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Environmental enrichment attenuates nucleus basalis lesion induced impairments to attention

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ENVIRONMENTAL ENRICHMENT ATTENUATES NUCLEUS BASALIS LESION INDUCED IMPAIRMENTS TO ATTENTION

A Thesis

Presented to the

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In Partial Fulfillment

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in

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ABSTRACT

The current experiment examined the effects of environmental enrichment on performance in an attention-dependent learning task in rats with selective lesions of the cholinergic nucleus basalis magnocellularis (NBM). To test the hypothesis that environmental enrichment would attenuate NBM lesion-induced impairments in attention-dependent learning, performance in NBM lesion and sham lesion control rats was assessed following rearing in either an enriched environment or in standard housing conditions. Results supported the hypothesis that compared to sham lesion controls, rats with NBM lesions (regardless of housing condition) would show impaired attention-dependent learning in the incremental attention task. This main effect, however, was largely a reflection of the severe impairment observed in the NBM lesion group raised in the standard housing condition. Results also supported the hypothesis that animals reared in an enriched environment (regardless of lesion condition) would show higher levels of performance in the attention-dependent learning task compared to animals reared in standard housing. Compared to all other groups,

the NBM lesion group reared in standard housing conditions showed persistent, low levels of performance. These results supported the main hypothesis that environmental enrichment would attenuate NBM lesion-induced impairments in attention-dependent learning. It was also found that sham lesion control rats reared in enriched environments showed better attention-dependent learning then those reared in the paired housed control condition; normal enriched animals showed near asymptotic levels of performance as early as the first block of training in Phase II of the incremental task, whereas control animals reared in the paired housing condition required all three blocks of training in order to reach comparable levels of performance. These findings demonstrate that environmental enrichment can enhance attention-dependent learning performance in normal animals as well as attenuating NBM lesion-induced impairments otherwise seen following rearing in standard conditions.

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CHAPTER ONE

ENVIRONMENTAL ENRICHMENT EFFECTS ON LEARNING AND BEHAVIOR

Introduction

As advancements in the medical field extend the average human lifespan, there is an increase in the number of people who will suffer from age-related cognitive defects such as dementia and Alzheimer's disease (AD). These disorders can have devastating effects on cognitive functions such as the ability to learn, remember, and reason. The precise causes of these disorders are not fully understood, although genetic (Bertram et al., 2000; Sleegers et al., 2004), environmental (Hertzog, Kramer, Wilson, & Lindenberger, 2008), and lifestyle factors (Hultsch, Hertzog, Small, & Dixon, 1999) play a role in both the likelihood of developing these disorders and the progression of these disorders once developed. Researchers are working towards a greater understanding of the causes of dementia and are actively developing effective treatments and preventative plans of action.

Current research suggests that cognitive enrichment can protect the brain against some of the damage caused by ageing. In humans, higher levels of education are correlated with a reduced likelihood of developing dementia (Anstey & Christensen, 2000). There is also evidence that suggests that people who have cognitively demanding careers and engage in mentally stimulating leisure actives such as reading, puzzle building and complex games early in life have higher levels of mental functioning later in life and show a decreased likelihood of developing dementia and AD (Bosma et al., 2003; Mulatu & Schooler, 1999; Wilson et al., 2002).

There is a clear link between cognitive enrichment and cognitive function later in life. However, the correlational data from human studies does not allow a distinction between the possibility that structural or functional changes occur in the brain as a result of enrichment, or if pre-existing structural differences led some to seek enrichment while others do not (Milgram, Siwak-Tapp, Araujo, & Head, 2006). Animal research has been critical in resolving this issue because it allows researchers to control for genetic and environmental factors. The enriched environment (EE) protocol developed

by Rosenzweig et al. (1966) partly models the phenomena of cognitive enrichment in humans, while allowing researchers to observe the precise changes occurring in the brain as a result of that enrichment. EE is a way of rearing animals that provides them with opportunities for exploration (e.g., tunnels, toys, and a regularly changing environment), exercise (e.g., running wheels and climbing opportunities) and social interactions afforded by group housing.

Environmental Enrichment and Changes in Brain Structure and Function

EE causes a variety of neurological changes. Enriched animals show increased cortical thickness and weight (Beaulieu & Colonnier, 1987; Mohammed et al., 2002; Sale, Berardi, & Maffei, 2008), tend to have longer dendritic spines and more dendritic branching (Greenough, 1973), show greater levels of synaptic connectivity than control animals (Mollgaard, 1971) and show an increased numbers of hippocampal neurons (Kempermann, 1997).

Enrichment also affects several neurotransmitter systems (Mora, Segovia, & del Arco, 2007). As a result of enrichment, higher levels of mRNA expression have been found in the serotoninergic 5-HT1A receptors located in

the dorsal hippocampus (Rasmuson et al., 1998). Turnover of the neurotransmitter serotonin in the hippocampus of enriched animals is increased relative to controls reared in standard housing conditions (Galani et al., 2007). Increases in dopamine levels normally associated with stress are lower in animals reared in an EE (Segovia, Del Arco, De Blas, Garrido, & Mora, 2008). Researchers have also found increased levels of glutamate and GABA in the hippocampus of rats reared in an enriched environment (Segovia, Yagüe, García-Verdugo, & Mora, 2006.)

Cholinergic Function, Learning and Memory, and Environmental Enrichment

The neurological changes that occur as a result of enrichment have been linked to advantages in learning and memory (Berardi, Maffei, & Sale, 2008), stress reduction (Segovia, Del Arco, De Blas, Garrido, & Mora, 2008; Del Arco et al., 2006, and attenuating aging-induced impairments in cognition (Arnaiz et al., 2004). Because the cholinergic system is damaged in AD, and AD patients appear to benefit from enrichment, animal research on the effects of EE has gained considerable attention. The effects of rearing rats in EE on the cholinergic system,

learning, memory, and stress have been evaluated in a number of different studies.

Del Arco and colleagues (2006) examined the effects of environmental enrichment on the release of dopamine and acetylcholine in the prefrontal cortex during both working memory performance and acute stress. Stress impairs performance in working memory tasks and both dopamine and acetylcholine neurons in the prefrontal cortex are activated during stress. Animals reared in an enriched environment show fewer behavioral impairments associated with stress compared to pair-housed animals. Del Arco et al. (2006) hypothesized that environmental enrichment would modulate the release of dopamine and acetylcholine in the prefrontal cortex in response to acute stress.

To test this hypothesis, these researchers randomly assigned 3-month-old male Wistar rats to either an enriched environment condition or a singly housed control condition for 12 months. The enriched environment rats were housed in large cages with 10-12 other rats. The cage had two running wheels, toys that were exchanged every 5-6 days and tunnels that could be rearranged to provide novel expiration routes. The control rats were

housed in small cages containing only bedding, food, and water. During the 12-month-housing period, rats from both groups were briefly handled once a week. At the end of this period, all rats were first tested for spontaneous motor activity in an open field, where photo beams were used to recorded locomotor activity.

Next, working memory was tested by training the rats from both housing conditions in a delayed alternation task in a water escape T-maze. The delayed alternation task consisted of 10-paired forced-choice trials. In the forced run, one arm of the T-maze is blocked. The rat is then placed in the center arm of the T-maze and allowed to swim only to one side of the maze to find the submerged escape platform. In the following choice run, both arms of the maze were open and the rat was placed in the center arm. Animals choose whether to swim left or right to locate the hidden platform. The platform in the choice run was always located in the arm that was blocked during the forced run. Normal rats learn to choose the opposite arm from the arm open during the forced run. If the rats choose the incorrect arm during the choice run a sliding door trapped the rat in the water for 10 seconds. The door was then opened and the rat was allowed to find

the platform on the other side. Approximately 15 percent of the rats did not reach performance criterion and were left out of the study. The effects of stress on working memory were tested by placing each rat in a brightly illuminated open field for 10 min before tested in the water escape T-maze task.

