Biconditional discrimination learning in rats with 192 IgG-saporin lesions of the nucleus basalis magnocellularis

Michael Ryan Kitto

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BICONDITIONAL DISCRIMINATION LEARNING IN RATS WITH 192 IgG-SAPORIN LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology:
General-Experimental

by
Michael Ryan Kitto
September 2006
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8-23-06
ABSTRACT

The current experiment tested the hypothesis that IgG-saporin lesions of the nucleus basalis magnocellularis (NBM) in rats would impair performance in a biconditional visual discrimination task, which requires configural association learning. In contrast, it was hypothesized that NBM lesions would not impair acquisition of simple visual discriminations, which require only simple association learning. In Problem 1, rats were trained in a T-maze to solve a simple visual discrimination between a food-reinforced black floor located in one goal arm (B+) and a non-reinforced white floor located in the opposite goal arm (W-) of the T-maze. During Problem 1, the walls of the maze were consistently striped (S). Next, in Problem 2, the reinforcement contingencies of the floor colors were reversed, and the wall color was changed to gray (G). Problems 1 (SB+ vs. SW-) and 2 (GW+ vs. GB-) require only simple association learning because the reinforcement values of the individual floor stimuli remain fixed and unambiguous throughout each problem. After training in Problems 1 and 2, rats were advanced to the biconditional visual discrimination task. In Problem 3, rats were presented with two alternating blocks of 6 consecutive trials of either Problem 1 or Problem 2 trial
types, whereas Problem 4 involved randomly inter-mixed presentations of these trial types. Because the reinforcement value of the floor stimuli could only be determined based on whether the walls of the maze were striped or gray, Problems 3 and 4 required a configural association solution. As hypothesized, results showed that groups did not differ on the simple association tasks of Problems 1 and 2, with the NBM lesion group showing significant performance impairments on the configural association tasks of Problems 3 and 4. These results support the hypothesis that the NBM is critically involved in configural but not simple association learning, and suggest that the NBM may be involved more generally in cognitive flexibility.
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TABLE OF CONTENTS

ABSTRACT ......................................................... iii
ACKNOWLEDGMENTS ............................................ v
LIST OF FIGURES ................................................ ix

CHAPTER ONE: INTRODUCTION

Introduction ..................................................... 1
Demographics .................................................... 1
Cognitive Impairments ....................................... 5
Neuropathology of Alzheimer’s Disease ..................... 18
The Cholinergic Hypothesis of Alzheimer’s Disease .......... 22
Nucleus Basalis of Meynert .................................. 29

CHAPTER TWO: ANIMAL MODELS OF ALZHEIMER’S DISEASE

Introduction ..................................................... 32
Selective Lesions of the Nucleus Basalis Magnocellularis .... 33
Behavioral Effects of Nucleus Basalis Magnocellularis Lesions .... 34
Attention Impairments ....................................... 34
Cognitive Inflexibility ....................................... 38
Configural Association Learning Impairments ............... 41

CHAPTER THREE: THESIS EXPERIMENTS

Introduction ..................................................... 45
Methods ......................................................... 51
Guidelines for Animal Use .................................. 51
Animals ......................................................... 51
CHAPTER FOUR: RESULTS

Introduction ............................................. 60

Problem 1: Simple Association Learning ...... 61
Problem 2: Simple Association Learning ...... 61
Problem 3: Configural Association Learning ............................................. 61

Total Number of Correct Responses ........ 61
Striped Trials ............................................. 64
Gray Trials ............................................. 64

Problem 4: Configural Association Learning ............................................. 68

Total Number of Correct Responses ........ 68
Striped Trials ............................................. 68
Gray Trials ............................................. 68
LIST OF FIGURES

Figure 1. Mean (+/- SEM) number of correct responses in the NBM lesion and control groups on striped trials during Problem 1. ...............62

Figure 2. Mean (+/- SEM) number of correct responses in the NBM lesion and control groups on gray trials during Problem 2. ..............63

Figure 3. Mean (+/- SEM) number of (A) total correct responses, (B) correct striped trial responses, and (C) correct gray trials responses in the NBM lesion and control groups during Problem 3. ..................66

Figure 4. Mean (+/- SEM) number of (A) total correct responses, (B) correct striped trial responses, and (C) correct gray trials responses in the NBM lesion and control groups during Problem 4. .....................70

Figure 5. Acetycholinesterase stained sections of the frontal cortex, the nucleus basalis magnocellularis (NBM), and the anterior hippocampus in a representative control (A, C, and E) and NBM lesion rat (B, D, and F) . . . 75
INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative syndrome and is the most common cause of dementia in the United States. Dementia caused by AD is identified as a progressively crippling malady of the brain and is experienced as a gradual loss of cognitive abilities that eventually becomes severe enough to interfere with daily functioning. The more arresting of the cognitive impairments resulting from AD dementia include memory loss (amnesia), language impairment (aphasia), memory failure (agnosia), as well as deficits in planning and decision-making. As those with the illness near the end of their lives they typically begin to experience coordination loss (ataxia) and mood swings. This chapter discusses the social, individual, and anatomical disease processes of AD.

DEMOGRAPHICS

The likelihood of suffering from AD dementia increases with advancing age. In the United States, it is estimated that the number of AD cases doubles every 5 years after the age of 60 (Cummings, 2002; Graves &
In addition to age, gender also seems to be a risk factor. According to Gao, Hendrie, Hall, and Hui (1998) AD is more common among women than men by a ratio of 3 to 2. Other risk factors include history of serious head injury, low levels of vitamin B12 and B6, elevated homocysteine levels, family history of AD, and low economic status (Gao et al., 1998).

The total prevalence of AD in the United States is estimated to be about 2.3 million (Brookmeyer, Gray, & Kawas, 1988; Cummings, 2002). Estimates project that these rates will nearly quadruple in the next 50 years (Von Strauss, Viitanen, De Ronchi, Winblad, Fratiglioni, 1999). The annual incidence is estimated to be about 360,000 (Bachman et al., 1993). Ernst and Hay (1994) estimated the prevalence rate of AD for 1991 to be 1.35 million cases in the United States population of 65 and older. Patients aged 45 to 64 were estimated at 245,000 persons (Brookmeyer et al., 1988). An estimate for nursing homes found that 23.5% of residents were diagnosed with AD (Schultz, O’Brien, Bookwala, & Fleissner, 1995). The estimated gross annual cost of mental hospitalization was $113,600 per patient in 1996 (Meek, McKeithan, & Schumock, 1998). Total annual unpaid caregiver costs were $33.3 billion, and the costs of disability and premature
mortality were $13.4 billion for persons diagnosed in 1991 (Ernst & Hay, 1994). In total, costs of AD in 1991 were $67.3 billion. Public health estimates suggest that if interventions could delay onset of AD by 2 years, the 50-year projection would decrease by nearly 2 million fewer cases (Brookmeyer et al., 1988).

In order to determine the severity of prevalence, Evans et al. (1989) assessed AD in an average United States community. Of 3,623 persons (80.8% of all community residents over 65 years of age) who had brief memory testing in their homes, a stratified sample of 467 persons underwent neurological, neuropsychological, and laboratory examination. Rates of prevalence for AD were calculated from the sample undergoing clinical evaluation. For those over the age of 65 years, an estimated 10.3% had AD. This prevalence rate was strongly associated with age. Of community residents with moderate or severe cognitive impairment, 84.1% had clinically diagnosed AD as the only probable diagnosis. These data suggest that clinically diagnosed AD is a common condition and that its public health impact will continue to increase with increasing longevity of a population.

On the other hand, despite the conclusion reached by Evans and colleagues in the preceding statement, treatment
and prevention of this disability continue to improve. According to Cummings (2002), detecting AD dementia during early development improves patient treatment and quality of life. Yet, early stage detection is rare and very difficult given the naive understanding of AD. What is more unfortunate is that under-diagnosing moderately severe dementia is relatively common in clinical practice (Callahan, Hendrie, & Tierney, 1995). According to the American Academy of Neurology, mental status screening instruments such as the Mini-Mental State Examination, are useful for detecting dementia and health care professionals should use them with greater frequency among populations at increased risk for dementia (Folstein, Folstein, & McHugh, 1975). In addition, asking patients or caregivers about abnormalities in learning, difficulty handling complex tasks, impaired reasoning ability, and changes in language or behavior can enhance detection during early stages of AD. Neurological imaging and functional imaging with positron emission tomography or single photon emission computed tomography has proven to be helpful when clinical features are ambiguous (Cummings, 2002).
Cognitive Impairments

AD patients experience retro- and anterograde amnesia, language disorders that start with anomia and progress to fluent aphasia, as well as dysfunctions in visuospatial ability. Interestingly, during the early stages, patients are typically unaware of memory or cognitive loss. Apathy also coincides with this early period; patients often show diminished interest in themselves and a general reduced concern. However, agitation becomes increasingly common as the illness advances. About half of AD patients experience depression and 25% suffer from delusions. During the last few years of the disease patients experience motor system abnormalities, gait changes, and seizures. Patients with AD usually survive 7 to 10 years after onset of symptoms, typically dying from bronchitis or pneumonia (Cummings, 2002).

Arguably, the most frustrating symptoms for patients with AD and their families are amnesia and learning impairments. Cognitive-behavioral and neuropsychological research in AD has largely adhered to a working memory model in formulating hypotheses for why AD causes these impairments in patients. Additionally, this model provides
a nomenclature that is helpful to researchers who use it to describe their scientific observations.

The working memory model is based on the information-processing model of memory and learning. This model, as it is relevant to the explanation of AD research, is comprised of four main components. The first part is input, where information is cognitively received. Next is the data processor, where data is evaluated, interpreted, or decoded. From here data is stored. Finally, when stored data is needed, it goes to the output component of the information-processing model. The effort involved in processing information through each of these stages is referred to as learning. Moving information through the input stage is termed acquisition; moving it into the processor and storage stages is known as retention; while information that is moved from storage to output is called retrieval (see McClelland, 1979).

