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Previous Spatial Memory Training and Nicotine Administration Alleviates Cognitive Deficits Produced by Medial Frontal Cortex Lesions in Rats.

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ABSTRACT

Previous Spatial Memory Training and Nicotine Administration Alleviates Cognitive Deficits Produced by Medial Frontal Cortex Lesions in Rats

by

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Rats were administered nicotine (0.3 mg/kg) for 11 consecutive days before and after an electrolytic medial frontal cortex lesion. Behavioral testing was arranged so that the rats were tested on the RAM 1 day after drug administration followed by behavioral testing on the MWT 19 days after drug treatment, or tested on the MWT 1 day after drug administration followed by testing on the RAM4 days after drug treatment. Results of MWT testing showed that regardless of the drug/behavioral testing interval, lesioned rats given nicotine demonstrated enhancement relative to saline-treated animals. Results of RAM testing showed that nicotine improved performance in non-lesioned rats compared to non-lesioned rats given saline. Four days after drug administration, nicotine improved performance in lesioned rats to levels of non-lesioned rats regardless of drug treatment. A second experiment was implemented to determine if the previous training on the MWT affected performance on the RAM.

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CHAPTER 1

INTRODUCTION

The primary focus of this project was to analyze the behavioral effects of the acetylcholinergic agonist nicotine on cognitive function in rats with medial frontal cortex (MFC) lesions as well as the nicotine's time-related effects on cognitive performance. It has been well-established that the MFC is involved in higher cognitive processes such as decision making and problem solving. Damage to this area has been shown to produce cognitive deficits on a various cognitive tasks (Brown, Gonzales, & Kolb, 2000; Bussey, Everitt, & Robbins, 1997; Mair, Burk, & Porter, 1998). The acetylcholinergic neurotransmitter system has been shown to be important in cognitive function (Abdulla et al., 1996; Levin & Simon, 1997), and nicotine acts to facilitate ACh function in a number of ways. Nicotine acts as an acetylcholinergic agonist by substituting for ACh at the nicotinic receptor site. It also increases ACh release from the presynaptic terminal and upregulates nicotinic receptors (Abdulla et al.; Belluardo, Mudo, Blum, & Fuxe, 2000).

Brain Injury: An Important Health Issue

A major health issue in the U. S. is traumatic brain injury (Dixon, Clifton, Lighthall, Yaghmai, & Hayes, 1991; Hamm, White-Gbadebo, Lyeth, Jenkins, & Hayes, 1992; Lighthall, Dixon, & Anderson, 1989). The brain can be damaged in many different ways such as trauma, disease, or stroke. The high incidence of brain trauma demands finding the means to repair damaged tissue and, thus, to improve behavioral function. According to Kolb (1995), there are at least three possible outcomes after brain injury. One result is compensation that occurs due to an adaptation to the loss. Another result could be partial restitution of the original behavior. Third is the possibility of complete restitution of the original behavior. However, another

possible outcome is no recovery of the original behavior. Nicotine may become a treatment for minor brain insults. The recovery of the behavior after the administration of nicotine would be defined as aiding in compensation due to brain injury.

Nicotine, an ACh Agonist

Nicotine is a natural drug obtained from tobacco leaves that stimulates acetylcholinergic nicotinic receptors (nAChRs). Nicotine receptors are present in the peripheral nervous system as well as in the brain in the cerebral cortex, striatum, and hippocampus, all of which are critical for cognitive function (Levin, 1992). Nicotine can stimulate or depress neural activity as well as induce the release of numerous neurotransmitters in addition to acetylcholine such as dopamine, norepinephrine, serotonin, and glutamate (Dani & Heinemann, 1996).

The results of several studies have shown that nicotine can enhance learning and memory (Abdulla et al.; Brown et al., 2000; 1996; Decker, Majchrzah, & Anderson, 1991; Levin, Briggs, Christopher, & Rose, 1992). Other studies have shown that several drugs in addition to nicotine that enhance ACh function produce enhancement of memory and learning (Girod & Role, 2001; Levin, Christopher, & Briggs, 1997; Levin & Simon, 1997). Therefore, a viable hypothesis is that nicotine enhances learning and memory by enhancing ACh function (Jones, Sahakian, Levy, Warburton, & Gray, 1992; Levin, 1992; Rinne, Myllykyla, Lonnberg, & Marjamaki, 1991).

Nicotine may also act to improve cognitive performance through its effects on neurotrophic factors. Neurotrophic factors are chemical messengers in the brain that act to help neurons grow as well as maintain synaptic connections. Nicotine has been shown to increase both nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Fumagalli, Santero, Gennarelli, Giorgio, & Riva, 2001; Kenny, File, & Rattray, 2001). Thus, nicotine's

effects on neurotrophic factors may be the basis of its possible neuroprotective benefits against neurodegenerative diseases and different types of neurotoxicity as well as its cognitive enhancing effects.

Nicotine has been shown to alleviate cognitive impairments produced by medial frontal cortex lesions. In a study by Brown et al. (2000), rats were treated with nicotine or vehicle 11 consecutive days before, after, or before and after a medial frontal cortex lesion. Behavioral testing began one day after the last injection. The results showed lesioned rats that received nicotine treatment had better performance during acquisition on the Morris water maze task than lesioned rats that received the vehicle treatment. However, they did not perform as well as rats given sham lesions. Rats that received nicotine treatment did perform to sham levels on the probe trial given at the end of training.

The Medial Frontal Cortex

Research has shown that damage to the medial frontal cortex produces cognitive deficits. For example, damage to the frontal cortex produces slowed tendencies to respond in delayed matching-to-sample and delayed non-matching-to-sample tasks (Mair et al., 1998). Approach tendencies were also slowed by medial frontal cortex lesions; however, the subjects were still able to discriminate between stimuli (Bussey et al., 1997).

It has been suggested that the medial frontal cortex plays a role in reward-based choice behavior. In a study by Walton, Bannerman, Alterescu, and Rushworth (2003), rats were trained on a T-maze cost-benefit task. Prior to lesions, all rats chose the high cost-high reward barrier for the larger reward. After MFC lesions, rats consistently chose the low cost-low reward barrier (Walton et al., 2003). This supports the idea that the MFC is involved in effort-based choice behavior. However, in an earlier study by Walton, Bannerman, & Rushworth (2002), when the

cost was reduced or the benefit was increased, the high-cost choice was restored. It has also been shown that the MFC plays a role in the deficits produced by normal aging (Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004).

The deficits that result from these lesions can be improved with the application of nicotine and nicotine agonists. Using the 8-arm radial maze, nicotine has been shown to significantly increase choice accuracy performance in brain-lesioned rats. Nicotine has been shown to reverse the impairment on RAM working memory that was a result of lesions to the medial frontal cortex (Levin & Rose, 1995). Hodges, Peters, Gray, and Hunter (1999) have also shown that nicotine reduced working memory errors resulting from by lesions of the basal forebrain. The basal forebrain is an area that contains cholinergic cells that project into the neocortex. It is hypothesized that the basal forebrain plays a role in maintaining an appropriate level of activity in the forebrain structures so that information can be processed. It plays an important role in attention, as well.

The Effects of Nicotine on Cognition

The effects of nicotine on cognitive performance have been observed in behavioral tests conducted with rats, monkeys, and humans (Levin & Simon, 1997). Most of these effects have been shown to be blocked by pretreatment with the nicotinic antagonists such as mecamylamine (Narahashi et al., 2000). Levin and Torry (1995) have shown that the significant improvements in choice accuracy in young rats that received chronic nicotine treatments were reversed by the administration of mecamylamine. It has been shown that mecamylamine blocks cognitive enhancement produced by nicotine on a variety of cognitive tests (Levin, 1992), indicating that the nicotinic receptor appears to be playing an important role in cognition.

The Nicotinic Receptor

The nicotinic ACh receptor (nAChR) is an ionotropic receptor to which the endogenous neurotransmitter ACh and the drug nicotine bind to regulate the flow of ions through the receptor pore. There are several subunits of the nicotinic receptor. Studies have shown that most of the cognitive effects of nicotine are mediated through the actions of the nicotinic receptor α4β2 and α7 subunits (Alturi et al., 2001). However, because nicotine acetylcholine receptors (nAChRs) of varying subtypes are widely distributed in the brain, any particular neuron can have a variety of nAChRs in any brain region. The subunit composition of the nAChRs and other local factors such as the interaction with other neurotransmitters affect different aspects of the response. Some of these are the speed of activation, ion current, rates of desensitization, and recovery from desensitization, pharmacology, and regulatory controls of the agonist response (Dani, Ji, & Zhou, 2001).

Nicotine, Neuroprotection, and Neurotrophic Factors

Nicotine has also been shown to increase the expression of neurotrophins and neurotrophic factors. Neurotrophic factors are a class of compounds that act to support growth and differentiation in developing neurons and may act to maintain neuronal functioning after a traumatic insult. Research indicates acute intermittent nicotine treatment leads to a substantial upregulation of neurotrophins in the cerebral cortex, hippocampal formation, the striatum and the ventral midbrain (Abdulla et al., 1996; Belluardo et al., 2000; French, Humby, Horner, Sofroniew, & Rattray, 1999). The increase in neurotrophins is thought to be mediated by increasing nAChR activity, specifically, the α7 nAChR subunit (Jonnala & Buccafusco, 2001). Upregulation of neurotrophic factors and neurotrophins could explain some of the compensatory effects nicotine exhibits in animals with learning and memory deficits.

The findings on neurotrophic factors are relevant to developing therapeutic agents acting on nAChRs, as these may be able to delay the onset and progression of chronic age related brain pathologies. Enhancing ACh activity neurotrophic factors may also play a role in improving cognitive abilities. These effects could help identify the molecular mechanisms of neural degeneration related to nAChR deficits occurring in normal aging or neurodegenerative diseases. Certain neurotrophins have also been shown to enhance ACh activity in the hippocampus (French et al., 1999). Belluardo et al. (2000) have shown that nicotine agonists may be therapeutic for Alzheimer's and Parkinson's Diseases. Alzheimer's patients given subcutaneous injections of nicotine or nicotine patches have shown improvement on cognitive and motor tasks (Jones et al., 1992; Rusted, Newhouse, & Levin, 2000). Nicotine and prototype nicotine agonists have also been shown to prevent experimental Parkinsonism in rodents (Maggio et al., 1998).

Nicotine and its Effects on Neurotransmission

Nicotine is a drug that acts on many neurotransmitter systems within the brain besides the ACh system. Research has shown that nicotine increases the presynaptic release of dopamine, norepinephrine, serotonin and glutamate through binding to nicotinic receptors located on the presynaptic terminal button. These effects on different neurotransmitters may be important in explaining nicotine's effects on memory. For example, studies have shown both the dopaminergic and glutaminergic systems are involved in the consolidation of memory for inhibitory avoidance learning (Souza et al., 2000). Glutamate appears to play a role in learning because of its excitatory characteristics and its effects on N-methyl-D-aspartate (NMDA) receptors, which have been shown to play a critical role in long-term potentiation (LTP), a possible cellular mechanism of learning and memory. Dopamine is also important with regards to learning because it is active in maintaining normal motor behavior and it has direct effects on

cognition. Dopamine has been shown to have important roles in the consolidation of memory in several areas of the brain.

