DEMONSTRATION AND THE IMPORTANCE OF THE MEDIAL SEPTUM AND DIAGONAL BAND (MSDB) FOR SPATIAL MEMORY AND LEARNING IN RATS

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ABSTRACT

The medial septum and diagonal band (MSDB) region of the brain has been shown to play a critical role in the cognitive deficits associated with Alzheimer's disease, anxiety disorders, and normal aging. The MSDB is known to be a direct source of the neurotransmitters acetylcholine, γ-aminobutyric acid, and glutamate for the hippocampus, a brain structure associated with learning and spatial memory. Loss of these neurochemical inputs, therefore, would be expected to produce deficits in learning and memory. To test this hypothesis, the medial septum (MS) and diagonal band (DB) of Broca were lesioned in male Sprague Dawley rats and the rats were then subjected to specific experimental protocols designed to test either spatial memory (the T-maze task) or learning (the eye blink test). Compared to sham-lesioned rats, MSDB-lesioned rats suffered a drastic deficit in spatial memory during the T-maze task and learning was impaired during the extinction, but not the acquisition, phase of the eye blink test. The MSDB therefore appears to play a critical but indirect role in learning and spatial memory, although the underlying mechanism of action for its effects on these cognitive functions is not yet clear. A better understanding of the functional role of the MSDB region should provide important insights regarding the cognitive impairments associated with Alzheimer's disease, anxiety disorders such as post-traumatic stress disorder (PSTD), and normal aging.

INTRODUCTION

The medial septum (MS) and the diagonal band (DB) of Broca are major afferent inputs to the hippocampus, a structure in the brain that has been associated with learning and memory (Amaral and Kurz, 1985; Jakab and Leranth, 1995). The MSDB projects to the hippocampus via the fimbria-fornix pathway, and transection of this pathway in animal models results in deficits in spatial learning and memory that are similar to those seen in human subjects with hippocampal damage (Kesner *et al*., 1986; Mizumori *et al*., 1990; Morris *et al*., 1982). Moreover, electrolytic damage to the medial septal area impairs spatial learning and memory tasks (Kesner *et al*., 1986) while temporary inactivation of this area results in a radical destruction of memory and loss of hippocampal theta (Θ) rhythm, an EEG activity pattern that is thought to be critical for memory formation (Givens and Olton, 1990). Previous studies in humans have suggested that the medial septal area plays a critical role in the cognitive impairments associated with Alzheimer's disease, anxiety disorders such as post-traumatic stress disorder (PTSD), and aging (Bartus *et al*., 1982; Coyle *et al*., 1983). Damage to the MSDB region impairs performance in several different learning and memory (especially spatial memory) tasks that are thought to be mediated by the hippocampus (Pang *et al*., 2010). These studies suggest strongly that MSDB inputs are crucial for normal hippocampal function and that damage to these inputs, either via trauma or aging, contribute to diseases characterized by deficits in learning and memory.

The specific neurochemical inputs from the MSDB that are required for learning and memory are not well known, however, neuronal projections to the hippocampus from the MSDB include both inhibitory (γ-aminobutyric acid, GABAergic) and excitatory (cholinergic, peptidergic, and glutamatergic) fibers (Amaral and Kurz, 1985; Colom *et al*., 2005; Freund, 1989). These neurochemical studies indicate that the MSDB projection provides the most critical excitatory cholinergic input to the hippocampus.

Several specific tests have been developed to assess spatial memory and learning in rats. For example, spatial memory can be tested using a T-maze task. In this test, the rat is trained to obtain a food reward by entering a specific arm of a capital T-shaped maze from a constant starting point. Once trained, this task can be used to determine whether rats learn food location by habit (e.g., turning consistently in only one direction to approach their goal, an egocentric non-spatial strategy) or by using external cues (e.g., turning in a particular direction using surrounding visual cues as a guideline to approach their goal, an allocentric spatial strategy) (Chang and Gold, 2003). The animals used in these studies are initially mildly food-deprived to motivate them for a food reward. The T-maze task can be performed in two different ways: the free test or the forced test. The forced test mode is designed to study alternation behavior. In this test, one of the arms is blocked to "force" the rat into a specific arm. Subsequently, the block is removed and the rat is allowed to choose either arm. Alternation behavior is produced by removing the food reward when the rat selects the same arm it was initially forced into, and allowing the rat to obtain the food reward when it selects the "alternate" (initially unblocked) arm (Bats *et al*., 2001).