After completion of the water escape T-maze component of the experiments, a microdialysis probe was implanted in the medial prefrontal cortex of rats in both groups. Microdialysis samples were collected from free moving rats for 3 hours to establish baseline concentration of acetylcholine and dopamine. Stress was then induced by gentle restraint for 40 min. Dopamine and acetylcholine samples were analyzed by HPLC.

Rats reared in an enriched environment showed a different pattern of stress induced dopamine and acetylcholine release in the prefrontal cortex as compared to control animals. Baseline levels of both dopamine and acetylcholine were not dependent on housing conditions. Stress increased dopamine in the prefrontal cortex in rats from both housing conditions in a similar way, while acetylcholine release in the prefrontal cortex was increased only in the control animals. Rats from both

housing conditions showed habituation in spontaneous motor activity, although the enriched animals habituated faster than the control animals. In regards to working memory, stress decreased performance equally for rats in both housing conditions in the water escape T-maze.

Del Arco et al. (2006) suggest that the hippocampus and amygdala work to inhibit cholinergic activation of the prefrontal cortex. Both of these brain areas are involved in stress response and are connected to the prefrontal cortex. Previous studies have shown that these brain areas undergo morphological and functional changes as a result of environmental enrichment.

Changes in cholinergic function resulting from environmental enrichment have also been demonstrated by Park, Pappas, Murtha and Ally (1992) who examined the effect of environmental enrichment on choline acetyltransferance (ChAT). 28-day-old male long evans rats were randomly assigned to either the enriched environment housing condition or the standard housing condition. The enriched environment rats were housed in groups of 8 to 10 in large cages that contained running wheels, toys and tunnels. Every three days the rats were moved to a completely different cage with a completely

different configuration. The rats in the standard housing control group were housed 2 per small barren cage. Every three days the standard housing control rats were handled in order to equalize handing effects in the two housing conditions. The rats remained in these conditions for 50 days.

On day 51 all rats were moved to single housing in order to make identification easier. 18 rats from both housing conditions were trained in the Morris water maze while the remaining 10 rats from each housing condition were left naive to serve as controls for the ChAT assay. The Morris water maze training consisted of 5 days of training with four trials per day in which the rats had to locate a hidden platform in the southwest quadrant. On the 6th day the rats were put in the pool for 60 seconds but the platform was removed. On the 7th day the platform was relocated and visible in the northwest quadrant. At the end of the 7 days of Morris water maze training all the rats brains were extracted and ChAT assays were done for the anterior cortex, the posterior cortex, the hippocampus and the caudate.

Rats reared in the enriched environment were able to find the hidden platform quicker than the rats in the

standard housing control group on every single test day. There were no differences between rats from different housing conditions in regards to their swimming pattern on day six when the platform had been removed or on day seven when the platform was visible.

Rats reared in the enriched environment condition and trained in the Morris water maze showed greater ChAT activity in the anterior cortex than animals reared in the standard housing condition and the untrained rats reared in the enriched environment condition. Maze training did not affect anterior cortex ChAT activity in the standard housed rats. They found no significant differences in the ChAT activity in the posterior cortex in any of the rats, which suggests that acetylcholine in the posterior cortex does not play a role in the type of spatial learning seen in the Morris water maze or in the biochemical changes seen in rats reared in enriched environments.

In the hippocampus maze trained enriched environment rats showed higher ChAT activity than the untrained rats from the same housing condition. There was no difference in ChAT activity found between maze trained or untrained rats from the standard housing condition in the

hippocampus. The ChAT assays in the caudate revealed no difference in activity between the maze trained or untrained rats in the enriched environment condition but it did show an increase in ChAT activity in the maze trained standard housed rats compared to the untrained rats from the same condition. Overall there was higher ChAT activity in the caudate of the rats raised in the enriched environment than those raised in standard housing.

These findings reflect an environmental enrichment priming effect in the cortex and hippocampus that causes an increase in acetylcholine in response to spatial learning. The increase in ChAT activity in the caudate of maze trained standard housed animals was believed to be due to the motoric demands of the Morris water maze. It was further theorized that maze training and environmental enrichment may have a similar effect on acetylcholine neurons in the caudate.

In addition to these biochemical and behavioral measures observed in enriched and standard housed animals, differential housing conditions have distinct effects on brain injured and normal animals. Thouvarecq, Caston, and Protais (2006) examined the effects of

rearing environments on spatial abilities and associative learning in mice at different ages. The spatial behavior of 3, 12, and 20-month-old mice reared in either an enriched or standard housing environment was assessed. An adaptation of the Morris water maze was employed in this study such that even the oldest mice could learn. Potential effects of cholinergic antagonism on learning in ageing mice were determined by treating animals with scopolamine hydrobromide, a muscarinic receptor antagonist.

Males or female mice were separated into either a standard housed rearing condition or an enriched environment rearing condition at 22 days of age. The standard house condition consisted of 5 mice in a small barren cage containing only food, water, and bedding. The enriched environments consisted of 10 mice kept together in two large cages which were connected and filled with wheels, pipes and small chambers, the configuration of these environments changed every two weeks in order to continually invoke exploration. The water and food locations in the enriched environment condition were also changed once a week.

The mice were divided into 16 groups that varied according to age, housing condition, and scopolamine dose. The water maze task used consisted of a circular pool filled with milky water with an escape platform hidden 1 cm beneath the water. This platform remained fixed throughout the experiment in a position relative to distal visual cues located on the outside of the maze. Because aged animals are known to have difficulty formulating a cognitive map, the mice were always given the same starting location, making this paradigm more associative in nature. Each mouse was trained to criteria on the water maze task, and after a seven days interval was given a retrieval test. An hour after this retrieval test, the mice were given 4 trials starting in different locations around the maze in order to assess memory for cognitive mapping of the maze environment.

Results showed that all the mice regardless of condition or age were able to find the platform faster across trials. Not surprisingly the number of trials needed to reach learning criteria was highest in the 20-month-old rat and the 3-month-old rats had the least number of trials to criteria. Once learning criteria was met the escape latencies were similar across ages. This

suggests that the increased trials needed in older animals are indicative of impairment in acquisition as opposed to impairment in memory.

All animals including those injected with scopolamine hydrobromide were able to reach learning criteria which Thouvarecq et al. attribute to the nicotinic receptors, which play a role in spatial cognition, being left intact. Though there was a significant delay in the learning of the animals injected with scopolamine hydrobromide as compared with controls, which is consistent with the idea that the cholinergic system plays an important role in this type of learning. Yet long-term memory was spared in all the scopolamine treated animals which was reflected in the fast escape latencies found in the retrieval task. This finding suggests that cholinergic hypofunction impairs spatial learning but not recall; it also indicates that the impairments seen in acquisition were not caused by memory defects. They also found that the effects of scopolamine hydrobromide increased with age.

In contrast to several other studies, results showed that the rearing of the animals had no effect on the scores of the control mice in this study. This may be due

to the nature of the enrichment and standard housed conditions used in this study, where common standard housed procedures in other studies houses only 2 animals per cage this study used 5 and where most enrichment studies change the environment on a weekly or bi-weekly biases this study only rearranged the enriched environments once every two weeks. Enrichment was found to have a positive effect on the 3-month-old scopolamine treated mice that showed faster acquisition than their standard housed counterparts. This suggests that enrichment can help restore abilities following compromise of the cholinergic system.

De Bartolo and colleagues (2008) also investigated the effects of environmental enrichment on rats with cholinergic damage. These researchers assessed performance in rats with basal forebrain lesions in a four choice serial learning task which requires cognitive flexibility. Basal forebrain lesions impair cognitive flexibility in standard housed rats (Cabrera, Chavez, Corley, Kitto, & Butt, 2006). De Bartolo et al. (2008) hypothesized that EE would protect rats with basal forebrain cholinergic lesions from these impairments in cognitive flexibility.

To test this hypothesis, 21-day-old male rats were randomly assigned to either an enriched environment or a standard housing condition. In the enriched environment housing condition, rats were housed in groups of 10 in a cage containing toys (exchanged twice a week), running wheels, small constructions, a shelter, feeding boxes and water bottles. In the standard housing condition, rats were housed in pairs in small-impoverished cage containing only bedding, food, and water.

