young rats and sham aged rats. Moreover, human volumetric studies of BF neurons show an increase in atrophy of the cholinergic system that is associated with normal aging (Grothe, Heinsen, & Teipel, 2012; 2013). All of these findings point to the importance of the cholinergic system in the maintenance of attention abilities into aging.

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Previous research has indicated that AD patients have significantly fewer BF ACh-releasing neurons when compared to age-matched controls (Nagai, McGeer, Peng, McGeer, & Dolman, 1983). One study found that fewer choline acetyltransferase-positive neurons in the BF may be one of the main underlying mechanisms that causes cognitive decline in AD patients (Coyle, Price, & DeLong, 1983). In combination, this research highlights the importance of BF ACh connections for cognitive aging and signifies that the cholinergic system is key in the maintenance of cognitive and attentional abilities in both normal and pathological aging.

Non-Cholinergic Neurons (Glutamate/GABA). Research has highlighted that the number of GABAergic neurons changes in both normally aged and AD patients compared with earlier in life. The quantity of GABAergic neurons throughout the brain increases with age with an even larger increase in AD patients (Bañuelos et al., 2013; Marczynshi, 1998). This increase in GABA neurons is due to an increase in activity of glutamic acid decarboxylase (GAD). GAD is the enzyme which converts glutamate into GABA. There are two forms of GAD within the brain, active and inactive. GAD needs a cofactor to be in its active form. Mitochondrial adenosine triphosphate production (ATP) and amino acids (ATP is probably the most critical with regard to aging) promote the inactive form of GAD (Martin & Rimvall, 1993). However, in aging, disruption of ATP activity leads to the disinhibition of active GAD activity meaning that GAD is more often in its active form (Beal, Hyman, & Koroshetz, 1993). This increase in active GAD activity decreases the number of glutamate releasing neurons while increasing the number of GABA releasing neurons (Martin, 1987). Glutamate and GABA have inverse functions throughout the brain. While glutamate is

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typically considered a major excitatory neurotransmitter, GABA is considered the major inhibitory neurotransmitter, although the specific actions depend on the receptors. Additionally, glutamate is a precursor to GABA and once GAD transforms a glutamate-releasing neuron into a GABA-releasing neuron it produces a combination of decreased excitatory and increased inhibitory neuron firing throughout the brain. It has also been shown that the down regulation of post-synaptic glutamate receptor activity plays a role in cognitive impairments associated with age (Burke & Barnes, 2010; Ménard, 2015) This change in ratio of neurons within the brain has been theorized to be one neurological cause of the cognitive decline found throughout normal aging (Mora, Segovia, & del Arco, 2007).

Collectively, there is a shift in increased GABA neurons and decreased cholinergic activity that happens with age which has been theorized to lead to cognitive decline. This gives rise to the importance of investigating the interaction between the GABAergic and cholinergic systems and how they may affect executive functions such as attention.

Aging and Plasticity

Plasticity is broadly defined as an organism's ability to adapt and change in accordance to changes in the demands placed on the organism such as an increase in irrelevant sensory information during target detection. Presumably, the flexibility that allows an organism to learn and adapt to these changes is due to modifications in brain connectivity including increases in brain size, cortical thickness, neuron size, dendritic branching, spine density, synapses per neuron and glial numbers (Kolb & Whishaw, 1998) otherwise referred to neuroplasticity. Kolb & Wishaw (1998) maintain that

neuroplasticity tends to be more limited with increased age. Increasing learning and neuroplasticity may potentially rescue some aspects of age related cognitive decline (i.e. Jones et at., 2006; Kramer & Willis, 2003; Nyberg et al., 2003).

Bherer and colleagues (2005, 2006) tested the flexibility of attentional control in older adults using a dual attention task. For this study, participants completed two attention tasks. In one task, participants were asked to indicate on a key board whether a B or a C was presented on the screen in front of them. In the second task, participants indicated whether a low or high pitch tone was played by pressing one of two other keys on a key board. In each session, there were blocks of solely visual or auditory trials and blocks where both were presented at once. Participants were first brought into the lab and completed a session, and then they were brought back into the lab for five training sessions where they were given continuous, adaptive feedback after each trial as to whether their performance was adequate or too slow. Feedback was only given for the five training sessions and not for the initial or final testing session. The results from this study indicated that although younger adults had overall faster reaction times and higher accuracy, improvement in performance was equivalent for both age groups by the final session. Furthermore, research conducted using the field of view task (defined as the visual area in which information can be acquired within one eye fixation) revealed that both older and younger adults successfully increase performance with training (Ball, Beard, Roenker, Miller, & Griggs, 1988). These findings support the theory that flexibility of attentional control is possible in older adults, as evidenced by task improvement with practice and points to potential neuroplasticity.

Previous studies have added a flashing houselight during the two-choice sustained attention task to examine the rat's ability to adapt and continue to perform with an increased cognitive load (McGaughy & Sarter, 1995). Demeter, Sarter, and Lustig (2008) investigated the translatability of manipulations to two-choice sustained attention task, typically used with rats, to human attention. For this study, the researchers, in separate experiments, trained rats on the two-choice sustained attention task and brought humans into a laboratory setting to complete a similar task on a computer. A distracter was introduced to the rats and humans by flashing a houselight and having the background flash on the computer screen, respectively. The data showed that although humans had overall higher performance on the task, both rats and humans showed a similar pattern of better performance at the longer signal durations without the addition of the distracter and decreased performance at the shortest signal duration after the addition of the flashing distracter. These results support the translatability of the sustained attention task features from rats to humans.

The addition of a flashing houselight during the task has also been shown to increase cognitive flexibility and ability to learn a new task (Hirsh & Burk, 2013). For this study, rats trained on the two-choice sustained attention task described by McGaughy and Sarter (1995) until they reached criterion performance. The rats were then randomly assigned to one of two conditions. One group continued on sessions with a consistently illuminated houselight and the other group had the addition of a flashing houselight as a distracter. After the initial distracter exposure of 12 sessions, blocks of a new light-location task were intermixed within the sustained attention task for both groups. For this new task, animals were required, after illumination of a right

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or left signal light, to press the lever under the light to receive water reward access. Results from this study showed that the initial introduction of the distracter decreased performance, however, with additional training both the distracter- and non-distracter-exposed animals had similar performance in the sustained attention task. The addition of 10% of new light-location task trials showed no difference in performance between groups for either task. However, at 40% of the new light-location task blocks, the rats in the distracter condition had increased performance in the light-location task beyond that of the non-distracter animals while maintaining similar performance in the sustained attention task. These results indicate that the ability to overcome increases the attentional demand (the flashing houselight) and regain good attentional performance therefore further increases the neuroplasticity and thus ability to adapt to and perform in the new light-location task without sacrificing performance in the two-choice sustained attention task.

Cholinergic System and Plasticity. A number of studies have investigated how the addition of a distracter in the form of the flashing houselight during the sustained attention task is associated with increased cortical ACh release (Gill, Sarter, & Givens, 2000; Newman & McGaughy, 2008). These studies indicate that the increased attentional demands due to the distracter stimulate cortical ACh release. Furthermore, neural changes within the cholinergic system may contribute to the animal's ability to overcome the additional load associated with the flashing houselight distracter and maintain attentional performance (Himmelheber, Sarter, & Bruno, 2000). This relationship was elucidated by the *in vivo* microdialysis measurements of ACh efflux within the frontoparietal cortex while the rats performed the sustained attention

task. The results from this experiment showed a complex relationship between ACh release and performance on the task. As aligned with previous studies, the initial distracter exposure decreased attentional performance. However, the recovery in performance was accompanied by an increase in ACh release. These findings suggest that the augmentation of attentional demand produced by the distracter and the compensation of the animal for that distracter to recover performance elicits further increases in ACh release.

The mechanisms through which ACh may influence neuroplasticity are beginning to be elucidated. A review by McKay, Placzek, and Dani (2007) cites multiple mechanisms through which the cholinergic system may affect neural plasticity. ACh can facilitate depolarization of postsynaptic neurons by blocking K^+ channels, thereby promoting NMDA-mediated glutamate release. Additionally, ACh may independently initiate plasticity through production of protein kinase II or other intracellular mechanisms. These modes through which ACh acts to alter neurotransmitter transmission indicates that the cholinergic system may key in the development of neuroplasticity.

Furthermore, there are now data documenting the effects of ACh on neuroplasticity with changes in behavior. Research has associated alterations to the cholinergic system to both neuroplasticity and behavioral flexibility (Conner, Culberson, Packowski, Chiba, & Tuszynski, 2003). The researchers found that BF cholinergic lesions are associated with decreased cortical plasticity as measured by electrophysiological stimulation to the sensory motor cortex and behavioral flexibility in a skilled motor task that required the rats to reach for food pellets. These lesions also

impaired learning in the skilled motor task. The lesions removed any prior increases in performance due to the learning of the task. Linking ACh neuroplasticity and behavioral flexibility further supports the theory of codependence between neurological and behavioral changes.

Current Studies and Hypotheses

Experiment 1. The goal of the first experiment is to build upon previous findings by testing whether there are interactions between cholinergic muscarinic receptors and GABAergic neurons in the BF that affect attentional processing in order to better understand one of the main symptoms of cognitive decline associated with aging. To produce surgical lesions, the animals received the selective GABAergic immunotoxin, GAT1-saporin. This is an immunotoxin that selectively binds to and destroys GABAergic neurons while preserving cholinergic neurons by only attaching to and deactivating the neurotransmitter transporter that is responsible for the movement of GABA across cell membranes (http://www.atsbio.com/catalog/toxins/it32.php).