The Radial-Arm Maze

Several behavioral tasks have been used to test the effects of nicotine on memory. One of the most common behavioral tests used to analyze nicotine's effects on cognitive performance is the 8-arm radial maze (see Figure 1). The 8-arm radial maze consists of an elevated central platform with eight arms radiating out from the center. Each arm is bordered by Plexiglas and contains a food well at the end. By baiting specific arms in the maze, working memory errors, reference memory errors, and response latency can be determined. A working memory error is defined as a return visit to an arm that was previously baited. A reference memory error is defined as a visit to an arm that was never baited. Because that the RAM is a spatial memory task, the rat must use extra-maze cues such as posters on the wall and other objects in the room to associate to food location (Levin & Simon, 1997).

In a study by Kim and Levin (1996), choice accuracy and response latency were measured after nicotine treatment. Response latency is the elapsed time it takes the rat to complete the maze. The nicotine antagonist mecamylamine produced a deficit in choice accuracy and response latency in the maze. The added administration of the muscarinic cholinergic antagonist scopolamine also produced significant deficits in choice accuracy. The study's results showed that both muscarinic and nicotinic ACh antagonists significantly increased working memory errors in performance on the RAM. Therefore, it appears that muscarinic receptors as well as nicotinic receptors affect spatial memory. This demonstrates the importance of these two receptor subtypes in RAM performance.

Nicotine's Effects on Radial-Arm Maze Performance

Research has shown that nicotine's enhancing effects appear to be specific to working memory but do not affect reference memory (Levin, Kaplan, et al., 1997). In a different study (Levin, Bettegowda, Weaver, & Christopher, 1998), the effects of nicotine on working memory errors and reference memory errors were investigated. In this study, 12 arms were baited in the 16-arm radial maze. Before each trial, nicotine and the glutamatergic NMDA antagonist, dizocilpine, were administered. Results showed that both nicotine and dizocilpine decreased working memory *and* reference memory errors. It has also been shown that coadministration of nicotine and MK-801, an NMDA antagonist, caused significant deficits in both working and reference memory errors. The coadministration blocked nicotine-induced improvements in working and reference memory. Glutamate is a neurotransmitter that causes excitatory responses in the brain. This suggests that glutamate plays a role in learning and memory, which is affected by the administration of nicotine (Levin et al., 1998).

The Morris Water Maze

Although the MWT (Morris, 1980) is referred to as a maze, it is actually an open-field spatial memory apparatus in which an animal must locate a platform "hidden" below the surface of the water. Thus, the word maze is a bit misleading, as this task is not a maze in the traditional sense. The "maze" consists of a round pool painted white; the water in the apparatus is made opaque with the addition of powdered milk to the water. A platform is located 1 cm below the water level. The rat is released into the pool from one of four different release points. Response latency is the time it takes the rat to reach the platform on each training trial, referred to as acquisition latency. Rats presumable use the visual cues in the environment in order to escape from the water (Brown, Gonzalez, Whishaw, & Kolb, 2001; Brown et al., 2000; Decker,

Majchrzah, & Arneric, 1993; Morris). In a probe trial given at the end of training, the platform is removed from the water, and the animal's swim patterns are recorded by a video camera mounted above the pool. Although several different dependent measures have been used to analyze the probe trial, the mean zone difference (MZD) score was used in this study. The MZD score is a ratio of the number of visits made to the former platform location versus three other zones of identical size in the pool. Treatments that result in a rat's inability to find the platform are assumed to demonstrate malfunction in the neural systems involved in spatial learning (Brown et al., 2001; Brown et al., 2000; Decker et al.; Gonzalez, Miranda, Gutierrez, Ormsby, & Bermudez-Rattoni, 2000).

Performance on the Morris water maze can be affected by many variables such as the size of the pool, testing procedure, and the characteristics of the rats (D'Hooge & De Devn, 2001). One variable that seems to affect Morris water maze performance is pre-training experience. Studies have shown that prior experience on spatial tasks, not necessarily the Morris water task, can improve Morris water maze performance (Caplan & Schooler, 1990; McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001; Swick, 1998). Rats with lesions of the nucleus basalis magnocellularis showed impaired acquisition on the Morris water maze when the exposure to the maze was only during testing. However, if the rats were given pre-training experience on a complete training trial or a partial training trial, lesioned rats performed to sham levels (Nieto-Escamez, Sanchez-Santed, & de Bruin, 2004).

Nicotine's Effects on Morris Water Task Performance

Decker et al. (1993) found lobeline, a nicotine agonist, has been shown to reduce acquisition latency in rats with septal lesions performing on the Morris water maze. In a study by Decker, Majchrzah, and Anderson, (1991), spatial discrimination in the Morris water maze

was defined as one of two visible platforms being stable for escape. Nicotine increased the number of correct platform choices and decreased acquisition latency.

Fimbria-fornix lesions have been shown to produce a deficit on the Morris water task (Brown et al., 2001; Hannesson & Skelton, 1998; Kleschevnikov, Sinden, & Marchbanks, 1994), as the fimbria-fornix is a major brain pathway that synapses in the hippocampus. Brown et al. (2001) found that nicotine administration significantly improved acquisition in animals with a fimbria-fornix lesion. Similar to the study in MFC-lesioned rats, Fimbria-fornix lesioned rats were given nicotine for 11 consecutive days before, after, or before and after a fimbria-fornix lesion. All rats that were treated with nicotine performed significantly better than controls administered saline on the Morris water task. The rats that were treated with nicotine before and after the lesion performed to sham levels on acquisition and the probe trial. All compensatory effects of nicotine were blocked by the co-administration of the antagonist mecamylamine.

The Current Study: Statement of the Problem

There is no known approved therapeutic treatment that has been shown to alleviate memory deficits produced by brain injury. Nicotine may have therapeutic potential for brain-injured patients because of its agonist action on the ACh system as well as its effects on neurotrophic factors, possibly producing neuroprotection against brain injury. Although nicotine therapy is applicable to humans for various neurodegenerative diseases, most nicotinic research on humans has limited external validity for several reasons. First, the population of nicotine users is self-selected. People decide whether or not to start using tobacco. Second, the acute effects of nicotine are harder to determine due to the chronic use of cigarettes or tobacco. To test for acute effects an individual would have to be taken off of nicotine for a long period of time, which would then cause withdrawal problems. Third, experiments with humans lack the proper

control in the administration of the drug (Levin, 1992). Most people are going to smoke when they feel it is necessary. This leaves the administration schedule randomly open to the individual. Also, there are evident health risks that result from smoking.

Still, nicotine research can be properly conducted on humans. The nicotine patch has been shown to reduce some motor and cognitive deficits that result from health problems such as Parkinson's Alzheimer's disease (Maggio et al., 1997; White & Levin, 1998) . Also, related drugs may be specific to cognition and avoid the possible negative effects of nicotine on the cardiovascular system as well as on brain development (Levin & Simon, 1997).

The current study was designed to analyze whether nicotine will alleviate cognitive deficits in rats given a medial frontal cortex (MFC) lesion. In addition, we also analyzed whether previous experience may aid in recovery. According to Hodges (1996), apparatus, sensory cues, task requirements, and motivation are all different for the RAM and the MWT. However, the mazes use a variety of processes that affect spatial learning. Finally, we are interested in whether nicotine produces long-term effects on recovery of function.

Studies have shown that priming and repetition of behavioral tasks alone can lead to an enhancement of performance (Caplan & Schooler, 1990; McIlwain et al., 2001; Swick, 1998). Results have shown that animals with a lesion of the nucleus basalis performed to control levels on the Morris water maze when they were pre-trained in an allocentric navigation task. The lesions did not produce deficits in acquisition of a new platform, nor in the animal's ability to remember where the new platform was located on successive trials (Nieto-Escamez et al., 2004). Santin et al. (2000) performed a series of studies on the effects of alcohol consumption on reference memory and working memory in rats. They found that animals that were submitted to several trials showed an improvement in working memory. This could be because performance

in previous trials created a "proactive interference" and improved performance on subsequent trials. Based on the results of Experiment 1, a second experiment was designed to determine if previous training on the MWT affected subsequent performance on the RAM.

Basic research with nicotine can lead to the understanding of the neurobiology of normal memory function. It can also help with the development of therapeutic agents for memory dysfunction, especially if the disease is degenerating the acetylcholine system (Rusted et al., 2000). The understanding of the involvement of nicotinic systems with cognitive function is crucial in the development of nicotinic therapies for cognitive impairments such as Alzheimer's disease.

Nicotine has been shown repeatedly to enhance memory and learning in normal animals through chronic and acute administration (Jones et al., 1992; Levin, Christopher, et al., 1997; Levin et al., 1992; Levin & Rose, 1995; Welz, Alessandri, Oettinger, & Battig, 1988). It has also been shown to alleviate deficits caused by lesions and naturally occurring neurodegenerative disease. The RAM and the MWT provide examples of spatial tasks but differ in terms of task demands. The MWT is an aversive spatial memory task. The RAM is an appetitive spatial memory task. The MWT requires animals to swim to a location that is placed in an open field, whereas on the RAM, animals must find food caches in a land task. If the platform position does not change during training on the MWT, this has been hypothesized to be a reference memory task in that the animal must locate a stable platform location. The RAM measures relatively stable asymptotic reference memory and working memory performance. In the initial experiment, Experiment 1, results showed nicotine enhancement on performance. However, nicotine improved behavioral performance on the RAM in lesioned rats only in animals that were first tested on the MWT. This result suggests that previous training may augment nicotine

induced behavioral recovery. Based on the results of Experiment 1, Experiment 2 was designed to test for any pre-training or priming effects that occurred in Experiment 1.

Experiment 1

Assignment to Groups. There were eight treatment groups: saline/sham/MWT-RAM, saline/MFC/MWT-RAM, nicotine/sham/MWT-RAM, nicotine/MFC/MWT-RAM, saline/sham/RAM-MWT, saline/MFC/RAM-MWT, nicotine/sham/RAM-MWT, and nicotine/MFC/RAM-MWT.

Research Design. Rats were administered nicotine tartarate (0.3mg/kg free base) or saline (0.9% concentration) for 11 consecutive days before and 11 consecutive days after an MFC lesion. Animals were tested either first on the MWT followed by the eight-arm RAM, or tested first on the eight-arm RAM followed by the MWT. In rats tested on the MWT as their first behavioral task, behavioral training on the MWT began one day after cessation of nicotine administration. Behavioral training on the RAM began four days after nicotine administration had ceased, although habituation began one day after nicotine administration had ceased. In rats tested on the RAM as their first behavioral task, behavioral training on the RAM began one day after nicotine administration had ceased. Behavioral training on the MWT began 19 days after cessation of nicotine administration. The research design for Experiment 1 is presented in Table 1.