Spatial memory may also be assessed using the Morris water maze (Morris, 1981). In this test, rats are placed in a circular tank of opaque water that contains a hidden platform. Various visual cues are arranged around the outside of the tank that the rat can use for orientation. During training sessions, the rat is placed in the tank and allowed to swim until it encounters and climbs onto a hidden platform (which is always in the same place with respect to the external visual cues) to escape from swimming. After an initial training period, a transfer test is performed in which the platform is removed and the amount of time the rat spends in each quadrant of the tank during a subsequent swimming session is recorded. Utilization of spatial memory is indicated when the rat spends the majority of the time swimming in the quadrant previously occupied by the hidden platform (Morris, 1981).

To study mammalian brain mechanisms underlying associative learning and memory, the Pavlovian (or classical) eye blink conditioning paradigm is commonly used (Lee and Kim, 2004). In this paradigm, a neutral stimulus (the conditioned stimulus [CS], often a tone) is paired with a response-inducing stimulus (the unconditioned stimulus [US], e.g. a periorbital eye shock). At first, the tone does not result in a behavioral response, but the periorbital eye shock results in a reflexive eye blink. After repetitive pairings of the tone and the eye shock, the tone presented alone eventually results in the reflexive eye blink response (the conditioned response [CR]) (Allen *et al*., 2002). Extinction behavior is often studied in conjunction with these conditioning studies. Extinction refers to the decrease in a CR after a CS is repeatedly presented in the absence of the US with which it was once originally paired.

Recent studies have utilized a combination of transmitter-specific lesions and spatial and associative learning tests to assess the importance of the MSDB region in learning and memory. For example, Yu *et al*. (1996) employed the immunotoxin 192-IgG-saporin to selectively target cholinergic neurons of the MSDB. This toxin consists of a rat antibody against the nerve growth factor receptor p75 that is conjugated to a ribosome-inactivating protein known as saporin. The toxin specifically targeted cholinergic MSDB neurons because these neurons are the only ones in this region to contain the p75 receptor. Injections of the 192-IgG-saporin immunotoxin into the MSDB caused complete and selective destruction of cholinergic neurons. However, despite the loss of the majority of cholinergic MSDB neurons following injection of the immunotoxin, the rats showed minimal deficits in spatial memory tasks. These results suggested that non-cholinergic neurons were responsible for the impairments in spatial memory and learning (Berger-Sweeney *et al*., 1994). Pang *et al*. (2010) used kainic acid to target GABAergic neurons in the MSDB of rats that were then tested in a water maze task (spatial working memory). Selective damage to GABAergic MSDB neurons impaired spatial working memory, while rats with damage to cholinergic neurons in this structure had mild or no impairment in these same spatial tasks. It was concluded that rats with GABAergic lesions exhibited impaired spatial working memory but had no deficits in the acquisition of spatial reference memory. Smith and Pang (2005) utilized an orexin-saporin neurotoxin to damage neurons expressing the hypocretin-2-receptor. A dose of 100 ng/μL selectively eliminated GABAergic septohippocampal neurons, while sparing the majority of the cholinergic neurons. At a dose of 200 ng/μL, however, GABAergic septohippocampal neurons were eliminated completely, as well as numerous cholinergic neurons. Spatial reference memory, as assessed by the water maze test, was impaired at both concentrations of orexin-saporin while spontaneous

alternation behavior was only impaired in rats treated at the higher dose. This suggested that orexin innervation of the MSDB might modify spatial memory by acting on both the GABAergic and cholinergic septohippocampal neurons.