The animals then received injections of the muscarinic receptor antagonist, scopolamine, prior to testing. By examining the effects of lesions of BF GABA neurons and the acute blocking of cholinergic muscarinic receptors, the results of this experiment will lead to a better understanding of how these two systems may interact to affect attentional performance. Based on the results from previous studies testing the effect of cholinergic and non-cholinergic BF neurons (Yi et al., 2014; Burk & Sarter, 2001; Lau & Salzman, 2008) it is predicted that there will be an interaction between

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the GABAergic BF neurons and cholinergic system that will decrease attentional performance.

Experiment 2. Previous research in our lab has revealed that, in young adult rats, the addition of a flashing houselight during a learned two-choice sustained attention task enhances rats' ability to learn a new light-location task (Hirsh & Burk, 2013). The authors concluded that the initial introduction of the distracter decreased performance, however, learning to overcome the distracter and maintain performance increased cognitive flexibility leading to faster acquisition of a light-location task. The present experiment was designed to test whether this form of plasticity extends to aged rats and if manipulations to the cholinergic system further alter that plasticity.

Rats were trained on a two-choice sustained attention task from ages 3 months until age 20 months (approximately equivalent to 50 human years; Sengupta, 2013). At 20 months half of the rats continued on the same task with no manipulations and half continued on the task with the addition of a flashing house light. After 20 sessions of this manipulation, blocks of a novel light-location discrimination task were added. Finally, to measure the effect of alterations to the cholinergic system, all animals then received injections of scopolamine (muscarinic receptor antagonist) and nicotine (nicotinic receptor agonist) prior to testing.

Sustained attention and the light-location discrimination performance were evaluated after each task manipulation. Based on the results from Hirsh & Burk (2013), it is predicted that those animals in the distracter group will initially show poorer attentional performance. However, they will be able to eventually compensate for the distraction and showing improved performance in the sustained attention and the light-

location discrimination tasks. Because these are aged animals, these effects may not be as strong as those seen in the previous experiment. Furthermore, it is predicted there will be accelerated learning for the distracter- exposed animals over the non-distracter-exposed animals for the light-location trials while maintaining performance in the sustained attention at the 40% and 70% light-location discrimination sessions. Regarding drug administration, because decreased muscarinic receptor stimulation has been shown to decrease attention and stimulating nicotinic receptors can improve attention (i.e. Rezvani & Levin, 2001), it is predicted that injections of scopolamine will decrease performance and, inversely, injections of nicotine will increase performance.

Method

Experiment 1

Subjects. Male FBNF1 hybrid rats ($N = 16$; National Institute of Aging Colony), aged 2 months at the beginning of the training, were used for this experiment. Rats were trained in the task daily beginning between the hours of 9:00 AM and 12:00 PM. When the animals were not being tested they were housed in plastic flat-bottom tubs in a temperature and humidity controlled room on a 14/10 hour light/dark cycle and were given food *ad libitum*. After completion of a training session rats were returned to their home cases and water access was given freely to the rats for 30 minutes. On days when the animals were not trained, water was given for one hour. All housing and testing methods were approved by the College of William and Mary Institutional Animal Care and Use Committee (IACUC).

Apparatus. Animals were trained in individual chambers, located within sound attenuating boxes with fans providing ventilation and background noise during testing. The testing chamber within each box contained one house light at the back which was consistently illuminated during the final stage of testing. Additionally, the chamber contained three panel lights at the opposite side of the box. Below the left and right panel lights there were two retractable levers and between the levers, a water dipper was used to provide access to 0.01 ml of tap water to the animal. All trials were controlled by MED-PC IV software.

Pre-surgical Training: Two Choice Sustained Attention Task. The rats were first shaped by receiving water access (dipper raised for 3 s) following every lever press, with the rule that, if the rat pressed the same lever five times, it had to press the other lever to receive reward. This rule was intended to minimize the development of a lever bias. After reaching a criterion of receiving 100 rewards for at least three testing session, rats were moved to the next training stage. The next training stage consisted of two types of trials, signal and non-signal. On the signal trials the central panel light would illuminate for 1 s and the response levers would then extend into the chamber. The animals were required to press one of the two levers in order to register a response. The correct response lever was counterbalanced between rats. For half of the animals, a right lever press on signal trials was considered correct and for the other half of the animals the left lever press was considered correct on signal trials. For non-signal trials, the panel light did not illuminate and the levers were extended into the chamber. For a correct response on these non-signal trials, rats were trained to press the opposite

lever than what was correct for the signal trials. After a correct response on a signal or non-signal trial the water dipper would raise into the chamber for 3 s as reward.

A rewarded lever press response for signal trials was considered a "hit" whereas a press on the opposite lever was considered a "miss". A rewarded response on non-signal trials was considered a "correct rejection", whereas a press on the opposite lever was considered a "false alarm". For both signal and non-signal trials, if the levers extended into the chamber and the animal did not press either lever within 3 s, then the trial was scored as an "omission". All incorrect response trials were followed by a correction trial that was identical to the previous trial. A forced trial followed if animals did not respond correctly on three consecutive correction trials. Forced trials consisted of illumination of the panel light (for signal trials) or no illumination (for non-signal trials) followed by an extension of only the correct lever into the box until the lever was pushed or 90 s elapsed. The intertrial interval (ITI) was 12+/- 3 s and the house light was not illuminated during this stage of training. Once animals reached 70% accuracy for both signal and non-signal trials for five consecutive days, they were moved to the next stage of training.

For the next stage of training, the rules of the task did not change, however some of the parameters were adjusted. First, the signal trials consisted of three different types of trials wherein the light would illuminate for 500, 100 or 25 ms in a semi-randomized order. Second, the correction and forced trials were removed. Third, the ITI was decreased to 9 +/- 3 s. These alterations were to minimize the likelihood that the rat could predict the type or timing of a trial before it was initiated, therefore requiring the animal to sustain attention throughout the entirety of the testing session

and not memorize the trial order. Each testing period contained 9 blocks of 18 trials (for a total of 162 trials). In each block, there were 9 signal trials (3 trials at each signal duration) and 9 non-signal trials. The entire session took approximately 40 minutes to complete. Once the animals reached a criterion of 70% accuracy on the 500ms signal and non-signal trials for seven consecutive days they were considered to have reached criterion performance. See *Figure 1* for portrayal of the final task.

Surgical Procedure. Once the rats reached criterion in the final task (on both signal and non-signal trials), they were considered ready to receive surgery. Each animal was anesthetized (90.0mg ketamine, 9.0 mg/kg xylazine, intraperitoneal injections) and placed in a stereotaxic surgical instrument. All surgical procedures took place under aseptic conditions. Holes were drilled over the appropriate locations and bilateral infusions made at AP: -1.3, ML: +/-2.7; DV: +2.5 (AP and ML relative to bregma; DV relative to IA). In each hemisphere, 0.5μl of saline ($N = 8$), or of GAT1-Saporin (0.4 μg/μl; Advanced Targeting Systems; $N = 8$) was administered through a 26-gauge cannula attached to a 1.0 μl Hamilton syringe. Saline or GAT1-Saporin infusions occurred over one minute and the needle was left in place for one minute to allow time for diffusion into the brain. After infusions, incisions were closed using surgical staples and animals were placed in their home cages to recover for one week. During this recovery, animals received *ad libitum* access to water containing acetaminophen (2.7 mg/ml) for three days following surgery and then tap water for 4 days.

Post Surgery Procedure. After the recovery period, water deprivation was reinstated, and the animals were then tested for at least 15 sessions on the same two-

choice sustained attention task that they were trained on prior to surgery. In accordance with McQuail and Burk (2006), the task was then modified so that the animals were placed in the testing chamber, but the attention task did not begin for 10 minutes. This was in order for the animals to become accustomed to delay prior to the addition of scopolamine injections, as this delay would allow time for the drug to cross the blood brain barrier before beginning the task.

Intraperitoneal injections (ip) of the muscarinic receptor antagonist, scopolamine, were then administered prior to testing sessions using a 1-ml syringe. Each rat received ip injections of saline, 0.05 mg/kg, 0.1 mg/kg, and 0.2 mg/kg of scopolamine (McQuail & Burk, 2006). The order of injections was randomized for each animal and there were at least two days of drug-free behavioral testing between each of the injection sessions.

Behavioral Measures. Accuracy on signal and non-signal trials was computed using the formulae hits/ (hits + misses) and correct rejections/ (correct rejections + false alarms), respectively. Total number of omissions was determined separately from measures of accuracy. Overall task performance was calculated using a Vigilance Index (VI; McGaughy, Kaiser & Sarter, 1996). Proportions were created for hits and false alarms divided by total signal and total non-signal trials respectively. VI was calculated by entering those proportions into the formula (hits - false alarms) / (2 X (hits + false alarms) − (hits + false alarms)^2) with values ranging from 1 (indicating 100% correct responses on signal and non-signal trials) to -1 (indicating a 100% incorrect responses on signal and non-signal trials).

Perfusion/ Histological Procedure. Rats received an ip injection of 100.0 mg/kg ketamine and 10.0 mg/kg xylazine. Once deemed sufficiently anesthetized, 10% sucrose and then 4% paraformaldehyde solutions were perfused intracardially throughout the circulatory system of the rat. The brain was then removed and placed into a 4% paraformaldehyde solution for at least 2 days and then transferred to a 30% sucrose solution in phosphate-buffered saline for cryoprotection. Sectioning was conducted using a freezing microtome (SM2000R; Leica Biosystems). Parallel sections (50 μM) were taken beginning at the formation of the anterior commissure and ending once the ventral portion of the hippocampus could be observed. After sections were taken they were stored at -20° C in an antifreeze solution until staining took place.