In order to detail the storage component of this model we must look to the working memory model, as it is particularly instrumental in explaining amnesia in AD. The working memory model proposes a memory system comprised of working memory, short-term memory, and long-term memory (Baddeley & Andrade, 2000; Baddeley & Hitch, 1974). This more complex model describes short-term memory as a
cognitive model that coordinates the temporary storage, simultaneous processing, and manipulation of information necessary in order to perform complex cognitive functions. Additionally, long-term memory can be subdivided into explicit memory (intentional recall) and implicit memory (memory resulting from repetitious behavior for example). Explicit memory, also called declarative memory, can be further subdivided into episodic memory and semantic memory. Many researchers would agree that episodic memory problems are significant in patients with mild to moderate AD (Balota, Burgess, Cortese, & Adams, 2002). However until recently, findings concerning the specific nature of learning and memory deficits in AD were unclear. As a result, researchers were unwilling to fully explain the impact of AD on each discrete phase of learning (i.e. acquisition, retention, and retrieval) (Baddeley, Logie, Bressi, Dellasala, & Spinnler, 1986). Recently, however, there is a trend among cognitive researchers to explain intellectual impairments of AD as it pertains to the working memory model of learning.

Initial research on the effects of AD on the phases of learning appeared to provide support for retrieval deficits among those with AD. For example, Cronin-Golomb, Keane, Kokodis, Corkin, and Growdon (1992) evaluated three
suspect areas of deficits in the retrieval of category information in AD. Researchers believed that impaired retrieval in AD was due to either an impaired ability to organize semantic knowledge, word difficulty, or limits on processing speed (such as how much or how quickly semantic information can be retrieved). To test their hypotheses, participants were given a category fluency test followed by a categorical exemplar ranking test. Their results showed that AD patients produced fewer items in a category fluency task and had longer reaction times in a category decision task. The pattern of performance across categories on both tasks in the AD group was similar to controls: the same categories elicited the most (or fastest) responses in both the control group and the AD group. AD patients showed normal performance in ranking of category. There was no evidence for differential accessibility by category of information (animate vs. inanimate). The authors concluded that a general factor or factors limit retrievability equally across all categories.

Alternative findings suggest that there is an encoding deficit in acquisition and not retrieval. For example, Greene, Patterson, and Xuereb (1996) assessed patients with early AD or matched controls on three
clinical tests of episodic memory, indexed by immediate and delayed recall, list-learning, and visual and verbal recall and recognition. On all tests, patients with AD were impaired in their ability to learn new information with little evidence of acquisition across trials. In addition, patients with AD showed lower performance than controls on delayed recall. However, when this was analyzed further, it was argued that this lowering in performance was due to the contribution of short-term memory to immediate recall but not delayed recall. Greene et al. (1996) proposed that immediate recall can be enhanced via short-term memory operating via the recency effect, whereas short-term memory does not contribute to delayed recall which requires retrieval from long-term memory. It was concluded that although the amount of information lost over a delay is greater in patients with AD, this is due to their greater reliance on short-term memory, rather than reflecting a more rapid loss of information from long-term memory.

A similar finding was made by Greene, Baddeley, and Hodges (1995). The goal of this research was to study anterograde episodic memory impairment in AD. The authors of this study report that the main function of episodic memory is to organize information and thoughts according
to how events are related to one another in order to learn more effectively. The measures used in this study included the immediate and delayed prose recall (IDPR) task, the consortium to establish a register in Alzheimer's disease (CERAD) test battery, and the doors people test of visual and verbal and recognition deficits. The IDPR was taken from the logical memory portion of the Wechler Memory Scale: this task required two paragraphs to be read out loud to the participant. After each administration, the participant was asked to provide as complete an account of the paragraph as they could. After 30 min, the participant is asked to do the same with the other paragraph. The CERAD was used specifically to measure the rate of learning and forgetting in AD. The doors and people test provides an overall episodic memory score. Results showed that participants with AD were impaired on the IDPR component of the logical memory test. In the CERAD test, findings showed that participants with AD forgot significantly more than non-AD participants during immediate recall but not delayed recall. No significant differences in forgetting were noted on the doors and people test. Contrary to the authors' hypothesis, there was no evidence that episodic memory impairment in AD is a deficit of retrieval of learned material. In fact, this
article lends support to evidence suggesting that AD impairs knowledge acquisition more so than knowledge retrieval and material-specific events. Accelerated forgetting among AD participants could not be substantiated either. A similar finding was made by Perry, Watson, and Hodges (2000). These researchers argued that once material is acquired or learned, patients with AD do not demonstrate accelerated forgetting from episodic long-term memory. Thus, it was concluded that the episodic memory deficit is a reflection of an impairment in the acquisition of new information, rather than a retrieval deficit.

Interestingly, the acquisition deficit, identified by Perry et al. (2000), seems to be restricted to the episodic subcategory of explicit memory; at least in the early disease process. This finding has been supported by Hodges and Patterson (1995), for example, who provided evidence that dysfunction of the transentorhinal region alone is not sufficient to result in reliable and significant impairments of semantic memory. Patients of this study were divided into three groups according to the severity of their dementia: minimal, mild, and moderate. Participants were subjected to a broad spectrum of tests that provided information about the state of participant's
semantic and episodic memory. The episodic memory measures included the Logical Memory Subtest from the Wechsler Memory Scale—Revised, the Recognition Memory Test, and the Rey Complex Figure Test. The semantic memory measures included The Semantic Memory Test Battery, Non-Verbal Semantic Memory Test, and tests of auditory verbal short-term memory and visuo-spatial ability. All patients showed a significant deficit in episodic memory. Impairment of semantic memory measures for the minimal group included category fluency, naming of line drawings, naming to verbal description, answering semantic feature questions and a non-verbal picture-picture matching. The mild and moderate groups shared these deficits and showed further impairment of semantic memory measures for picture sorting and word-picture matching tests. While some patients showed a consistent impairment across all of the semantic memory tests, others were impaired only on a section of these tests, and still others performed perfectly.

Hodges and Patterson (1995) point out that these results are evidence that the transentorhinal region has a consistent role in causing symptoms of very mild AD dementia. Researchers of this article hypothesized that damage of this site results in a disengagement of the
hippocampus, leading to consistent episodic memory impairment. Yet, from their findings, these authors deduced that because some of these same patients did not show impairment of semantic memory, damage to the transentorhinal region alone is not enough to produce a significant impairment of semantic memory.

A possible contradiction to this conclusion comes from evaluating the relationship between semantic memory and language functions (Martin & Fedio, 1983). Researchers found evidence for stereotypical AD dementia language disorders associated with deficits in semantic knowledge. Patients with suspected AD and a control group were administered name and fluency production tests and tests for comprehension of single words (semantic knowledge). Findings showed AD participants exhibited perseveration of vocabulary and target word similarities, difficulty with retrieval of object names and verbal fluency, and semantic but not phonemic paraphasias on object-naming. Martin and Fedio (1983) suggested that these findings could result from semantic knowledge impairment requiring comprehension and retrieval of specific words. Moreover, Martin and Fedio (1983) indicate that perseveration in this case may be due to an inability to retrieve and properly utilize specific attributes of broader categorical information.
In addition to the evidence of episodic memory impairments, scientists have also observed implicit memory deficits in AD (Bondi & Kaszniak, 1991). The purpose of this research was to determine if participants with different degenerative brain diseases exhibit different dissociations in performances on various explicit and implicit tasks. This research examined differing neuropathologic features associated with AD and Parkinson's disease (PD). It was hypothesized that the identification of patient's impairments in solving the tasks would provide evidence of the particular brain area afflicted in AD and PD and implicit or explicit memory. This reasoning is based on the differing brain lesion patterns of the two diseases resulting in their respective impairments. Therefore, observations of any differences between the groups in implicit or explicit memory may provide researchers verification of the involvement of the associated brain area. Tasks used included the fragmented pictures test, word stem completion priming, pursuit-rotor learning, and motor-reading tasks. The function of the fragmented pictures test was to derive an index for the learning of the configuration of the stimuli (performance over practice). The word stem completion priming provided a test to examine whether additional implicit learning
tasks dissociate among themselves. The purpose of the pursuit-rotor learning test was to demonstrate improvement of motor skills despite being unable to show explicit recall of having performed the task. The motor-reading task tested the nature of skill acquired as a result of reading transformed words (reading generalization). Results indicate that those with AD tend to have a disruption of conceptual relationships within their semantic memory relating to information retrieval. AD patients were also impaired in skill learning stemming from damage to the neostriatum. Other selective deficits included mirror reading and pursuit-rotor tracking. Given the deficits and likely areas of brain damage, Bondi and Kaszniak (1991) concluded that these data supported the theory that there are different neuroanatomical circuits involved in implicit and explicit memory.

Sebastian, Menor, and Elosua (2001) studied the short-term memory deficit in AD patients, hypothesizing that this deficit could be explained by a pattern of errors that indicate dysfunction of either individual or multiple memory systems within the working memory model. AD and control participants were administered the Brown-Peterson task. Results showed that the rate of forgetfulness was similar in the two groups. On the other
hand, a qualitative analysis of AD participant responses indicated that errors made were confusions, perseverations, omissions, and order alterations. These researchers suggest that this pattern is indicative of problems originating in the central executive system.

Alternative cognitive approaches to understanding AD have also been proposed in several articles. For example, in an effort to determine the context of AD learning impairment Freed, Corkin, Growdon, and Nissen (1989) examined picture recognition in AD patients using a procedure that matched AD and control groups for initial performance. The groups did not differ significantly in overall forgetting, although some patients displayed improved recognition performance 72 hr after learning. These same patients were impaired in a test of attentional focusing. A predictive experiment involving different patients with AD confirmed the initial findings; a subgroup of patients displayed improved recognition performance 72 hr after learning and impairments in attentional focusing. Neuropsychological tests thus aid in identification of a subgroup of AD patients with impaired selective attention, perhaps related to locus coeruleus neuropathology.
A final area of learning impairment in AD is perseveration. Perseverative behavior is the tendency to maintain a given response or problem solving behavior despite evidence that contingencies for the response have changed. This behavior can continue even in the face of diminishing returns and consequences. Unlike other learning impairments, perseverative behavior in AD is not typically discussed in a working memory model. Instead it is more often viewed in relation to other AD symptoms. For example, in a study by Lamar et al. (1997), evidence was provided in support of the predictions that 1) perseverative behavior is hierarchically arranged in terms of specific levels of cognitive complexity and the particular pattern of deficits, 2) the type of perseverative behavior in those with AD and those with subcortical ischemic vascular dementia (IVD) would be related to the overall pattern of cognitive deficits that present themselves, and 3) the degree of perseverative behavior is contingent on the extent of damage to the prefrontal area. Lamar et al. (1997) utilized the Graphical Sequence Test in order to induce and measure perseverative behavior. This was followed by a neuropsychological assessment of motor functioning, frontal systems functioning, language-semantic
functioning, visuoconstructional functioning, and declarative memory assessments. Data showed that IVD participants made more total perseverations than did AD participants and that perseverations made by AD participants were correlated with deficits on tests of semantic knowledge. Perseverations made by IVD participants were correlated with motor and frontal systems tests. The data were consistent with the researchers' hypothesis that perseverative behavior is hierarchically arranged in terms of specific levels of cognitive complexity and the overall pattern of cognitive deficits associated with each type of dementia.