With respect to associative learning, Fontan-Lozano *et al*. (2005) demonstrated the importance of cholinergic septohippocampal neurons for trace eye-blink classical conditioning tasks. Rats with MSDB 192-IgG-saporin-induced lesions exhibited a marked deficit in the acquisition, but not the retrieval, period of the classical eye-blink conditioning. The acquisition deficit was later reversed by systemic administration of carbachol, a muscarinic acetylcholine receptor agonist, but not by lobeline, a nicotinic acetylcholine receptor agonist. These results suggested that muscarinic agents may be useful for the improvement of some of the associative learning deficits observed in patients during the early stages of Alzheimer's disease. Another study in mice (Tseng *et al*., 2004) demonstrated that eye-blink conditioning was an associative learning task that depends on the hippocampus. Dorsal hippocampal neurons were lesioned by exposure to 0.1% ibotenic acid. The control group exhibited >60% conditioned responses, while the ibotenic acid-treated group showed significantly fewer (35-45%) responses. These data indicate that dorsal hippocampal neurons play a critical role in eye-blink conditioning.

The present study was conducted to determine the extent of spatial memory and learning impairment following a lesion in the MSDB region of rats. The lesion specifically targeted GABAergic and cholinergic septohippocampal neurons, respectively, thereby indirectly affecting hippocampal function. To assess disruption in spatial memory, a T-maze in forced test mode was utilized. It was predicted that rats with lesions in GABAergic MSDB neurons would exhibit deficits in the acquisition of spatial memory. To assess disruption in associative learning, a classical eye-blink conditioning paradigm was used. In this case, it was predicted that cholinergic MSDB neuronal lesions would produce deficits in the acquisition phase of associative learning.

METHODS

Spatial Learning and Memory

Subjects: Male Sprague-Dawley rats (n = 22; 9 sham and 13 GABA (GAT)-lesioned) with initial body weights of 250-350g at the start of the study were tested for spatial learning and memory in a T-maze task. The rats were placed on a food-restricted schedule such that their body weights were reduced to and then maintained at 85% *ad libitum*. All rats were housed individually on a 12:12 hour light:dark cycle with lights on at 7:00 a.m. Training and testing was performed only during the light phase. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the IACUC of the Veterans Affairs Medical Center in East Orange, New Jersey.
Surgical Procedures*:* Rats were anesthetized with sodium secobarbital (50 mg/kg initially, supplemented as necessary throughout the surgical procedure). Scopolamine methyl bromide (0.02 mg/kg) was administered to reduce cranial secretions. Body temperature was maintained at 37 ^{o}C with a Deltaphase isothermal pad. When sufficiently anesthetized, each rat was placed in a stereotaxic apparatus and the head was leveled to ensure that bregma and lambda were in the same horizontal plane. Four holes (1-1.5 mm diameter) were drilled through the skull over the MSDB region. The tip of a Hamilton syringe was inserted into the MS (0.6 mm anterior and ± 0.5 mm lateral to bregma, and 6.2 mm ventral to the brain surface) and 0.3 µl of saline (for sham-lesioned rats) or 100-300 ng/µl of orexin-saporin (for GAT-lesioned rats) was injected into each of the sites over a five-minute period. In addition, two injections of 0.4 µl each were administered into the left and right horizontal limbs of the diagonal band of Broca (DB) (0.6 mm anterior and ± 0.5 mm lateral to bregma, and 7.8 mm ventral to the brain surface). Injections were made at a rate of 0.05-0.1 µl/min and the drugs were allowed to diffuse from the site of injection for five minutes before the needle was removed. Following surgery, the scalp was sutured and the rat was monitored on an hourly basis for two days. Rats were allowed to recover for 12-14 days before the testing phase began.
Apparatus*:* A nonmatching-to-position (NMTP) spatial task was conducted using a T-maze. The maze was made of black-painted wood with sidewalls 12 cm high, two goal arms of equal length (55 cm) and a long arm with a 66 cm start position. The maze was elevated 80 cm from the floor. A manually operated black Plexiglas guillotine door served to separate the start box (SB) and the two goal boxes (GB) from the choice area. Broken pieces of Kellogg's Froot Loops® were used as positive reinforcers.