Sections were prepared for parvalbumin (PV) staining with three rinses of phosphate buffered saline (PBS). The sections then underwent a 10 minute period in 0.5% H_2O_2, 0.3% Triton X-100 in PBS. Following PBS rinses the sections were immersed in 2% bovine serum albumin (Sigma; St. Louis, MO, USA), 5% donkey serum, and 0.3% Triton X-100 for 90 minutes and were then put into a mouse anti-PV solution (Sigma; 1:2000) and were left at room temperature on an orbital shaker over night. The next morning sections received PBS rinses and were incubated for 90 minutes in a donkey anti-mouse secondary antibody (Chemicon, Temecula, CA, USA; 1:50), and were rinsed with TRIS buffered saline. The sections were then exposed to mouse peroxidase anti-peroxidase complexes for 90 minutes followed by the addition of 1.5 ml 3,3'-diaminobenzidine (DAB), 13.5 ml TRIS buffer, and 50 ml nickel ammonium sulfate.

Acetycholinesterase (AChE) staining protocol was developed through the modification of method detailed by Tago, Kimura, & Maeda (1986). Sections were first placed into three 0.1M phosphate buffer (pH 7.4) rinses and were then incubated for 30 minutes using a 0.1% H_2O_2 solution. Sections then received three maleate buffer (MAL; pH 6.0) rinses and were then immersed into an incubation of 200 ml MAL, 15 mg acetylthiocholine iodide, 0.75 ml sodium citrate solution (0.03 g/ml), 1.5 ml cupric sulfate solution (0.007 g/ml) and 1.5 ml potassium ferricyanide solution (0.02 g/ml) for 30 minutes. After 30 mM TRIS buffer (TRIS; pH 7.6) rinses the sections were placed into a solution of 50.0 mg of DAB and 0.375 g nickel ammonium sulfate in 125.0 ml of 50.0 mM TRIS buffer (pH 6.2). About 12 drops of 0.1% H_2O_2 solution were then added to the sections until the tissue turned black. The sections then received three more TRIS rinses and were mounted on gelatin-coated slides and then cover slipped for examination.

Statistical Analyses. Statistical analyses were conducted using a series of mixed model analysis of variance (ANOVA) that included group (sham, lesion), scopolamine dose (Saline, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg) and, for signal trials, signal duration (500 ms, 100 ms, 25 ms) as factors. An alpha level was set to .05 and all analyses were conducted using IBM SPSS version 21 statistical software.

Experiment 2

Subjects/ Apparatus. FBNF1 male hybrid rats (NIA Colony; $N = 20$), 3 months at the beginning of the experiment, were used in this experiment. All housing, testing conditions and apparatuses were the same as in experiment 1.

Two-Choice Sustained Attention/ Light-Location Discrimination Tasks.

Rats were shaped and trained on the two choice sustained attention task as detailed in experiment 1. Subjects continued to train until age 20 months. At that age, rats were divided into two experimental groups. The distracter- exposed group (n = 10) had the addition of a flashing house light (0.5 sec on; 0.5 sec off) while performing the task. The non-distracter-exposed group (n = 10) continued training in the sustained attention task with a continuously illuminated house light, as previously trained.

After 20 sessions of these distracter or non-distracter conditions, trials of a light-location discrimination task were randomly intermixed within blocks of the sustained attention task for both groups. For this light-location discrimination task, either the right or the left panel lights would illuminate for 500 ms. The two levers below the lights would then extend into the box. A correct response for these trials was a press on the lever below the light that was just illuminated. With the addition of the light-location discrimination task, the number of trials per block was increased from 18 to 20 for a total of 180 trials per testing session. See *Figure 2* for portrayal of light-location discrimination task.

For 20 consecutive sessions 40% of the trials within each block contained this light-location discrimination and 60% of trials contained the standard sustained attention task. These sessions contained 8 light-location discrimination trials (4 with left light illumination and 4 with right light illumination) and 12 sustained attention trials (6 signal, 2 each with 500, 100, 25 ms signals, and 6 non-signal) per block. The number of trials containing the light-location discrimination task was then increased to 70% per block and 30% of trials were the sustained attention task. These 70% trials

contained 14 light novel discrimination trials (7 with left light illumination and 7 will right light illumination) and 6 sustained attention trials (3 signal, 1 at 500, 100, 25 ms, and 3 non-signal) per block.

Drug Administration. After 20 sessions with 70% light-location discrimination trials and 30% sustained attention trials, a 10 minute wait was introduced similar to that in experiment 1 in order to allow animals to acclimate to the time needed for the drugs to fully cross the blood brain barrier. Subjects received ip injections of scopolamine with a 1-ml syringe prior to a testing session. All rats received doses of saline, 0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg of scopolamine in a randomized, counterbalanced order. To minimize the likelihood that there were carryover effects of the drug, there were at least two days of drug-free behavioral testing between each injection day.

Rats were then given ip injections of nicotine prior to testing sessions. The schedule and dosage was similar to that of scopolamine (saline, 0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg; Grilly, Simon, & Levin, 2000) and administration order was randomized with at least two days of behavioral testing in between each injection day.

Behavioral Measures. Performance on the two choice sustained attention task was determined by accuracy of hits for signal trials [hits / (hits + misses)] and correct rejections for non-signal trials [correct rejections/ (correct rejections + false alarms)]. For the light-location discrimination task, performance was determined by number of correct lever presses over total number of light-location discrimination trials when the animal pressed a lever (correct trials / correct trials + incorrect trials). Omissions were calculated separately for both tasks.

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Statistical Analysis. Statistical analyses for this experiment were conducted using IBM SPSS version 21 statistical software and alpha level was set to .05. Mix model ANOVAs were conducted to analyze the data collected.

Following distracter exposure (or not), performance in the sessions prior to drug injections was averaged into four blocks, each with five sessions (Block 1: Sessions 1-5, Block 2: Sessions 6-10, Block 3: Sessions 11-15, Block 4: Sessions 16-20). For the two choice sustained attention task, ANOVAs were conducted including factors such as distracter conditions (distracter, non-distracter), block (1, 2, 3, 4), and signal duration for signal trials (500, 100, 25 ms). Performance for the light-location discrimination task was analyzed using ANOVAs containing distracter condition (distracter, non-distracter) and block (1-4) as factors. Performance on the 40% light-location discrimination and 70% light-location discrimination sessions were analyzed separately.

Sustained attention and light-location discrimination data for scopolamine and nicotine sessions were analyzed separately according to the ANOVAs described above with the addition of scopolamine or nicotine dose as a factor (Saline, 0.1 mg/kg, 0.2 mg/kg, 0.4 mg/kg).

Results

Experiment 1

Behavioral Results. Analysis of post surgical performance for 15 sessions after recovery did not indicate any significant differences between the sham and lesioned animals (all $p > .06$). One lesion animal was excluded from final data analysis because of high omission rates.

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A group (sham, lesion) x scopolamine dose (Saline, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg) x signal duration (500 ms, 100 ms, 25 ms) ANOVA was conducted for VI for the post drug injection sessions. There was a significant effect of group on VI value $(F(1, 13) = 5.02, p = .04)$ which indicated that lesions animals performed worse that sham animals. Observation of the data indicated that lesioned animals tended to perform worse following scopolamine injections, although the dose x group interaction was not significant. To explore this observation, separate one way ANOVAs with dose as a factor were conducted for sham and lesioned animals. There was no main effect of dose for rats in the sham group $(F(3, 18) = 2.02, p = .15;$ *Figure 3*) however, there was a significant main effect of dose for the lesion group animals $(F(3, 21) = 3.61, p = .03;$ *Figure 4*) which was driven by a significant difference in performance when comparing the saline to the 0.2 mg/kg dose condition when averaging across signal duration $(t(7) = 2.57, p = .04)$. There were no differences involving group as a factor for hits or correct rejections (all $p > .23$). There was no difference between the two groups for omissions at any dose (all $p > .15$).

Additionally, there was a significant main effect of scopolamine dose on VI $(F(3, 39) = 4.80, p = .01;$ *Figure 5*). Paired samples t-tests revealed that all animals had significantly poorer performance after the 0.2 mg/kg dose when compared to the saline dose $(t(14) = 2.93, p = .01)$. The effects of scopolamine on VI were primarily due to decreased signal detection performance, as evidenced by a significant main effect of dose on percentage hits $(F(3, 39) = 6.26, p < .01;$ *Figure 6*) although there was also a trend for decreased correct rejection performance for all animals $(F(3, 39) = 2.22, p = .10;$ *Figure 7*). Follow up paired samples *t-* tests indicated that there was a difference in

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hits performance between the saline and 0.1 mg/kg dose ($t(14) = 2.61, p = .02$) and the saline and the 0.2 mg/kg dose ($t(14) = 3.15, p < .01$) for all animals.

Histology Results. For several animals, the quality of the histological processing did not permit quantitative evaluation of the lesions, thus limiting this analysis to a qualitative evaluation. Infusion of GAT1-saporin into the BF appeared to decrease the density of PV-positive neurons in the area, compared to the PV-positive fiber density in sham lesioned animals (*Figure 8*). This suggests that there was loss of GABAergic neurons within the BF as a result of bilateral infusions of GAT1-saporin.

Acetycholinesterase (AChE) staining revealed that there were similar numbers of AChE-positive fibers within the parietal cortex for both GAT1-saporin and sham lesion animals (*Figure 9*). This suggests that there was no loss of cortical cholinergic connections due to GAT1-saporin lesions.