In general, the examination of cognitive impairment caused by AD has been instrumental in aiding researchers in their effort to understand the AD disease process and the roles of the affected brain areas. The effort to gain an even greater understanding in this disease has led to the research of neuroanatomical structures and mechanisms thought to be damaged or dysfunctional in AD.

Neuropathology of Alzheimer’s Disease

Degenerative dementia is only manifested in about 15 to 25% of early AD cases (McKeith et al., 1996). However, many of the physiological features of AD tend to develop
stereotypically across patients. As such, post mortem examination reveals definitive AD lesions believed to be causal in cognitive deficits. Dickson (2001), for example, provided a recent survey of clinical features and pathology associated with various forms of dementia including AD, Lewy body disease, ischemic dementias, Pick’s disease and Creutzfeldt-Jakob disease. It was found that the brains of cadavers with AD typically have widespread atrophy throughout the cortex. At the microscopic level, there is loss of neurons within the cerebral cortex, the hippocampus, amygdala, and the basal forebrain. The loss of these cells correlates with the degree of cognitive impairment before death (Reisberg, 1983). Another pathological marker is the presence of paired helical filaments in the brain. These filaments are intraneuronal structures comprised of tau protein (Ball & Nuttall, 1981). When these structures accumulate in neuronal cell bodies they produce lesions called neurofibrillary tangles. When they accumulate in neuronal processes they are called senile plaques (Ball & Nuttall, 1981).

Perl (2000) reviewed common neuropathological brain features of AD and related conditions leading to dementia. A critical point made by Perl is that a confirmed
diagnosis of AD requires a postmortem neuropathological examination. The diagnosis of AD is based on the microscopic examination of the brain and the recognition of an abnormal pattern that indicates AD. Primary among the patterns that histo-technologists look for during this examination include plaques and tangles. One example of these includes the neurofibrillary tangle. This appears as bundles of coarse fibrils that surround the nucleus of the neuron and extend outwards toward the dendrite (Wisniewski, Narang, & Terry, 1976). Studies have shown that tangles are composed of much smaller filaments referred to as tau (Masters et al., 1985). Additionally, brains with AD also tend to have senile plaques that, relative to tau filaments, are large and complex. The primary component of these plaques is an extracellular accumulation of a 4-kD amyloid protein, typically identified as β-amyloid (Masters et al., 1985). Yet another pattern includes granulovacuolar bodies which consist of a cluster of small vacuoles within the perikaryal cytoplasm, each containing a dense basophilic granule. These lesions are found almost exclusively in the large pyramidal cell of the hippocampus and are encountered predominantly at the junction between the CA2 and CA1 regions (Vallet et al., 1992). Pearson, Esiri,
Hiorns, Wilcock, and Powell (1985) observed that the loss of synapses in cases of AD and that the extent of this loss correlated with the degree of impairment. Davis et al. (1999) found that in early stage cases, neocortical cholinergic function remained undiminished in multiple neocortical areas. This finding suggests that the cholinergic deficit associated with AD represents a late-emerging phenomenon.

Unfortunately, exact causes (or even a non-invasive, definitive diagnosis) at present remain elusive to clinicians and researchers. However, evidence generated by researchers within the last two decades of study of the nucleus basalis reveals much about the pathology of AD. Data suggest that there are strong correlates between AD related learning deficits and decrements of acetylcholine (ACh) levels within neocortices (for example, Araujo, Chan, Winka, Seymour, & Milgram, 1988; Lowes-Hummel, Gertz, Ferszt, & Cervos-Navarroa, 1989; Muir, 1997; Perl, 2000; Samuel, Terry, De Teresa, Butters, & Masliah, 1994; Whitehouse et al., 1982). Decreases in neuronal ACh activity can be traced to damage of the basal forebrain that supplies the cortex with ACh.

In rodents, the nucleus basalis magnocellularis (NBM), a structure with extensive cholinergic projections
to cortex and amygdala, has been at the center of attention in animal models of AD. Much of the interest in the NBM stems from the "cholinergic hypothesis". This hypothesis asserts that the decline of cognitive functioning in AD in humans can be linked to the progressive degeneration of the ACh producing cells of the cholinergic basal forebrain (Araujo et al., 2004; Lowes-Hummel et al., 1989; Muir, 1997; Perl, 2000; Perry et al., 1978; Samuel et al., 1994).

The Cholinergic Hypothesis of Alzheimer's Disease

The premise of the cholinergic hypothesis is that memory and learning deficits associated with AD and aging are attributable to degeneration of the cholinergic magnocellular neurons of the nucleus basalis of Meynert (nBM). According to Francis, Palmer, Snape, and Wilcock (1999) the antecedents that led to the cholinergic hypothesis began in the late 1960s and early 1970s with the biochemical investigation of the brains of AD cadavers. These early efforts to identify the neurochemical imbalances characteristic of AD were conducted with the hope of providing the basis for the development of a medical therapy. Advances in this approach were followed by subsequent discoveries that
persons with AD often have low levels of choline acetyltransferase (the enzyme responsible for the synthesis of ACh), reduced choline uptake, and decreased ACh release (Whitehouse et al., 1982). These findings aided in the understanding of how loss of cholinergic perikarya from the nbM affected brain function in persons with AD. According to Collerton (1986), pharmacological studies manipulating cholinergic function have provided much evidence in support of the cholinergic hypothesis. Collerton suggests that persuasive evidence for the cholinergic hypothesis comes from human studies that show deficits in complex behavioral tasks following the administration of anti-cholinergic drugs such as scopolamine. Both the research from animal data and findings from human studies contributed to the proposal that deterioration of cholinergic neurons in the basal forebrain and the resulting loss of cholinergic neurotransmission in the cerebral cortex is a significant factor in the process by which AD patients experience a loss in cognitive function (Davies & Maloney, 1976).

The cholinergic hypothesis has undergone only subtle changes as a consequence of neurological discoveries made over the last 20 years. Much of the exploration of the cholinergic hypothesis during this period has facilitated
the preclinical and clinical development of cholinomimetic drugs for the treatment of AD (focusing on acetylcholinesterase inhibitors). This effort also resulted in several alternative hypotheses explaining cortical ACh reduction and the role of ACh in cognitive impairment in AD. One alternative is the "amyloid cascade hypothesis". This idea explains that β-amyloid protein formations observed in the brains of AD patients are necessary and sufficient to cause cognitive decline. Many researchers who support this model acknowledge the relationship between high levels of β-amyloid and reductions in both choline uptake and ACh release (Lee et al., 2004). However, they suggest that cholinergic involvement in cognitive decline is secondary to the effects of β-amyloid (Ladner & Lee, 1998). Other studies have shown that the phosphorylation of tau, a step in the formation of tangles, may also be influenced by the phospholipase C second messenger system (Iqbal et al., 1994). Further testing is necessary to determine whether second generation cholinomimetic drugs would improve cognitive function. However, if the cholinergic hypothesis is correct, one could infer that they will be effective (Francis et al., 1999).
According to Ladner and Lee (1998) ACh exerts its effects by the interaction of nicotinic and muscarinic protein receptors. Since the discovery of these receptors, researchers have found at least five distinct muscarinic receptor proteins (M1, M2, M3, M4 and M5) (Dörje et al., 1991). The cholinergic receptor shown to be necessary for learning and memory is the M1ACh receptor (M1AChR) (Drachman & Leavitt, 1974).

Pharmacological blockade of this receptor by antagonists impairs short-term memory. For example, Drachman and Leavitt (1974) demonstrated that scopolamine impairs short-term human memory. Furthermore, other studies have shown that the M1AChR-selective antagonist, pirenzepine, impairs working memory in rats (Bymaster, Heath, Hendrix, & Shannon, 1993; Ohno, Yamamoto & Watanabe, 1994). Interestingly, scopolamine deficits were prevented by coadministration of muscarinic agonists (Ohno et al., 1994). In sum, these studies provide evidence that the deficiencies in the muscarinic cholinergic system are involved in AD.

Studies such as these have guided the development of two basic classes of cholinomimetics for the treatment of AD: direct muscarinic agonists and direct nicotine agonists. In general, persons with AD who are administered
direct muscarinic agonists will have small but noticeable improvements in cognition (Clader & Wang, 2005). Hammer, Berrie, Birdsall, Burgen, and Hulme (1980) suggest that subsets of the AD population are more responsive to these drugs such as those with the Lewy Body variant of AD, patients with ApoE4 genotype, and women. Side effects of direct muscarinic agonists may include nausea, vomiting, hepatotoxicity, agitation drowsiness or dizziness, muscle soreness, confusion, and decreased coordination or balance (Hammer et al., 1980).

A problem commonly observed in many AD drugs was that they were not selective enough to avoid triggering unrelated ACh mechanisms in the brain. In some instances, these drugs would simultaneously increase ACh activity within synapses in one region of the brain while decreasing ACh activity within synapse located elsewhere in the brain (Lander & Lee, 1998). In response to these issues a number of pharmaceutical companies have developed acetylcholinesterase-resistant exogenous cholinergic agonists targeted specifically for the primarily postsynaptic M1AChR (Lander & Lee, 1998). Results from clinical trials of xanomeline, a selective agonist of the M1AChR, have shown some improvement over other direct muscarinic agonists in the treatment of AD. At the time of
this writing, xanomeline remains the only M1AChR-selective agonist to reach clinical trials, although several others are in development (Stern, Sano, & Mayeux, 1988).