Training Phase*:* Prior to testing, the rats were handled for one hour per day for one-week. During this period, the rats were habituated and trained to eat on the maze by being allowed to move freely about the maze with food pieces distributed evenly throughout.
Testing Phase*:* The testing period was ten days in duration with twelve trials per day. Each NMTP trial consisted of a forced run (50% right arm and 50% left arm) followed immediately by a choice run. On the forced run trial, the rats were placed in the SB and the door to the SB was raised to allow entry to the choice area. In this case, one arm was blocked while one arm remained open. After the rat entered the forced GB, the door was lowered and it was allowed to consume the positive reinforcer.. The choice run was identical to the forced run except that both goal boxes remained open. Since alternation behavior was desired, if the subject entered the GB opposite to that of the previous forced run, the door was lowered and the rat received the positive reinforcement reward. If, however, the rat entered the same GB as the previous forced run, the door was lowered and the positive reinforcement was immediately removed. Each trial was approximately two minutes long: 30 seconds forced run, 60 seconds SB habituation, and 30 seconds choice run (Roland and Savage, 2007).
Histology*:* Following testing, each rat was deeply anesthetized and perfused through the heart with 250 mL of 0.9% saline followed by 500 mL of chilled 4% paraformaldehyde in 0.1M phosphate buffer (PB). After the skull was removed, the brain was post-fixed in 4% paraformaldehyde overnight at 4^{o}C. The brain was then transferred to a 30% sucrose solution in 0.1M PB, pH 7.4, for two days.

Following cryoprotection in sucrose, the brains were frozen over dry ice, sectioned (50μm) in the coronal plane on a cryostat and collected in 0.1M PB. Sections were washed (3x5 min) in 0.1 M PB and every fifth section was incubated in mouse anti-parvalbumin (anti-PV) antiserum (1:1,000 dilution) containing 1.0% normal donkey serum, 0.5% Triton X-100, and 0.1% sodium azide in 0.1M PB. Adjacent brain sections were incubated in goat anti-choline acetyltransferase (ChAT) antiserum (1:500 dilution) containing 1.0% normal donkey serum, 0.1% sodium azide in 0.1 M PB. Following incubation overnight at room temperature, the sections were washed (3x5 min) in 0.1M PB, then incubated for two hours at room temperature with either biotinylated donkey anti-mouse IgG (PV) or biotinylated donkey anti-goat IgG (Chat) secondary antibody diluted 1:200 in 0.1M PB with 1.0% normal donkey serum, 0.5% Triton X-100, and 0.1% sodium azide. Sections were then washed (3x5 min) in 0.1M PB, incubated in an avidin/biotin solution for two hours, washed (3x5 min) in 0.1M PB, and incubated in 0.06% nickel chloride enhanced with 0.05% 3,3' diaminobenzidine (DAB) for twenty minutes. Following incubation, 0.0005% hydrogen peroxide was added immediately to the nickel-DAB solution for ten minutes to allow full visualization of the cells. Finally, the sections were washed (6x5 min) in 0.1M PB. Free-floating tissue sections were mounted on gelatin-coated microscope slides, dehydrated in alcohol, cleared in xylene, and coverslipped using Permount. Histological sections were scanned using a microscope to assess the accuracy of the injection sites. Only rats with accurate targeting of the MSDB were included in the data analysis.

Associative Learning
Subjects*:* Male Sprague-Dawley rats (250-350 g, Sham, *n*=8, GAT, *n*=9, SAP, *n*=8) were tested for the acquisition of associative learning using a classical eye-blink conditioning paradigm. Animal husbandry for this set of rats was identical to that described above.
Surgical procedures*:* Rats were anesthetized with a 30 mg/kg ketamine/2.5 mg/kg xylazine intraperitoneal injection. Supplemental injections were provided as needed. When the rat was sufficiently anesthetized, two intracerebral guide cannulae were implanted near the MSDB complexes and anchored firmly to the skull with dental acrylic resin and two stainless steel screws. Infusions of 0.5-μL of either 192-IgG-saporin (0.2 mg/mL in artificial cerebrospinal fluid), orexin-saporin (200 ng/μL), or vehicle (artificial cerebrospinal fluid only) were then made into the MSDB region to produce a cholinergic or GABAergic lesion, or to serve as an injection control, respectively. The stereotaxic coordinates were: MS: +0.6mm and +1.5mm lateral from bregma, -6.6mm from brain surface and 15^{o} toward the midline; DB: +0.6mm and +0.5mm from bregma and -1.8mm from the brain surface. Microinjections were made using a 0.5 μL syringe at a rate of 0.1 μL/min. Following each injection, the syringe was kept in place for ten minutes to allow diffusion of the injected substance into the target area.