Experiment 2

Effects of Distracter Exposure. Prior to the introduction of the distracter, a group x signal duration ANOVA revealed that there were no significant group differences in performance on the two-choice sustained attention task prior to the introduction of the distracter. The mean percent hits for the distracter- exposed animals the three days prior to the addition of the distracter were as follows; $M_{500} = 87.32$ ($SEM = 0.55$), $M_{100} = 66.24$ ($SEM = 1.76$), $M_{25} = 44.59$ ($SEM = .75$), $M_{CR} = 87.67$ ($SEM = 0.85$). The mean percent hits for the non-distracter-exposed animals on the same three days were as follows; $M_{500} = 84.62$ ($SEM = 0.65$), $M_{100} = 75.06$ ($SEM = 0.90$), $M_{25} = 44.25$ ($SEM = 1.50$), $M_{CR} = 85.99$ ($SEM = 0.15$). To evaluate performance after the introduction of the distracter but before the addition of the light-location

discrimination task, a block (Block 1: Sessions 1-5, Block 2: Sessions 6-10, Block 3: Sessions 11-15, Block 4: Sessions 16-20) x signal duration (500ms, 100ms, 25ms) x condition (distracter, non-distracter) ANOVA was conducted to evaluate hits performance on signal trials. There was a significant block x signal duration x condition interaction ($F(6, 108) = 2.74, p = .02$).

Independent samples t-tests were conducted, comparing the distracter- and non-distracter- exposed animals at each block, to determine the basis for the significant three-way interaction. These analyses showed that the percentage of hits by the distracter animals was lower when compared to the non-distracter rats for the 100 ms signal duration trials during block 1 ($t(18) = 2.74, p = .01$) and for the 500 ms trials ($t(18) = 2.58, p = .02$) and 100 ms trials ($t(18) = 4.46, p = .049$ during block 2 (*Figure 10; Figure 11*). There were no significant differences in hits at any signal duration between the two groups during block 3 (all $p > .16$) or block 4 (all $p > .10$). Overall, there was decreased performance across the first 10 sessions (blocks 1 and 2) after the addition of the distracter but performance recovered during the final 10 sessions (blocks 3 and 4).

Furthermore, a block x signal duration x condition ANOVA conducted for VI performance did not yield a significant three way interaction ($F(6, 108) = 2.13, p = .06$). For non-signal trials and omissions, block x condition ANOVAs did not yield any significant interactions (all $p > .09$).

Task Performance Following Inclusion of Light-Location Discrimination Task. After the introduction of the light-location discrimination task, a block (Block 1: Sessions 1-5, Block 2: Sessions 6-10, Block 3: Sessions 11-15, Block 4: Sessions 16-

20) x signal duration (500 ms, 100 ms, 25 ms) x condition (distracter, non-distracter) ANOVA was executed to evaluate VI and hits for the sustained attention task and block x condition ANOVAs were conducted to evaluate performance on non-signal trials and the light-location discrimination task. There were no significant differences between distracter and non-distracter group performance on any measure for the sustained attention task and the light-location discrimination task during the sessions with 40% trials of the light-location discrimination task (all $p > .09$).

During the 70% light-location discrimination sessions, there was a significant signal duration x condition interaction when assessing relative hits on the sustained attention task ($F(2, 36) = 4.05, p = .03$). This interaction was due to a significant difference in performance between the distracter and non-distracter rats for the 25 ms trials ($t(18) = 3.26, p < .01$). The distracter-exposed animals had a higher percentage of hits than the non-distracter animals (*Figure 12*). There were no differences in performance between the groups when evaluating VI, correct rejections or performance on the light-location discrimination task for the 70% sessions (all $p > .15$)

Scopolamine Administration. The effects of scopolamine along with distracter condition on sustained attention performance was analyzed using dose (Saline, 0.1 mg/kg, 0.2 mg/kg) x signal duration (500 ms, 100 ms, 25 ms) x condition (distracter, non-distracter) ANOVAs for the signal, and non-signal (without the signal duration factor) trials. The 0.4 mg/kg dose was removed from analyses because of the high omission rate ($M = 163.50$). There was a significant effect of scopolamine dose on signal trial performance for all animals ($F(2, 36) = 5.67, p = .01$; *Figure 13*) and on VI ($F(2, 22) = 3.52, p = .047$). These effects were mainly due to a significant difference

between hits after the saline and 0.2 mg/kg scopolamine injections ($t(9) = 2.99$, $p = .02$).

There were no significant effects involving condition or scopolamine dose as factors for correct rejections on the sustained attention task (all $p > .09$). A scopolamine dose (Saline, 0.1 mg/kg, 0.2 mg/kg) x condition (distracter, non-distracter) ANOVA was conducted to evaluate performance on the light-location discrimination task which indicated no significant difference in performance depending on scopolamine dose or condition (all $p > .46$).

Nicotine Administration. ANOVAs were conducted to evaluate the effect of nicotine on the distracter and non-distracter animals. Sustained attention task performance was assessed using nicotine dose (Saline, 0.1 mg/kg, 0.2 mg/kg) x signal duration (500 ms, 100 ms, 25 ms) x condition (distracter, non-distracter) ANOVAs for signal trials, and similar ANOVAs (without the signal duration factor) for non-signal and light-location discrimination trials. Because of high omission rates the 0.4 mg/kg nicotine dose condition was not included in data analysis ($M = 176.10$).

There was a significant effect of nicotine dose on signal trials ($F(2, 36) = 4.28$, $p = .02$; *Figure 14*) for all animals. Follow-up paired samples t-tests revealed that there was a difference in hits when comparing the saline and the 0.2 mg/kg dose ($t(19) = 2.77$, $p = .01$). Performance on the non-signal trials revealed a significant main effect of dose ($F(2, 36) = 8.17$, $p < .01$; *Figure 15*). There was a significant difference in performance on non-signal trials when comparing saline to 0.1 mg/kg trials ($t(19) = 2.05$, $p = .054$) and 0.2 mg/kg trials ($t(19) = 3.37$, $p < .01$). In all of these cases performance was worse after nicotine injection when compared to saline. In

combination, there was also a main effect of nicotine dose on VI ($F(2, 10) = 5.38$, $p = .03$). Follow-up paired samples t-tests reveled a significant difference in performance when comparing the saline to the 0.2 mg/kg dose condition ($t(15) = 2.80$, $p = .01$).

A nicotine dose x condition ANOVA indicated that there was no significant main effect of nicotine dose when comparing the distracter-exposed and non-distracter-exposed animals for the light-location discrimination task ($F(2, 36) = 1.92$, $p = .16$).

Discussion

The main purpose of the present experiments was to investigate the neurobiological factors that contribute to age-related changes in attention and cognitive flexibility. Therefore, these experiments may provide a stepping stone to better understand these potentially life altering declines.

Experiment 1: Interactions Between Basal Forebrain GABAergic Neurons and Cholinergic Muscarinic Receptors

The main results from experiment 1 reveal an interaction between BF GABAergic neurons and the cholinergic system that affects attentional task performance. There were no differences in attentional performance between the GABAergic lesion group and the sham lesion group prior to scopolamine injections. However, with the addition of injections of scopolamine, GABAergic lesioned animals exhibited lower VI (signifying decreased attentional performance on both signal and non-signal trials) scores compared to sham rats. This group difference was driven largely by decreased attentional performance by lesioned animals when comparing the saline to the 0.2 mg/kg dose of scopolamine. When analyzing the trial types individually, the deficit could not be specified to hits or correct rejections. Exploratory

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analyses indicated that the difference in the effect of scopolamine on VI between the two groups indicates that lesioned animals were more susceptible to the attentional deficits caused by the blocking of muscarinic receptors. There were also no significant differences between the two groups when analyzing the omission data which supports that the lesion and drug-induced changes in task performance are not due to changes in motivation or motoric abilities. Overall the results from experiment 1 indicate that lesioning BF GABAergic neurons and blocking the muscarinic receptor system causes a decrease in attentional performance.

Results from experiment 1 also indicated a decrease in VI in both the sham and lesion groups as scopolamine dose increased. This effect was mainly driven by a significant main effect of dose on decreased signal detection performance (percent hits). These results are consistent with findings of previous studies that highlight the successful use of scopolamine to decrease attentional performance on signal trials through the blocking of muscarinic receptors (i.e. Johnson & Burk, 2006; McQuail & Burk, 2006).

The results from experiment 1 are complementary to the findings of Burk and Sarter (2001), Lau and Salzman (2008) and Yi and colleagues (2014) by highlighting the importance of non-ACh neurons within the BF on attention. However, this experiment goes beyond these findings through selectively lesioning GABAergic neurons in the BF and then acutely blocking the muscarinic receptors. By being able to differentially render these systems inactive we are better able to understand how each system moderates attention individually and then in combination with one another. The lesioned animals did not differ in performance from the sham animals immediately

after surgery. However, there was a main effect of group once drug administration procedures began. The data suggest that there was a trend for group differences post lesion that became significant with drug administration. The results from this experiment indicate that although the loss of GABAergic connections in the BF independently does not lead to a decrease in attentional performance, the loss of GABAergic BF neurons makes an organism more vulnerable to the negative attentional effect due to the blocking of muscarinic receptors within the cortex.

Interestingly, the results from GABAergic lesions and scopolamine injections in this experiment differentially affected attentional performance beyond what has been shown in previous studies. Research that has solely lesioned BF ACh neurons has typically shown a pattern of attentional deficits through decreased hit rate (poorer performance on signal trials). Moreover, Burk and Sarter (2001) performed indiscriminate lesions of both GABAergic and cholinergic neurons using ibotenic acid. The resulting attentional deficit was highlighted through an increase in false alarms (indicating incorrect responses to non-signal trials). In experiment 1, differences between the lesion and sham groups after scopolamine injections were observed when analyzing VI (a combination of both hits and correct rejections). The discrepancy in attentional deficits indicates a difference in the neurobiological responses to the three types of lesions. In experiment 1 there was an initial loss of GABAergic neurons due to the lesion while ACh neurons were preserved. was not until the muscarinic receptors were blocked that accuracy significantly declined for both signal and non-signal trials. This indicates that disrupting these two systems caused a decreased ability to

differentiate between the signal and non-signal indicators, possibly reflecting a limited capacity to apply the proper rules for each trial type.