In addition to the aforementioned techniques used to determine the role of ACh in learning and memory researchers have also used specialized equipment to observe the function of ACh in the human brain. For example, Bentley, Vuilleumier, Thiel, Driver, and Dolan (2003), used event-related fMRI, while biochemically altering attention and emotionality with physostigmine. The goal in this study was to examine human brain areas of cholinergic modulation. Bentley et al. (2003) predicted that cholinergic afferents would influence both selective attention and emotional processing. During the experiment, face or house pictures appeared at task-relevant locations, with the alternative picture type at irrelevant locations. Faces had either neutral or fearful expressions. Results showed that physostigmine increased relative activity within the anterior fusiform gyrus for faces at attended, versus unattended, locations, but decreased relative activity within the posterolateral occipital cortex for houses in attended, versus unattended, locations. It was argued that cholinergic innervations enhanced neuronal responses within the middle
fusiform gyrus to fearful faces. On the other hand, physostigmine influenced responses in the orbitofrontal, intraparietal and cingulate cortices to fearful faces when faces occupied task-irrelevant locations. In other words, physostigmine resulted in the attenuation of the differential responses to attended vs. unattended stimuli. Bentley and colleagues (2003) concluded that ACh may modulate both selective attention and emotional processes through independent, highly region-specific effects. In addition, Bentley and colleagues (2003) also found that systemic increases in cholinergic transmission may have limited cognitive effects.

Since the inception of the cholinergic hypothesis, significant progress has been made in AD research. Particularly important is the wider acceptance of animal models for studying AD, which has been crucial in providing insight into the potential causes. The preceding portion of this literature review provided an extensive presentation of evidence for deficient levels of the neurotransmitter, ACh, in memory loss and learning impairment for those with AD dementia. The remainder of this review will focus on the animal lesion model of AD with specific attention given to ACh depletion in the
cortex of rats and evidence of resulting learning and memory impairments.

Nucleus Basalis of Meynert

Doucette and colleagues (1986) examined the degree of neuronal loss from the nbM in two groups of patients with AD that differed in their degree of cognitive impairment. Significant cell loss from the nbM was found only in the more severely demented group of patients. Mean cell counts were compiled separately for the anterior, intermediate, and posterior subdivisions of the nbM in three groups of subjects: Group 1 was severely demented and was untestable on the Extended Scale for Dementia (ESD) for at least the last two years of life; Group 2 was less demented and had completed at least one ESD test within 12 months of death; Group 3 had died of non-neurological causes. In Group 2 there was a small (but nonsignificant) trend toward cell loss in the anterior subdivision, and a normal complement of neurons in both the intermediate and posterior subdivisions of the nbM. There was, however, significant cell loss from all subdivisions in Group 1. As such, Doucette et al. (1986) found that cell loss in the nbM relate to the severity of the dementia.
Whitehouse et al. (1982) examined antecedents leading to the pathology of the basal forebrain in AD. Immunocytochemical techniques were used to examine the cellular localization of the amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptor subunits GluR1 and GluR2/3 in the basal forebrain of non-demented elderly human subjects (66-102 years). For each participant, GluR1-positive magnocellular cells were darkly labeled within all main divisions of the basal forebrain (Ch1-Ch4). Double-labeling immunohistochemical techniques verified the cholinergic character of these neurons. In contrast, GluR2/3 immunolabeling on magnocellular neurons was relatively faint or nonexistent. The latter observations were most apparent in cases of advanced age. In addition to the elderly subjects, GluR1 and GluR2/3 immunostaining was examined in the nbM of younger cases. GluR1 and GluR2/3 labeling on nbM neurons was found in the Ch 4 region only. On average, the distribution of labeled cells and intensity of immunoreaction were comparable between GluR1 and GluR2/3. The presence of GluR2/3- and GluR1-labeled neurons in the Ch4 region of younger cases, but primarily GluR1 in cases of advanced age, suggested an age-related decrease in neurons containing GluR2/3 receptors.
Lastly, Allen, Dawbarn, and Wilcock (1988) challenged the observation that there was a loss of large cells in the cholinergic nbM in patients with AD. They hypothesized that there may be neurons in the nbM that become atrophied in AD dementia. As such, the task of distinguishing neuroglia by size becomes increasingly difficult. Using a neuron-specific antiserum directed toward neuron-specific enolase (NSE), these researchers stained sections of nbM and measured the neuronal cross-sectional area. A profile of neuronal distribution was determined by image analysis and compared in controls and patients with AD. A significant 29% overall loss of neurons was found in AD with a greater loss (61%) of large neurons and a concurrent increase (59%) in small neurons. Analysis of variance supported this reduction in mean cross-sectional neuronal area. It was thus argued that large neurons are not completely lost in nbM in patients with AD; many cells are shrunken and were likely excluded from the previous studies of large cells counted in Nissl-stained material. Because there is partial preservation of these neurons, it is more likely that cholinergic dysfunction, characteristic of AD, will be sensitive to neurotrophic influence.
CHAPTER TWO
ANIMAL MODELS OF ALZHEIMER'S DISEASE

Introduction

This chapter discusses how animal models have served to advance our understanding of the relationship between the learning impairments caused by AD and the neuropathology characteristic of this disorder. An important class of animal model involves manipulations of brain structures in rats that are homologous to those damaged in humans with AD. The review of literature in this chapter focuses on the research that has used selective lesion models of AD and the behavioral effects of these lesions. Lesion studies of the cholinergic projections from the NBM to the neocortex have been utilized extensively as the basis of an animal model of dementia. Such studies have been instrumental in determining causal relationships between specific learning and memory impairments and the cortical cholinergic denervation resulting from the degeneration of the cholinergic neurons of the NBM.

However, not all researchers agree that the NBM lesion model serves as an effective tool in understanding AD. Fibiger (1991), for example, questions the validity of the cholinergic hypothesis. In his review, he states that
although there is substantial pharmacological evidence that unspecified cholinergic systems in the brain play important roles in some forms of learning and memory, recent findings in humans indicate that antimuscarinic drugs do not model the deficits seen in AD. In addition, he points out that the goal of elucidating the functions of the basal forebrain neurons in animals has yet to be achieved. As such, he concludes, the cognitive and behavioral consequences of cholinergic pathology in AD remain unknown and under these circumstances, attempts by researchers to develop cholinergic pharmacotherapies for AD are based on questionable assumptions.

Selective Lesions of the Nucleus Basalis Magnocellularis

Many criticisms of the lesion models of AD, and consequently the cholinergic hypothesis, are in response to early lesion experiments that (partially or wholly) failed to achieve impairments approximating the dysfunction observed in AD (Wrenn & Wiley, 1998). In response to these criticisms, more sophisticated means of producing NBM lesions have been conducted using a selective cholinergic immunotoxin, 192 IgG-saporin. These studies resulted from the hypothesis that neurotoxins
(such as ibotenic acid) that were thought to be highly selective; were in fact damaging non-cholinergic structures in the vicinity of the NBM (Dunnett, Everitt, & Robbins, 1991). However, infusion of 192 IgG-saporin into the NBM causes more consistent and accurate representations of AD-like lesions by isolating cellular destruction to cholinergic neurons (Torres et al., 1994). 192 IgG-saporin exclusively effects p75 neurotrophin receptors of the cholinergic neurons. Once the toxin enters the cells it disrupts protein synthesis, resulting in cellular necrosis (Wiley, Oeltmann, & Lappi, 1991).

Behavioral Effects of Nucleus Basalis Magnocellularis Lesions

Animal models of AD involving 192 IgG-saporin lesions of the NBM have primarily focused on lesion-induced deficits in incremental attention, selective and sustained attention, strategy switching and set shifting, and configural association learning to address changes in learning, memory, and attention following NBM damage. Attention Impairments

Attention, the cognitive process of selectively concentrating on one thing while ignoring other things, is impaired as a result of 192 IgG-saporin lesions of NBM ACh
neurons. This seems to mirror attention impairments present in mild to moderate AD. As such, this provides further evidence that the animal lesion model of AD is an accurate representation of the disease process of human AD. This was exemplified in the research conducted by McGaughy, Turchi, and Sarter (1994) who have taken an unorthodox approach of conceptualizing aspects learned in human studies and applying the concepts in this effort to the animal model. In this experiment, a task of a cross-modal divided attention was developed for rats to investigate pharmacological manipulation of multi-tasking and information processing. Rats were trained consecutively in operant auditory and visual conditional discrimination tasks. The rules for this task were: if presented with constant light stimulus, press the left lever; if presented with flashing light stimulus, press the right lever. For the auditory task, if presented with constant tone, rats would be reinforced for pressing the right lever; if rats were presented with a pulsing tone, the left lever was correct. The final task consisted of two consecutive blocks of 20 trials per modality (auditory or visual), followed by 60 trials comprising a semi-randomized sequence of both stimuli modalities. The mixed condition was hypothesized to challenge the animals'
divided attention. The longer response latencies of the mixed condition versus the "uni-modal condition" seemed to confirm this. Rats in the experimental conditions were administered scopolamine (a muscarinic ACh receptor antagonist) or chlordiazepoxide (a benzodiazepine GABA inhibitor).

Results demonstrated that scopolamine produced the greatest increase in response latencies in the modality uncertainty condition. Based on cost-benefit analyses, both drugs produced qualitatively similar divided attention costs; however, scopolamine was more potent in increasing the absolute divided attention costs than chlordiazepoxide. The findings support the hypotheses that benzodiazepine receptor ligands mediate attentional abilities through their effects on cortical ACh release on brain information processing capacity.

Similarly, Baxter, Bucci, Holland, and Gallagher (1999), examined the conditioned behavior of rats with 192 IgG-saporin lesions of the hippocampus and neocortex. These combined lesions disrupted both incremental and detrimental changes in CS processing in a serial conditioning procedure. Rats with lesions also showed more general impairments in conditioned responding. The results indicate two different findings. First, the neural systems
for increasing and decreasing attentional processing may be independent. Second, combined loss of hippocampal and neocortical cholinergic input may produce behavioral impairments that are synergistic since these affects are not apparent after either lesion alone.

Furthermore, Baxter and Chiba (1999) examined the role of the basal forebrain cholinergic neurons in attention, memory, and cortical information processing in an AD model that used selective cholinergic immunolesion via 192 IgG-saporin. Results from this study indicate that cholinergic neurons that project to cortical and limbic structures serve a modulatory function in cognition, by optimizing cortical information processing and influencing attention.

Finally, Chiba, Bucci, Holland, and Gallagher (1995) examined the ability of rats with selective 192 IgG-saporin lesions of cholinergic neurons to modulate attention within an associative learning task. Each rat was exposed to conditioned stimuli (CS) that were either consistent or inconsistent predictors of subsequent cues. Intact control rats showed increased CS associability when that cue was an inconsistent predictor of a subsequent cue, whereas rats with cholinergic lesions were impaired in their ability to associate cues when its established
relation to another cue was modified. In a separate experiment designed to test latent inhibition, removal of the cortical cholinergic neurons spared a decrement in associability that occurs when rats are extensively preexposed to a CS prior to conditioning. These data indicate that the cholinergic innervation of cortex is necessary for incrementing, but not for decrementing attentional processing.

