TURNING BACK THE CLOCK: CGP55845 REVERSES MILD COGNITIVE IMPAIRMENT IN FISCHER 344 RATS

A Senior Scholars Thesis

by

JEFFREY DANIEL MAYSE

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Turning Back the Clock: CGP55845 Reverses Mild Cognitive Impairment in Fischer 344 Rats. (April 2009)

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Mild cognitive impairment (MCI) is a rapidly growing problem, especially in light of the vast increase in the population of the elderly. MCI is characterized by a lack of frank neural loss, unlike Alzheimer's disease, yet the patient has behavioral impairments in memory, olfactory functioning, and information processing abilities. This study examines the efficacy of CGP55845, a $GABA_B$ receptor antagonist, at reversing the effects of odor discrimination deficits in male aged (n = 8 impaired, n = 10 unimpaired) and young (n = 10) F344 rats. Animals were given CGP55845 40 minutes prior to testing in the odor discrimination task. This task is particularly useful for pharmacological studies, since it allows for a within subjects design. Although only a subset of aged rats were impaired on saline trials (trials-to-criterion with saline: aged-impaired, 8.4833; aged-unimpaired, 18.023; control young, 10.15 SD 3.22), those aged-impaired rats showed significant improvement in the task when injected with the drug compared to saline. Performance deficits were not due to inability to detect odors. This

study illustrates that CGP55845 is effective at treating age-related cognitive deficits and supports the hypothesis that the GABAergic system is involved in age-related changes in learning and memory.

DEDICATION

This thesis is dedicated to my parents for their unending support, advice, and guidance. Without them, I would not have the ambition or the dedication to complete what I have so far and especially this research. Also, I would like to dedicate this thesis to my brothers – Chris, Jon, and Richard – for always keeping me grounded, giving me something to work for, and (in Jon and Richard's cases) pushing me to stay ahead.

ACKNOWLEDGMENTS

First of all, I must acknowledge all of my fellow undergraduate researchers for their help in writing, editing, and revising this thesis. Also, Rebecca Simmons deserves special credit for her invaluable help in running the behavioral task – thank you for not only helping me, but teaching me all the various tricks and methods I needed to be effective. I would like to recognize Candi LaSarge-Jendro's guidance, help, and patience. Without you, Candi, I would never have hoped to complete this thesis. I would like to acknowledge Nick Simon and Ian Mendez' general help and guidance. All of my fellow undergraduates in Bizon-Setlow Lab deserve credit for assisting with care and feeding, behavioral tasks, and keeping things interesting at all times. Finally, and most importantly, Drs. Bizon and Setlow deserve my acknowledgement and respect for guiding me to this point academically. Without both of you, I would not be where I am right now.

NOMENCLATURE

ACh	Acetylcholine
AChe	Acetylcholinesterase
AD	Alzheimer's disease
BF	Basal Forebrain
CBF	Cholinergic Basal Forebrain
GABA	γ-amino butyric acid
GBF	GABAergic Basal Forebrain
i.p.	Intraperitoneal
MCI	Mild Cognitive Impairment

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CHAPTER I

INTRODUCTION

Age-related cognitive disorders such as mild cognitive impairment (MCI) represent an increasingly dire problem in modern society. Americans aged sixty-five years or older numbered thirty-five million as of the 2000 U.S. Census, comprising slightly over twelve percent of the total United States population. Additionally, with the baby-boom generation nearing retirement, the elderly population in America will experience its own boom in the next ten years. The increased survival of aged individuals imposes a heavy social and financial toll on society, as the amount of people susceptible to MCI and other age-related cognitive disorders continues to rise. MCI is characterized by loss of neuron density in several brain regions and by a progressive decline in cognitive functioning, working memory (memory for newly acquired information and the ability to distinguish this information from previously learned information), episodic memory (memory for people, places, and events), reference memory (memory for information which is constant over a specific time span) and memory retrieval (Whitwell et al. 2007). However, unlike neurodegenerative disorders such as Alzheimer's disease (AD), MCI does not require frank neural loss as a diagnosis criterion. One critical neuroanatomical region which contributes to MCI is the basal forebrain (located in the medial septum), specifically the nucleus basalis of Meynert and the diagonal band of Broca (Whitwell et

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This thesis follows the style of Psychopharmacology.