Experiment 2: The Effects of Introducing a Visual Distracter on Plasticity in Aged Rats

The goal for experiment 2 was to measure the effects of the addition of a distracter (flashing houselight) on attentional performance in a learned sustained attention task and a new light-location discrimination task in aged rats. The effects of the initial distracter exposure decreased hits are longer signal durations during the first 10 sessions for the distracter-exposed group compared to the non-distracter-exposed rats. This effect was found at the longest signal durations which indicates that the difference in performance between the two groups is not due to a deficit in perception of the signal. Rather, it suggests a deficit in the processing of and application of the rules following that signal. If this is the case, the 500 ms signal would be where the errors would occur because it is the most likely signal duration to be detected, but perhaps errors occurred in the inability for the animals to apply the rules after the detection of the signal. During blocks 3 and 4 there was no difference between the distracter- and non-distracter-exposed groups on sustained attention task performance. These results indicate that although there was an initial decrease in hits due to the flashing houselight, the animals were able to compensate for the added distracter and regain attentional performance similar to that of the non-distracter-exposed animals.

The two groups did not differ on sustained attention or light-location discrimination task trials after the modification of 40% blocks of the light-location discrimination task. However, there was a difference during the 70% blocks of the

light-location discrimination task between the two groups on the sustained attention task. Specifically, the distracter-exposed rats had a higher percentage hit rate at the 25 ms signal duration compared to the non-distracter-exposed rats. There were no differences between the groups on any other measure of sustained attention or the light-location discrimination task indicating that the distracter-exposed animals were able to surpass the non-distracter-exposed animals at the shortest signal duration on the sustained attention task while maintaining performance on other measures of attention. These results suggest that the animals' ability to compensate for the addition of the distracter increased behavioral or cognitive flexibility. The higher level of hits at the shortest (the most attentionally taxing) signal duration by the distracter-exposed animals above the non-distracter-exposed animals while not sacrificing performance on any other measure of attention, suggests that these animals were able to maintain higher levels of attention compared with non-distracter-exposed rats.

This effect is similar to but not as pronounced as the results found in Hirsh and Burk (2013). In the previous study, the distracter-exposed rats showed improved performance beyond the non-distracter-exposed rats in both the sustained attention and the light-location discrimination tasks whereas the aged animals in the current experiment only displayed better performance during the sustained attention task at the 25 ms signal duration. Also, it took the distracter-exposed animals 10 sessions to regain attentional performance similar to the non-distracter-exposed animals. Previous research showed that younger animals regained attentional performance after 1 session (Hirsh & Burk, 2013). These discrepancies indicate that although aged animals do have

sufficient neuroplasticity to benefit from overcoming an increase in attentional demand, the effects are not as robust as what has been seen in younger animals.

There was a decrease in hits for both groups after the administration of the highest dose of scopolamine. This further confirms the negative effects that muscarinic receptor blockade has on attentional performance. Moreover, there were no differences between the distracter-exposed and non-distracter-exposed animals' performance after scopolamine administration. Therefore, the addition of the distracter did not interact with scopolamine dose to change attentional performance. Scopolamine did not have a significant effect on performance on the light-location discrimination task. This selective effect of scopolamine may implicate muscarinic receptors in certain aspects of attention, such as switching between signal and non-signal trials, that are necessary for the two-choice sustained attention task but not for the light-location discrimination task for aged animals.

There was also a decrease in hit (correct responses to a signal trial) and correct rejection (correct responses to a non-signal trial) percentage following injections of nicotine for both distracter- and non-distracter-exposed rats. This is counter to previous research that has found that the stimulation of nicotinic receptors increases attention in both animals and humans (i.e. Rezvani & Levin, 2001). This could be due to the rat's age (beyond 20 months). It also indicates that, similar to scopolamine, the addition of the distracter did not alter the effects of nicotine and there was no significant effect of nicotine on performance on the light-location discrimination task.

Limitations and Future Directions

There are multiple ways to build upon the findings from experiment 1. First, the ip injections of scopolamine used in this experiment blocked muscarinic receptors throughout the brain and periphery. Future research could focus on altering the cholinergic system in precise cortical areas. Blocking muscarinic receptors in the prefrontal cortex may be of particular interest because previous research has indicated that the prefrontal cortex is abundant in ACh neurons and controls executive functioning (Fuster, 1988).

Furthermore, it would be beneficial to investigate how blocking the different types of cholinergic receptors would affect attentional performance. ACh binds to nicotinic and five different types of muscarinic receptors (M_1-M_5). Previous research as shown that blocking M_1 receptors using dicyclomine significantly decreases attentional performance in the same sustained attention task as used in the present experiments (Robinson, Mangini, & Burk, 2012). The results from this study showed that blocking M_1 receptors decreased performance at the longest signal duration during the two-choice sustained attention task with and without the presence of a flashing houselight distracter. Therefore, selectively blocking this class of receptors may have differing effects on attentional performance and further specify which aspects of the cholinergic system interact with the GABAergic system to affect attention.

Because of the decrease in glutamate neurons associated with aging and the inverse relationship between glutamate and GABA neurons (Masliah, Hansen, Alford, Deteresa, & Mallory, 1996) it would be of interest to go beyond the findings of Experiment 1 to investigate the potential interaction between BF glutamate neurons

and the cholinergic system that may affect attention. This could be carried out through the development of an immunotoxin that could be used to surgically lesion glutamate neurons within the BF and then pharmacologically manipulating the cholinergic system through ip injections of scopolamine and nicotine.

Further studying the effects of GABAergic lesions in aged animals would provide an important extension of experiment 1. An increase in GABA activity has been cited as a factor in cognitive decline for both normal aging and in AD (Marczynski, 1998). Therefore, perhaps lesioning GABAergic neurons within different brain regions could be protective against this increase in GABAergic neurons associated with aging.

Future studies could also investigate the relationship between the GABAergic and cholinergic systems and its effect on attention in aged animals. For example, an experimenter could administer GABAergic lesion early in life, in early adulthood or in advanced age and observe the effects of cholinergic manipulations due to nicotine and scopolamine injections on attentional performance once the animals have aged. A potential difference in the pattern in attentional performance due to the timing of the lesions may reveal important data on the age that vulnerability is highest for the effects of changes within GABA and glutamate systems to interact with the cholinergic system to contribute to attentional cognitive decline. For example, perhaps lesions that occur earlier in life will force the cholinergic system to compensate more for the loss of these neurons throughout life and rely more on these connections to perform in attention demanding tasks. Therefore, there would be a more drastic effect of manipulations to the cholinergic system on attention if the lesions occurred at an earlier age.

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There are also experimental manipulations that could further the findings from experiment 2. One future study could explore the effect of the addition of a flashing houselight distracter in combination to ip injections of scopolamine and nicotine injections on attentional plasticity in younger animals. The results from this study would be comparable to the results from Hirsh and Burk (2013) by investigating how pharmacological manipulations to the cholinergic system may change plasticity at a younger age and to the current experiment by examining if cholinergic manipulations have a different effect on younger animals as compared to aged animals. Young animals would most likely show the typical effects of nicotine and benefit from nicotine administration counter to the results found in experiment 2 where nicotine decreased attentional performance in aged animals.

Additionally, future studies could surgically lesion BF cholinergic neurons using 192 IgG-saporin in both young and aged animals and compare performance on the sustained attention task and light-location discrimination task after the addition of the distracter. During experiment 2 manipulations to the cholinergic system were very selective and did not completely render the cholinergic system inactive (scopolamine only blocking muscarinic receptors and nicotine only activating nicotinic receptors). The use of 192 IgG-saporin would produce a more general inactivation within the cholinergic system within one brain region through destroying ACh neurons within the area of infusions. Rather than acutely manipulating specific receptors within the cholinergic system throughout the entirety of the brain this study would selectively lesion cholinergic connections projecting from the BF and investigate their effect on behavioral flexibility. Furthermore, these immunotoxic lesions could be administered

to specific cortical areas, for example the prefrontal cortex, to see how cholinergic connections within that region interact with the flashing houselight distracter to affect attention.

Because of the preceding cholinergic and GABAergic theories of cognitive decline, future studies could integrate the methods from experiment 1 and experiment 2 to look at the interaction between of the cholinergic and GABAergic systems that may lead to changes in both neuroplasticity and attention. This can be carried out in a number of ways. Altering the timing of the pharmacological and task manipulations could provide further insight into the interaction between these two neurotransmitter systems and attention and neuroplasticity. For example, the lesion to the BF GABAergic system could occur first followed by the addition of the distracter. Then the light-location discrimination task would be introduced the cholinergic system manipulated through ip injections of scopolamine and nicotine. Also, the addition of the distracter could take place first followed by the BF GABAergic lesions and then the introduction of the light-location discrimination task with pharmacological cholinergic manipulations. These methods would test whether the ability to compensate for the distracter and maintain attentional performance prior to the loss of BF GABAergic neurons could be protective against the attention deficits.