At 3 mouths of age, rats from both housing conditions were randomly assigned to either an intact control group or basal forebrain cholinergic lesion group. At 90 days of age, animals in the lesioned groups received bilateral injections of the immuotoxin 192 IgG-saporin into the basal forebrain. These immunotoxian lesions damage the cholinergic neurons projecting from the basal forebrain to the neo cortex and hippocampus. Cholinergic activity in both of these brain areas plays a role in learning and memory.

Upon recovery, rats underwent 4 days of pre-training in a serial learning task. The apparatus used consisted of a straight alley divided into five compartments. Each compartment was separated by a panel containing two

unidirectional swing doors that could be locked or unlocked. The final compartment contained a food reward. On the first day of pre-training, pairs of rats were allowed to freely explore the apparatus with inter panels removed. On the second day of pre-training, rats were tested individually. On day three of pre-training, single rats were placed in the apparatus with the 1st and 4th panels in place and all doors unlocked. On the fourth day of pre-training, all panels were in placed with all doors unlocked. This pre-training was designed to allow animals experience-pushing doors open for reinforcement.

After the extensive pre-training rats from all four conditions underwent 10 days of training which consisted of 12 trials per day. During a given trial rats were placed at the beginning of the alley facing the panels. The trial ended when the rat reached the reward in the fifth compartment. Cognitive Flexibility was measured as a rat's ability to learn a new sequence of unlocked doors to reach his food reward in the fifth compartment with as few errors as possible. The sequence remained the same for all 12 trials in a day but changed daily. The changing response requirements were designed to tax cognitive flexibility.

Learning rate and total errors, defined as attempts at opening locked doors, were measured. The number of correct choices made, position errors, and preservative errors were also measured.

Lesioned animals from both housing conditions had a greater number of total errors compared to their respective control groups. The standard housed lesioned animals had shorter series of correct responses than all other groups, although enriched lesioned animals were also impaired relative to controls. Although control animals in both housing conditions reduced preservative responding across trials, neither lesion group decreased preservative responding. These results show that rearing in an enriched environment can attenuate impairments to cognitive flexibility normally following basal forebrain lesions.

Another study demonstrating the effect of enrichment on animals with damage to the cholinergic system was conducted by Paban and colleagues (2005). These researchers investigated the effects of damage to the basal forebrain cholinergic system on the behavior of rats across time. Four behavioral tasks were used; a nonmatching-to-position task and an object-recognition

test both of which assess the animals recognition-memory, an object-location test, and an open-field activity test. All rats received either a basal forebrain lesion using the cholinergic antagonist 192 IgG-Saporin (SAP) or a sham control lesion in the same area done with saline at 3 months of age. The rats were randomly divided into either a group that was tested 7 days after surgery, a group that was tested one month after surgery or a group that was tested one year after surgery. All the rats in this first part of the experiment were kept in a standard house condition. The standard housed condition consisted of 2 or 3 rats in a small cage.

In addition, Paban and colleagues explored the effects of environmental enrichment on a separate group of rats that received either the SAP lesion or the saline lesion at three months of age. These rats were housed for 9 months in the same standard house condition mentioned above and were moved to an enriched environment where they stayed for 3 months before they underwent behavioral testing. The enriched environment consisted of 8 to 9 rats housed together in two large interconnected cages filled with plastic tubes, a running wheel, nesting material, and toys.

As excepted enrichment had a positive effect on rat's behavior regardless of lesion type although this benefit was most pronounced in the rats with the SAP lesions, a finding that is promising in terms of therapeutic programs for people with cholinergic damage. It was found that the damage caused by the lesions varied across time and task. In the nonmatching-to-position task no deficit was observed in the rats tested 7 days after surgery, 192 IgG-SAP-treated rats tested 1 month after surgery had a longer acquisition phase than that of controls, rats from the 1 year after surgery condition showed a greater difficulty learning the task than their 7 day and one month post surgery counterparts, with the 192 IgG-SAP lesion animals again showing significant impairment when compared to that of controls from the same condition. In the object-recognition task, greatest impairment was found in the 192 IgG-SAP-treated rats tested at 1 year after surgery. The 192 IgG-SAP lesion animals tested 7 days post surgery only showed a minor impairment that seemed to reflect a problem with short-term memory or attention. The authors interpret these results as indicating that the few defects seen in the 7 day after surgery group was cause by cholinergic

depletion, but the defects seen in the 1 month and 1 year after surgery groups was a product of other neuronal systems in the brain being altered. There was no difference in the performance of the rats in the open field test as a result of lesions showing that the rats did not differ in terms of activity levels.

In general these experiments have demonstrated the beneficial effects enrichment has on both the cholinergic system and the ability of animals with cholinergic damage similar to that seen in AD to perform a variety of learning and memory tasks. Understanding the specific nature of these benefits is critical to the search for effective treatments for people suffering from these disorders.

CHAPTER TWO

THE ROLE OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN LEARNING, MEMORY, AND ATTENTION

The basal forebrain cholinergic system supplies the neurotransmitter acetylcholine (Ach) to areas known to be involved in learning, memory and in mediating attention. These brain areas include the hippocampus, which receives cholinergic projections from the medial septum (MS), and the neocortex, which receives projections from the nucleus basalis magnocellularis (NBM). The NBM is of special interest to researchers because it is comparable to the nucleus basalis of Meynert in humans, one area known to be invoked in Alzheimer's disease. In Alzheimer's the cells of the nucleus basalis are damaged leading to a decrease in projected Ach to the neocortex causing some of the impairments seen in learning and memory. Thus much NBM research has focused on trying to understand the role that this area plays in learning and memory.

The cholinergic projections from the NBM are not critical for simple associative learning but do play a role in more complex forms of learning such as configural

learning which requires the subject to be able to make associations based on the relationship between two or more stimuli in order to receive reinforcement. This was demonstrated by Butt and Bowman (2001) when they tested rats with bilateral 192 IgG-saporin lesions of the NBM and sham lesion controls in a paradigm known as transverse patterning. The transverse patterning task is designed to be able to test both simple associative learning and configural learning. This task has three phases, in the first phase rats are presented with a simple association in which stimuli A is always reinforced and stimuli B is never reinforced, in phase two the rats are still given the first set of stimuli but they are also given stimuli B paired with stimuli C so in the second phase A is always reinforced, B is reinforced half of the time and C is never reinforced. Finally in phase three the rats are given every combination of stimuli and must understand the associative relationships between them in order to receive reinforcement. Thus phases one and two require simple associative learning and phase three requires configural learning. Butt and Bowman hypothesized that rats with NBM lesions would be able to learn the first two phases of the task normally

but would be impaired when compared to controls in their ability to acquire the third phase of the task.

As hypothesized NBM lesioned rats where unimpaired during the first two phases of the task showing that cholinergic projections from the NBM are not critical for simple associative learning, and as hypothesized these same lesioned rats were impaired during the third phase of this task suggesting that the NBM is critically involved in configural learning. Butt and Bowman suggest that these impairments represent a disrupted ability to attend to multiple discriminative stimuli at the same time. Further impairment in the NBM lesioned group was found unexpectedly in the ability of these rats to retain their high performance for problem 1 once they were confronted with problem two, which may be indicative of problems shifting between response strategies as the stimulus conditions changed.

Further support for the NBM's involvement in configural learning comes from Butt, Schultz, Arnold, Garman, George, and Garraghty (2003) who examined the effects of NBM lesions on rats during performance in an operant appetitive-to-aversive transfer task another task designed to test configural association learning. Rats

were trained to bar press for a food reward in response to a tone during the appetitive phase of this task, once the rats had learned the appetitive task they then had to learn the tones new association as a predictor of shock during the aversive phase. Butt et al. hypothesized that the rats with NBM lesions would be unimpaired during the appetitive phase of this task because it requires only simple associative learning which cholinergic projection from the NBM is not critical for. Furthermore it was hypothesized that the NBM lesioned animals would be impaired in their ability to learn the changing association of the tone during the aversive phase of this task. To control for potential confounds associated with the nature of aversive conditioning a separate group of both NBM lesioned and sham control animals were trained in the aversive task alone.