Following placement of the injection cannulae, four Teflon-coated stainless steel wires were implanted subcutaneously into the right upper eyelid. The tip of the wires were exposed, bent into a V-shape, and hooked onto the orbicularis oculi muscles. Of the four wires, two were used to record the differential electromyogram (EMG), and the remaining two were used to administer a mild periorbital shock. The other ends of the four wires, along with a ground wire attached to one of the anchoring screws on the skull, were soldered to the pins of a socket cemented to skull. Rats were allowed seven days of postoperative recovery.

Apparatus: Eye-blink conditioning was conducted in four modular operant test chambers. Each chamber was placed inside a sound-attenuating chest within a Faraday field. The floor grid (composed of eight stainless steel bars) and base pan of each chamber were washed thoroughly with soap and tap water and completely dried before each experiment. Stimulus presentations were controlled, and EMG data were collected simultaneously, using custom-written software (LabVIEW; National Instruments). The tone stimulus (2.8 kHz sine wave, 82 dB) was produced using a function generator and presented through a speaker mounted on the chamber. The periorbital shock (50 Hz square wave pulses) was delivered using a stimulus isolator. Shock intensity was adjusted daily for each rat to elicit a reliable and consistent eye-blink response. EMG activity from the eyelid muscle was amplified (1000x) using a differential AC amplifier, filtered (300-5000 Hz), digitized (10 kHz sampling rate), and stored to a computer for off-line analysis. Electronic leads were connected to a cable-commutator assembly mounted to the ceiling, which relayed EMG signals and delivered the US to the freely moving rat.

Training and Testing: Each rat was placed in the operant chamber two days prior to testing, with the headstage connected to the cable-commutator assembly, and allowed to habituate to this environmental set-up for one hour. Training consisted of six days of 100 trials, arranged into 3 sessions of 10 blocks each. Each session included 10 CS-US paired trials (trial block). The trials were delivered every 60 seconds and consisted of a five-second CS tone with a co-terminating two-second US shock pairing. Extinction was studied on the seventh day of training and consisted of five blocks of regular CS-US paired trials followed by five trials of only the CS tone.

Data Analysis

Data were analyzed with a 2-way repeated measures analysis of variance (ANOVA) or an unpaired Student's t-test (α=0.05). For the T-maze tests, the percentage of "correct" responses was calculated for all subjects of each treatment group (sham *vs* GAT) on each day of the 10 day testing period. A correct response was designated as a choice by the rat during the free choice phase of a forced test T-maze task in which the rat exhibited alternation behavior. These data were subjected to a 2-way repeated measures ANOVA (factors: treatment, day). For the eye-blink analysis, the frequency of the conditioned response (eye-blink) was based on the raw EMG signals for each trial. Trials with unstable baseline EMG activity during the pre-CS period or tone-induced startle EMG activity (immediately following the CS) were excluded from data analysis. Data from each session were subjected to a 2-way repeated measures ANOVA (factors: treatment, block number). Analysis of the first 4 blocks of session 3 was performed separately from blocks 5-10 (extinction period).

RESULTS

T-maze test

The mean percent correct responses of the sham-lesioned and GABAergic-lesioned (GAT) group of rats for the non-matching to position T-maze task is shown in Figure 1. The percentage of correct choices in the T-maze test, indicative of alternation behavior, was significantly lower in the GAT group compared to the sham-lesioned group on days 2-3 and 5-10 of the 10-day experimental period.