Cognitive flexibility, a second mental function that is impaired in 192 IgG-saporin studies; has been defined as the degree a subject adapts its strategies to more effectively respond to changing problems. Impairments of this nature in AD often take the form of perseverative behaviors (Connor et al., 1998). When animals display similar behaviors following brain lesions these behaviors are referred to as behaviors of cognitive inflexibility.

Cognitive Inflexibility

Cognitive inflexibility due to NBM lesions have been demonstrated in a number of animal studies. In a recent study by Bailey, Rudisill, Hoof, and Loving (2003) cognitive inflexibility was examined in a task shifting exercise. In this study, rats with bilateral 192 IgG-saporin lesions to the NBM were tested on olfactory discrimination learning set (ODLS), olfactory
discrimination reversal learning set (DRLS), and open field activity. Control animals demonstrated learning set in both the ODLS and DRLS tasks. The NBM-lesioned animals showed initial acquisition impairment in learning set in the ODLS task but eventually demonstrated learning set in both ODLS and DRLS tasks. There were no group differences in open-field activity. Results suggest that removal of the NBM cholinergic system through 192 IgG-saporin lesions impairs early acquisition of learning set compared to control animals, but does not prevent later use of learning set formation.

Cabrera, Chavez, Corley, Kitto, and Butt (2006) tested the hypothesis that lesions of the NBM in rats would result in cognitive inflexibility. In other words, it was anticipated that cholinergic innervation of the NBM is necessary for cognitive flexibility in rats. In this study, male rats were given 192 IgG-saporin lesions of the NBM and were trained to solve a serial reversal task during subsequent extinction testing via operant discrimination. Animals that had lesions were compared to normal animals' level of perseveration to the task (i.e., cognitive inflexibility). Testing had three phases: acquisition, serial reversal, and extinction. The acquisition phase was the presentation of a
food-reinforced tone (T+) and a non-reinforced light (L-). This phase was followed by a reversal phase consisting of four serial reversals of the original operant discrimination. Lastly, the extinction phase consisted of no reinforcement for any response.

Results showed that the NBM lesion and control groups did not differ in the acquisition phase since no demands were placed on cognitive flexibility. However, the NBM lesion group did perseverate during the serial reversal and extinction phases of testing due to the demands placed on cognitive flexibility.

Cognitive inflexibility has also been demonstrated in an article by Butt and colleagues (2003). These researchers hypothesized that NBM lesions would not affect performance in an appetitive task, but that performance would be impaired if it were followed by a transfer to an aversive task. This hypothesis was engendered from the proposition that the NBM is not necessary for simple association learning that does not tax attention. The appetitive phase of the transfer task is argued not to tax attention as it depends on simple association learning. Consequently, performance in this task was predicted to be spared following NBM lesions. On the other hand, complex, associative learning is argued to depend on the NBM since
it is attention demanding. The results supported the researchers' hypothesis; the NBM lesion group acquired the appetitive response normally, but they showed impaired performance following transfer to the aversive conditioning phase of the transfer task.

**Configural Association Learning Impairments**

A final learning process that has been shown to be affected by 192 IgG-saporin is configural association learning. There are two broad categories of associative learning elemental or “simple” association learning and relational or “configural” association learning (Pearce & Wilson, 1990; Rescorla, 1972; Sutherland & Rudy, 1989). A simple association can be described as having an unchanging and explicit contingency associative link between an individual stimulus element and its reinforcement outcome. A configural association, on the other hand, has a complex or relational associative link between a unique representation of two or more stimulus elements and that unique representation’s reinforcement outcome. For example, in a configural task, two simultaneous events may signal a cue for a given behavior and two other simultaneous events indicate a different behavior. Butt and Bowman (2002) and Sutherland and Rudy (1989) have argued that simple and configural association
learning rely on distinct learning and memory systems. These authors have explained that the NBM is necessary for configural association learning but not for simple association learning. Evidence of the disassociation of these two brain systems has been provided by Wagner, Logan, and Haberlandt (1968) who have demonstrated that subcortical brain areas alone are sufficient for animals to learn simple association tasks.

In order to test animals' ability to make configural associations, several tasks have been devised that explicitly challenge configural association learning. Common configural association tasks include negative patterning, transverse patterning, and biconditional discrimination. The following literature describes negative patterning and transverse patterning tasks used in the evaluation of configural association learning in rats.

Evidence that the NBM is critically involved in complex configural association learning has been provided by Butt and Bowman (2002). This study tested the hypothesis that the cholinergic NBM is necessary configural association learning, but not for simple association learning. Rats with 192 IgG-saporin lesions of the NBM and sham-operated controls were tested in a
transverse patterning task. Rats were trained in phases to progressively solve three different visual discriminations; first Problem 1 (A+ vs. B-), then Problem 2 (B+ vs. C-), then Problem 3 (C+ vs. A-). Problem 1 and Problem 2 could be solved using simple associations, whereas solving Problem 3 required learning configural associations. The results showed that the NBM lesion group solved the simple discriminations but showed impaired configural association learning. As such, the NBM may be critically involved in solving configural associative problems but not problems that can be solved using simple associations.

In a study similar to the aforementioned experiment, Butt, Noble, Rogers, and Rea (2002) trained rats in either a simple discrimination paradigm assessing simple association learning or a negative patterning paradigm assessing configural association learning. Subject groups were comprised of rats with 192 IgG-saporin lesions of the NBM and rats that received a sham-operation. In the simple discrimination task, rats were reinforced for responding to a light but were not reinforced for responding to a tone. In the negative patterning discrimination task, rats were reinforced for responding to either a light or a tone presented alone, but were not reinforced for responding to
both stimuli presented simultaneously. Results showed that simple discrimination learning was not affected, whereas acquisition of negative patterning was impaired due to NBM lesions. Impaired configural association learning may reflect a loss in the ability of rats with NBM lesions to attend to multiple sensory stimuli or to cope with different reward contingencies.
CHAPTER THREE

THESIS EXPERIMENTS

Introduction

Patients with AD experience significant deterioration of the nbM (Doucette et al., 1986; Muir, 1997; Whitehouse, Price, Clark, Coyle, & Delong, 1981). This damage results in a reduction of ACh release in the neocortex (Whitehouse et al., 1982). The loss of this neurotransmitter within the cortex contributes to the profound learning and memory impairments seen in AD (Perry et al., 1978; Samuel et al., 1994). Previous research using the selective immunotoxin 192 IgG-saporin to damage the cholinergic cells of the rat NBM, the brain structure analogous to the nbM in humans, has provided a useful animal model of both the neuropathology and behavioral impairment characteristic of human AD (see Butt & Bowman, 2002; Butt & Hodge, 1997; Butt et al., 2002). As demonstrated in these studies, NBM lesions impair specific learning and memory abilities including the ability to form configural associations. The purpose of the current experiment was to further test the hypothesis that complex or configural association learning is impaired in this rat model of AD. In the present study, the cortically project ing cholinergic neurons of the NBM rats were damaged with 192 IgG-saporin and potential
changes in both simple and configural association learning were subsequently assessed.

The position argued in this thesis is that the NBM is involved in complex or "configural" associative learning, but is not necessary for "simple" association learning. Simple association learning is defined as having a fixed and unambiguous contingency between a stimulus and its associated reinforcement outcome. On the other hand, in configural association learning, animals are required to solve learning problems where the solution depends on learning a relationship between two or more stimulus events in order to predict reinforcement outcomes. Butt and colleagues have previously demonstrated that rats with NBM lesions were impaired in configural but not simple association learning using both the negative patterning (Butt & Hodge, 1997) and the transverse patterning paradigms (Butt & Bowman, 2002).

Butt and colleagues (2002) tested separate groups of rats with 192 IgG-saporin lesions of the NBM and sham-operated controls trained in an operant discrimination paradigm assessing simple association learning or in a negative patterning task assessing configural association learning. These authors showed that NBM lesions did not affect simple discrimination learning.
As expected, however, NBM lesions did impair acquisition of configural association learning in the negative patterning task. In a report by Butt and Bowman (2002), rats with 192 IgG-saporin lesions of the NBM and sham-operated controls were tested in the transverse patterning problem, which provides a test of both simple and configural association learning. Results again showed that the NBM lesion group solved the simple discrimination tasks but showed impaired configural association learning.

The present experiment further tested the hypothesis that NBM damage spares simple association learning but impairs the ability to learn configural associations. Rats with 192 IgG-saporin lesions of the NBM were tested in a T-maze using a series of four instrumental visual discrimination problems, including problems that required either simple or configural solutions. The particular configural task chosen in these experiments was a biconditional visual discrimination problem that explicitly required configural association learning in the late phases of the paradigm.

In Problem 1, rats learned to discriminate between two visual cues (black floor vs. white floor in the arms of a T-maze) in the presence of a common, constant background visual cue (black and white striped maze
walls). Here, rats were food reinforced (+) for selecting the black maze floor (B), regardless of which position (left or right) the floor was in the T-maze. Selection of the white maze floor (W) was not reinforced (-). This contingency applied whenever the maze walls were striped (S).

Next, in Problem 2, a different, distinctive background visual cue (gray maze walls) was introduced and the reinforcement contingencies from Problem 1 were reversed. That is, in the presence of gray walls (G), selecting the white floor (W) was reinforced (+) whereas selecting the black floor (B) was not (-).

In Problem 3, trial blocks from Problems 1 and 2 were combined to form a biconditional discrimination problem that required a configural association solution. In this problem, the reinforcement values of the two floors (B vs. W) were ambiguous because either stimulus may or may not have been reinforced on a given trial within a session during Problem 3. This potential ambiguity was eliminated when animals learned that the reinforcement value of a given floor stimulus (B or W) was determined by which background wall stimulus (G or S) was present on any given trial. Specifically, the black floor was reinforced when the maze walls were striped (SB+), but the white floor was
reinforced when the maze walls were gray (GW+). The incorrect floor stimulus was also determined by the specific background configuration and the floor stimuli (i.e. SW-, GB-). This associative learning problem is known as a biconditional discrimination because two conditions (i.e., floor color and wall color) determine reinforcement contingencies. The biconditional discrimination task required rats to learn specific relationships among cues in order to determine the reinforcement outcomes. Because of this requirement, the biconditional discrimination task provides a critical test of the ability to learn configural associations.