Table 1

Experiment 1: Research Design

Pre-treatment	Surgery	Post-treatment	1st task	2nd task
Saline (n = 9)	Sham	Saline	MWT	RAM
Saline (n = 6)	Lesion	Saline	MWT	RAM
Nicotine (n = 7)	Sham	Nicotine	MWT	RAM
Nicotine (n = 7)	Lesion	Nicotine	MWT	RAM
Saline (n = 5)	Sham	Saline	RAM	MWT
Saline (n = 6)	Lesion	Saline	RAM	MWT
Nicotine (n = 8)	Sham	Nicotine	RAM	MWT
Nicotine (n = 9)	Lesion	Nicotine	RAM	MWT

Hypothesis 1. Normal rats that receive nicotine treatment will perform significantly better than normal rats that receive saline.

Hypothesis 2. Rats that do not receive a MFC lesion will perform significantly better than rats that receive a MFC lesion.

Hypothesis 3. Rats that receive nicotine treatment and a MFC lesion will perform significantly better than rats that receive saline treatment and a MFC.

Hypothesis 4. Rats that do not receive MWT training prior to RAM will perform significantly worse than rats that receive MWT training prior to RAM.

Experiment 2

Assignment to Groups. There were eight treatment groups: saline/sham/MWT Day 19, saline/MFC/MWT Day 19, nicotine/sham/MWT Day 19, nicotine/MFC/MWT Day 19, saline/sham/RAM Day 4, saline/MFC/RAM Day 4, nicotine/sham/RAM Day 4, and nicotine/MFC/RAM Day 4.

Research Design. Rats were administered nicotine tartarate (0.3mg/kg free base) or saline (0.9% concentration) for 11 consecutive days before and 11 consecutive days after an MFC lesion. Animals were tested on either the MWT or the eight-arm RAM only. Behavioral testing on the MWT began 19 days after the cessation of nicotine administration. Behavioral testing on the RAM began 4 days after nicotine administration has ceased. The research design for Experiment 2 is presented in Table 2.

Table 2

Experiment 2: Research Design

Pre-treatment	Surgery	Post-treatment	Behavioral Task
Saline (n = 7)	Sham	Saline	MWT Day 19
Saline (n = 5)	Lesion	Saline	MWT Day 19
Nicotine (n = 6)	Sham	Nicotine	MWT Day 19
Nicotine (n = 5)	Lesion	Nicotine	MWT Day 19
Saline (n = 4)	Sham	Saline	RAM Day 4
Saline (n = 6)	Lesion	Saline	RAM Day 4
Nicotine (n = 2)	Sham	Nicotine	RAM Day 4
Nicotine (n = 4)	Lesion	Nicotine	RAM Day 4

Hypothesis 1. Rats that receive nicotine treatment will perform significantly better than rats that receive saline.

Hypothesis 2. Rats that do not receive a MFC lesion will perform significantly better than rats that receive a MFC lesion.

Hypothesis 3. Rats that receive nicotine treatment and a MFC lesion will perform significantly better than rats that receive saline treatment and a MFC.

CHAPTER 2

METHODS

Experiment 1

Rationale

Studies have shown that the administration of nicotine improves performance on behavioral tasks in non-lesioned animals (Jones et al., 1992; Levin, Kaplan, et al., 1997; Levin et al., 1992; Levin & Rose, 1995; Welz, Alessandri, Oettinger, & Battig, 1988). Studies have also shown that nicotine administration alleviates deficits produces by a medial frontal cortex lesion (Hodges, Peters, Gray, & Hunter, 1999; Kolb & Whishaw, 1994; Levin & Rose, 1995;). The current experiment was designed to analyze whether nicotine will alleviate cognitive deficits in rats given a medial frontal cortex (MFC) lesion as well as whether nicotine produces long-term effects on recovery of function.

Subjects

Fifty-seven male Long-Evans hooded rats were housed 1-2 to a cage in a climate-controlled vivarium with a 12h on/12h off light/dark cycle. All rats weighed 250 – 300gms at the beginning of the drug treatment. Food and water were available at ad libitum prior to surgery and during MWT testing. During RAM testing, the rats were weighed and given food to maintain 75% of their original body weight.

Drugs

Nicotine tartarate (0.3 mg/kg free base) was dissolved in 0.9% NaCl (saline) and injected in a volume of 1 mg/kg. All vehicle injections contained 0.9% NaCl (saline).

Apparatus

Radial-Arm Maze Apparatus. The radial-arm maze (RAM) was elevated 94cm above the floor with each arm and central platform supported by wooden planks. The entire maze was constructed of wood and painted white with eight arms radiating from a central platform. The arms were 76cm in length and 8.9cm in width. The central platform was an octagon 25cm in diameter (see Figure 1). The central platform and all of the arms were bordered by Plexiglas. At the end of each arm was a food well depressed 1cm below the surface of the maze. Extra-maze cues included the experimenter, several pieces of office furniture, stored operant boxes, and various types of lab equipment.

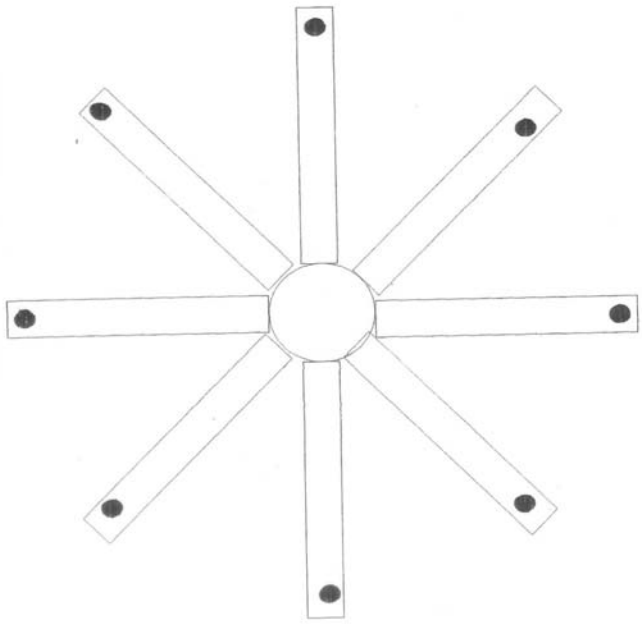


Figure 1. Radial Arm Maze

Morris Water Task Apparatus. The Morris Water Task (MWT) was a round tank approximately 145 cm in diameter and 58.4 cm in height. The inside of the pool was painted

white and filled to a height approximately 40 cm with water that was 19-21°C. A round, white platform made of white PVC pipe 12.7 cm in diameter was placed centrally in the southwest quadrant and remained there throughout training. Its top surface was approximately 1cm below the surface on the water. Powdered milk was dissolved in the water so that the animal was presumably unable to see the platform. Extra-maze cues included the experimenter, posters on the wall, and a video monitor. The context in which the MWT was performed was different from that of the RAM.

Procedure

Groups and Drug Administration. Rats were divided into four treatment groups. Rats were given one injection in the morning and one injection in the afternoon and were administered the nicotine (NIC) or saline (SAL) according to group assignment: NIC-SHAM, SAL-SHAM, NIC-LES, or SAL-LES. All rats received two subcutaneous (sc) injections of either nicotine or saline for 11 consecutive days before and 11 consecutive days after a medial frontal cortex lesion. Rats received the same drug treatment in the morning and afternoon as well as before and after surgery. There were no injections given the day after surgery due to surgical stress, and all injections ceased 24h before behavioral testing began.

Rats were tested in one of two conditions: MWT training followed by RAM training (MWT-RAM) or RAM training followed by MWT training (RAM-MWT). In the MWT-RAM condition, the MWT was used as the first behavioral task, and animals began behavioral testing on the RAM 4 days after nicotine administration had ceased, or 1 day after the completion of the MWT. In the RAM-MWT condition, the RAM was used as the first behavioral task, and animals began behavioral training on the MWT 19 days after nicotine administration had ceased, or 1 day after RAM testing was completed (see Table 1).

RAM Behavioral Testing. Rats underwent 4 consecutive days of habituation in the RAM. For habituation, all of the arms were baited with three Noyes sucrose pellets (Lancaster, NH) down each arm. The habituation trial was 5 minutes in length, and the number of arms visited was recorded. Habituation trials were terminated when either the animal visited all eight arms or 5 minutes had elapsed. Beginning the following day, rats began cognitive testing on the RAM that once daily for 12 consecutive days. Four arms were randomly selected for each rat and baited with one sucrose pellet. The same four arms were baited on each trial. Rats were released from the central platform and arm visits were recorded. Two types of errors were recorded. **Working memory errors** were revisits to arms that had been previously baited on the same testing trial. **Reference memory errors** were visits to any of the four arms that had never been baited during the testing trials. The testing was considered complete when all four pellets were consumed, all eight arms had been visited, or 5 minutes had elapsed.

MWT Behavioral Testing. Rats were given eight trails per day for 3 consecutive days, for a total of 24 training trials. On each training trials, the rat was released from one of four different release points (N,W,S,E) and allowed 60s to locate the platform. Trial blocks consisted of four training trial each, and a total of six trial blocks were administered. The time to locate the platform was recorded as acquisition latency. If the rat did not locate the platform on a particular trial, it was place there by the experimenter. All animals spent the last 10s of each trail on the platform, regardless of whether the animal reached the platform on its own or was placed there by the experimenter. The inter-trial interval was approximately 4-5 minutes. Immediately after the last training trial and on the third day of testing, rats were given a 60s probe trial. Animals were released from only the N release point. This trial was recorded by a video camera mounted above the pool and swim patterns were later analyzed on videotape. The dependent

variable measure used on the probe trial was the mean zone difference score, which is the ratio of the number of visits made to the former platform location versus three other zones in the pool. The formula for the mean zone difference score is as follows:

$$\frac{(\text{Target} - W) + (\text{Target} - X) + (\text{Target-Y})}{3}$$

Lesion Procedure. For surgery, rats were anesthetized with ketamine (100mg/kg) and xylazene (50mg/kgml) and placed into a stereotaxic apparatus. A constant flow of isofluorane and oxygen mixture was administered to the rats from an anesthesia machine (JD Medical, Phoenix, AZ) at a rate that could be manipulated by the experimenter during surgery through tubes attached to the nose bar. A Kopf stereotaxic mask was attached to the anesthesia machine and delivered the isoflurane/O2 mixture to the nose and mouth of the animal. Once the animal was anesthetized and placed into the ear bars of the stereotaxic apparatus, the scalp was shaved and a midline incision was made. Two drill holes were made on the surface of the skull with a dental drill. The drill holes were made at the following locations relative to bregma: 1.3mm anterior, 1.5mm lateral and 3.6mm ventral, 1.5mm anterior, .05mm lateral, and 3.3mm ventral. An insulated electrode was placed at each of these coordinates and a cathodal current was passed for 40s at each site to create the lesion. Rats that received sham surgery received an incision and anesthesia.