Overall, these studies have helped to advance the understanding of two major symptoms of cognitive decline associated with age: decline in attention and in neuroplasticity. Understanding the neurobiological processes that underlie these two processes can help to better understand their origins and causes. Much of the focus of the neurobiological causes of AD is targeted at the formation of β-amyloid plaques and

neurofibrillary tangles. Additionally, both ACh and GABAergic transmission have been implicated the formation of β-amyloid. Previous research has shown that activation of M_1 receptors can lead to decrease β-amyloid (Hock, 2001) and β-amyloid plaques disrupt glutamatergic and GABAergic transmission within the brain (Nava-Mesa, Jiménez-Díaz, Yajeya, & Navarro-Lopez, 2014). Perhaps a more holistic understanding of the underlying mechanisms (both neurotransmitter and neurobiological changes) through which decreases in attention and neuroplasticity manifest will lead to a more complete and better understanding of AD as well as normal age related cognitive decline.

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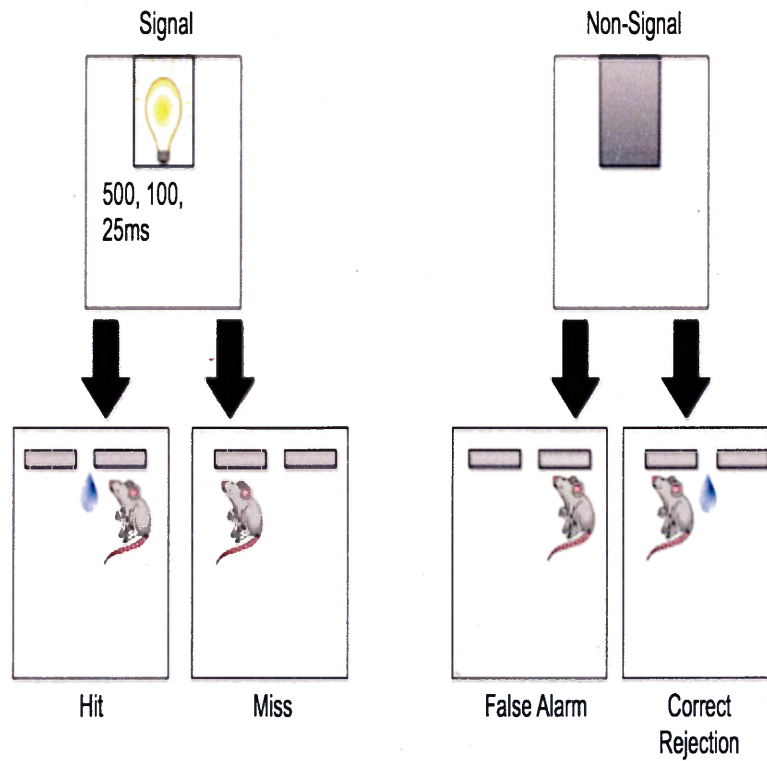
Figure 1. A visual representation of the two-choice sustained attention task. Animals received water access after correctly pressing the trained lever after to signal or non-signal prompts. The correct response lever on each trial type was counter balanced for half of the rats.
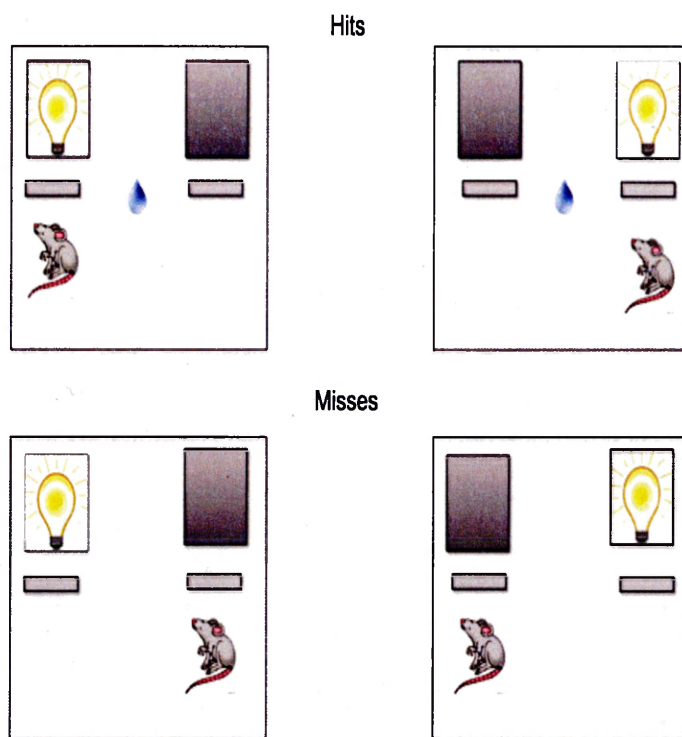
Figure 2. A visual representation of the light-location discrimination task used in experiment 2. For this task, rats had to indicate which of two signal lights illuminated by pressing the lever under the light to receive a reward of water.

Sham VI Performance
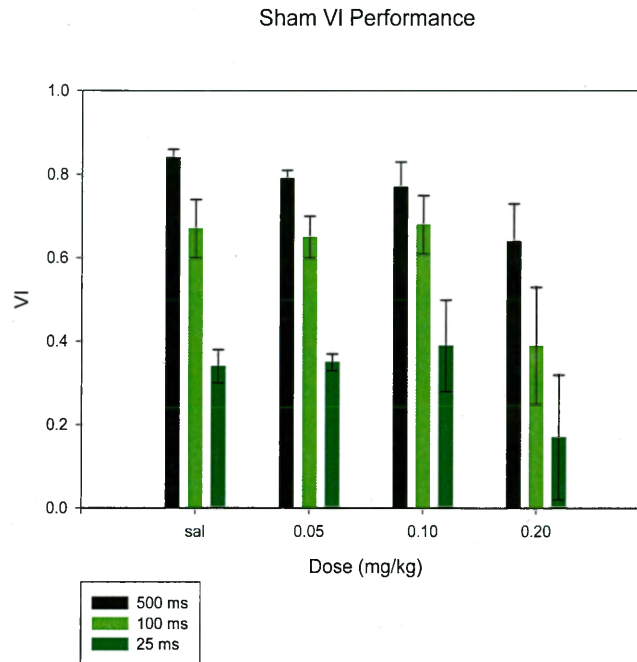
Figure 3. There is a trend of decrease performance as scopolamine dose increases and signal duration decreases for the sham animals. However, there is no significant difference in performance depending on scopolamine dose; $p = .15$

Lesion VI Performance
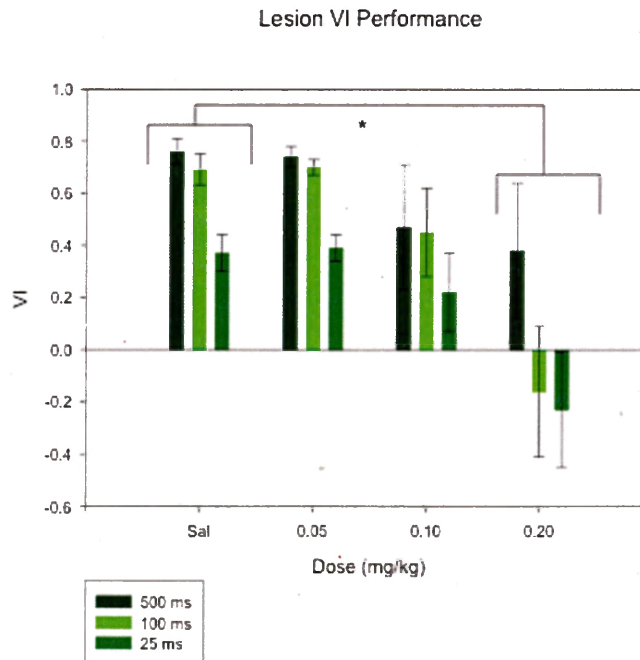
Figure 4. When comparing to the sham graph above there is a similar trend of decreased performance at higher doses of scopolamine and shorter signal trials. However, there is a significant decrease in performance after the 0.2 mg/kg dose; $p = .03$.

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Effects of Scopolamine on VI
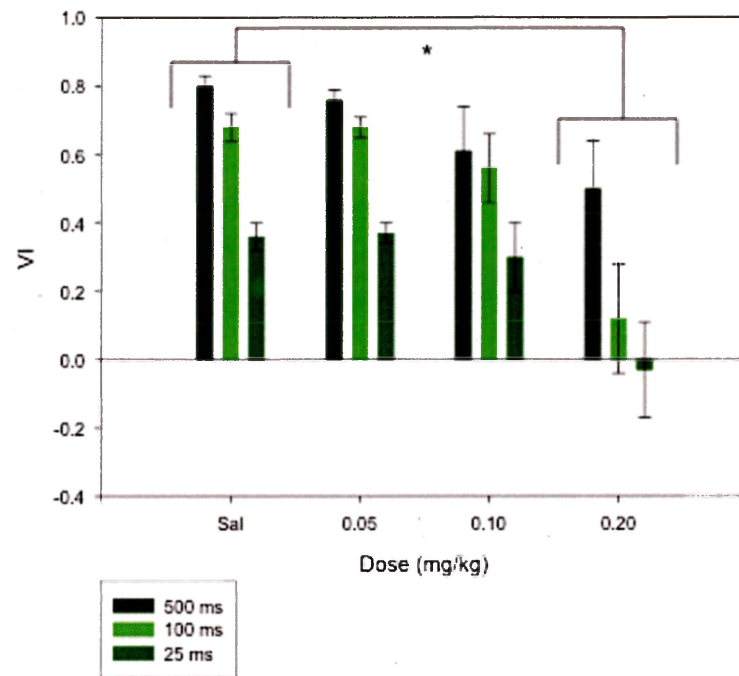
Figure 5. All animals (both sham and lesion) displayed a significant decrease in VI as scopolamine dose increased; $p = .01$.