al., 2007). This region is implicated in attention, learning, and memory and is densely populated with cholinergic and GABAergic cell bodies (Whitwell et al., 2007). Acetylcholine, one of the most diffuse modulatory neurotransmitters in the central nervous system, is generally excitatory. Cholinergic cell bodies in the basal forebrain project to the CA1, CA3, and dentate gyrus regions of the hippocampus, providing modulatory innervation to the limbic system, a region integral for the formation and retrieval of memories (Lei, S. and McBain, C., 2003; Son, J. and Winzer-Serhan, U., 2008). γ-amino butyric acid, or GABA, is an inhibitory neurotransmitter that also projects from the BF to the limbic system, inhibiting neurons in this region. In the septalhippocampal pathway, GABA assists with cognition and memory formation (Mufson et al., 2003; Son, J. and Winzer-Serhan, U., 2008). Together, the GABA-ACh interactions in the limbic system play a crucial role in the formation of and retrieval of memories. A marked loss in neuron density and size (but not overall number per se) in both the cholinergic and GABAergic neuron populations in the basal forebrain is observed in MCI (Whitwell et al. 2007). These neuroanatomical changes are hypothesized to contribute many of the symptoms of MCI, including memory loss and cognitive decline.

Pharmacologically reversing cognitive impairment

CGP55845, a $GABA_B$ receptor antagonist, and Donepezil (trade name Aricept), an acetylcholinesterase (AChe) inhibitor, have been shown previously to enhance cognition in aged-impaired rats when administered separately (Hernandez et al. 2006, LaSarge, et

al. 2007). The mechanism of action for CGP55845 involves decreasing the GABAergic tone in the BF and the structures it projects to, most prominently by preventing the action of GABA at the synapse by occupying the $GABA_B$ receptor on the postsynaptic neuron. $GABA_B$ receptors are metabotropic (that is, coupled to an internal G-protein complex) and produce many long-lasting changes in neurons, generally through activation of the $G_{\alpha i}$ protein complex, which results in the inhibition of adenylyl cyclase and decrease in cyclic adenosine monophosphate (a second-messenger) production. Donepezil enhances cognition by increasing the overall levels of ACh in the synapse via inhibition of AChE, the primary enzyme involved in destruction of ACh. AChE is a synaptic enzyme which decomposes ACh into acetate and choline, which are then taken back up into the presynaptic neuron and re-synthesized into ACh. By inhibiting the function of AChE, Donepezil increases the time ACh spends in the synapse, therefore increasing the effective concentration of ACh and increasing its duration of action.

The basal forebrain and MCI

The BF and the limbic system, notably the hippocampus, are linked via a bundle of neural fibers called the fimbria-fornix. The fimbria-fornix, BF, and hippocampus form part of the septalhippocampal circuit (Packard and McGaugh, 1992). The BF receives input from the hippocampus and sends information back to the hippocampus through the fornix. In MCI, the BF is diminished in volume and neuron density, and, as such, the hippocampus receives diminished input from the BF. However, selective damage to cholinergic basal forebrain neurons has not been shown to be sufficient for causing the

deficits seen in MCI (Baxter et al., 1995; Frick et al., 2004). Similarly, selective lesions of GABAergic basal forebrain neurons have not been shown to cause deficits of the severity observed in MCI (Pang et al., 2001). However, lesions of both the GABAergic and cholinergic outputs of the basal forebrain have been shown to elicit severe deficits in cognition and memory formation (Pang and Nocera, 1999; Pang et al., 2001; Yoder and Pang, 2005). Also, several studies have implicated BF degeneration in cognitive dysfunction and learning impairments (Eichenbaum and Mathews, 1989; Packard and McGaugh, 1992). This dysfunction may be tested through an odor discrimination task in which rats learn to distinguish between a reward-paired odor and a non-reward paired odor, and through the Morris Water Maze task, a test of spatial memory (Eichenbaum and Mathews 1989, LaSarge et al. 2007).