In Problem 3, animals received six consecutive trials of either type (i.e., Problem 1 or 2 trials) followed by six trials of the alternate type. The sequence of Problem 1 and 2 trial blocks on a given day was pseudo-randomized such that on half of the days, Problem 1 was be presented first, and on the other half of the days, Problem 2 was presented first.

In Problem 4, animals were placed in a random interspersed trial block version of the biconditional discrimination task encountered in Problem 3. In Problem 4, reinforcement contingencies were identical to those for Problem 3, but instead of receiving trial blocks of six
consecutive trials of the same type, animals received a pseudo-random, intermixed sequence of Problem 1 and 2 trials. This interspersed trial sequence placed maximum demands on the ability to perform using the biconditional rule (SB+ vs. SW-; GW+ vs. GB-). Pilot testing in our laboratory revealed that presenting animals with long strings of trials of the same type is necessary to train normal rats to solve the biconditional problem; immediately imposing randomly interspersed Problem 1 and 2 trials leads to chance performance or to the systematic biasing towards one problem type at the expense of the other. By initially training rats using large sequential trial blocks of a given problem type, we have successfully been able to advance trained rats to a randomized trial type presentation while maintaining adequate levels of performance in both Problems 1 and 2.

Problem 1 of this experiment was a simple associative task and it was hypothesized that NBM lesions would not interfere with acquisition in this task. Likewise, Problem 2 could be solved using simple association solutions. Thus it was predicted that there would be no impairment in the lesion group performing this problem. Problems 3 and 4, on the other hand, required configural association learning because trials from both Problems 1 and 2 were
interspersed within the same testing session. Here, each stimulus element was ambiguous with respect to its associative value when both problems were intermixed. As such, it was predicted that rats with NBM lesions would disrupt configural association learning in Problems 3 and 4.

Methods

Guidelines for Animal Use

All of the following procedures involving research animals met the requirements set by the Guidelines for Ethical Conduct in the Care and Use of Animals (American Psychological Association, 2005) and the California State University, San Bernardino Animal Care and Use Committee.

Animals

Twenty-two male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing approximately 300 g were housed individually under a reversed 12-hour light cycle (lights on 1800 hours) for a period of two weeks prior to behavioral and surgical procedures. Rats were allowed free access to food and water and were handled for 5 min daily during this time. One week prior to maze habituation, rats were gradually reduced to and then maintained at approximately 85% of their ad libitum

**Apparatus**

Behavioral testing was conducted in two identical T-mazes. Each maze was constructed from ¼ inch thick clear Plexiglas. The stem measured 17.78 cm wide, 19.05 cm high, and 63.5 cm from the top to the bottom of the maze. The arms were 17.78 cm wide, 19.05 cm high, and 77.47 cm from end to end. Small holes were bored into both ends of the right and left goal arms for the delivery of reinforcement (wheat cereal) to the rats for correct responses. Four goal inserts for each maze were constructed. Goal inserts were painted either black or white and stem inserts were painted black-and-white striped or gray. Sliding guillotine doors made of clear Plexiglas separated the start and goal inserts from the rest of the T-maze. The guillotine doors of the start arms were painted gray on one side and painted black and white striped on the other. At the beginning of each trial, the start arm doors were positioned to match the visual pattern inside the start box inserts. The sliding doors of the goal arms were clear.
Surgery

Rats were randomly assigned to either a bilateral 192 IgG-saporin NBM lesion group (n = 10) or to a control group (n = 12). The control group consisted of non-operated rats (n = 5) and sham-operated rats (n = 7) that received Dulbecco’s sterile saline injected into the NBM (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995). Before surgery, rats were anesthetized with sodium pentobarbital (55 mg/kg ip; Butler Co., Dublin, OH) and placed in a stereotaxic frame (Kopf Stereotaxic Instruments, Tujunga, CA), with the incisor bar set at -3.5 mm relative to the interaural line. The scalps of the subjects were incised and the tops of their skulls were exposed. Then, four small holes were drilled so that a cannula could be passed into the brain. The craniotomies were bilaterally located -0.75 mm posterior from bregma, +/- 2.3 mm and +/- 3.3 mm lateral to midline. Animals in the lesion group received bilateral infusions of 192 IgG-saporin (Chemicon, Temecula, CA) at a concentration of 0.4 μg/μl in Dulbecco’s sterile saline solution (Sigma, St. Louis, MO). A volume of 0.2 μl of the 192 IgG-saporin was infused through a 28-gauge cannula at a rate of 0.1 μl/min into each hemisphere. For the medial sites, infusions were made at -7.8 mm and -8.1 mm below the
surface of the skull. Once lowered, the cannula was left in place for 30 S to allow the brain to settle around the cannula prior to infusion. Following each infusion, the cannula was left in place for an additional 3 min to allow diffusion from syringe into the brain. Surgical procedures were identical for rats in the sham-operated control group, with the distinction that these subjects received infusions of saline solution without IgG-saporin. Following surgery, incisions were cleaned and sutured. Subjects were given two weeks to recover before pre-training.

**Behavioral Procedures**

*Pre-training.* Rats were habituated to the maze and pre-exposed to the food reinforcement for 7 days. During this time, the rats were placed in the start arm of a maze with clear start guillotine doors closed and without the stem or goal inserts. In addition, the door to one of goal arms was opened while the other was closed. The location of closed and opened opposing goal arms was reversed after each complete trial. This procedure was performed to prevent side bias and to facilitate the animals' entry into both goal arms on future trials. When the rats entered one of the goal arms from the start of the maze they were trapped and rewarded with food. If an animal did
not enter the goal arm within 3 min it was removed from the maze and the run was scored as a non-trial; not as an error. During this phase of the study, both goal arms were dusted with equal amounts of ground up cereal to help the rats overcome their fear of the goal arms.

**General Discrimination Training Procedures.** Before any animals were placed inside the maze the guillotine door at the start arm was closed. Upon placing a rat in the start arm of the maze, at the beginning of each trial, researchers allowed 10 s to elapse. This ensured that the rats had an opportunity to visually observe the wall stimulus. After the 10 s time period, the guillotine door was raised and the rat was allowed to proceed to and enter one of the goal arms. After the animal made a selection, defined as placing all four paws on the goal insert floor, the researcher slid the guillotine door closed behind the rat. Rats that made an incorrect response were trapped for 30 s with no reinforcement. However, rats that chose correctly were trapped and given two pieces of cereal as reinforcement. Afterward, a 30 s timer was started, if the rat finished consuming the reinforcement before the 30 s it was left in the goal box until the time elapsed. Otherwise, the animals were given up to 180 s to completely finish the reinforcement. Failing this, they
were removed and placed back in their home cages and the reinforcement was discarded. In such eventualities, additional but temporary dietary restrictions were implemented.

In each problem, goal inserts were pseudo-randomly assigned to either the left or right arms of the maze to prevent the animals from developing side biases. As a variation on the Y-maze discussed in Murray and Ridley (1999), rats were trained in the T-maze to solve a series of four visual discrimination problems over four successive phases of training.

Problem 1 Procedures. Problem 1 involved training the rats to learn a simple discrimination between black and white goal inserts in the T-maze. During this stage of training, subjects completed the task as discussed in the general procedure for trials. The black and white striped stem insert was held constant and the rats were rewarded for choosing the black goal insert, but were not reinforced for choosing the white inserts (SB+ vs. SW-). This was a 5 day procedure with 12 trials per day.

Problem 2 Procedures. In Problem 2, subjects completed the task as discussed in the general procedure for trials. However, the goal arm reinforcement contingencies were reversed (the rats were rewarded for
choosing the white insert but not the black insert) and
the stem inserts were changed to gray (GW+ vs. GB-). This
was a 10 day procedure with 12 trials per day.

Problem 3 Procedures. In Problem 3 the aforementioned
tasks, Problem 1 and 2, were presented within the same
testing session each day (SB+ vs. SW-; GW+ vs. GB-). In
this procedure, half of the trials were Problem 1 trials
and the others were Problem 2 trials. The animals
underwent blocks of six consecutive trials of either
striped or gray trials followed by six consecutive trial
runs of the other trial type (gray or striped). This was a
10 day procedure. As before, there were 12 trials per day.

Problem 4 Procedures. Problem 4 provided a random
presentation of equal numbers of Problem 1 and Problem 2
trial types (SB+ vs. SW-; GW+ vs. GB-). Problem 4 was
continued for 10 test days with 12 trials per day.

Statistical Analyses

In order to compare the lesioned and control rats,
the data for Problems 1, 2, 3 and 4 were analyzed
separately using repeated measures analyses of variance
(ANOVA) for mixed designs. On Problems 1 and 2, the total
number of correct responses across days (5 days on Problem
1, 10 days on Problem 2) was analyzed. On Problem 3, data
were grouped into blocks of 4 days, resulting in 10 blocks
(40 days) of data for analyses. The number of correct responses on gray trials, the number of correct responses on striped trials, and the total number of correct responses (gray and striped trials combined) were each analyzed separately for Problem 3. Data from Problem 4 were similarly analyzed, with separate analyses for number of correct responses on gray trials, striped trials, and total correct responses across 10 days of testing. Prior to conducting analyses between the NBM and control groups, analyses were conducted comparing the sham lesion control group (n = 7) to the intact control group (n = 5) to ensure that these groups were not different.

In order to determine potential differences in performance as a function of transfer effects occurring in the transition from one problem to the next, repeated measures ANOVA were performed comparing performance on a given trial type (i.e., Striped or Gray) on the last day of a given problem (i.e., Problems 1 through 4) to performance on the first day of the subsequent problem.