Experiment 2

Rationale

The results of Experiment 1 indicated a priming effect from the Morris water task to the radial arm maze. Studies have shown that priming and repetition of behavioral tasks alone can lead to an enhancement of performance (Caplan & Schooler, 1990; McIlwain et al., 2001; Swick,

1998). This study was designed to compare performance on the behavioral tasks at the same time intervals used in the first experiments but without the first behavioral task. The rats were tested on either the MWT beginning on Day 19 after drug administration had ceased or the RAM beginning on Day 4 after the cessation of drug administration.

Subjects

Thirty-seven male Long-Evans hooded rats were housed 1-2 to a cage in a climate-controlled vivarium with a 12h on/12h off light/dark cycle. All rats weighed 250-300 at the beginning of the drug treatment. Food and water were available at ad libitum prior to surgery and during MWT testing. During RAM testing, the rats were weighed and given food to maintain 75% of their original body weight.

Drug

Drug treatment was identical to Experiment 1.

Apparatus

Radial-Arm Maze Apparatus. RAM apparatus was identical to Experiment 1.

Morris Water Task Apparatus. MWT apparatus was identical to Experiment 1.

Procedure

Groups and Drug Administration. Rats were divided into four treatment groups. Rats were given one injection in the morning and one injection in the afternoon and were administered the nicotine (NIC) or saline (SAL) according to group assignment: NIC-SHAM, SAL-SHAM, NIC-LES, or SAL-LES. All rats received two subcutaneous (sc) injections of either nicotine or saline for 11 consecutive days before and 11 consecutive days after a medial frontal cortex lesion. Rats received the same drug treatment in the morning and afternoon as well as before and after surgery. There were no injections given the day after surgery due to

surgical stress, and all injections ceased 24h before behavioral testing began.

Rats were tested in one of two conditions. Rats were then tested on either the MWT or the RAM. Behavioral testing on the MWT began either 1 or 19 days after the cessation of nicotine administration. Behavioral testing on the RAM began 1 or 4 days after nicotine administration had ceased (see Table 2).

RAM Behavioral Testing. RAM behavioral testing was identical to Experiment 1.

MWT Behavioral Testing. MWT behavioral testing was identical to Experiment 1.

Lesion Procedure. The lesion procedure was identical to Experiment 1.

CHAPTER 3

RESULTS

Experiment 1

Histological Results for Experiment 1 and 2.

A picture of a representative medial frontal cortex lesion is presented in Figure 13. All lesioned animals had significant but not complete damage to the Cg1, Cg2, and Cg3 areas of the frontal cortex. There was no damage to the striatum in any case.

MWT-RAM Behavioral Results

MWT analysis. A two-way mixed ANOVA for acquisition latency revealed a significant main effect of group $F(3,23) = 8.45$, $p<.01$, trial block $F(3,5) = 45.27$, $p<.01$, and a significant interaction of Drug Treatment x Trial Block $F(3,15) = 3.71$, $p<.01$. Fisher's PLSD post hoc revealed that overall the nicotine/sham group had significantly lower latencies that the saline\sham group throughout training. The nicotine/lesion group had significantly lower latencies than the saline-treated lesion group at trial blocks 4, 5, and 6. There were no other significant effects (see Figure 2).

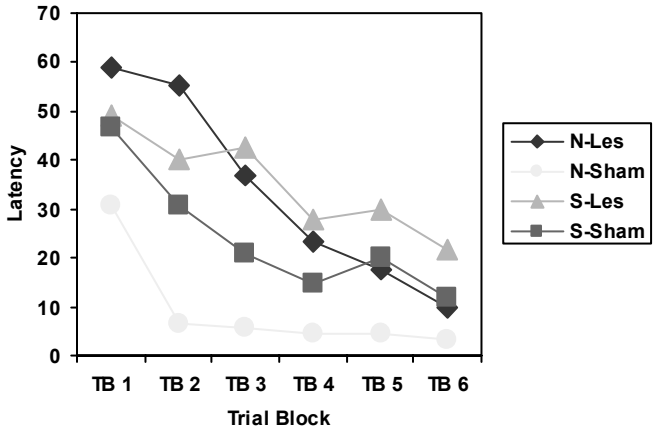


Figure 2. MWT-RAM: Acquisition Latency

A one-way ANOVA for mean zone difference (MZD) on the probe trial revealed a significant effect of group $F(3,23) = 3.58$, $p<.02$. Fisher's PLSD post hoc showed that the saline-treated lesioned animals showed significant deficits compared to all other groups, demonstrating that nicotine alleviated deficits produced by MFC lesions on the probe trial. There were no other significant effects (see Figure 3).

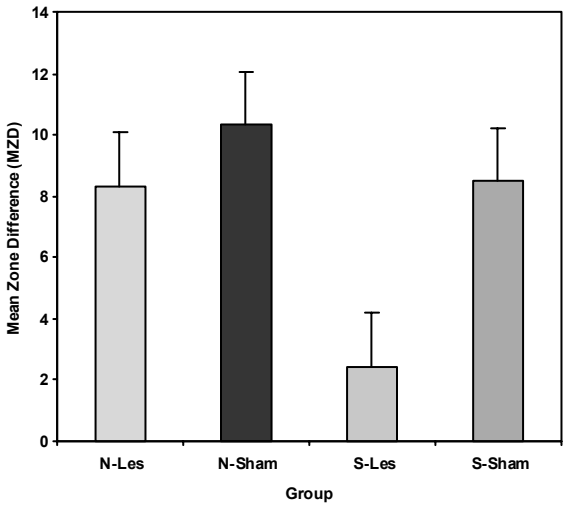


Figure 3. MWT-RAM: Mean Zone Difference

RAM analysis. A two-way ANOVA for working memory errors revealed a significant main effect of group $F(3,25) = 8.54$, $p>.01$. Fisher's PLSD post hoc revealed the nicotine treated groups made fewer working memory errors than the saline treated groups regardless of whether the animal received an MFC lesion. There were no other significant effects (see Figure 4).

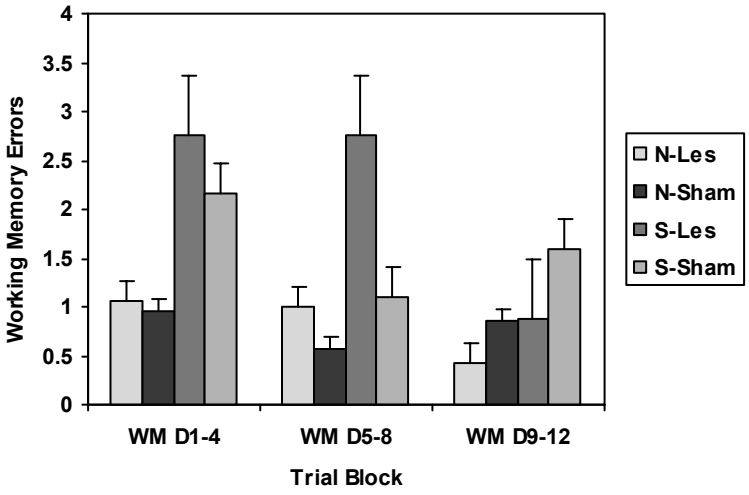


Figure 4. MWT-RAM: Working Memory Errors

A two-way ANOVA for reference memory errors revealed a significant main effect of group $F(3,25) = 11.13$, $p<.01$ and day of training $F(3,2) = 8.09$, $p<.01$. Fisher's PLSD post hoc showed that the nicotine treated animals made fewer reference memory errors than the saline treated groups regardless of whether they received an MFC lesion. There were no other significant effects (see Figure 5).

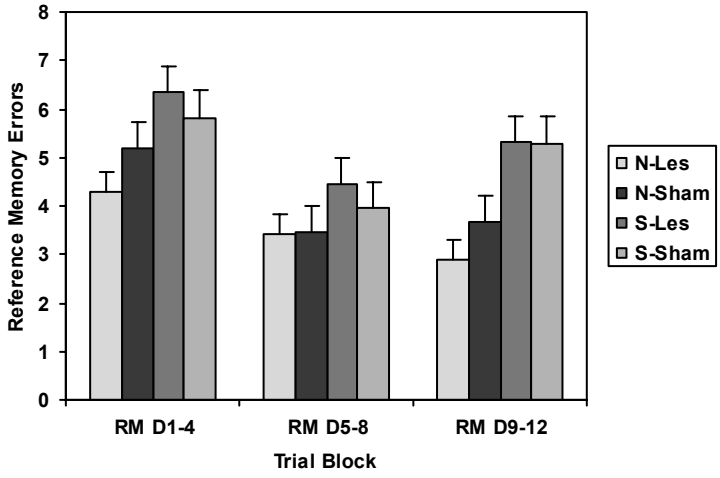


Figure 5. MWT-RAM: Reference Memory Errors

RAM-MWT Behavioral Results

RAM analysis. A two-way ANOVA for working memory errors revealed a significant main effect of day of training $F(3,2) = 5.73$, $p<.01$ and a significant interaction of Group x Drug Treatment $F(3,7) = 2.20$, $p<.02$. Fisher's PLSD post hoc indicated that the nicotine/shams made fewer working memory errors than all other groups and the number of working memory errors decreased over time for all groups. There were no other significant effects (see Figure 6).

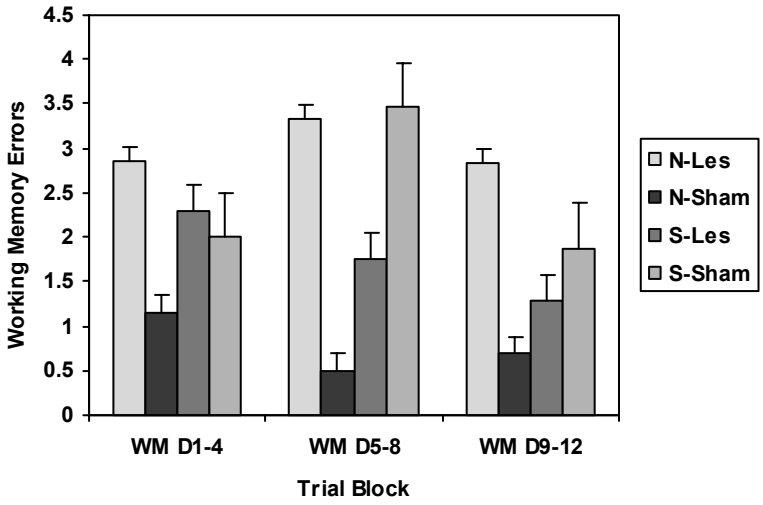


Figure 6. RAM-MWT: Working Memory Errors

A two-way ANOVA for reference memory errors revealed a significant main effect of group $F(3,24) = 4.01$, $p<.01$. Fisher's PLSD revealed that the nicotine sham group made fewer reference memory errors that the other treatment groups. However, nicotine did not improve RAM performance in lesioned animals in this condition. There were no other significant effects (see Figure 7).