As hypothesized the lesioned rats did not differ from controls in the acquisition or performance of the initial appetitive phase or in the aversive only group, but NBM lesioned rats were found to be significantly impaired when the predictive value of the tone changed from a predictor of food to a predictor of shock during the aversive phase of this task. These findings are

consistent with the evidence suggesting that the cholinergic projections from the NBM are not necessary for simple associative learning but do in fact play a role in more complex types of learning. The cognitive demands of the appetitive-to-aversive transfer task are similar to those required in the incremental attention task described below in the experiments done by Chiba et al. (1995). The change in the predictive value of the tone in the appetitive-to-aversive task requires rats to re-attend to a stimulus that was previously consistent, this arguably requires greater attention. So it is not unlikely that the impairments seen in the NBM lesioned rats during this experiment are a reflection of damage to attentional processes. The posterior parietal cortex which receives its cholinergic projections from the NBM may be one of the major players in attentional processes. Another possibility is that these impairments reflect a difficulty in NBM lesioned rats to change behavioral strategies, more precisely that the lesioned rats are at a disadvantage when it comes to switching between an appetite strategy and an aversive one. This interpretation lends support to the cognitive flexibility argument.

Studies examining the effects of cholinergic lesions of the NBM have been varied, the impairments found in each study depending greatly on the type of behavioral task employed. According to Cabrera, Chavez, Corley, Kitto, and Butt, (2006) the pattern of results indicate a specific type of impairment, one to cognitive flexibility, a term they define as: the ability to shift attention, to shift cognitive set, to respond to different stimulus configurations or conditions in different ways as particular tasks demand, or to adapt to changing response rules. To test this theory rats with IgG Sap lesions of the NBM and rats with saline sham lesions of the NBM were tested using a serial reversal task and then tested in an extinction task.

It was found that although there was no difference in the ability of the rats to acquire the association for the original discrimination, NBM lesioned rats were impaired in the serial reversal task. The NBM lesioned rats also showed a tendency to be slower at extinction than the sham control rats. These findings are consistent with the idea that cholinergic projects from the NBM do in fact play an important role in cognitive flexibility.

The prefrontal cortex, an area long known to be involved in attentional processes is one of the areas that receive its primary cholinergic inputs from the NBM. Cholinergic projections from the NBM to the neocortex play a major role in modulating attention. Chiba, Bucci, Holland, and Gallager (1995) performed lesions to the substantia innominata and the NBM of rats using the immunotoxin 192 IgG-saporin in order to examine the role the cholinergic projections in modulating attention. Two experiments were performed; the first was done in order to test a rat's ability to improve the associability of a cue when its previously established relationship to a different cue was changed. The second experiment looked at the natural inclination to decrease the associability of a cue when it has been extensively presented, a phenomenon known as latent inhibition.

A complex behavioral paradigm known as incremental attention was employed for experiment 1. In the first phase of the incremental attention task rats are exposed to a series of trials half of which consisted of light-tone-food pairings with the other half of the trials consisted of light-tone pairings the presentation of which is random. During this phase normal rats will

learn that the tone predicts the food and that the light's only predictive value comes from its pairing with the tone, thus a normal rat will learn to dis-attend the light, since it is a poor predictor of the food US, while paying great attention to the tone. In the second phase of task the rats are randomly assigned to one of two conditions. The first condition is known as the Consistent Prediction condition because the animals in this condition receive the same pattern of trials as they received in phase I. The second group of rats is assigned to the shift condition, in this condition the light-tone-nothing trials are replaced with the presentation of the light only. This is designed to change the predictive value of the light since it is no longer consistently followed by the tone causing a normal rat from the shift group to return attention to the light that it previously ignored.

Attention to the light is tested in the third phase of this task in which all animals are presented with light-food pairings. Normal rats from the shift group are expected to acquire this new association (light predicts food) faster than those animals from the Consistent Prediction condition, which is exactly what Chiba et al.

found. By contrast rats with SI/NBM lesions showed less conditioned responding to the light after the shift treatment than that seen from the Consistent Prediction, which supports the theory that cholinergic projections from the NBM are indeed involved in attention. There were no differences seen between the lesioned rats and the control rats from the Consistent Prediction condition which suggests that the lesions did not affect attention when there was no violation of assumptions.

In the second experiment rats were exposed repeatedly to one of two visual stimuli. This was done to reduce the associability of the pre-exposed CS. Then the rats were presented each visual stimuli followed by a food US. A normal rat will show reduced conditioned responding to the CS which was previously presented without the food reinforcement. This pattern of responding was found in both the SI/NBM lesioned animals and the intact control animals. This suggests that cholinergic projections from the NBM do not play a critical role in latent inhibition.

The above articles supported the theory that cholinergic projections from the NBM are involved in various types of learning, attention and complex

cognitive functions. These behaviors are often negatively affected in people with AD a disorder characterized in part by damage to the cholinergic projections from the NBM. Despite the findings reviewed in this chapter, there is still much that is not known concerning the precise role of the NBM in areas such as attention and learning. More research is needed to fully understand the complex role the NBM plays in these processes.

CHAPTER THREE

THESIS EXPERIMENTS

Introduction

The concept of "cognitive reserve" has been used to explain the finding that many people with high levels of educational attainment show preserved cognitive function despite the presence of widespread neuropathology such as that which occurs in Alzheimer's disease (Milgram, Siwak-Tapp, Araujo, & Head, 2006; Stern, 2002). A variety of adaptive brain changes are likely contributing to the assumed cognitive reserve, including the proliferation of cells in the hippocampus and the development of increased synaptic connectivity among both hippocampal and neocortical circuits (Milgram et al., 2006).

The cognitive reserve phenomenon can be modeled in animals reared in an enriched environment where there are abundant opportunities for perceptual, physical, and social stimulation (Jankowski et al., 2005). Rearing in an enriched environment causes dramatic increases in synaptic plasticity within the hippocampus (Bayer, Yackel, & Puri, 1982) and neocortex (van Praag, Kempermann, & Gage, 2000). Furthermore, compared to rats

reared under standard housing conditions, rats reared in an enriched environment show superior learning in a variety of behavioral tasks, including two-way active avoidance (Escorihuela et al., 1994), contextual conditioning (Duffy, Craddock, Abel, & Nguyen, 2001; Rampon et al., 2000), delayed alternation (Winocur & Greenwood, 1999), and spatial learning (Park, Pappas, Murtha, & Ally, 1992; Wainwright, Levesque, Krempulec, Bulman-Fleming, & McCutcheon, 1993).

Together, these findings show that environmental enrichment leads to profound modifications in neural processes associated with superior learning and memory. Furthermore, these enrichment-induced brain changes can provide later protection against the cognitive impairments normally associated with cholinergic damage of the basal forebrain (e.g., De Bartolo et al., 2008). The cortically projecting basal forebrain cholinergic system undergoes progressive degeneration in human Alzheimer's disease, where the extent of cholinergic deficit is correlated with the degree of impairment in cognition, learning, and memory (Jellinger & Bancher, 1998; Muir, 1997; Wenk, 1997). Damage to the analogous structure in rats, the NBM, also produces impairments in

a wide variety of behavioral learning tasks (Butt & Bowman, 2003; Cabrera et al., 2006; Wenk, 1997), especially including tasks that explicitly require attentional processing (for review see Sarter & Bruno, 1997).

Although recent research strongly suggests that enrichment can attenuate behavioral impairments in a variety of learning paradigms, it is not yet known if enrichment-dependent neuroprotection from NBM lesion-induced impairments extends to deficits in attention-dependent learning in particular. This question is important because the NBM is critically involved in attention processes (Sarter & Bruno, 1997), and the question of specificity of enrichment-induced effects on NBM-dependent learning and memory mechanisms has not yet been adequately addressed. The current experiment therefore examined the effects of environmental enrichment on performance in an attention-dependent learning task in rats with selective cholinergic NBM lesions.