Effect of Scopolamine on Hits
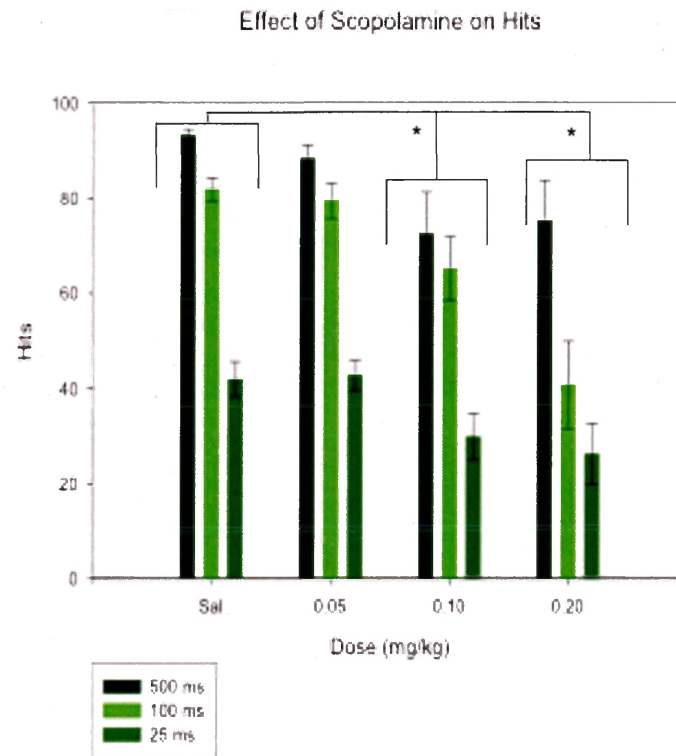
Figure 6. As scopolamine dose increased, all animals showed a significant decrease in the hit rate; $p = .001$.

Effects of Scopolamine on CR
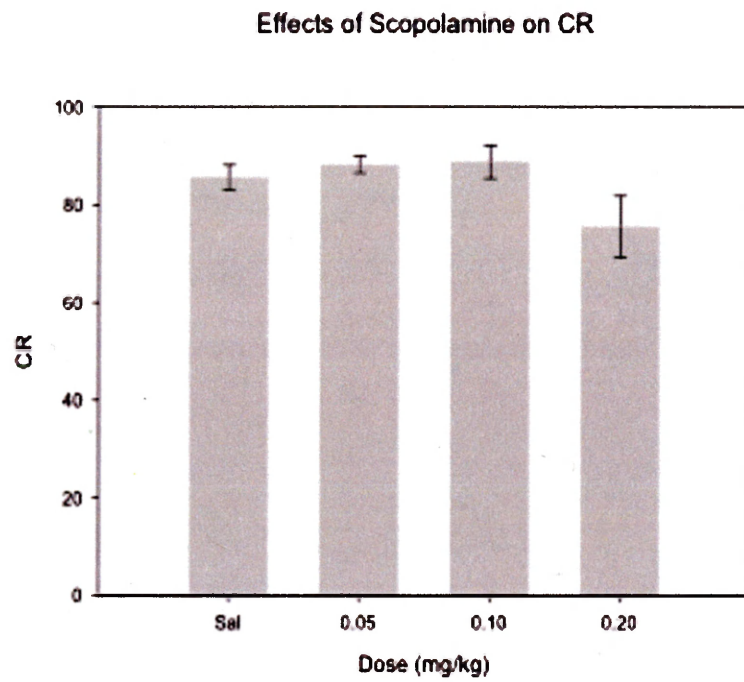
Figure 7. As scopolamine dose increased, all animals showed a non-significant trend of decreased accuracy on non-signal trials; $p = .08$.
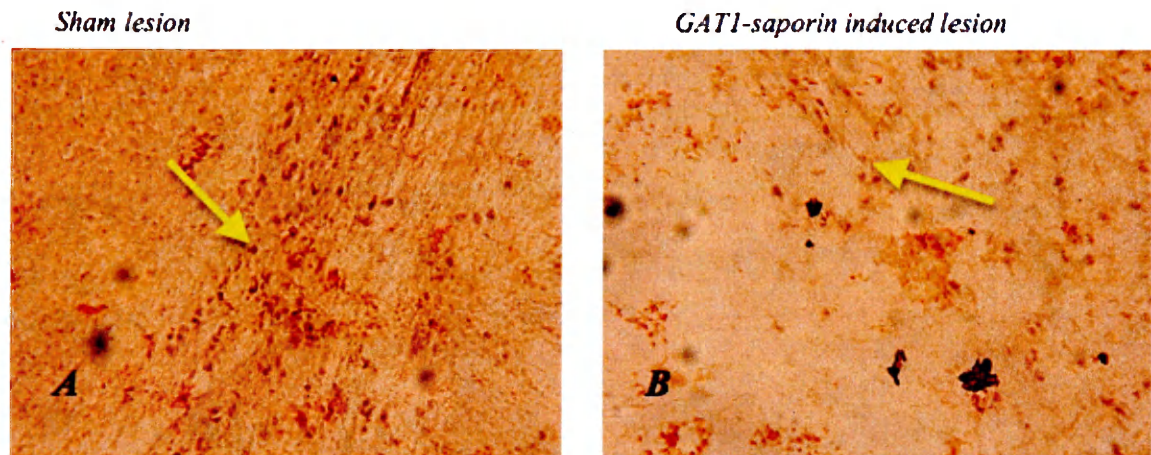
Figure 8. This figure depicts parvalbumin (PV) stained BF neurons after A) sham lesion and B) GAT1-saporin lesion to bilateral hemispheres at 40x magnification from experiment 1. The GAT1-saporin lesion shows a decrease in PV-positive neurons compared to the sham lesion.

Sham lesion

GAT1-saporin induced lesion
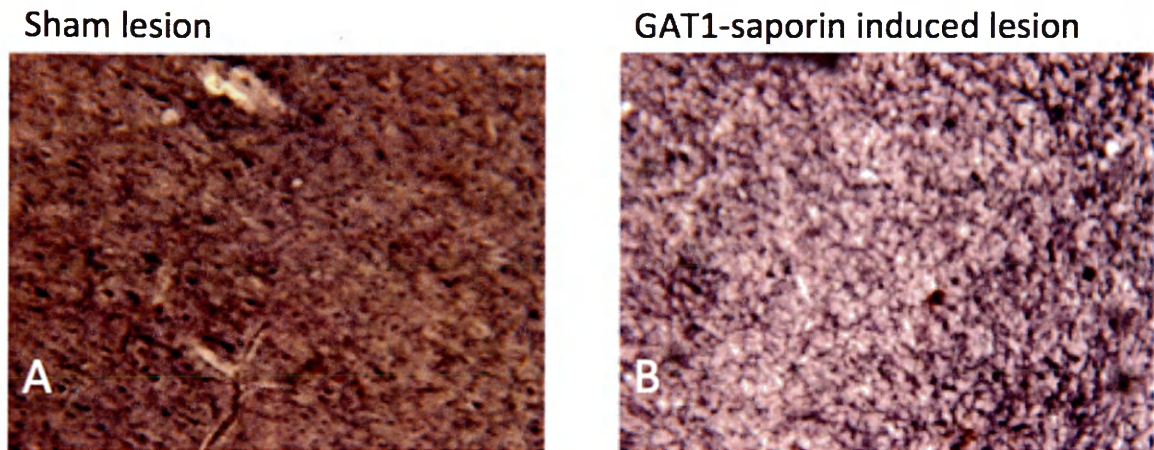
Figure 9. This figure depicts acetycholinesterase (AChE) staining within parietal cortex in a A) sham lesioned and B) GAT1-saporin lesioned animal at 20x magnification from experiment 1. These figures show similar preservation AChE positive neurons within the cortex for both the sham and lesion groups.
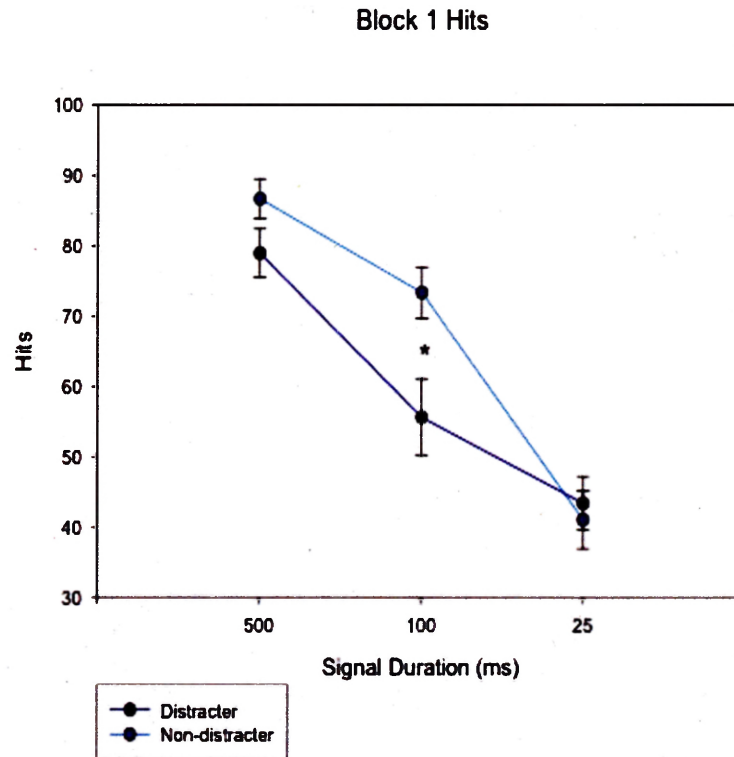
Block 1 Hits