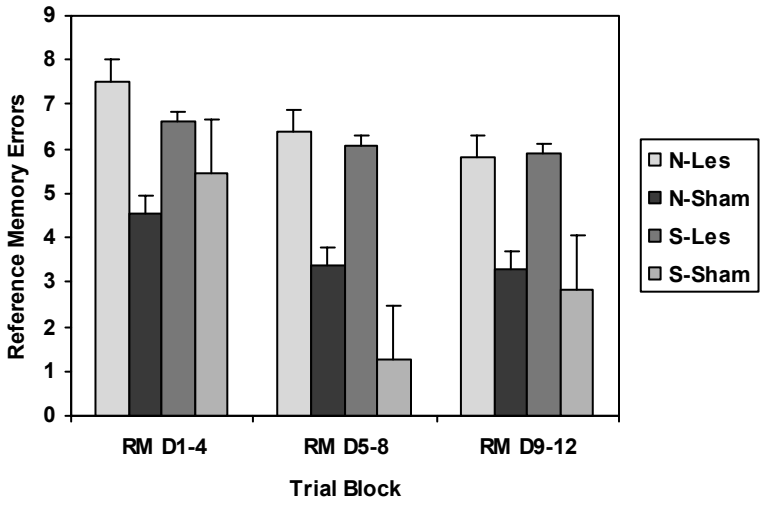


Figure 7. RAM-MWT: Reference Memory Errors

MWT analysis. A two-way ANOVA for acquisition latency revealed a significant main effect of group $F(3,26) = 3.83$, $p<.02$, trial block $F(3,5) = 38.93$, $p<.01$, and significant interaction Drug Treatment x Trial Block $F(3,15) = 1.64$, $9<.04$. Fisher's PLSD post hoc revealed that the nicotine/lesion group had significantly lower latencies than the saline treated lesion group at trial blocks 3, 4, 5, and 6. There were no other significant effects (see Figure 8).

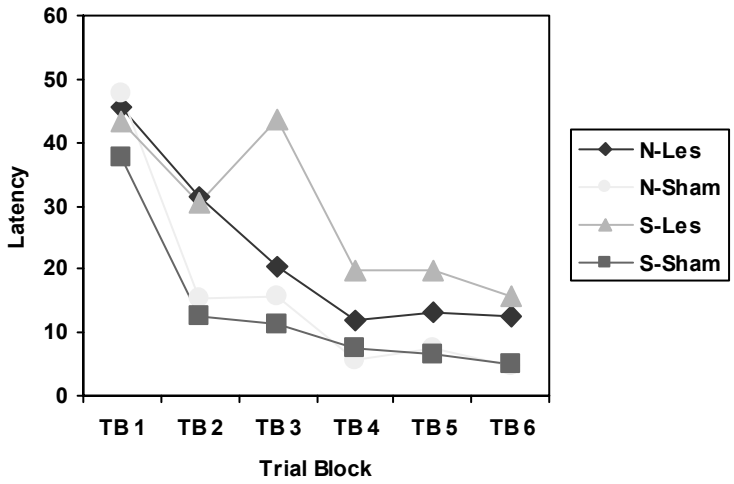


Figure 8. RAM-MWT: Acquisition Latency

A one-way ANOVA for the mean zone difference (MZD) on the probe trial revealed no significant effect $F(3,26) = 1.18$, $p<.33$. There were no other significant effects (see Figure 9).

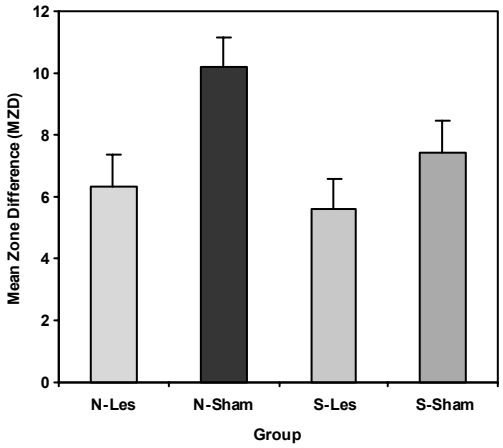


Figure 9. RAM-MWT: Mean Zone Difference

Experiment 2

Behavioral Results

MWT analysis. A two-way ANOVA for acquisition latency revealed a significant main effect of lesion $F(1, 19) = 9.19$, $p<.007$, for trial block $F(3,19) = 33.36$, $p<.0001$. Fisher's PLSD post hoc revealed that the medial frontal cortex lesion produced an overall deficit in acquisition latency that was not alleviated by nicotine treatment. There were no other significant effects (see Figure 10).

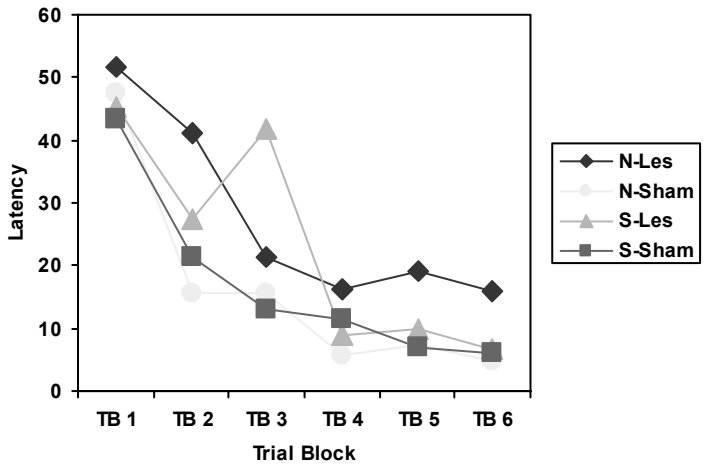


Figure 10. MWT: Acquisition Latency

A one-way ANOVA for the mean zone difference (MZD) revealed a significant main effect of lesion $F(3,23) = 8.57$, $p<.005$ on the probe trial. Again, Fisher's PLSD post hoc showed that the MFC lesion produced a significant deficit on the MZD score that was not alleviated by nicotine. There were no other significant effects (see Figure 11).

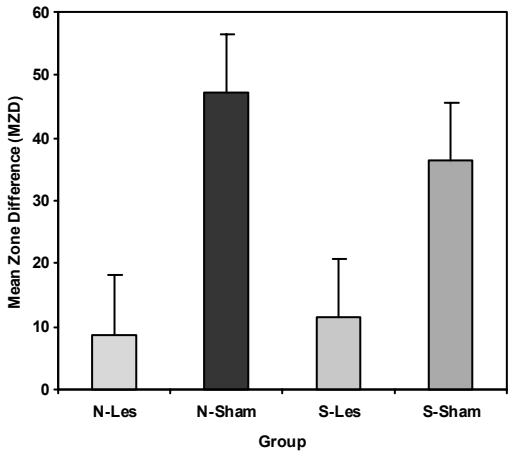


Figure 11. MWT: Mean Zone Difference

RAM analysis. A two-way ANOVA for working memory revealed a significant main effect of drug $F(1,12) = 8.01$, $p<.015$, a significant main effect of lesion $F(1,12) = 5.42$, $p<.038$. Although nicotine improved performance overall, and the MFC lesion produced a deficit, Fisher's PLSD post hoc revealed that nicotine did not alleviate the significant decrease in working memory errors produced by the MFC lesion. There were no other significant effects (see Figure 12).

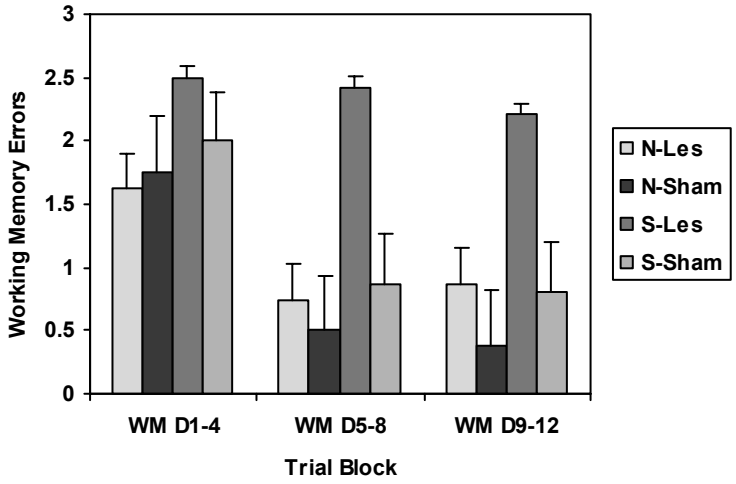


Figure 12. RAM: Working Memory Errors

A two-way ANOVA for reference memory revealed a significant main effect of drug F(1, 17) = 18.35, p<.005, lesion F(1,17) = 16.72, p<.001, and a significant two-way interaction of Drug x Lesion F(2, 17) = 4.62, p<.04. Fisher's PLSD post hoc showed that the MFC lesion produced a significant deficit in reference memory that was alleviated by nicotine. There were no other significant effects (see Figure 13).