Hypothesis and Research Design

To test the hypothesis that environmental enrichment will attenuate NBM lesion-induced impairments in attention-dependant learning, incremental attention performance across blocks of training trials was compared in groups of rats reared either in an enriched environment or in standard housing conditions and subjected to either lesions of the NBM or sham lesion control surgeries (CON). The attention-dependent associative learning task selected for these experiments, the incremental attention paradigm, was used in this experiment because previous research has shown that this paradigm is specifically sensitive to lesions of the NBM (see Chiba, Bucci, Holland, & Gallagher, 1995).

The incremental attention task is a three-phase paradigm that typically involves two training conditions. The first condition, the Consistent Prediction condition, involves exposure to an unchanging relationship among cues throughout the first two phases of the task. In this condition, a light CS is followed by a tone CS, where this sequence is followed by a sucrose pellet US on half of all trials. In normal rats, this procedure leads to a decrease in associability of the first cue in the

sequence, the light CS. This decrement in attention to the light CS occurs because compared to the noise CS, the light CS is a poorer predictor of the US. The noise CS achieves relatively greater control over behavior as a function of its close temporal proximity to the US. Moreover, as the relationship between light and the noise becomes better established, animals will pay progressively less attention to the light (Holland & Gallagher, 2006; Wilson et al., 1992).

Animals in the other training condition, the Predictive Shift condition, are exposed to the same constant relationship among cues during phase I as in the Consistent Prediction condition. However, during phase II of the incremental attention task, animals in this condition encounter a surprising shift in the previously established predictive relationship among the cues. In this phase, attention to the light CS is increased by changing its relationship to the noise CS, where this violation of condition expectation leads to an increase in associability of the Light CS.

Finally, the changes in associability resulting from the surprising prediction error experienced in Phase II are assessed in Phase III of the task when the light is

paired directly with the US (light-US). Rats in the Predictive Shift condition typically show faster conditioning to light compared to rats in the Consistent Prediction condition. This increment in attention to the Light CS is due to the modulating effect of surprise (i.e., violation of conditioned expectations) experienced during Phase II. In contrast, rats in the Consistent Prediction condition do not undergo this increase in attention and therefore tend to ignore the light CS during Phase III. Rats trained in this condition therefore learn more slowly than rats in the Predictive Shift condition during Phase III.

Chiba and colleagues (2005) showed that selective cholinergic lesions do not impair Phase III acquisition performance in animals trained in the Consistent Prediction condition. These authors suggest that the NBM is critical for processing resulting in increments in attention but not for processing necessary for decrements in attention. Findings from Chiba et al. (2005) also demonstrate that the NBM is not necessary for serial Pavlovian conditioning per se, as rats with NBM lesions showed normal acquisition of the CR to the Light CS during Phase III. Because the focus of the current

experiment is to explore the effects of environmental enrichment on attention-dependent learning performance in a task known to be sensitive to selective cholinergic NBM lesions (see Chiba et al. 2005), the current study tested animals only in the Predictive Shift condition of the incremental attention task.

It is hypothesized that rearing in an enriched environment will facilitate attention-dependent learning (Duffy et al., 2001; Escorihuela et al., 1994; Park et al., 1992; Rampon et al., 2000; Wainwright et al., 1993; Winocur & Greenwood, 1999). Specifically, compared to rats that are reared in a standard-housing condition, rats that are reared in an enriched environment will show superior attention-dependent learning. We also hypothesize that NBM lesions will impair attention-dependent learning. In general, compared to rats with a sham lesion, rats with NBM lesions will show poorer attention-dependent learning. Furthermore, based on previous environmental enrichment studies (e.g. DeBartolo et al., 2008), it is hypothesized that environmental enrichment will attenuate NBM lesion-induced impairments in attention-dependent learning. For the NBM-lesion rats, it is expected that

those that are reared in a standard-housing condition will show poorer attention-dependent learning than those that are reared in an enriched environment. For the sham-lesion control rats, it is expected that those that are reared in a standard-housing condition will also show poorer attention-dependent learning than those reared in an enriched environment. However, the differences in attention-dependent learning between the standard housing and enriched environment rats will be less pronounced for the sham-lesion rats than the NBM-lesion rats.

CHAPTER FOUR

METHODS

Introduction

The design of this experiment involved testing NBM lesion and sham lesion control rats in an incremental attention task across three blocks of trials following rearing in either an enriched environment or in standard housing environment. The experimental design is a 2 x 2 x 3 mixed factorial design. The between-subjects variables included lesion condition (NBM lesion, Sham lesion) and housing condition (Standard housing, Enriched environment). The within subjects variable was training block, where each block consisted of 5 trials.

The dependent variable consisted of a difference score reflecting the duration of conditioned responding. Visual conditioned stimuli predicting food delivery evoke a CR characterized by initial rearing and orienting towards the visual cue, followed by the eventual approach to the food cup. Consequently, snout entry into the food cup is delayed until late in the CS interval. Therefore, to assess CR acquisition during Phase III in the current experiment, responding (i.e., food cup approach) during

the latter 6 s of the 10 s Light CS presentation was measured. To account for baseline levels of food cup approach in the absence of the visual CS, baseline responding was subtracted from responding during the last 6 s of CS presentation. The resulting difference score served as the measure of conditioned responding in Phase III of the incremental attention paradigm.

Subjects

Eighty male Long-Evans rats, 1 month of age were randomly assigned to either the enriched environment condition or the paired housing control condition upon arrival. Following the enrichment (or standard housing) period, subgroups (n = 20) from each rearing condition were randomly assigned to either the NBM lesion or CON groups. The methods for standard and enriched environmental rearing were adapted from those used by De Bartolo and colleagues (2008), in their demonstration of spared cognitive performance in NBM-lesioned rats following environmental enrichment.

Rats assigned to the enriched environment condition were housed in groups of 10 in a large cage (100x50x70 cm) with multiple levels. The cage contained bedding, a

running wheel, shelters (small boxes), colored plastic toys, and a variety of tubes that could be used to explore different parts of the cage. Throughout the 3-month enrichment period, the "old" toys were exchanged for novel toys, and the positions of the tubes were changed twice a week. The location of feed bins and water bottles shifted to different points in the cage twice a week to encourage foraging and explorative behaviors. Rats reared in the standard housing environment were housed in pairs in standard cages (40x26x18 cm) containing bedding but no objects. Feed bins and water bottles remained in the same position throughout the experiment.

Regardless of condition, all rats were housed in the animal facility in the Social & Behavioral Sciences Building at California State University, San Bernardino, under a 12-hour light/dark cycle with *ad libitum* water and food prior to the experiment. After surgery, rats were placed in individual housing and food deprived for 10 days before behavioral testing, specifically each rat was given enough food to maintain 85% of their normal body weight to motivate conditioned food approach in the incremental attention behavioral paradigm. The

experimental protocol has been approved by the Institutional Animal Care and Use Committee at California State University, San Bernardino.

Apparatus

Testing was conducted in computer-controlled operant chambers (Coulbourn Instruments, Allentown, PA) equipped with a speaker for generating the white noise CS, a light CS and a pellet dispenser for delivering the sugar pellet US into a food cup (magazine). Rat snout entries into the magazine (i.e., CRs) were detected and recorded electronically using Coulbourn photobeam response detectors positioned in front of the food magazine. The duration of snout entries into the food magazine were recorded via computer interface (Coulbourn, Allentown, PA) both during each inter-trial interval and during the 10 second Light CS interval.

Surgery

Surgical procedures were derived from Baxter et al. (1995) and employed by Butt and colleagues (Butt & Bowman, 2003; Cabrera et al., 2006). Lesions of the NBM were made by infusing the immunotoxin 192 IgG-saporin (SAP). Prior to surgery, rats were anesthetized with

sodium pentobarbital (65 mg/kg i.p.; Sigma, Chemicals, St. Louis, MO), the scalp was shaved and cleaned, and rats were placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA).