Research aims

This study examines the efficacy of CGP55845 and donepezil at reversing MCI. This aim has two benefits: first, the clinical applications are immediately evident. The results of this study could used to justify the implementation of human drug trials for CGP55845 in treating MCI and donepezil for treating non-Alzheimer's dementia. Secondly, we hypothesize that CGP55845 and donepezil both modify the functioning of the BF. Therefore, simultaneously decreasing the GABAergic tone and increasing the cholinergic tone of the BF and the structures it projects to should reverse the cognitive deficits observed in MCI. Observation of this effect strengthens the hypothesis that BF degeneration contributes significantly to MCI. Understanding the role of the BF in MCI

and other age-related disorders may potentially lead to a clearer understanding of what causes these disorders, how to treat them, and how to prevent them. Future benefits of this study will include possible clinical relevance and application of CGP55845 and donepezil and a greater understanding of the mechanisms, both on a systems-level and a molecular-level, which contribute to MCI and other age-related disorders.

CHAPTER II

METHODS

Young (Exp. 1: n=10; aged 6 months; Exp 2: n=4; aged 6 months; Exp 3: n=20; aged 6 months) and aged (Exp 1: n=17; aged 24 months; Exp 22: n=4; aged 24 months) male Fischer 344 rats were obtained from the National Institute of Aging. Rats were housed individually in the Psychology Department vivarium (Texas A&M University, College Station, TX). The vivarium was maintained at 25° Celsius with a 12:12h light/dark cycle. All testing occurred during the light cycle. Rats were trained on a two-choice odor discrimination task and the Morris water maze task. The rats were food deprived to 85% of their free-feeding weight during the odor discrimination task and given ad libidum access to water. All animals were screened daily for health problems and sentinel rats were kept in the vivarium and further screened for diseases.

Water maze testing

Apparatus

Young and aged rats were assessed for spatial learning abilities on the Morris water maze task using a protocol modified from Gallagher et al. The maze consisted of a circular tank (diameter 183 cm, wall height 58 cm) painted white and filled with water (27 ◦C) made opaque with the addition of non-toxic white tempera paint. A retractable white escape platform (12 cm diameter, HVS Image, UK) was submerged 2 cm below the water's surface near the center of the southwest quadrant of the maze. The maze was

surrounded by black curtains, to which were affixed large white geometric designs that provided extramaze cues. Data were analyzed using a computer-based video tracking system (Water 2020, HVS Image, UK).

Procedure

Rats received three trials a day over eight consecutive days with a 60 s intertrial interval. On each training trial, rats were placed in the water facing the wall and permitted to swim until finding the escape platform or until 90 s elapsed, at which time they were guided to the platform by the experimenter. Rats remained on the platform for 30 s before removal from the maze and the start of the intertrial interval. The starting position for each trial varied pseudorandomly among four equally spaced positions around the perimeter of the maze (north, south, east, or west). Every sixth trial was a probe trial on which the escape platform was retracted to the bottom of the tank for the first 30 s of the 90 s trial. Training and probe trials assessed spatial acquisition and search strategy, respectively. To assess rats' sensorimotor abilities and motivation to escape the water independent of spatial learning ability, rats received one session with six trials of cue training after the last day of spatial training. In this session, rats were trained to escape to a visible black platform that protruded 2 cm above the water surface and that was moved to a different maze quadrant on each trial. On each trial, rats were given 30 s to reach the platform and were allowed to remain there briefly before a 30 s intertrial interval. In the hidden platform task, accuracy of performance was assessed using two proximity measurements. A *cumulative search error* measurement was computed from training

trials, and a *learning index score* was calculated from probe trials. For both measures, the distance of the rat from the platform was sampled 10 times/s during each trial and these distances were averaged into 1 s bins. Cumulative search error is the sum of these 1 s averages across training trials minus the optimal path from the start position to platform location. The spatial learning index score was calculated from the average proximity (cumulative search error divided by the length of the probe trial) on the second, third and fourth probe trials. Scores from these probe trials were weighted and summed to provide an overall measure of spatial learning ability. Lower spatial learning indices indicate a more accurate search. More traditional measurements of escape latency and swim path length during both hidden and visible platform training were also recorded. A one-way ANOVA was used to compare spatial learning indices from probe trials and mean platform latency averaged across cue training trials. In all cases, $p < 0.05$ was considered significant.

Classification of impairment

In this study, rats are referred to as "spatially unimpaired" or "spatially impaired". Spatially impaired rats had learning indices outside the range of young rat performance (>250), whereas spatially unimpaired rats had indices within the range of young rats (<250). A two-way ANOVA (spatial learning group × trial block) confirmed that the spatially impaired and spatially unimpaired subgroups were different with respect to spatial learning.

Odor discrimination

These methods are identical to those described in LaSarge et al.