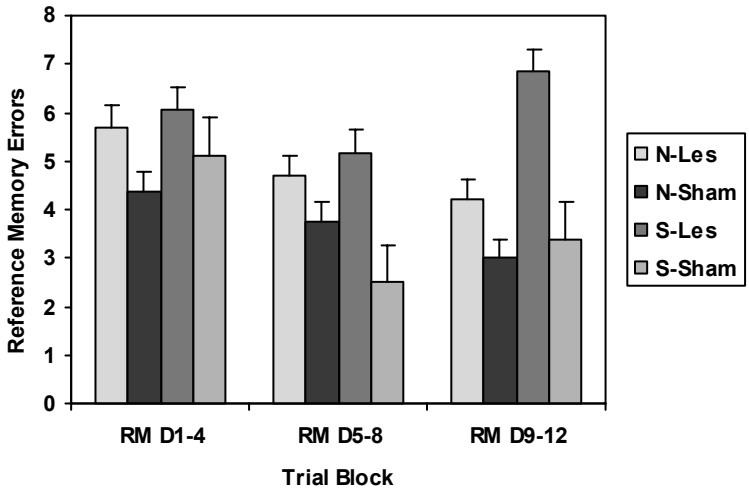
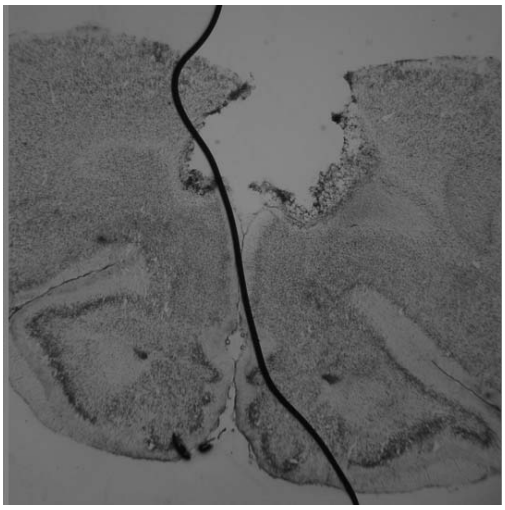


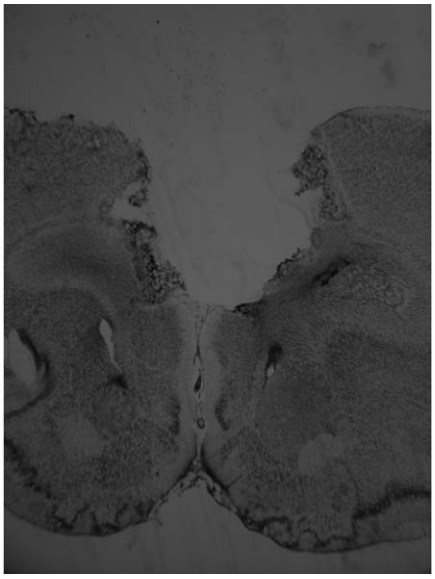
Figure 13. RAM: Reference Memory Errors

Figure 14. Histological Results. Below are representative sections of MFC lesions and their corresponding brain areas in controls. The left slide is representative of the most anterior portion of the MFC lesions, whereas the right slide is representative of the most posterior portion of the MFC lesion. Note that neither lesion is below the cortical level.

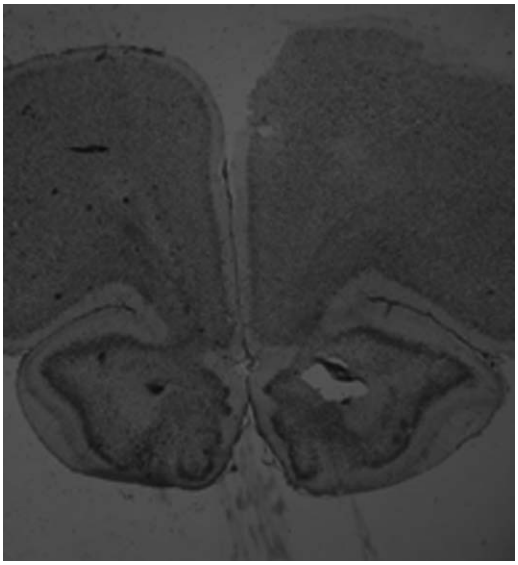
MFC Lesion Anterior: MFC Lesion Posterior:

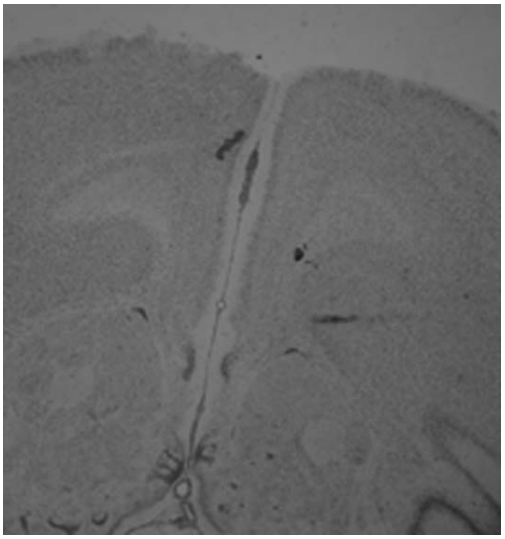
Note: Line across slide is cover slip solution.





MFC Control: Anterior. MFC Control: Posterior.





CHAPTER 4

DISCUSSION

Summary of Results

The results of this study showed that (a) rats given medial frontal cortex lesions were impaired on both the MWT and RAM; (b) chronic nicotine administration given for 11 consecutive days before and 11 consecutive days after a medial frontal cortex lesion improved acquisition on the MWT to sham control levels at trial blocks 4, 5, and 6; (c) chronic nicotine treatment improved probe trial performance in rats given medial frontal cortex lesions to sham control levels; (d) chronic nicotine administration and previous experience on the MWT partially alleviated the increase in working memory errors in medial frontal cortex lesioned rats behaviorally tested on the RAM; (e) chronic nicotine administration and previous experience on the MWT improved reference memory performance on the RAM in rats given medial frontal cortex lesions. (f) In Experiment 2, MFC lesioned animals administered nicotine demonstrated an improvement in RAM performance. However, the improvement dissipated again by 19 days in rats tested on the MWT. This study demonstrates previous training on the MWT in addition to chronic nicotine administration improves performance on a subsequent spatial memory task, and that the improving effects of nicotine appear to improve RAM performance more so than MWT performance, an interesting result. Additionally, it appears that priming from previous experience on the MWT may have acted synergistically with nicotine to enhance RAM performance.

Previous Research

Damage to the MFC has been shown to produce cognitive impairment on spatial memory tasks (Brown et al., 2000; Bussey et al., 1997; Mair et al., 1998). The impairment of acquisition

on the MWT after a medial frontal cortex lesion is consistent with previous reports (Brown et al., 2000; Brown et al., 2001; Decker et al., 1993; Gonzalez et al., 2000). Lesions of several areas of the brain have been shown to produce deficits on the MWT, but this task appears to be most dependent upon integrity of the hippocampus formation and prefrontal cortex. It has been hypothesized that the MFC plays a role in reward-based decision making, which is a critical aspect of the MWT (Walton et al., 2003; Walton et al., 2002). The MWT is a spatial memory task that requires integrity of neurological systems that underlie cognitive performance. Research has shown that the hippocampus plays a primary role in performance on this task (Hodges et al., 1999; Whishaw et al., 1995), although several other studies have shown that the medial frontal cortex also plays an important although somewhat mediary role (Brown et al., 2000; Bussey et al., 1997; Mair et al., 1998). In the current study, deficits in a rat's inability to locate the hidden platform are assumed to demonstrate disruption in the medial frontal cortex circuits that are involved in spatial learning (Brown et al., 2000; Brown et al., 2001; Decker et al.; Gonzalez et al.).

The improvement in acquisition on the MWT after chronic nicotine administration is also consistent with previous research. Chronic nicotine administration has been shown to enhance learning and memory in several studies in non-lesioned animals (Abdulla et al., 1996; Decker et al., 1991; Levin et al., 1992). Additionally, previous work from this laboratory has shown that nicotine alleviates cognitive impairments produced by MFC lesions but extends these findings to include the RAM and the effects of previous training on a spatial memory task.

The improvement in working and reference memory on the RAM after chronic nicotine administration and previous training on the MWT supports the idea that priming and pre-training on a spatial task can improve behavioral performance in rats. Priming and repetition of

behavioral tasks alone have been shown to enhance performance (Caplan & Schooler, 1990; McIlwain et al., 2001; Swick, 1998). For example, pre-training in rats with a lesion of the nucleus basalis of Meynert has resulted in performance to control levels on the MWT (Nieto-Escamez et al., 2004). Previous training on the MWT could lead to an improvement in working and memory errors on the RAM because the MWT is arguably a more difficult spatial task (Hodges et al, 1999; Whishaw et al., 1995). The increase of difficulty on the MWT is based on the fact that this task is an open field spatial memory task, and animals must locate a particular location within this open-field. In contrast, when the animal is behaviorally trained on the RAM, the animal can make one of eight possible choices to forage for food, making the task demands of the RAM much different from those of the MWT, and possibly a simpler task. Additionally, when an animal is placed into the MWT, stress levels are undoubtedly increased, as the animal is placed into a cool water environment, whereas the stress levels of the RAM are likely due to the slight food deprivation to motivate animals for the task. Thus, the more acute increases stress levels of the MWT also makes this task more demanding than that of the RAM. It could be that previous training on a more difficult spatial memory task may enhance performance on a task that is not an open-field spatial task, such as the RAM. This may be especially true in the present study, as the hippocampus has been shown to be important in working memory performance on the RAM, and both the RAM and MWT are well-known to be spatial memory tasks.

Although the pre-training on the MWT improved performance on the RAM, the effects of chronic nicotine administration still did not extend for the entire 19 days. Thus, there are limits to the priming effect produced by previous training as well as nicotine administration. This result was expected, as the effects of chronic nicotine administration on receptor levels and other

biological markers have been shown to drastically decrease over time. In fact, Collins and colleagues (1990) have shown that chronic nicotine administration produces nicotinic receptor upregulation that persists for approximately 7 days before decreasing to control levels. Thus, it appears that the effects of nicotine in this study, as well as the effects of previous training, have limited effects as the delay interval increases.

Possible Mechanisms

There are several possible mechanisms that could be underlying the effects observed in the present study. For example, nicotine has been shown to enhance neurotrophic factor production after chronic nicotine administration in several studies (Brown et al., 2005; French et al., 1999; Kenny et al., 2000; Maggio et al., 1997, 1998). Neurotrophic factors are chemical messengers that are involved in synaptic development and maintenance and are especially important in development and maintenance of septohippocampal circuitry. Infusion of either nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) into the hippocampus has been shown to enhance cognitive performance on both the MWT (Pelleymounter et al. 1996) and the RAM (Jakubowska-Dogru et al., 2005). Additionally, both acute and chronic nicotine treatment has been shown to lead to a significant increase of neurotrophins in several areas of the brain, including NGF and BDNF in the hippocampus (Abdulla et al., 1996; Belluardo et al., 2000; French et al., 1999). Although we do not have data on neurotrophic factors in this study, we hypothesize that nicotine's effects may be mediated by these important chemical messengers. A recent study from our laboratory has shown that nicotine administration using a similar treatment regimen as the present study given twice daily over a 14-day period to non-lesioned rats produced a significant increase of both hippocampal NGF and BDNF in female rats (Brown et al., 2006).