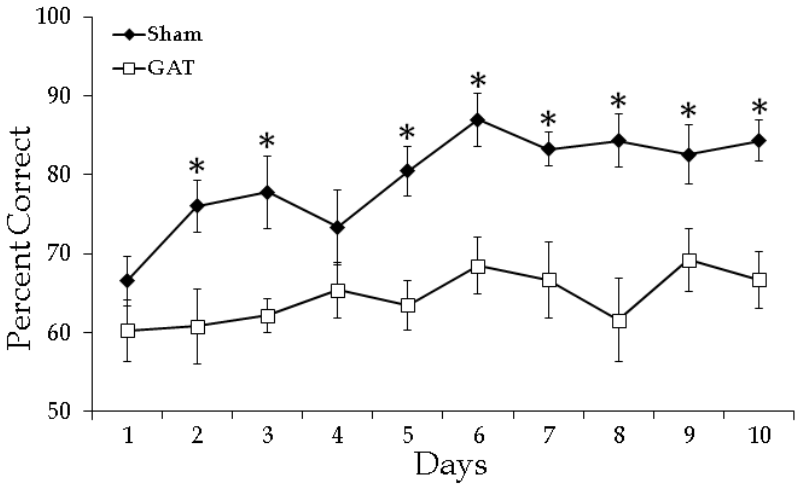

Figure 1. Percent correct responses (± standard error) during a T-maze spatial memory task for sham-lesioned (Sham) and GABAergic-lesioned (GAT) rats tested on each day of a 10-day experimental period. *p<0.05 between the sham and GAT groups. Sample sizes: Sham, n=9; GAT, n=13.

The percent of correct responses for all of the rats in the Sham and GABAergic-lesioned (GAT) groups were also calculated and compared using a Student's unpaired t-test (Figure 2). Similar to the ANOVA analysis, a significant decrease in the percentage of correct responses in the GAT-lesioned group compared to the sham-lesioned group was observed.

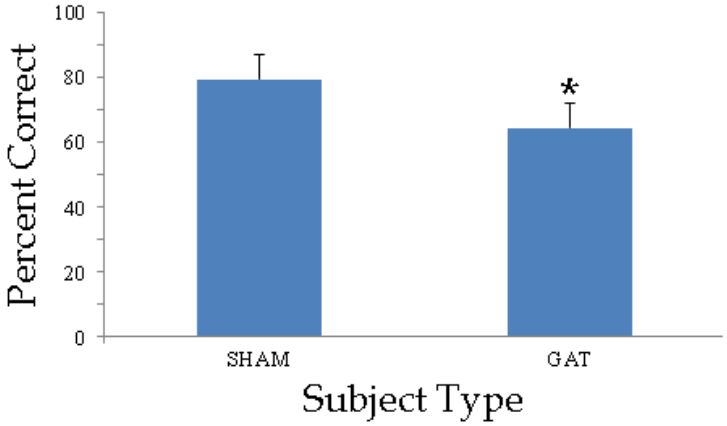

Figure 2. Comparison of the overall mean percentage correct choices (± standard error) for the sham-lesioned (Sham) and GABAergic-lesioned (GAT) rats (n=13) during a 10-day testing period. There was a significant decrease in the percentage of correct responses in the GAT *vs* the sham groups *p<0.05. Sample sizes: Sham, n=9; GAT, n=13.

Eye-blink test

The frequency of conditioned responses in the delay eye-blink test were similar between the sham-lesioned, cholinergic neuron-targeted 192-IgG-saporin-lesioned (GAT), and GABAergic neuron-targeted orexin-saporin-lesioned (SAP) groups of rats during the first two acquisition sessions (Figure 3). In addition, no differences between these three groups were observed during the first 4 blocks of session 3. However, during the extinction phase of the experiment, a significant decrease in the frequency of conditioned responses was seen in the SAP group relative to sham-lesioned controls in blocks 9 and 10, and a significant decrease was also observed in the GAT group (compared to Sham) in block 10. However, there were no significant differences between the SAP and GAT groups in block 10.