Apparatus

The discrimination task was conducted in an open translucent plastic box (measurements 49 cm x 33 cm x 28 cm). The front and side walls were covered with black paper affixed to the exterior surface of the box so as to make them opaque. A video camera mounted externally and connected to a TV monitor and a DVD recorder recorded the trials and allowed for observation through the remaining translucent wall without disturbing the animals. An opaque white Plexiglas square (33 cm x 28 cm) separated the test chamber (33 cm) from the start compartment (16 cm). Two terra cotta flower pots (11 cm diameter at top, 10 cm high) were placed side-by-side against the back (translucent) wall and affixed to the box floor with Velcro pads.

Procedure

Shaping

Rats were first food-restricted to 85% of their free-feeding weight over 5 days. Pots identical to those in the test apparatus were filled with clean home cage bedding and placed in the rats' home cages with two quartered Froot Loops (Kellogg's, Battle Creek, MI) buried in the bedding in each pot. The pots were left in the cage overnight to reduce neophobia for the food reward and the pots and to prime the rats for digging to retrieve a reward. Shaping took place in the test apparatus and consisted of training the rats to dig

in two pots, each filled with clean home cage bedding and a food reward (1/4 of a Froot Loop) buried at varying depths and with or without bedding over it. On each trial, the rat was placed in the start compartment and the barrier was raised to allow access to the test compartment. Rats were considered shaped when they would reliably retrieve both rewards in less than one minute.

Odor discrimination

On the day following shaping, rats began discrimination problems. For each problem, only one pot contains a reward. Crushed Froot Loops were sprinkled over both pots so as to disguise the scent of the reward. The position of the reward pot was varied pseudorandomly across trials. For the first four trials of every new discrimination problem, the rats were allowed to dig in both pots until the obtained the reward. On these self-correcting trials, only the rats' first choice was scored. On every trial thereafter, rats were removed from the test chamber after their initial dig (either correct or incorrect). A dig was scored if the rat displaced the digging medium with either its paws or nose. For the odor discrimination problems, a small drop of odorant (approx. 20 μl) was applied to the rim of each pot, and the reward was consistently associated with one odor. The odorants were oils obtained from The Bath Junkie and The Body Shop. On non-olfactory discrimination problems, rats were trained to discriminate between two different substances (digging media) filling the pots. Reward was consistently associated with only one media (see fig. 1). The following discrimination pairs were used for each rat: odor: rose and citrus, hazelnut and peppermint; digging medium:

Styrofoam and sequins, shredded latex gloves and shredded tissue paper. The positive and negative stimulus in each pair of discriminanda and the sequence of discrimination problems were randomized across rats, although each rat received alternating odor- and medium discrimination problems (either odor-medium-odor-medium or medium-odor-medium-odor). Rats were considered to have acquired a discrimination problem when they achieved six consecutive choices of the correct (baited) pot, after which they immediately began the next problem in the sequence. Both the numbers of trials and the number of errors to criterion were recorded and used as measures of performance.

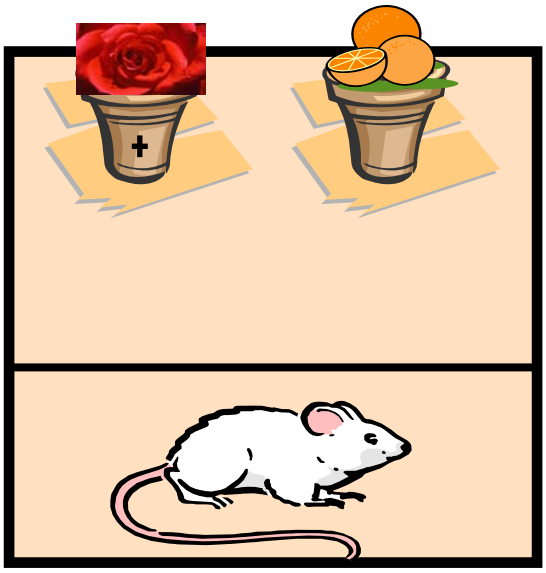

Figure 1. – A diagram of the odor discrimination apparatus. The pot with the rose odorant contains the reward, indicated by the "+" and the pot with the citrus odorant contains no reward.