Another important mechanism may be through nicotine's effects on the acetylcholinergic system. Nicotine acts as an agonist to the acetylcholinergic nicotinic receptor The acetylcholinergic system plays an important role in learning and memory, and disruption of cholinergic circuitry in the hippocampus and frontal cortex have been shown to produce impairment in both MWT and RAM performance (Abdulla et al., 1995; Nieto-Escamez et al., 2002). It has been shown that drugs that enhance ACh function produce enhancement of learning and memory, and based on the fact that nicotine acts as an agonist at acetylcholinergic nicotinic receptors, this could be the basis of the improvement in spatial memory performance observed here (Girod & Role, 2001; Levin, 2002; Levin, Kaplan et al., 1997; Levin & Simon, 1997). However, when animals were behaviorally tested in the present study, nicotine was not in the blood stream during testing, and animals were actually experiencing withdrawal to the drug. Withdrawal to chronic nicotine treatment has been shown to significantly increase corticosterone levels in female rats, indicating that these animals are in a stressed state when behaviorally tested. Paradoxically, chronic nicotine treatment has also been shown to increase neurotrophic factors, even 48h after nicotine treatment has ceased (Brown et al., 2005; French et al., 1999). Additionally, chronic nicotine treatment has been shown to produce an improvement in both MWT (Adbulla et al., 1993, 1996) and RAM performance even when animals are tested during nicotine withdrawal, but the underlying mechanism has not been elucidated. Past research suggests that if nicotine increases neurotrophic factor production, there is a positive correlation with increases in acetylchoinergic activity. If BDNF and NGF Trk A and B receptors are located on cholinergic neurons in the septohippocampal pathway, thus, chronic nicotine increases neurotrophic factor production that increases acetylcholinergic activity. Ultimately this results in enhanced cognitive performance. It has been hypothesized that by enhancing ACh, nicotine

enhances learning and memory (Jones et al., 1992; Levin, 1992; Rinne et al., 1991). The enhancement of performance in the current study could be attributed to nicotine's enhancing affects on the acetylcholinergic system.

The effects of the two learning and memory tasks could also play a role in enhancement of learning and memory in the current study. The RAM and MWT are two very different spatial memory tasks. The RAM is a maze that tests special memory by using extra-maze cues to help the rat navigate the arms of the maze. The RAM uses food reward for motivation. Rats make one of eight choices as they search for food. Working and reference memory errors are used to analyze cognitive performance on this maze. This task is not as physically or mentally demanding as the MWT. The MWT is also a spatial memory task, but it is very different from the RAM. Unlike the RAM, the rat searches for a "hidden" platform in an open field, and choices are not restricted. The MWT also requires the rats to be placed into the water, and they must swim, which means the animal is likely undergoing a significant increase of acute stress while trying to find the platform, whereas on the RAM, the stress is more constant due to food deprivation. Thus, it would seem that the water maze is probably a more complex task than the RAM.

Limitations of the Current Study and Suggestions for Future Research

Although the current study provides many insights into to the causes and possible treatments for brain insult and brain injury, there are several limitations. First, the underlying mechanisms of the behavioral effects observed here were not identified. As mentioned above, the precise mechanism for nicotine influence on cognitive performance has not been elucidated by our laboratory and not been entirely discovered in the research literature, although several mechanisms, which were reviewed above, have been posited (for review, see Levin & Simon,

1999). Another weakness is that it is not known how nicotine may interact with the injured brain, as its actions and enhancing effects on the injured brain may be very different from its actions in normal subjects. For example, when brain injury occurs, it is well-known that neurotrophic factors are increased to aid the brain in compensation for the injury. Precisely how nicotine may enhance or even prolong this process is not known. What is known at this point is that nicotine can enhance compensatory mechanisms in the brain when insult occurs, suggesting that nicotine may have therapeutic potential (Sacco et al., 2004).

A second weakness is the precise influence of priming of spatial performance from the MWT to the RAM. Obviously, the MWT is a task that involves spatially navigating to a place of refuge through a water environment, whereas the RAM is a dry land maze in which a rat must locate caches of food located at the end of one of eight arms. Based on the fact that the task demands are so different, it is surprising that any priming could occur from one task to the next. It appears that spatial memory training on the MWT may be able to prime performance on the RAM, and this is the first study to demonstrate such a phenomenon. Importantly, studies have shown that ablation of the hippocampus in rats or humans produce a significant deficit on both tasks, or as is tested in humans, virtual versions of these two tasks. Therefore, it appears that there is a common link between both tasks in that the hippocampus plays a primary role in both the MWT and RAM.

Application of the current study

There has now been plenty of research evidence to demonstrate that nicotine has therapeutic potential in cases where the brain has been compromised. Nicotine has been shown to have most of its therapeutic potential it appears, when brain areas that mediate cognitive performance are compromised (Brown et al., 1999, 2000; Decker & Anderson, 1994; Levin et

al., 1998; Yamazaki et al., 1998). Recent studies from Newhouse, Levin, and colleagues have shown that nicotine has therapeutic potential in ADHD (Potter & Newhouse, 2004; Newhouse, et al., 2004), Tourette's syndrome (Hayslett & Tizabi, 2005; McEvoy & Allen, 2002), and schizophrenia (Kumari & Postma, 2005).

The MWT is also a spatial memory task, but it is not a traditional maze. It is an open-field task that requires the rat to locate a platform "hidden" below the surface of the water. The rat must use visual cues in the environment to escape from the water, and this task is physically demanding on the rat as well as produces a significant increase in stress. In addition to swimming in the pool, the rat must continuously search for the visual cues that must be used to locate the platform. Although both the RAM and MWT both test learning and memory, the task demands differ greatly. Though the radial maze and water maze are designed to assess the spatial learning abilities, one cannot generalize that these mazes use the same environmental visuospatial cues to form cognitive maps, rather the mazes differ in their search strategies and tap a variety of sources of non spatial information in addition to the visuospatial cues for the construction of spatial maps (Hodges, 1996; Jarrard, 1986; Sutherland et al., 1988). Water maze uses an aversive learning strategy when compared to the reward task in radial maze. The nature of errors committed is also different in both the tasks. The radial arm maze explicitly requires working memory, and the hippocampus is essential for mediating working memory. The Morris water maze helps to study the spatial localization component of hippocampal functioning. Accordingly, the behavioral performances of rats differ in these mazes and cannot be correlated (Jarrard; Sutherland et al.). Such studies however help one to look into brain structure function relationships although such dissociations in maze tasks make for complexity (Hodges, 1996).

The priming effect that was created by pre-training on the MWT that extended to the

RAM could be explained by the higher level of difficulty on the MWT. Studies have shown that previous training on a more complex but related task may more often prime performance on a simpler task (Domjan & Burkhard, 1993). After practice on a maze that required more physical and mental exercise on the part of the rat, performance on the RAM increased because the task was less demanding than the previous task. The learning and memory skills obtained on the MWT could have carried over to the RAM.

Because there are so many mechanisms at work to produce the results of this study, it is impossible to determine one single mechanism for the improvements of performance. This study supports the previous findings where nicotine administration improved learning and memory on the MWT and RAM in rats with MFC. However, the priming effects deserve future examination to determine if training on behavioral tasks can affect future performance on other behavioral tasks.

REFERENCES

Abdulla, F. A., Bradbury, E., Calmine, M. R., Lippiello, P. M., Wonnacott, S., Gray, J. A., et al. (1996). Relationship between up-regulation of nicotine binding sites in rat brain and delayed cognitive enhancement observed after chronic or acute nicotine receptor stimulation. *Psychopharmacology, 124,* 323-331.

Akaike, A., Gamura, Y., Yokota, T., Shimohama, S., & Kimura, J. (1994). Nicotine-induced protection of cultured cortical neurons against N-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. *Brain Research, 644,* 181-187.

Alturi, P., Fleck, M., Shen, Q., Mah, S., Stadfelt, D., Barnes, W., et al. (2001). Fundtional nicotinic acetylcholine receptor expression in stem and progenitor cells of the early embryonic mouse cerebral cortex. *Developmental Biology, 240,*143-156.

Bancroft, A., & Levin, E. D. (2000). Ventral hippocampal α4β2 nicotinic receptors and chronic nicotine effects on memory. *Neuropharmacology, 39,* 2770-2778.

Belluardo, N., Mudo, G., Blum, M., & Fuxe, K. (2000). Central nicotinic receptors, neurotrophic factors and neuroprotection. *Behavioral Brain Research, 113,* 21-34.

Brown, R. W., Gonzales, C. L. R., & Kolb, B. (2000). Nicotine improves Morris water task performance in rats given medial frontal cortex lesions. *Pharmacology, Biochemistry, and Behavior, 67,* 473-478.

Brown, R. W., Gonzales, C. L. R., Whishaw, I. Q., & Kolb, B. (2001). Nicotine improvement of Morris water task performance after fimbria-fornix lesion is blocked by mecamylamine. *Behavioral Brain Research, 119,* 185-192.

Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1997). Dissociable effects of cingulated and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian

autoshaping procedure for the rat: Implications of neurobiology of emotion. *Behavioral Neuroscience, 111,* 908-919.

Caplan, L. J., & Schooler, C. (1990). Problem solving by reference to rules or previous episodes: the effects of organized training, analogical models, and subsequent complexity of experience. *Memory and Cognition, 18,* 215-27.

Ciamei, A., Aversano, M., Cestari, V., & Castellano, C. (2001). Effects of MK-801 and nicotine combinations on memory consolidation in CD1 mice. *Psychopharmacology, 154,* 126-130.

Collins A. C., Romm E., & Wehner J. M. (1990). Dissociation of the apparent relationship between nicotine tolerance and up-regulation of nicotinic receptors. *Brain Research Bulletin, 25,* 373-9.

Costa, G., Abin-Carriquiry, J. A., & Dajas, F. (2001). Nicotine prevents striatal dopamine loss produced by 6-hydroxydopamine lesion in the substantia nigra. *Brain Research, 888,* 336-342.

D'Hooge, R., & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Brain Research Review, 36,* 60-90.

Dani, J. A., & De Biasi, M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacology, Biochemistry, and Behavior, 70,* 439-446.

Dani, J. A., & Heinemann, S. (1996). Molecular and cellular aspects of nicotine abuse. *Neuron 16,* 905-908.

Dani, J. A., Ji, D., & Zhou, F. (2001). Synaptic plasticity and nicotine addiction. *Neuron, 31,* 349-352.

Decker, M. W., Majchrzah, M. J., & Anderson, D. J. (1991). Effects of nicotine on spatial

memory deficits in rats with septal lesions. *Brain Research, 572,* 281-285.

Decker, M. W., Majchrzah, M. J., & Arneric, S. P. (1993). Effects of lobeline, a nicotinic receptor agonist, on learning and memory. *Pharmacology, Biochemistry, and Behavior, 45,* 571-576.

Dixon, C. E., Clifton, G. L., Lighthall, J. W., Yaghmai, A. A., & Hayes, R. L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods, 39,* 253-262.

Duncan, J., & Owen, A. M. (2000). Common regions of the human frontal lobe recruited by diverse cognitive demands. *Trends in Neuroscience, 23,* 475-483.

Elrod, K., Buccafusco, J. J., & Jackson, W. J. (1988). Nicotine enhances delayed matching-to-sample performance by primates. *Life Sciences, 43,* 475-483.

French, S. J., Humby, T., Horner, C. H., Sofroniew, M. V., & Rattray, M. (1999). Hippocampal neurotrophin and tck receptor mRNA levels are altered by local administration of nicotine, carbachol, and pilocarpine. *Molecular Brain Research, 67,* 124-136.

Fumagalli, F., Santero, R., Gennarelli, M., Giorgio, R., & Andrea Riva, M. (2001). Decreased hippocampal BDNF expression after acute systemic injection of quinpirole. *Neuropharmacology, 40*, 954-957.