Histology

Upon completion of behavioral testing, rats received a lethal dose of barbiturate followed by a cardiac perfusion with 0.9% saline solution for 5 min. This was followed by 4% phosphate-buffered formalin solution for 30
min followed by 5% phosphate-buffered sucrose solution for 5 min. Perfused brains were extracted and placed in 10% phosphate-buffered sucrose solution for 24 hr before being transferred to a 25% phosphate-buffered sucrose solution for another 24 hr prior to being frozen and sectioned (60 μm). Sections were stained for AChE using the method described by Baxter et al. (1995). AChE-stained sections were examined to verify the placement and extent of the NBM lesions.
CHAPTER FOUR

RESULTS

Introduction

Sham-lesion (n = 5) and non-operated control animals (n = 7) were compared to confirm the absence of group differences prior to combining these groups for analyses. No between-group differences or group by day interactions occurred on Problems 1, 3, or 4. Group differences did, however, occur on Problem 2. The non-operated group acquired Problem 2 at a faster rate compared to the sham-lesion group; ANOVA yielded a between-group main effect of $F(1, 10) = 13.59$, $p < .05$, and a group by day interaction of $F(9, 90) = 20.24$, $p < .001$. However, these effects were attributable to differences occurring only on the first few days of testing in Problem 2, with no differences in performance across the last five days of testing. Because the sham-lesion and non-operated control groups differed only on five days out of a total of sixty-five days of testing, and because these groups did not differ in terms of their asymptotic level of performance on any problem, these groups were combined for all subsequent analyses. This combined group is hereafter referred to simply as the control group.
Problem 1: Simple Association Learning

In Problem 1 (SB+ vs. SW-), both the NBM lesion and control groups made progressively more correct responses across test days (see Figure 1). ANOVA confirmed these observations, yielding a significant within-group main effect of test day of $F(4, 80) = 18.59, p < .001, \eta^2 = .482$. There was no significant between-group main effect of lesion type, nor was there a significant group by day interaction.

Problem 2: Simple Association Learning

In Problem 2 (GW+ vs. GB-), both the NBM lesion and control groups made progressively more correct responses across test days (see Figure 2). ANOVA confirmed these observations, yielding a significant within-group main effect of test day of $F(9, 180) = 41.66, p < .001, \eta^2 = .676$. There was no significant between-group main effect of lesion type, nor was there a significant group by day interaction.

Problem 3: Configural Association Learning

Total Number of Correct Responses. In Problem 3, where both problem types (SB+ vs. SW-; GW+ vs. GB-) were presented in blocks of 6 consecutive trials of a given problem type, both the NBM lesion and control groups improved performance, making progressively more total
Figure 1. Mean (± SEM) number of correct responses in the NBM lesion and control groups on striped trials during Problem 1. Performance in both groups improved across test days (p < .001), with no between-group differences occurring in the acquisition of this simple association discrimination problem.
Figure 2. Mean (± SEM) number of correct responses in the NBM lesion and control groups on gray trials during Problem 2. Performance in both groups improved across test days (p < .001), with no between-group differences occurring in the acquisition of this simple association discrimination problem.
correct responses (on striped and gray trials combined) across blocks (4 days/block) of test days (see Figure 3A). ANOVA confirmed these observations, yielding a significant within-group main effect of test block of $F(9, 180) = 12.40, p < .001, \eta^2 = .383$. There was no significant between-group main effect of lesion type, nor was there a significant group by day interaction.

**Striped Trials.** Both the NBM lesion and control groups made progressively more correct responses on striped trials across 4 day blocks of test days (see Figure 3B). ANOVA confirmed these observations, yielding a significant within-group main effect of test block of $F(9, 180) = 39.26, p < .001, \eta^2 = .663$. There was no main between-group effect of lesion type, nor was there a group by day interaction.

**Gray Trials.** Both the NBM lesion and control groups showed variability in the number of correct responses on gray trials across blocks of test days. Although there was no progressive improvement in either group (see Figure 3C). ANOVA, yielded a significant within-group main effect of test block of $F(9, 180) = 3.90, p < .001$. Visual inspection of the data indicated that this within-group mean difference was due to variability of responses not progressive improvement across days. There was no
significant between-group main effect of lesion type, nor
was there a significant group by day interaction.
A

Total Correct

Correct

0 2 4 6 8 10 12

Block (4 Days)

- CON
- NBM

B

Striped Trials

Correct

0 1 2 3 4 5 6

Block (4 Days)

- CON
- NBM

C

Gray Trials

Correct

0 1 2 3 4 5 6

Block (4 Days)

- CON
- NBM
Figure 3. Mean (± SEM) number of (A) total correct responses, (B) correct striped trial responses, and (C) correct gray trial responses in the NBM lesion and control groups during Problem 3. Both test groups improved performance across blocks of test days under total correct responses and striped trial responses (p < .001). There were no significant mean differences between the groups.
Problem 4: Configural Association Learning

Total Number of Correct Responses. In Problem 4, where both problem types (SB+ vs. SW-; GW+ vs. GB-) were pseudorandomly intermixed, both the NBM lesion and control groups made progressively more total correct responses (on striped and gray trials combined) across test days (see Figure 4A). ANOVA confirmed these observations, yielding a significant within-group main effect of test day of $F(9, 180) = 5.13, p < .001, \eta^2 = .204$. However, the NBM lesion group made significantly fewer total correct responses compared to controls. As a result, there was a between-group main effect of $F(1, 20) = 8.10, p < .01, \eta^2 = .228$. There was no significant group by day interaction.

Striped Trials. Both the NBM lesion and control groups showed variability in their response performance on striped trials across days (see Figure 4B). This variability was reflected by a within-group main effect of test day; $F(9, 180) = 4.34, p < .001, \eta^2 = .178$. Visual inspection of the data indicated that this within-group mean difference was due to variability of responses not progressive improvement across days.

Gray Trials. Both the NBM lesion and control groups made progressively more correct responses on gray trials across blocks of test days (see Figure 4C). ANOVA
confirmed these observations, yielding a significant within-group main effect of test day of F(9, 180) = 2.63, p < .01, η² = .116. Between-group differences in the number of correct responses on gray trials approached significance. There was no significant group by day interaction.

Although no significant between-group differences occurred when analyzing the number of correct responses on gray trials across all ten test days, inspection of the data suggested that the control group was performing better than the NBM lesion group during the latter days of testing. Between-group differences in the number of correct responses on gray trials during the last 5 days of testing were significant; F(1, 20) = 5.53, p < .05, η² = .217.

**Problem-to-Problem Transfer**

**Problem 1 to Problem 2 Transfer.** Both groups showed a significant decrease in the number of correct responses when comparing the last day (Day 5) of Problem 1 (striped trials, M = 11.5) training to the first day of Problem 2 (gray trials, M = 2.59) training. ANOVA confirmed this observation: F(1, 20) = 13.30, p < .001. No between-group or group by day interactions occurred.
Figure 4. Mean (± SEM) number of (A) total correct responses, (B) correct striped trial responses, and (C) correct gray trials responses in the NBM lesion and control groups during Problem 4. Both test groups improved performance across blocks of test days under total correct responses (p < .001) and gray striped trial responses (p < .01). Under total correct responses, the NBM lesion group made fewer total correct responses across days compared to controls (p < .01). On gray trials, the NBM lesion group made fewer correct responses over the last 5 days of testing compared to controls (p < .05).
Problems 1 and 2 to Problem 3 transfer. Both groups showed a significant decrease in the number of correct responses when comparing the last day (Day 5) of striped trial performance in Problem 1 \((M = 11.5)\) to the first day of striped trial performance in Problem 3 \((M = 1.73)\). ANOVA confirmed this observation: \(F(1, 20) = 678.39, p < .001\). No between-group or group by day interactions occurred. Both groups showed a similar decrease in the number of correct responses when comparing the last day (Day 10) of gray trial performance in Problem 2 \((M = 9.82)\) to the first day of gray trial performance in Problem 3 \((M = 3.26)\). ANOVA confirmed this observation; \(F(1, 20) = 105.42, p < .001\). Additionally, the NBM lesion group made fewer correct responses after the transition between problems on gray trials than controls; ANOVA yielded a significant between-group main effect, \(F(1, 20) = 5.21, p < .05\). Student’s t-test analyses showed that gray trial performance did not differ between groups on the last day of Problem 2. The T-test analysis of gray trial performance on Day 1 of Problem 3 showed a tendency toward a between-group differences, although their difference did not reach statistical significance.

Problem 3 to Problem 4 Transfer. Striped trial performance remained high in both groups upon transfer from Problem 3.
to 4, with neither group showing decreased performance when comparing Day 40 of problem 3 to Day 1 of Problem 4. Accordingly, ANOVA showed that there were no significant between-group differences or within-group differences, nor was there a group by day interaction for performance on striped trials during the transition from Problem 3 to Problem 4.

Both groups showed a significant decrease in the number of correct responses on gray trials when comparing the last day (Day 40) of Problem 3 ($M = 3.73$) to the first day of Problem 4 ($M = 1.03$). ANOVA confirmed this observation; $F(1, 20) = 27.18$, $p < .001$. No significant between-group differences or group by day interactions occurred.

**Histology**

Clear differences in AChE-positive staining were observed between the cortices of rats in the NBM lesion and sham lesion control groups (see Figure 5). Compared with controls, brains from the NBM lesion group showed a loss of AChE-positive fibers and cell bodies in the vicinity of the NBM, in addition to the depletion of AChE throughout the cortex. SAP lesions of the NBM did not deplete the hippocampus or amygdala of AChE, suggesting that the cholinergic medial septal projections as well as
the basoamygdaloid cholinergic pathways were not damaged by the 192 IgG-saporin lesion.
Figure 5. Acetylcholinesterase (ACHE) stained sections of the frontal cortex, the nucleus basalis magnocellularis (NBM), and the anterior hippocampus in a representative control (A, C, and E) and NBM lesion rat (B, D, and F). ACHE was depleted throughout the cortex of the NBM lesion brain, confirming the effectiveness of the 192 IgG-saporin lesion.
Rats with selective 192 IgG-saporin lesions of the NBM were tested in a biconditional visual discrimination T-maze task in order to test the hypothesis that the NBM is necessary for configural association learning but not necessary for simple association learning. The biconditional task was comprised of four successive visual discrimination problems. Problem 1 required rats to discriminate between a food-reinforced visual cue (i.e., a black floor in the arm of the T-maze) and a non-reinforced cue (i.e., a white floor) in the presence of a constant background cue, striped walls (SB+ vs. SW-). Problem 2 required rats to solve a task with reversed contingencies of the floor stimuli from Problem 1, but in the presence of a different background cue, gray walls (GW+ vs. GB-). Problems 1 and 2 can be solved using simple associations, as the reinforcement contingencies associated with the floor color in each problem is fixed and unambiguous across testing sessions. Problems 3 and 4, however, critically depend on the ability to form configural associations between the floor colors and the wall color or pattern (SB+ vs. SW-; GW+ vs. GB-).
Problem 1 and 2 trial types were presented within the same session, in blocks of 6 trials for each problem type. Problem 4 consisted of randomly intermixed presentations of Problem 1 and 2 trial types. Problems 3 and 4 both require a configural association solution because the reinforcement value of the individual floor stimuli can only be determined if the animal forms unique representations of each floor color in conjunction with the background cue (i.e., wall color) present on a given trial. Problem 4 poses a relatively greater cognitive challenge than Problem 3 because of the requirement in Problem 4 to frequently shift from the correct strategy for Problem 1 (SB+ vs. SW-) to the strategy for Problem 2 (GW+ vs. GB-) as successive trials are presented. It was hypothesized that NBM lesions would not impair performance on Problems 1 or 2 because the NBM is argued to be unnecessary for the simple association learning required in these problems. In contrast, it was hypothesized that NBM lesions would impair performance in Problems 3 and 4 because the NBM is presumed to be critically involved in the configural association learning required in these tasks.