After incising and deflecting the surrounding skin, a stereotaxic drill (David Kopf Instruments, Tajunga, CA) was used to make four craniotomies bilaterally above the NBM at the following coordinates: -0.75 mm posterior to bregma at +2.3 and +3.3 mm lateral to midline. Using a microinjection unit (David Kopf Instruments, Tajunga, CA), the SAP solution was infused via a 28-gauge, blunt-tip syringe (Hamilton, Reno, NV) at a rate of 0.1 ul/min, bilaterally into the NBM site referenced above. A volume of 0.2 ul SAP solution was infused into each medial NBM site at a depth of -7.8 mm below the surface of the level skull, and into each lateral NBM site at a depth of -8.1 mm below the surface of the level skull. The cannula was left in place for an additional 3 min following each NBM infusion.

Surgical procedures were identical for rats in the sham-operated control group, with the critical distinction that these animals received infusions of Dulbecco's sterile saline only. Following surgery, the

incision was cleaned and sutured and rats were returned to their home cages. Rats received analgesic (Ketoprofin, 2 mg/kg s.c.; Sigma, St. Louis, MO) immediately after the surgery, and were allowed 10 days for recovery before testing.

Upon completion of behavioral testing, rats were administered a lethal dose of sodium pentobarbital (80 mg/kg, i.p.; Sigma, St. Louos, MO). Brains were extracted, sectioned, and stained for acetylcholinesterase to verify the effectiveness of the lesions.

Behavioral Methods

Two days before the start of behavioral testing each rat were given 20 sucrose pellets in their home cages in order to reduce their naturally neophobic responses to the pellets. The next day, rats underwent magazine training, this was done in order to familiarize the rats with the testing chambers as well as to teach them where the sucrose pellets were located in these chambers. During magazine training each rat was placed in the same chamber they were assigned to during their behavioral testing with 10 sucrose pellets placed in the food cup

for a duration of 1 hour or until they have consumed all 10 of the sucrose pellets. The following day, rats began phase I of the incremental attention task. Each rat received an hour of baseline in the testing chamber before the conditioning trials of each day begins.

In Phase I, every day for 10 days rats were given 60 serial conditioning trials consisting of a 10 second visual CS (light) followed immediately by a 10 second auditory CS (white noise), 30 of these trials consisted of the light-noise presentations followed immediately by a sucrose pellet US while the other 30 of these daily trials were just the light-noise presentation with no sucrose pellet US. The presentation of these trials were pseudo random with no more than three trials of the same type occurring consecutively. The inter-trial interval (the time between trials) was variable with an average of 40 seconds.

Day 11 of behavioral training begins Phase II. In this phase, which only lasts 1 day, the rat's attention to the light was manipulated by altering its relationship to the white noise. This was accomplished by replacing the 30 trials from phase I that were non-reinforced light-noise pairings with the presentation of light alone

trials. The rats still receive the 30 light-tone-sucrose trials pseudo-randomly presented along with these new light alone trials in the same way that they did in phase I.

Day 12, the final day of behavioral testing begin Phase III in which rats received 15 trials where the light CS is paired directly with the sucrose pellet US (light US). These trials were separated by a 100 second inter-trial interval.

Data Analysis

Difference scores from individual trials in Phase III were summed for each of the three blocks of 5 trials each, yielding three blocked difference scores for each rat. Consequently, Phase III data was analyzed using a 2 x 2 x 3 analysis of variance for mixed design.

CHAPTER FIVE

RESULTS

This experiment examined the effects of rearing environment and selective damage to the NBM on attention-dependant learning. Data were analyzed using a 2 (Housing condition) X 2 (Lesion condition) X 3 (Block) ANOVA for mixed design, with housing condition and lesion condition as two between-subjects variables, and block as a within-subjects variable. Not all 80 animals survived surgery and/or completed all three phases of behavioral testing (due to isolated equipment failures). The final numbers of animals in the four treatment combinations of aired Housing NBM Lesion, Enriched Environment NBM Lesion, Paired Housing Sham Lesion, and Enriched Environment Sham Lesion, were 13, 14, 18, and 19, respectively. Table 1 summarizes the results of the experiment.

Main Effects of Housing and Lesion Conditions

Rats reared in an enriched environment showed significantly greater levels of performance (i.e., CR difference scores) in attention-dependent learning compared to rats reared in a standard, paired housing

Table 1. Mean (SD) Conditioned Response Difference Scores: Housing x Lesion x Blocks

	Block			
	Block 1	Block 2	Block 3	Total
NBM Lesion				
Paired Housing	-103.99	14.26	55.83	-33.90
(n = 13)	(182.59)	(209.12)	(264.58)	(486.78)
Enriched Environment	103.34	209.56	256.53	569.43
(n = 14)	(169.71)	(207.24)	(174.01)	(419.30)
Total (Paired + Enriched)	3.51	115.53	159.90	278.94
(n = 27)	(202.32)	(227.04)	(240.61)	(540.03)
Sham Lesion				
Paired Housing	53.50	174.77	306.23	534.49
(n = 18)	(170.42)	(163.27)	(187.77)	(359.08)
Enriched Environment	178.07	222.56	293.12	693.76
(n = 19)	(199.81)	(208.33)	(198.69)	(476.75)
Total (Paired + Enriched)	117.46	199.31	299.50	616.27
(n = 37)	(194.06)	(186.75)	(190.87)	(425.49)
Paired Housing				
Total (NBM + Sham)	-12.55	107.46	201.22	269.13
(n = 31)	(189.82)	(197.69)	(252.50)	(499.13)
Enriched Environment				
Total (NBM + Sham)	146.37	217.04	277.60	641.01
(n = 33)	(188.58)	(204.70)	(186.67)	(450.74)
Total (n = 64)	69.39	163.96	240.61	473.96
	(204.03)	(207.22)	(222.57)	(502.00)

environment (M_{EE} = 641.01, SD_{EE} = 450.74; M_{PH} = 269.13, SD_{PH} = 499.13; $F(1, 60) = 11.92$, $p = .001$). Additionally, rats with NBM lesions showed significantly lower levels of performance compared to Sham Lesion Control rats ($M_{NBM\text{-}Lesion}$ = 278.94, $SD_{NBM\text{-}Lesion}$ = .540.03, $M_{Sham\text{-}Lesion}$ = 616.27, SD $M_{Sham\text{-}Lesion}$ = 425.48; $F(1, 60) = 9.83$, $p = .003$).

Housing Condition by Lesion Condition Interaction

A significant interaction between Housing and Lesion Conditions was found ($F(1, 60) = 4.04$, p = .049). As shown in Figure 1. In the NBM lesion condition, pair-housed rats showed much poorer performance than the enriched environment rats (NBM Lesion: M_{PH} = -33.90, SD_{PH} = 486.78; M_{EE} = 569.43, SD_{EE} = 419.30). In the Sham Lesion control condition paired housed rats performed poorer then the enriched housed rats (Sham Lesion: M_{PH} = 534.49, SD_{PH} = 359.08; M_{EE} = 693.76, SD_{EE} = 476.75); however the difference between group means (159.27) is not as pronounced as that seen in the NBM lesion condition (603.33).