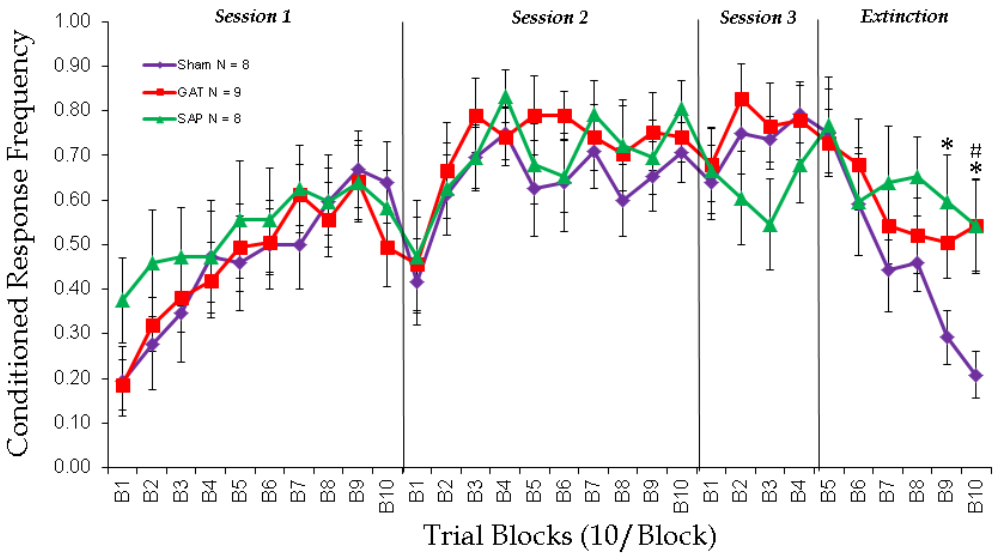

Figure 3. Mean frequency (± standard error) of the conditioned response (reflexive eye blink) for sham-lesioned (Sham), GABAergic neuron-lesioned (SAP) and cholinergic-lesioned (GAT) rats during 100 trials during the acquisition (sessions 1 and 2, session 3, blocks 1-4) and the extinction (session 3, blocks 5-10) periods of the delayed eye-blink test. *p<0.05, Sham vs GAT; #p<0.05, Sham vs SAP. Sample sizes: Sham, n=8; GAT, n=9, SAP, n=8.

DISCUSSION

In the present study, lesions specifically targeting MSDB GABAergic hippocampal neurons resulted in the loss of spatial memory function as assessed by the T-maze task, while lesions targeting both MSDB GABAergic and cholinergic hippocampal neurons resulted in the loss of associating learning function during the extinction phase of the delayed eye-blink test. These results support previous experiments using rodent models and provide further evidence for the importance of the MSDB region for learning and memory and potentially for Alzheimer's disease. Septohippocampal GABAergic medial septal neurons appear to be critical for spatial memory, in agreement with previous studies that have used similar experimental procedures. For example, Dwyer *et al*. (2007) concluded that non-cholinergic MSDB neurons (but not cholinergic MSDB neurons) were critical for spatial memory interference and the hippocampal theta rhythm. In addition, the most prominent non-cholinergic MSDB neurons were GABAergic septohippocampal neurons that, in combination with cholinergic inputs, comprise 80-90% of the septohippocampal pathway (Kiss *et al*., 1997). Therefore, the primary non-cholinergic MSDB neurons responsible for mediating spatial memory are thought to be GABAergic, consistent with the present study.

The present study also indicates that septohippocampal GABAergic and cholinergic medial septal neurons are critical for associative learning. Associative learning is a form of learning in which a new response becomes associated with a particular stimulus. In the case of eye-blink conditioning, a reflexive eye blink becomes associated with a periorbital eye shock (Weinberger, 2008). Similar to this study, selective cholinergic neuron lesions in the MSDB resulting from exposure to 192-IgG-saporin resulted in deficits in classical eye-blink conditioning, whereas selective GABAergic neuron lesions did not (Fontan-Lozano *et al*. 2005).