Girod, R., & Role, L. W. (2001). Long-lasting enhancement of glutamatergic synaptic transmission by acetylcholine contrasts with response adaptation after exposure to low-level nicotine. *The Journal of Neuroscience, 27,* 5182-5190.

Grilly, D. M., Simon, B. B., & Levin, E. D. (2000). Nicotine enhances stimulus detection performance of middle- and old-aged rats: A longitudinal study. *Pharmacology,*

Biochemistry, and Behavior, 65, 665-670.

Gonzalez, C. L., Miranda, M. I., Gutierrez, H., Ormsby, C., & Bermudez-Rattoni, F. (2000). Differential participation of the NBM in the acquisition and retrieval of conditioned taste aversion and Morris water maze. *Behavioral Brain Research, 116,* 89-98.

Hamm, R. J., White-Gbadebo, D. M., Lyeth, B. G., Jenkins, L. W., & Hayes, R. L. (1992). The effect of age on motor and cognitive deficits after traumatic brain injury. *Neurosurgery, 31,* 1072-1077.

Hannesson, D. K., & Skelton, R.W. (1998). Recovery of spatial performance in the Morris water maze following bilateral transection of the fimbria/fornix in rats. *Behavioral Brain Research, 90,* 35-56.

Hodges, H. (1996). Maze procedures: the radial-arm and water maze compared. *Brain Reseach Cognitive Brain Research, 3,* 67-81.

Hodges, H., Peters, S., Gray, J. A., & Hunter, A. J. (1999). Counteractive effects of a partial (Sabcomeline) and a full (RS86) deficits in radial maze performance induced by S-AMPA lesions of the basal forebrain and medial septal area. *Behavioral Brain Research, 99,* 81-92.

Humphreys, G. W., & Riddock, M. J. (2000). One more cup of coffee for the road: Object-action assemblies, response blocking, and response capture after frontal lobe damage. *Experimental Brain Research, 133,* 81-93.

Jakubowska-Dogru E., & Gumusbas U. (2005). Chronic intracerebroventricular NGF administration improves working memory in young adult memory deficient rats. *Neuroscience Letters, 382(1-2),* 45-50.

Joel, D., Tarrasch, R., Feldon, J., & Weiner, I. (1997). Effects of electrolytic lesions of he

medial prefrontal cortex or its subfields on 4-arm baited 8-arm radial maze, two-way active avoidance and conditioned fear tasks in the rat. *Brain Research, 765,* 37-50.

Jones, G. M., Sahakian, B. J., Levy, R., Warburton, D. M., & Gray, J. A. (1992). Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology,108,* 485-494.

Jonnala, R. R., & Buccafusco, J. J. (2001). Relationship between the increased cell surface alpha7 nicotinic receptor expression and neuroprotection induced by several nicotinic receptor agonists. *Jouranl of Neuroscience Research, 66,* 565-72.

Kenny P. J. , File S. E. , & Rattray M. (2001). Nicotine regulates 5-HT(1A) receptor gene expression in the cerebral cortex and dorsal hippocampus. *European Journal of Neuroscience,* 13, 1267-71.

Kim, J. S., & Levin, E. D. (1996). Nicotinic, muscarinic, and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: Effects on spatial working memory in rats. *Brain Research, 725,* 231-240.

Kleschevnikov, A. M., Sinden, J. D., & Marchbanks, R. (1998). Fimbria-fornix lesions impair spatial performance and induce epileptic-like activity but do not affect long-term potentiation in the CA1 region of rat hippocampal slices. *Brain Research, 656,* 221-228.

Kolb, B. (1995). Brain plasticity and behavior. city, NJ: Lawrence Earlbaum Associates.

Levin, E. D. (1992). Nicotinic systems and cognitive function. *Psychopharmacology, 108,* 417-431.

Levin, E. D., Bettegowda, C., Weaver, T., & Christopher, C. (1998). Nicotine-dizocilpine interactions and working and reference memory performance of rats in the radial-arm maze. *Pharmacology, Biochemistry, and Behavior, 61,* 335-340.

Levin, E. D., Briggs, S. J., Christopher, N. C., & Rose, J. E. (1992). Persistence of chronic nicotine-induced cognitive facilitation. *Behavioral and Neural Biology, 58,* 152-158.

Levin, E. D., Christopher, N. C., & Briggs, S. J. (1997). Chronic nicotinic agonist and antagonist effects on T-maze alternation. *Physiology and Behavior, 61,* 863-866.

Levin, E. D., Kaplan, S., & Boardman, A. (1997). Acute nicotine interactions with nicotinic and muscarinic antagonists: Working and reference memory effects in the 16-arm radial maze. *Behavioral Pharmacology, 8,* 236-242.

Levin, E. D., & Rezvani, A. H. (2000). Development of nicotinic drug therapy for cognitive disorders. *European Journal of Pharmacology, 393,* 141-146.

Levin, E. D., & Rose, J. E. (1995). Acute and chronic nicotinic interactions with dopamine systems and working memory performance. *Annals of the New York Academy of Sciences, 757,* 245-252.

Levin, E. D., & Simon, B. B. (1997). Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology, 138,* 217-230.

Levin, E. D., & Torry, D. (1995). Acute and chronic nicotine effects on working memory in aged rats. *Psychopharmacology, 123,* 88-97.

Levin, E. D., Torry, D., Christopher, N. C., Yu, X, Einstein, G., & Schwartz-Bloom, R. D. (1997). Is binding to nicotinic acetylcholine and dopamine receptors related to working memory in rats? *Brain Research Bulletin, 43,* 295-304.

Lighthall, J. W., Dixon, C. E., & Anderson, T. E. (1989). Experimental models of brain injury. *Journal of Neurotrama, 6,* 83-97.

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of

behavioral test batteries: Effects of training history. *Physiological Behavior, 73,* 705-17.

Maggio, R. G., Riva, M., Vaglini, F., Molteni, R., Armogida, M., Racagni, G., et al. (1998). Nicotine prevents experimental parkinsonism in rodents and induces striatal increase of neurotrophic factors. *Journal of Neurochemistry, 71,* 2439-2446.

Mair, R. G., Burk, J. A., & Porter, M. C. (1998). Lesions of the frontal cortex, hippocampus, and intra laminar thalamic nuclei have distinct effects on remembering in rats. *Behavioral Neuroscience, 112,* 772-792.

Morris, R. G. (1980). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12,* 239-261.

Narahashi, T., Fenster, C. P., Quick, M. W., Lester, R. A., Marszalec, W., Aistrup, G. L., et al. (2000). Symposium overview: Mechanism of action of nicotine on neuronal acetylcholine receptors, from molecule to behavior. *Toxological Science, 57,* 193-202.

Nieto-Escamez, F. A., Sanchez-Santed, F., & de Bruin, J. P. (2004). Pretraining or previous non-spatial experience improves spatial learning in the Morris water maze of nucleus basalis lesioned rats. *Behavioral Brain Research, 148,* 55-71.

Pelleymounter M. A., Cullen M. J., Baker M. B., Gollub M. , & Wellman C. (1996). The effects of intrahippocampal BDNF and NGF on spatial learning in aged Long Evans rats. *Molecular Chemistry Neuropathoogy, 29(2-3),* 211-26.

Perry, E. K., Perry, R. H., Smith, C. J., Dick, D. J., Candy, J. M., Edwardson, J. A., et al. (1987). Nicotinic receptor abnormalities in Alzheimer's and Parkinson's diseases. *Journal of Neurology, Neurosurgery, and Psychiatry, 50,* 806-809.

Puttfarcken, P. S., Jacobs, I., & Faltynek, C. R. (2000). Characterization of nicotinic acetylcholine receptor-mediated [3H]-dopamine release from rat cortex and striatum,

Neuropharmacology, 39, 2673-2680.

Ridderinkhof, K. R., Ullsperger, M., Crone, E. A., & Nieuwenhuis, S. (2004). The role of the medial frontal cortex in cognitive control. *Science, 306,* 443-7.

Rinne, J. O., Myllykyla, T., Lonnberg, P., & Marjamaki, P. (1991). A postmortem study of brain nicotinic receptors in Parkinson's and Alzheimer's disease. *Brain Research, 547,* 167-170.

Rusted, J. M., Newhouse, P. A., & Levin, E. D. (2000). Nicotinic treatment for degenerative neuropsychiatric disorders such as Alzheimer's disease and Parkinson's disease. *Behavioral Brain Research, 113,* 121-129.

Sacco K. A., Bannon K. L., & George T. P. (2004). Nicotinic receptor mechanisms and cognition in normal states and neuropsychiatric disorders. *Journal of Psychopharmacology, 18,* 457-74.

Souza, T. M., Vianni, M. R. M., Rodrigues, C., Quevedo, J., Moleta, B. A., & Izquierdo, I. (2000). Inducement of the medial precentral prefrontal cortex in memory consolidation for inhibitory avoidance learning in rats. *Pharmacology, Biochemistry, and Behavior, 66,* 615-622.

Swick D. (1998). Effects of prefrontal lesions on lexical processing and repetition priming: An ERP study. *Brain Research Cognitive Brain Research, 7,* 143-57.

Walton, M. E., Bannerman, D. M., Alterescu, K., & Rushworth, M. F. (2003). Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. *Journal of Neuroscience, 23,* 6475-9.

Walton, M. E., Bannerman, D. M., & Rushworth, M. F. (2002). The role of rat medial frontal cortex in effort-based decision making. *Journal of Neuroscience, 22,* 10996-1003.

Whitehouse, P. J., Martino, A. M., Wagser, M. V., Price, D. L., Mayeux, R., Atack, J. R., et al. (1988). *Neurology, 38,* 720-723.

Welzl, H., Alessandri, B., Oettinger, R., & Battig, K. (1988). The effects of long-term nicotinic treatment on locomotion, exploration, and memory in young and old rats. *Psychopharmacology, 96*, 317-323.

Whishaw, I. Q. (1995). A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. *Physiolgical Behavior, 58,* 687-93.

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Thacker, S.K., Flanigan, T.J., Thompson, K.N., Norris, R.L., & Brown, R.W. (2003). Early developmental cognitive deficits in a rodent model of schizophrenia. Southeastern Psychological Association Annual Meeting, New Orleans, LA.

Norris, R.M., Click, I.A., Thacker, S.K., Baisden, R., & Brown, R.W. (2002). Nicotine enhancement after medical frontal cortex lesions: behavioral and temporal parameters. Society for Neuroscience Annual Meeting, Orlando, FL.

Brown, R.W., Gass, J.T., Click, I.A., Thacker, S.K., Norris, R.L., & Kostrzewa, R.M. (2001). Ontogenetic quinpirole treatment produces spatial memory deficits and reaching accuracy enhancement in rats. Society for Neuroscience Annual Meeting, San Diego, CA.