Pharmaceutical injections

All injections were intraperitoneal (i.p.). After the rats completed all four simple discrimination problems, drug trials began. Rats were injected with saline or CGP55845 (.01 or .1 mg/kg). After injection, rats were left for 40 minutes to allow acclimation to the room and to allow time for the injected drug to effectively circulate. After 40 minutes, rats were then tested with two more discrimination problems and their scores on each of the discriminations were recorded. The odor pairs used for these discriminations were sandalwood vs. cucumber and red apple vs. thyme. Rats were given at least 48 hours for the drug to completely wash out of their systems before injection and testing with other drugs was performed.

Odor detection threshold testing

After completion of discrimination testing, rats were tested for their ability to detect and respond to decreasing concentrations of odorants. The first discrimination problem in this task uses a new odor pair at full strength (20 µl) versus 20 µl of mineral oil applied directly to the rims of two pots filled with clean home cage bedding. The food reward was in the pot with the odorant, and rats were trained until they reached criterion. As in the odor discrimination problems, trials and errors to criterion were recorded. This data was analyzed using separate one-way ANOVAs. Testing continued with three further discrimination problems using decreasing concentrations of the same odorant as the first problem (1:10, 1:100, and 1:1000 in mineral oil) versus mineral oil alone. New pairs of pots were used for each problem. Rats were given 16 trials at each dilution, and the

number of correct choices out of 16 was recorded. A group x odorant concentration repeated measures ANOVA was used to compare performance of spatial learning groups and $p<0.05$ was considered significant.

Correlational analyses

Correlations were performed with an $n = 20$ (Exp 1: $n = 10$ aged, $n = 10$ young, Exp 3: $n = 10$ lesion, $n = 10$ sham) except experiment two ($n = 4$ young, $n = 4$ aged). To assess test-retest reliability of performance on the odor discrimination task, Pearson's correlations were performed on both trials and errors to criterion across the three odor discrimination problems in aged rats. To directly compare performance on spatial learning and odor discrimination tasks, a Pearson's partial correlation was conducted on corrected data (spatial learning index on the water maze task and trials and errors to criterion on the odor discrimination task). For each rat in experiment three, performance values were zeroed to their respective group means (the group mean was subtracted from their individual value). This controlled for the lesion differences present across groups. Correlations were performed on these corrected data. Performance measures (errors and trials to criterion) of spatial learning groups were compared by two-way repeated measures ANOVAs (discrimination problem x spatial learning group). Repeated measures ANOVAs were also used to compare trials and errors to criterion across odor and medium discrimination problems (to assess relative difficulty). In all cases, $p<0.05$ was considered significant.

CHAPTER III

RESULTS

Odor discrimination

The trials-to-criterion for the two odor discrimination problems when rats were injected with saline were averaged. The young (n=10) had an average trials-to-criterion of 10.15 and a standard deviation of 3.22. The rats labeled as aged odor-unimpaired were those that had an average of trials-to-criterion below the average of the young plus one standard deviation, 13.35; those aged rats with an average of saline trials-to-criterion above 13.35 were labeled as aged odor-impaired. The impairment groups were significantly different from each other ($F(2,26) = 21.923$, $p < 0.001$) with the odor-impaired age group (n=7, average = 8.4833) significantly different from both the young (n=10, average = 10.150) and odor-unimpaired group (n=10, average = 18.023) , post hoc of $p < 0.001$, which were not significantly different from each other.

Odor detection threshold testing

Odor threshold testing was used to both ensure that none of the subjects were anosmic and to eliminate the possibility that the effect of drug administration was simply an improvement in olfactory rather than cognitive ability. There was no significant difference between the young and aged groups during anosmia testing when rats were injected with saline ($F (1,8) = 4.325$, n.s.) or CGP55845 ($F (1,8) = 2.760$, n.s.) and thus no rats exhibited general smelling impairments. Thus, age groups were grouped together

to compare saline to CGP55845 during anosmia testing. There were no difference between saline or CGP55845 ($F(1,18) = 0.941$, n.s.), demonstrating that the CGP55845 did not affect rats' ability to detect the odor, and that the group differences seen were due to the ability to perform an odor discrimination. Additionally, there was a significant difference in the dilution of the odorant ($F(2,36) = 3.404$, $p<0.05$; seen in Figure 2); and although rats did in fact get less of the 1:1000 dilution problems correct compared to the 1:100 dilution ($p < 0.05$), all rats still performed above chance level indicating that they could indeed smell the odorant.