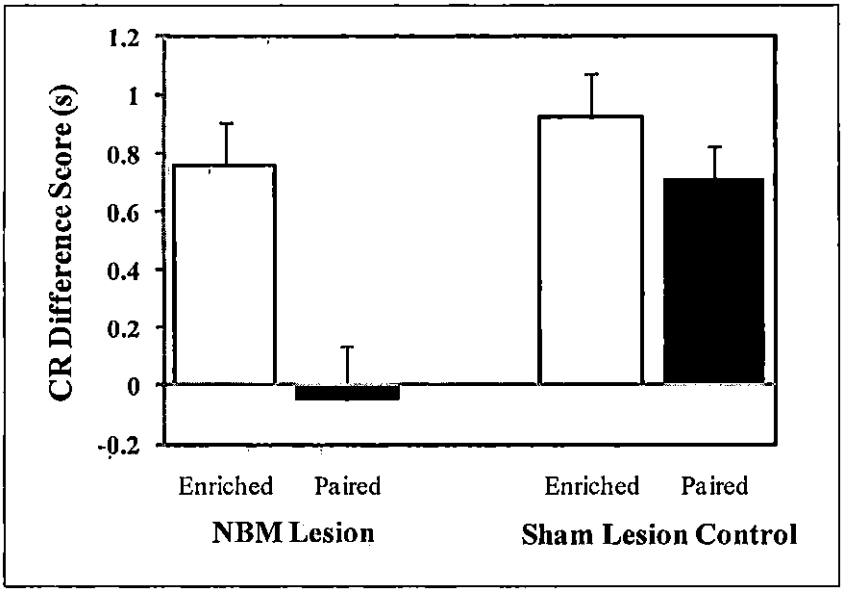

Figure 1. Mean (+/- SEM) Conditioned Response Difference Scores Collapsed Across Training Blocks in Rats with Nucleus Basalis Magnocellularis Lesions and Sham Lesion Controls Reared in a Standard Paired Housing Condition or Reared in an Enriched Environment

Within-Group Effect of Block

A significant main effect regarding learning block was observed, indicating that attention-dependent learning did occur, ($F(2, 120) = 17.93$, $p < .001$). In general, a steady increase in rats' performances was observed from Block 1 to Block 3 ($M_{B1} = 69.39$, $SD_{B1} = 204.03$; $M_{B2} = 163.96$, $SD_{B2} = 207.22$; $M_{B3} = 240.61$, $SD_{B3} = 222.57$).

Inspection of the data shown in Figure 2 reveals an initial acquisition performance that was relatively low on Block 1 in all groups except the Sham Lesion control group reared in the enriched environment condition. Upon completion of training, all groups reached comparably high levels of performance except the NBM lesion group reared in the paired housing condition, which continued to show impairment.

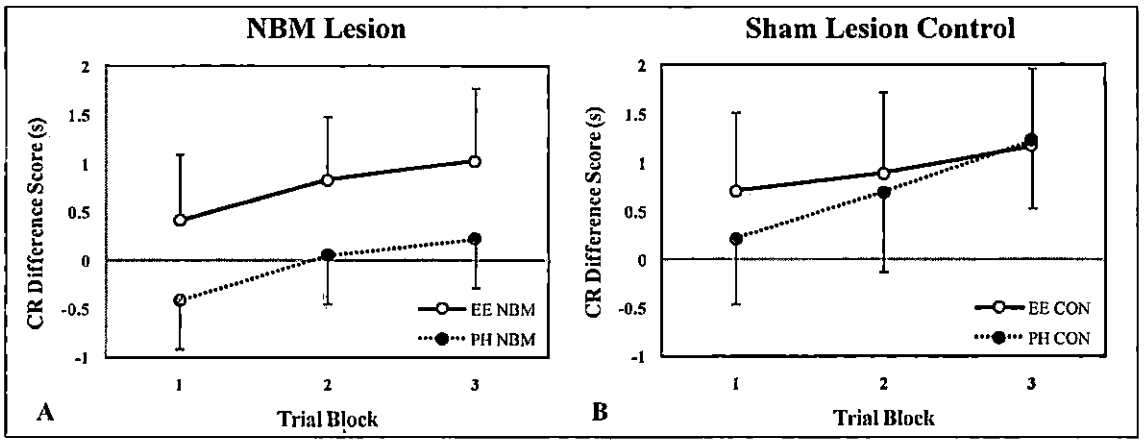

Figure 2. Mean (+/- SEM) Conditioned Response Difference Scores in Rats with Nucleus Basalis Magnocellularis Lesions (A) and Sham Lesion Controls (B) Reared in a Standard Paired Housing Condition (PH: Filled Circles) or Reared in an Enriched Environment (EE: Open Circles)

CHAPTER SIX

DISCUSSION

The findings from this experiment confirmed the hypothesis that rearing in an enriched environment facilitates attention-dependent learning. A significant main effect of housing was found; rats reared in enriched environments, regardless of their lesion condition, showed superior attention dependant learning when compared to paired house controls. This finding is unique in that it demonstrates for the first time that the cognitive benefits of enrichment extend to include benefits to attention dependant learning.

As hypothesized NBM lesions did impair attention-dependent learning. A significant main effect of lesion was found indicating that compared to rats with sham lesions, rats with NBM lesions showed poorer attention-dependent learning.

As expected a significant interaction between housing and lesion was found indicating that environmental enrichment does in fact attenuate NBM lesion induced impairments to attention dependant learning. Specifically the rats reared in the enriched

environment with NBM lesions showed a sparing of attention dependent learning when compared to the rats with NBM lesions reared in the standard paired housing condition.

Environmental Enrichment Effects on Learning

Control animals from this study reared in the enriched environment condition showed superior near asymptotic performance as early as Block 1 of training, whereas control animals reared in the standard paired housed control environment began at a lower level of performance on block 1 before reaching enriched environment control levels by Block 3 of training. The finding that control rats reared in an enriched environment showed superior attention dependent learning when compared to paired house control animals is consistent with a growing body of research showing that environmental enrichment provides benefits to a verity of cognitive tasks, including stress reduction (Segovia, Del Arco, De Blas, Garrido, & Mora, 2008; Del Arco et al., 2006), several types of learning and memory (Berardi, Maffei & Sale, 2008), including two-way active avoidance (Escorihuela et al., 1994), contextual conditioning

(Duffy, Craddock, Abel, & Nguyen, 2001; Rampon et al., 2000), delayed alternation (Winocur & Greenwood, 1999), and spatial learning (Park, Pappas, Murtha, & Ally, 1992; Wainwright, Levesque, Krempulec, Bulman-Fleming, & McCutcheon, 1993).

The Effect of Nucleus Basalis Magnocellularis Lesions on Attention Dependent Learning

The NBM lesion induced impairments in attention-dependent learning observed in the paired housing NBM lesion group in the current study replicate those found in the Chiba et al. (2005) experiment. These authors reported that selective cholinergic lesions impaired Phase III acquisition performance in animals trained in the Surprise condition of the incremental attention task (Chiba et al., 2005). These findings are also consistent with a study conducted by Holland and Gallagher (2006), where the NBM of rats were temporary inactivated during Phase II or Phase III of the "surprise" condition of the incremental attention paradigm. They found that the NBM is critical for the expression of enhanced attention to the CS during acquisition of Phase III of the incremental attention task. However, the NBM was found to not be necessary for

establishing the surprise-induced enhancement of attention during Phase II. The current study provided additional evidence that the cholinergic projections from the NBM are critically involved in attention dependent learning.

Environmental Enrichment Attenuates Nucleus Basalis Magnocellularis Lesion Induced Impairments in Learning

The observation that environmental enrichment attenuated NBM lesion-induced impairments in attention-dependent learning in the current study is consistent with previous research findings (e.g., DeBartoli et al., 2008). Results from the current study show that environmental enrichment contributes to the brain's ability to tolerate damage to the cholinergic projections to the neocortex. By extension, these results suggest that social programs such as the First 5 program, aimed at enriching preschooler's intellectual growth, might contribute to a neuroprotective effect in humans that in turn could reduce the impact of age-related deficits in cognition including those caused by dementia.

Limitations and Future Research

One limitation of this study is that the specific aspect or aspects of environmental enrichment that were responsible for the observed sparing of attention-dependent learning in the NBM lesioned animals cannot be identified in the current experiment. Several factors associated with the enriched environment might contribute to the observed sparing of performance in the attention-dependent learning paradigm. These factors include social contact and play, exercise, exploration, and novelty. Future experiments where these variables are isolated experimentally will improve our understanding of these various factors on attenuation of NBM lesion-induced learning impairments.

The underlying neurobiological mechanisms of attenuation of NBM lesion-induced impairments are not fully understood. It is possible that compensatory neurotransmitter systems such as norepinephirine, or serotonin are strengthen when animals are reared in an enriched environment, such that when damage does occur the rest of the brain is able to compensate for the damaged system, thus reducing behavioral impairments traditionally associated with NBM damage.

Future research utilizing micro-dialysis techniques would allow researchers to measure the levels of possible compensatory neurotransmitters in the brains of NBM lesioned animals that had been reared in enriched environments. This type of study would provide more evidence for the cognitive reserve theory.