The potential link between classical eye-blink conditioning and Alzheimer's disease should be mentioned. Acetylcholine depletion due to MSDB cholinergic neuron lesions results in disturbances to the eye-blink response that are similar to those found in patients diagnosed with Alzheimer's disease (Bartus *et al*. 1982). The idea that cholinergic MSDB neurons are essential for the associative learning eye-blink conditioning task is therefore reasonable. During the early stages of Alzheimer's disease, cholinergic neurotransmission is impaired, and according to the cholinergic hypothesis, the debilitating

effects of the disease ultimately results from a severe reduction in the synthesis of acetylcholine thanks to the death of a high concentration of cholinergic neurons (Francis *et al*., 1999). However, the use of acetylcholinesterase inhibitors such as donepezil, galantamine, and rivastigmine, although effective at minimizing the depletion of brain acetylcholine levels in Alzheimer's patients, appears to only mildly delay the progress of the disease (Raschetti *et al*., 2007). Additional research needs to be done to further understand the effects of acetylcholine depletion on Alzheimer's disease and how this can be reversed.

Alzheimer's disease has been studied frequently with rodent models. Previous studies have found that rats with electrolytic lesions of the medial septum exhibit order memory deficits in spatial tasks involving 8-arm radial mazes (Kesner *et al*., 1986). Rats with large medial septal lesions, and thus large acetylcholine depletions in the hippocampus, displayed large memory deficits in completing an ordered task. However, rats with minor medial septal lesions, resulting in a small level of acetylcholine depletion, were only impaired during the beginning, but not the ending, components of the ordered task. These findings were compared with the symptoms of Alzheimer's disease and various similarities were found (Kesner *et al*., 1986). Subsequently, Kesner *et al*. (1989) compared performance on spatial tasks of college students and elderly individuals diagnosed with mild to moderate Alzheimer's with rats that received small to large lesions of the medial septum (MS), the dorsal hippocampal formation (DHF), or the nucleus basalis magnocellularis (NBM). Equivalent patterns of memory deficits were found between patients with Alzheimer's disease and the rats with the small or large MS or DHF lesions. However, rats with NBM lesions exhibited no deficits and therefore no similarities to the patients diagnosed with Alzheimer's. The symptoms of patients with Alzheimer's disease could therefore be mimicked by rats with MS and DHF lesions, providing further evidence that these regions are vital for learning and memory functions that are disrupted by this disease.

The hippocampus is the primary structure that has been implicated for mediating extinction behavior in eye-blink conditioning. Rabbits with a bilateral hippocampectomy exhibited a complete resistance to extinction of the eye-blink following acquisition of the response during eye-blink conditioning (Moyer *et al*., 1990). In the present study it was found that after the conditioned (periorbital eye shock) stimulus was removed from the experiment, the conditioned (reflexive eye blink) response was significantly decreased during the extinction period in rats with lesions in MSDB GABAergic or cholinergic septohippocampal neurons. Myers and Ermita (1998) hypothesized that cholinergic septohippocampal neurons were critical for regulating the amount of information stored in the hippocampus and influenced the extent of extinction behavior that occurred during eye-blink conditioning. However, the results presented here showed that rats with either cholinergic or GABAergic septohippocampal neuron lesions exhibited a sharp deficit in extinction behavior. Both groups of rats were less able to learn that the conditioned stimulus was no longer present. Therefore, both groups of lesioned rats continued to display the unconditioned response, while the sham (control) rats displayed a sharp decrease in reflexive eye-blink behavior after the conditioned stimulus was removed.

The role of MSDB GABAergic and cholinergic septohippocampal neurons in memory and learning need to be studied further. Although the present findings are generally consistent with previous studies aimed at understanding the importance of GABAergic and cholinergic neurons for spatial memory and associative learning, respectively, further research is needed to clearly delineate how these chemically defined pathways impact human disorders such as Alzheimer's disease, post-traumatic stress syndrome, and other anxiety disorders. In particular, further research is needed to determine if GABAergic septohippocampal MSDB neurons are critical for extinction behavior. Additional studies may lead to a better understanding of the underlying mechanisms through which the MSDB functions in learning and memory.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Jessica Roland and Dr. Kevin Pang for graciously allowing me to participate in their research in the Neurobehavioral laboratory at the Veterans Affairs Medical Center in East Orange, New Jersey and for providing their outstanding knowledge and guidance.

I would also like to thank Professor W. S. Klug for organizing this valuable research internship at The College of New Jersey and for providing his knowledge and assistance throughout the process.

I would also like to thank Professor Jeffery Erickson for providing me with his expertise and guidance and helping me edit, amend, and finalize this thesis paper.