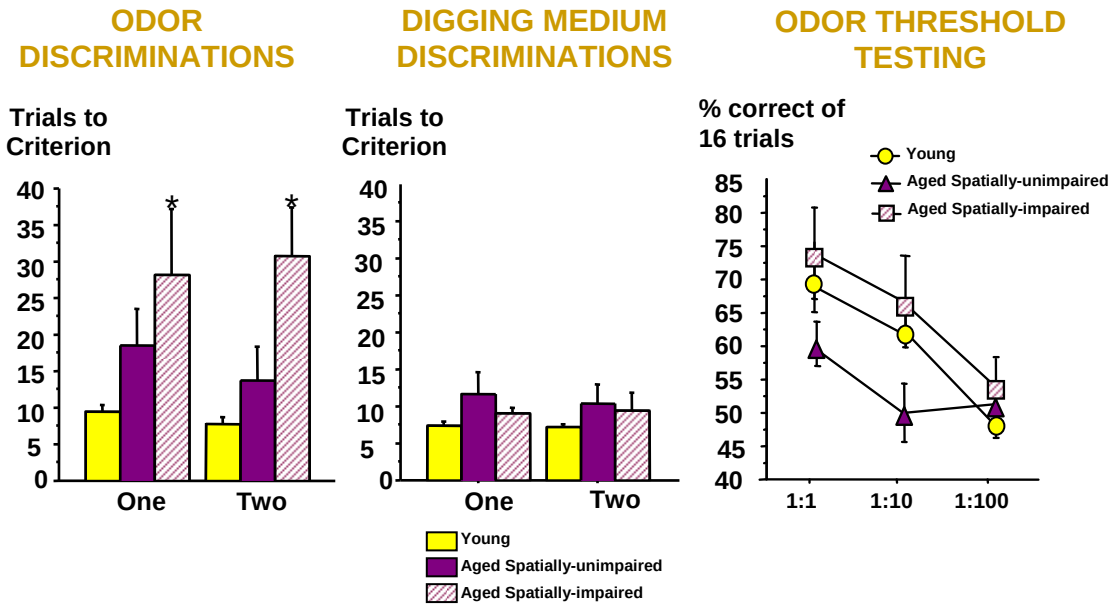

Figure 2. Left hand graph: Aged-impaired rats (light purple) have significantly more trials to criterion than aged-unimpaired (dark purple) or young control (yellow) rats, indicating impairment in making odor discriminations. Middle-graph: All three cohorts required similar trials to criterion in a medium-discrimination task, indicating discrimination abilities are present. Right-hand Graph: All three cohorts responded similarly in an anosmia test, indicating equivalent odor-sensing abilities for each group.

Pharmacological testing

Each of the aged subjects was tested on their odor discrimination learning ability after injection with either saline or the CGP55845 at 0.01 or 0.1 mg/kg doses. For CGP55845 testing, the aged rats were sub-grouped by cognitive status into young, odor-unimpaired aged, and odor-impaired aged subjects. A repeated measures ANOVA revealed a significant interaction between the cognitive status and dose of CGP55845 ($F(4,48) = 3.263$, $p<0.05$), as well as a main effect of cognitive status ($F(2,24) = 4.066$, $p<0.05$). Post hoc tests revealed a significant difference between odor-unimpaired and the odor-impaired group, as well as a significant difference between the saline injection and the 0.01 dose of CGP55845 (p's<0.05). However, there was no difference between the 0.01 and 0.1 dose of CGP55845, signifying the low dose as effective at ameliorating cognitive impairment in the odor-impaired group (p=n.s.). Furthermore, there was a clear improvement observed in the performance as measured by trials-to-criterion and errors-to-criterion of aged odor-impaired subjects after administration of both .01 and .1 mg/kg doses of CGP5584, while the performance of their young and aged unimpaired cohorts was not significantly affected by administration of CGP5584. These results were also observed when using errors-to-criterion for both the interaction between dose and cognitive status ($F(4,48) = 3.680$, $p < 0.05$), as well as the main effect of cognitive status ($F(2,24) = 6.458$, $p< 0.01$); post hoc tests also showed a significant difference between odor-impaired and odor-unimpaired errors-to-criterion and a significant difference between saline and 0.01 mg/kg of CGP55845 (p's < 0.05).