Figure 10. Hit rate on the two-choice sustained attention task for block 1. There was a significant difference in hits for the 100ms trials where the non-distracter rats performed better than the distracter animals; $p = .01$.
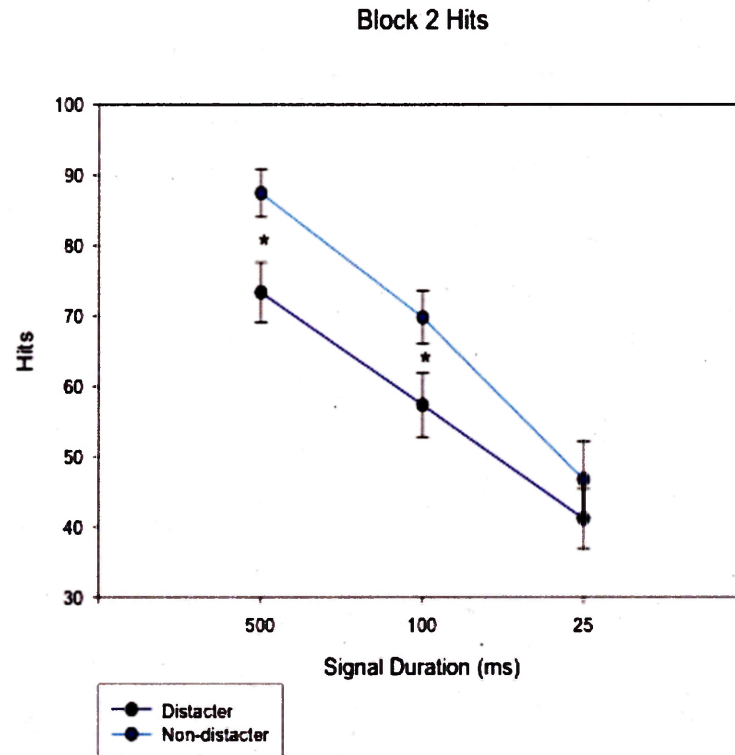
Figure 11. Hit rate in the two-choice sustained attention task for block 2 of this is illustrated here. Non-distracter animals had a significantly higher percentage of hits for both the 500 and 100 ms trials than the distracter animals; all $p < .05$.
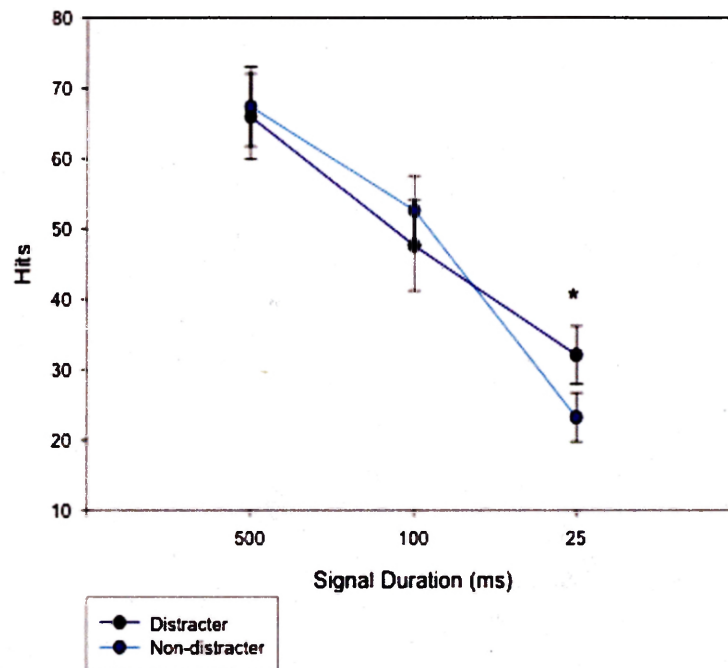
Sustained Attention Hits at 70% Novel Task

Figure 12. Hits performance on the two-choice sustained attention task during the 70% light-location discrimination task. During these sessions, the distracter animals had a significantly higher percentage of hits during the 25ms signal duration trials, *p* = .01.
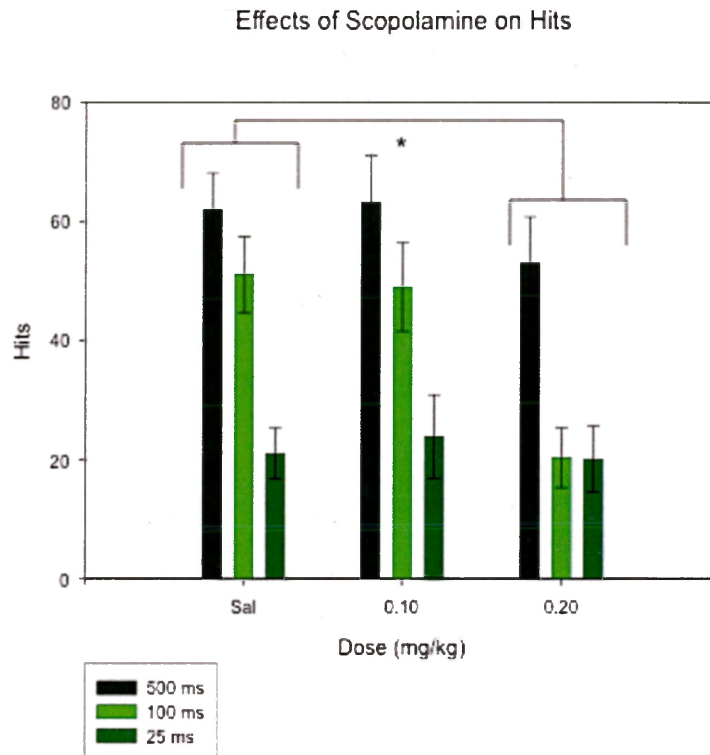
Effects of Scopolamine on Hits

Figure 13. There was a significant effect of scopolamine dose on hits performance for all animals, $p = .01$, which was mainly due to a difference in performance between the saline and 0.2 mg/kg doses.
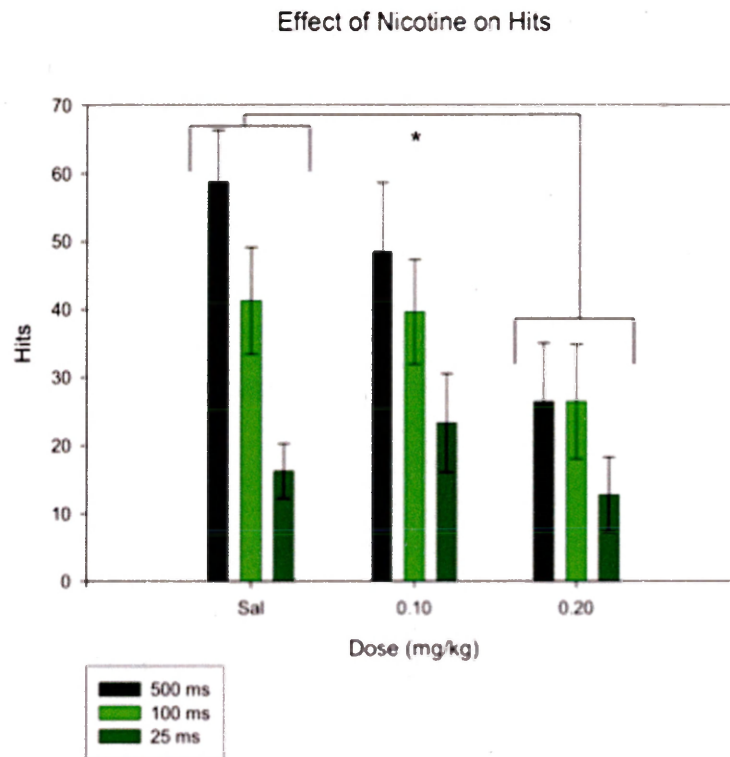
Effect of Nicotine on Hits

Figure 14. There was a significant effect of nicotine dose on hits performance for all animals, $p = .02$, which was mainly due to a difference in performance between the saline and 0.2 mg/kg doses.

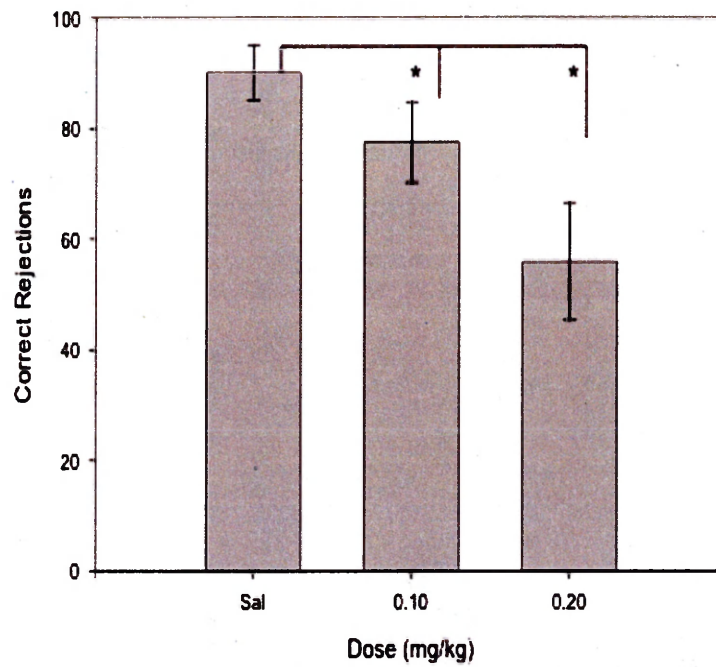Effects of Nicotine on Correct Rejections

Figure 15. Nicotine dose had a significant effect on non-signal trials, $p < .01$ where saline performance was significantly higher than both 0.10 mg/kg and 0.20 mg/kg performance.