Results from Problem 1 showed that both groups improved performance and made progressively greater
numbers of successful responses on striped trials across test days. There were no differences in the number of correct responses in the NBM lesion and control groups. Similarly, in Problem 2, both groups made progressively greater numbers of correct responses on gray trials across days, and again groups did not differ in their performance. These results support the hypothesis that NBM lesions would spare simple association learning.

Despite the absence of significant between-group differences, visual inspection of the data from the gray trials on Problem 3 suggest that the control group performed at levels superior to the NBM lesion group, especially during the latter part of training during Problem 3. Between-group differences in the number of correct responses on gray trials on the last 5 blocks of testing approached statistical significance.

Therefore, in contrast to the sparing of simple association learning in Problems 1 and 2, NBM lesions produced an observable impairment in configural association learning in Problem 3. The impairment observed in the NBM lesion group reflected a diminished ability to manage the increase in associative and cognitive demands imposed on both groups when they were advanced from the simple association learning tasks in Problems 1 and 2 to
the configural association learning tasks in Problems 3 and 4.

Analyses of the data showed that when animals were advanced from the simple association learning tasks (Problems 1 and 2) to the configural task in Problem 3, performance on both striped trials (Problem 1) and gray trials (Problem 2) initially suffered in both the NBM lesion and control groups. Both groups had reached a maximum level of performance of greater than 90% correct on striped trials by the end of testing in Problem 1, and greater than 80% correct on gray trials by the end of testing in Problem 2. However, when confronted with the biconditional discrimination in Problem 3, where both gray and striped trials were presented within the same testing session, performance fell in both groups to less than 60% correct on both trial types. Both groups re-attained high levels of performance (greater than 90% correct) on striped trials, but not on gray trials. The control group reached a final level of performance of only 45% correct, and the NBM lesion group reached only 42% correct on gray trials by the end of training in Problem 3. Although neither group performed well on the gray trials during Problem 3, the control animals tended to show greater average levels of performance across the last five blocks
of training compared to the NBM lesion group (although these differences failed to reach statistical significance).

A similar pattern of results occurred in Problem 4, where performance on gray trials again suffered in both the NBM and control groups when they were transferred from Problem 3 to Problem 4. In Problem 4, both striped and gray trials were randomly intermixed (whereas they were presented in blocks of 6 consecutive trials in Problem 3). Although both Problems 3 and 4 are configural association tasks, the intermixing of problem types in Problem 4 requires a comparatively greater degree of cognitive flexibility. In particular, animals were required to switch strategies (i.e., Problem 1 or Problem 2 strategies) each time the trial type (i.e., striped or gray trials) shifted. Here, both groups again continued to perform well on striped trails, with no between-group differences. On gray trials, however, both groups again showed a decrease in the number of correct responses on the first day of Problem 4 training compared to the last day of Problem 3 training. It appears that, when confronted with the added difficulty of Problem 4, both groups resorted to the simple associative learning strategy of solving only one of the two problems (i.e.,
striped trials) at the expense of solving the other problem (i.e., gray trials).

It is interesting to note that both the NBM lesion and control groups tended to choose the black floor (i.e., to use the Problem 1, striped trial solution) when faced with the challenge of the biconditional discrimination in Problems 3 and 4. A potential explanation of the bias towards the black surface lies in rats' innate preference for darkness over light (e.g., Matsuo & Tsuji, 1989; Stratton, Kastin, & Coleman, 2003). Although rats appeared to prefer black, both groups demonstrated excellent acquisition in Problem 2, where rats had to choose the white floor. Consequently, the poor performance observed in both groups on gray trials during Problems 3 and 4 cannot be attributed to an overwhelming preference for black or to an excessive aversion to the light surface of the white floor. Instead, it appears that the cognitive demands of biconditional discrimination, where rats had to act on two competing response strategies, led rats to default to acting on a single response strategy and the strategy animals chose was to select the black floor (i.e., solving Problem 1 striped trials).

The tendency to choose the black floor in the biconditional tasks was gradually overcome in the control
group to a greater extent than in the NBM lesion group. In particular, the control group made a greater number of correct gray trial responses than the NBM lesion group during the latter half of training in Problem 4. Moreover, the control group made significantly more total correct responses (ie., striped and gray trials combined) compared to the NBM lesion group. These data suggest that the control group learned to cope with the increased cognitive demands of switching strategies as a function of the stimulus configuration present on a given trial more effectively than the NBM lesion group when the two trial types (striped and gray) were presented at random within the testing session.

These data suggest that NBM lesions compromise the ability either to learn the configural associations necessary to solve Problems 3 and 4, or compromised the ability to efficiently switch between the two conflicting simple association-based solutions to Problems 1 and 2. It is also possible the NBM lesions impaired both configural association learning and disrupted cognitive flexibility in the biconditional discrimination task. In this regard, it is noteworthy that the NBM lesion group was not impaired in the simple association learning tasks of
Problems 1 and 2; simple association learning does not require cognitive flexibility.

The data from Problem 4 are also consistent with the more recent argument put forth by Butt and colleagues (Cabrera et al., 2006) suggesting NBM involvement in mediating cognitive flexibility. These researchers showed that 192 IgG-saporin lesions of the NBM disrupt serial reversal learning in an operant discrimination task. Although the NBM lesion group was able to solve a simple discrimination between a food-reinforced tone and a non-reinforced light, they did not perform as well as controls when the reinforcement contingencies were reversed. In the current experiment, the NBM lesion group appears to have had a similar problem switching between competing reinforcement contingencies, especially in Problem 4 when contingencies switched several times within a single testing session.

The construct of cognitive flexibility has been described by Butt and colleagues (Cabrera et al., 2006) as including "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." The essential features of this definition include
the ability to adapt to changes in the stimulus
environment or in the reinforcement contingencies in
effect within that environment (Cabrera et al., 2006).

The biconditional discrimination task used in the
current experiment may be especially taxing on cognitive
flexibility. As described above, cognitive flexibility is
necessary when learning situations require animals to
respond to different stimulus configurations in different
ways, and is also necessary to adapt to changing response
rules. Both of these aspects of cognitive flexibility are
required to learn the biconditional discrimination in the
current experiment. In Problems 3 and 4, each floor
stimulus has contradicting reinforcement values (depending
on which wall color or pattern is present), such that rats
must be able to respond flexibly to a given stimulus (e.g.,
the black and white maze floors) depending on the
configuration of wall and floor colors present. This
requirement first appears in Problem 3, where the NBM
lesion group showed moderate impairment. Additionally,
Problem 4 of the biconditional discrimination task requires
an additional form of cognitive flexibility in that animals
must repeatedly switch between response strategies for
Problems 1 and Problem 2. In Problem 3, rats were required
to switch between strategies only once within each testing,
whereas in Problem 4 rats had to switch strategies repeatedly within the testing session. The relatively greater degree of impairment observed in the NBM lesion group in Problem 4, as compared to Problem 3, suggests that the ability to readily switch strategies was impaired in the NBM lesion group.

A possible role for the NBM in mediating cognitive flexibility is further suggested by the finding that cholinergic activation of the prefrontal cortex by NBM neurons in rats is critical to meet the mental demands of frequently switching between response strategies (Ragozzino, Wilcox, Raso, & Kesner, 1999). Similarly, according to Wise, Murray, and Gerfen (1996), the medial prefrontal cortex is involved in reversal learning and in shifting between perceptual dimensions or strategies. These reports, along with the results from the current experiment, strongly suggest that NBM lesions disrupt cognitive flexibility.

Conclusion

The finding of spared simple and impaired configural association learning in rats with NBM lesions is consistent with past research on association learning in rats with 192 IgG-saporin of the NBM (e.g., Butt & Bowman,
In Butt and Bowman (2002), 192 IgG-saporin lesions of the NBM did not affect simple association learning in a visual discrimination problem. However, NBM lesions did impair acquisition of configural association learning in a transverse patterning task. Similarly, Butt and colleagues (2002) found intact simple association learning in rats with 192 IgG-saporin lesions of the NBM in a test of simple operant discrimination, but found impairments in configural association learning in a test of negative patterning. Collectively, these past data and the results from the current experiment (where no group differences were observed in simple association learning in Problems 1 and 2) strongly support the hypothesis that the NBM is critically involved in configural association learning but is not necessary for simple association learning. The greater degree of configural association learning performance impairment observed in the NBM lesion group in Problem 4 compared to Problem 3 further suggests an impairment in cognitive flexibility. These findings are consistent with those reported by Cabrera et al. (2006) where rats with 192 IgG-saporin lesions of the NBM showed impairment in the serial reversal of an operant discrimination.
The present findings support the hypothesis that cholinergic innervation of the neocortex by the NBM is necessary for rats to solve simple but not complex discrimination tasks. This research also confirmed previous findings that NBM modulation of cortical ACh is required for cognitive flexibility. Future research should explore the degree to which the NBM is involved in switching strategies in general. This future research should also investigate whether NBM lesions disrupt performance when animals must switch strategies but do not have to operate on conflicting response rules. For example, a future study might explore the effects on NBM lesions on the ability to perform two separate simple association learning problems involving unique stimuli for each problem, where stimuli do not have ambiguous or conflicting reinforcement histories. When the two discriminations are intermixed within the same testing session, animals would have to switch strategies depending on which discrimination problem was presented, but would not have to operate on configural representations. Such an experiment would help unravel potential NBM lesion impairments in cognitive flexibility in configural association learning.
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