CHAPTER IV

SUMMARY AND CONCLUSIONS

The current study examined the efficacy of CGP55845 administrations in reversing cognitive deficits due to MCI. First, the study established a strong correlation between two hippocampus-dependent tasks, the Morris Water Maze and a two-choice odor discrimination task. This allows for the classification of rats into aged-impaired and aged-unimpaired, where impairment refers to dysfunction in the Morris Water maze and predicts performance in a two-choice odor discrimination task. Secondly, the current study established that aged-impaired rats are impaired significantly when compared to aged-unimpaired and young controls in a two-choice odor discrimination task. This effect was not due to an inability to form discriminations or to an inability to detect odors. Third, the current study established that administration of CGP55845 would improve performance of the aged-impaired cohort to the level of the young and aged-unimpaired cohorts in the odor discrimination task. MCI as demonstrated in this experiment and others results in significant impairment in tasks requiring intact cognitive function, working memory, reference memory, and episodic memory (Eichenbaum and Mathews 1989, LaSarge, et al. 2007, Whitwell et al. 2007). Furthermore, this impairment correlates significantly with reduced cholinergic input to the hippocampus from the basal forebrain (Whitwell et al. 2007, Zhang et al. 2006). However, this impairment cannot be reproduced by selective cholinergic lesions of the basal forebrain alone, nor can it be reproduced by selective GABAergic lesions of the

basal forebrain alone (Baxter et al. 1995, Frick et al. 2004, Pang et al. 2001). Lesions of both the GABAergic and cholinergic neurons in the BF will reproduce these deficits, however, implicating an interaction between the BF GABAergic, BF cholinergic, and hippocampal neurons (Pang and Nocera, 1999; Pang et al. 2001; Yoder and Pang, 2005). In fact, such a modulatory circuit has been identified and studied previously (Manseau, et al. 2005) and is outlined below (See Fig. 3):