Implications for Therapeutic Interventions

Environmental enrichment has been shown to reduce impairments caused by NBM damage, since the NBM is also damaged in human Alzheimer's, cognitive enrichment should be considered an important treatment option. Early and continued access to education could potentially reduce the number of people suffering from age-related cognitive impairments later in life.

REFERENCES

Anstey, K., & Christensen, H. (2000). Education, activity, health blood pressure and apolipoprotein E as predictors of cognitive change in old age: a review. *Gerontology, 46,* 163-177.

Arnaiz, S. L., D'Amico, G., Paglia, N., Arismendi, M., Basso, N., & del Rosario Lores Arnaiz. M. (2004). Enriched environment, nitric oxide production and synaptic plasticity prevent the aging-dependent impairment of spatial cognition. *Molecular Aspects of Medicine, 25*(1-2), 91-101.

Beaulieu, C. & Colonnier, M. (1987) Effect of the richness of the environment on the cat visual cortex. *Neurology, 266, 478-494*

Bertram, L., Blacker, D., Mullin, K., Keeney, D., Jones, J., Basu, S., Yhu, S., McInnis, M. G., Go, R. C., Vekrellis, K., Selkoe, D. J., Saunders, A. J., & Tanzi, R. E. (2000). Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science, 290,* 2302-2303.

Bosma, H., Martin, P. J., van Boxtel, R. W. H., Ponds, W. H. M., Houx, P. J., Burdorf, A., & Jolles, J., 2003. Mental work demands protect against cognitive impairment: MAAS prospective cohort study. *Experimental Aging Research, 29*, 33-45.

Butt, A. E., & Bowman, T. D. (2002). Transverse Patterning Reveals a Dissociation of Simple and Configural Association Learning Abilities in Rats with 192 IgG-Saporin Lesions of the Nucleus Basalis. *Neurobiology of Learning and Memory*, 77, 211-233.

Butt, A. E., Schultz, J. A., Arnold, L. L., Garman, E. E., George, C. L., & Garraghty P. E. (2003). Lesions of the rat NBM disrupt appetitive-to-aversive transfer learning. *Integrative Physiological & Behavioral Science, 38*(4), 253-271.

Cabrera, S. M., Chavez, C. M., Corley, S. R., Kitto, M. R., & Butt, A. E. (2006). Selective lesions of the nucleus basalis magnocellularis impair cognitive flexibility. *Behavioral Neuroscience, 120*, 298-306.

Chiba, A. A., Bucci, D. J., Holland, P. C., & Gallagher, M. (2005). Basal forebrain cholinergic lesions disrupt increments but not decrements in CS processing. *The Journal of Neuroscience, 75*(11), 7315-7322.

De Bartolo, P., Leggio, M. G., Mandolesi, L., Foti, F., Gelfo, F., Ferlazzo, F., & Petrosini, L. (2008). Environmental Enrichment Mitigates the Effects of Basal Forebrain Lesions on Cognitive Flexibility. *Neuroscience, 154,* 444-453.

Del Arco, A., Segovia, G., Garrido, P., de Blas, M., & Mora, F. (2006). Stress, prefrontal cortex and environmental enrichment: Studies on dopamine and acetylcholine release and working memory performance in rats. *Behavioural Brain Research, 176,* 267-273.

Galani, R., Berthel, M. C., Lazauars, C., Majchrzak, M., Barbelivien, A., Kelche, C., & Cassel, J. C. (2007). The behavioral effects of enriched housing are not altered by serotonin depletion but enrichment alters hippocampal neurochemistry. *Neurobiology of Learning and Memory, 88,* 1-10.

Greenough, W. T., et al. (1973). Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Experimental Neurology, 41,* 371-378.

Hultsch, D. F., Hertzog, C., Small, B. J., & Dixon, R. A., (1999). Use it or lose it: engaged lifestyle as a buffer of cognitive decline in aging? *Psychology of Aging, 14,* 245-263.

Jellinger, K. A., & Bancher, C. (1998). Neuropathology of Alzheimer's disease: A critical update. *Journal of Neural Transmission, 54,* 77-95.

Kempermann, G. (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature, 386,* 493-495.

Milgram, N. W., Siwak-Tapp, C. T., Araujo, J., & Head, E. (2006). Neuroprotective effects of cognitive enrichment. *Ageing Research Reviews, 5,* 354-369.

Mohammed, A. H., Zhu, S. W., Darmopil, S., Hjerling-Leffler, J., Ernfors, P., Winblad, B., Diamond, M. C., Eriksson, P. S., & Bogdanovic, N. (2002). Environmental enrichment and the brain. Progress in Brain Research. *Elsevier,* pp. 109-133.

Mollgaard, K. (1971). Quantitative synaptic changes with differential experience in rat brain. *International Journal of Neuroscience, 2,* 113-127.

Mora, F., Segovia, G., & del Arco, A. (2007). Aging, plasticity and environmental enrichment: Structural changes and neurotransmitter dynamics in several areas of the brain. *Brain Research Review, 55,* 78-88.

Muir, J. L. (1997). Acetylcholine, aging and Alzheimer's disease. *Pharmacology Biochemistry, & Behavior, 56,* 687-696.

Mulatu, M. S., & Schooler, C., 1999. Longitudinal effects of occupational, psychological, and social background characteristics on health of older workers. *Annual New York Academy of Sciences, 896,* 406-408.

Paban, V., Jaffard, M., Chambon, C., Malafosse, M., & Alescio-Lautier, B. (2005). Course Of Behavioral Changes Following Basal Forebrain Cholinergic Damage In Rats: Environmental Enrichment As A Therapeutic Intervention. *Neuroscience, 132,* 13-32.

Park, G. A. S., Pappas, B. A., Murtha, S. M., & Ally, A. (1992). Enriched environment primes forebrain choline acetyltransferase activity to respond to learning experience. *Neuroscience Letters, 143,* 259-262.

Rasmuson, S., Olsson, T., Henriksson, B. G., Kelly, P. A. T., Holmes, M. C., Seckl, J. R., & Mohammed, A. H. (1998). Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. *Molecular Brain Research, 53,* 285-290.

Rosenzweig, M. R. (1966) Environmental complexity, cerebral change, and behavior. *American Psychology, 21,* 321-332.

Sale, A., Berardi, N., & Maffei, L. (2008) Enrich the environment to empower the brain. *Trends in Neurosciences, 32*(4), 233-239.

Sarter, M., & Bruno, J. P. (1997). Cognitive functions of cortical acetylcholine: Toward a unifying hypothesis. *Brain Research Reviews, 23,* 28-46.

Segovia, G., Del Arco, A., De Blas, M., Garrido P., & Mora, F. (2008). Effects of an enriched environment on the release of dopamine in the prefrontal cortex produced by stress and on working memory during aging in the awake rat. *Behavioral Brain Research, 187,* 304-311.

Segovia, G., Yagüe, A. G., García-Verdugo, J. M., Mora, F. (2006). Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats. *Brain Research Bulletin, 70,* 8-14.

Sleegers, K., Roks, G., Theuns, J., Aulchenko, Y., Rademakers, R., & Cruts, M. (2004). Familial clustering and genetic risk for dementia in a genetically isolated Dutch population. *Brain: A Journal of Neurology, 127*(7), 1641-1649.

Thouvarecq, R., Caston, J., & Protais, P. (2007). Cholinergic System, Rearing Environmental and Trajectory Learning During Aging in Mice. *Physiology & Behavior, 90,* 155-164.

Wenk, G. (1997). Review: The nucleus basalis magnocellularis cholinergic system. One hundred years of progress. *Neurobiology of Learning and Memory, 67,* 85-95.

Wilson, R. S., Bennett, D. A., Bienias, J. L., Aggarwal, N. T., Mendes de Leon, C. F., Morris, M. C., Schneider, J. A., & Evans, D. A. (2002). Cognitive activity and incident AD in a population based sample of older persons. *Neurology, 59,* 910-1914.