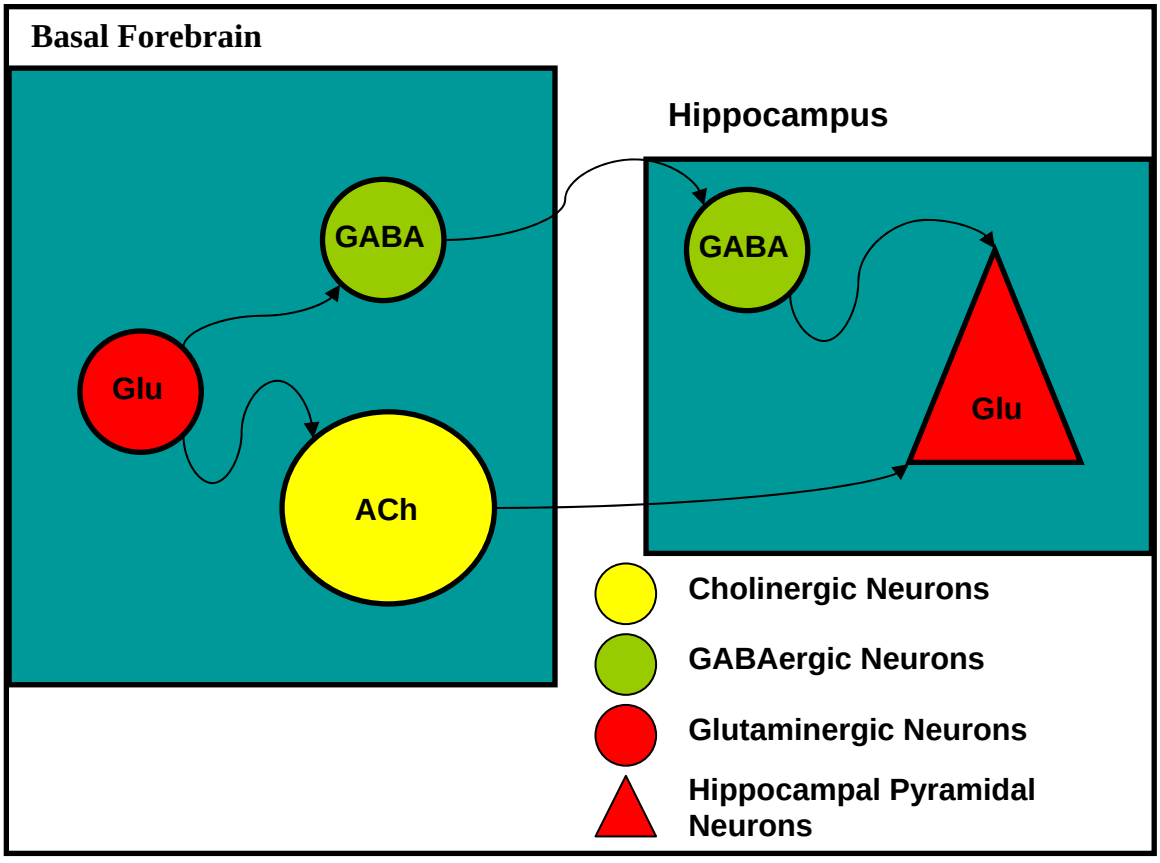

Figure 3. A simplified neuron circuit diagram of the BF ACh/GABA and hippocampus interactions. Both ACh and GABA neurons in the BF are stimulated by glutamate, an excitatory neurotransmitter. This results in the BF GABAergic neurons inhibiting hippocampal GABAergic neurons. The net effect of this Glu-GABA-GABA-Pyramidal neuron circuit is disinhibition of the hippocampal pyramidal neurons. The BF ACh neurons are then free to excite the hippocampal pyramidal neurons maximally. Adapted from Manseau et al. 2005.

Finally, the current experiments show that pharmacological reversal of MCI is possible with CGP55845, a $GABA_B$ receptor antagonist. This result may seem contradictory, as selective lesions of GABAergic BF neurons are not sufficient to produce the deficits observed in MCI (Pang et al. 2001). However, it should be noted that in MCI, degeneration of cholinergic neurons in the BF has progressed, albeit in the absence of frank neural loss (Whitwell et al. 2007). Furthermore, aged Fischer 344 rats have been shown to have an increased effect of GABA on single neurons *in vitro* (Griffith and Murchison 1995). Combined, these two effects may result in increased inhibition of and decreased stimulation of hippocampal pyramidal neurons, resulting in a net decrease in activation. To speculate further, this hypothetical decrease in hippocampal pyramidal neuron activation would contribute significantly many of the cognitive and memory deficits observed in MCI. CGP55845 potentially alleviates this effect by blocking the $GABA_B$ receptor type, resulting in a net decrease in inhibition of GABAergic target neurons. This net decrease in inhibition may allow the damaged BF cholinergic neurons in the aged-impaired population to more effectively stimulate hippocampal pyramidal neurons. Compounding the fact that cholinergic neurons degenerate over time, evidence that post-synaptic receptors for acetylcholine, namely the M1-subclass of cholinergic metabotropic receptors, function less effectively in two rat strains, Fischer 344s and Long-Evans rats has been recently published (Zhang et al. 2007). This effect has been observed in human prefrontal cortex, which may contribute to the cognitive deficits observed in MCI (Zhang et al. 2007).

Future research aims

Direct pharmacological applications of this study are likely to involve coupling treatment with a CGP55845-like drug with a drug which affects the cholinergic system to utilize the ACh-GABA circuitry in the BF and hippocampus. In fact, Donepezil, a cholinesterase inhibitor, would be a likely candidate. The method of action of Donepezil involves inhibiting the enzymes which degrade ACh in the synapse, referred to as acetylcholinesterases (Hernandez et al. 2006). By inhibiting the action of these enzymes, Donepezil increases the duration of action and effectiveness of ACh in the synapse (Hernandez et al. 2006). This alleviates in part the diminished size and density of cholinergic neurons in the BF and the potential decreased efficiency of the post-synaptic M1 receptors (Hernandez et al. 2006; Zhang et al. 2007). Co-administration of a drug similar to CGP55845 and Donepezil would not only increase the effectiveness of both drugs, but would also allow for lower doses of Donepezil to be used. Donepezil has been shown previously in clinical use, and also in laboratory settings, to be neurotoxic at high doses and patients quickly develop a tolerance to the drug, necessitating increasingly higher doses (Hernandez et al. 2006). By administering CGP55845 with Donepezil, both drugs could be used for longer periods of time, extending the treatment window for MCI. Future experiment in this vein will examine the efficacy of co-administering sub-threshold doses of CGP55845 and Donepezil in reversing the cognitive effects of MCI.

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