A NOVEL SENSITIVE METHOD OF DETECTING MNEMONIC DECLINE IN MOUSE MODEL OF ALZHEIMER'S DISEASE

A Junior Scholars Thesis

by

REBECCA KAYE SIMMONS

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

UNDERGRADUATE RESEARCH SCHOLAR

April 2009

Major: Psychology

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Approved by:

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

A Novel Sensitive Method of Detecting Mnemonic Decline in Mouse Models of Alzheimer's Disease. (April 2009)

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Deficits in transferring generalized information of past learning to new problems are related to mild hippocampal atrophy in the elderly and appear to be an early marker of age-related cognitive decline. This inability to transfer information could be used as an early diagnostic tool for such decline, and a rodent model that is sensitive to this deficit could be valuable in the search for therapies to prevent or reverse such impairments. The goal of this study is to develop an analogous animal model to be used for assessing cognitive abilities in a variety of transgenic mouse models of Alzheimer's disease (AD). Such a rodent model will be valuable for identifying specific biomarkers associated with early age-related cognitive decline and should prove useful for developing and testing therapies directed at preventing or reversing such impairments.

Three month old APP+PS1 mice were not impaired in initial discrimination learning or on the ability to ransfer this learned information to the altered context. In contrast, at 12 months of age, APP+PS1 mice learned the initial concurrent discriminations on par with

NTgs but were impaired when required to "transfer" this learning into a new configuration/context. There were no differences in Morris water maze performance between the APP+Ps1 and NTgs at 12 months of age. These data are the first to demonstrate deficits associated with reconfiguration of stimuli or transfer learning thought to be dependent on the hippocampal formation is impaired in a mouse model of AD. Moreover, these data suggest that this deficit may precede or is more sensitive in detecting deficits than water maze in this model.

DEDICATION

I would like to dedicate this thesis to Matthew Dillion. Thank you for helping me realize what I want to do with my life and for teaching me so much.

ACKNOWLEDGMENTS

I would like to acknowledge Karienn Montgomery for allowing me to work on this project and for being there every step of the way. It has truly been amazing working with you for these past few years.

Dr. Jennifer Bizon and Dr. Barry Setlow for introducing me to the neuroscience field and helping me get started and also for being there to help answer any questions and for teaching me how to be a better researcher.

All the graduate students and undergraduates in the lab, thank you for all your help, advice, and teachings. Work would not be the same without you.

NOMENCLATURE

AD	Alzheimer's Disease
APP	Amyloid Precursor Protein

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

According to the National Institute of Aging, Alzheimer's disease affects as many as 4.5 million Americans and the numbers are continuing to climb[1]. Current research suggests that Alzheimer's disease is caused by a build up of β-amyloid protein caused by a mutation in the of β-amyloid precursor protein (APP). This is commonly referred to as the amyloid hypothesis[2]. However, the amyloid hypothesis is lacking in detail and there is evidence to suggest that observations do not fit easily with the hypothesis[2]. Despite current efforts and research to determine the neurobiological causes of this disease, sensitive behavioral assays that identify individuals at early preclinical stages remain elusive.

Functional changes in the medial temporal lobe system and the hippocampus is among the earliest associated with AD[3,4,5]. One example of early neuropsychological change is delayed paragraph recall, which has about a 90% accuracy rate in distinguishing which among a group of non-demented elderly will progress into cognitive decline[4,6]. Previous animal models have suggested that some memory tasks are sensitive to hippocampal region damage[7]. However, current cognitive screens in both humans and rodents are not

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This thesis follows the style of *Journal of Geriatric Psychiatry and Neurology.*

sufficiency sensitive or selective to detect functional decline at early ages.

If hippocampal atrophy is indeed a predictor of cognitive decline and AD, then it ought to be possible to estimate hippocampal atrophy by behavior alone. Myers, Gluek, and colleagues have developed a computational model of hippocampal function that appears sensitive to age related dysfunction. This computational model has suggested that the hippocampus is critically involved in encoding new information during learning so as to support subsequent transfer when familiar information is presented in novel recombination[7,8]. Recently, a computer based task has been used to test some of these predictions in humans[9]. The task involves nondemented patients on a series of eight concurrent discriminations with each pair consisting of two objects that vary in color or two colors that vary in shape. Therefore, each pair consists of a relevant and irrelevant feature. Patients discriminate between a pair of figures presented on a computer screen until they reach criterion. After reaching criterion, recombinations of the shape and color featured in the concurrent learning phase are presented. During recombination, the irrelevant feature is changed while the same feature still predicted the correct choice. For example, a patient would be presented with two triangles, one yellow and one red during the concurrent learning phase. The patient would learn that the red circle is positive. During the transfer phase, the triangles, which are the irrelevant feature, would be changed to circles however the red circle would still be correct. Thus, a set of response rules that emphasized the relevant features in the learning phase would perfectly predict the reward stimuli in the transfer phase.

Results showed that patients with or without hippocampal atrophy learned the initial discriminations however patients with mild hippocampal atrophy had difficulty during the transfer phase of the task. This suggests that patients with mild hippocampal atrophy have trouble combining learned associations to make novel combinations.

The computational predictions were recently confirmed in a group of amnesic patients with bilateral hippocampal damage. Patients could learn the basic discrimination pairs but performed at near chance during the transfer test component[10]. Similarly, non-demented elderly individuals with and without hippocampal atrophy show a similar pattern on the transfer test. There is preserved learning of the discrimination pairs, followed by poor transfer when irrelevant information is altered[7]. Interestingly, these individuals were not impaired relative to non-atrophied controls on the delayed paragraph recall, suggesting that transfer performance may be a more sensitive or an earlier marker of hippocampal dysfunction. In fact, a small scale longitudinal study suggests that , in non-demented elderly individuals, poor performance on the transfer portion of this task may be predictive of short term cognitive decline[10]. This task may offer a selective and sensitive memory assessment relevant to hippocampal function and has predictive value for determining long-term cognitive health in humans.

There is a similar need for sensitive assays of age related hippocampal impairment in rodent models of AD as the water maze (the primary task used to evaluate hippocampal

function in rodents) yields varied results with relatively minimal changes in transgenic models of the disease[11,12,13,14,15]. The water maze task is also not ideal for pharmacological studies and a more sensitive task is needed to evaluate hippocampal function in rodent models of AD.

The goal of the present study is to develop an analogous mouse version of the human concurrent discrimination task with feature irrelevant transfer task and to asses the task during a longitudinal study. A naturalistic odor discrimination task was modified for the mouse model with odors and digging media used as the discriminada[16]. In the first experiment, the experimental design and procedures were validated using young adult C57B6J mice. In experiment 2, aged APP+PS1 mice expressing a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and mutant human presenilin 1 (PS1-dE9) and NTg mice were assessed in the task[17,18]. In experiment 3, APP+PS1 and aged-matched controls were longitudinally studied in the transfer generalization task and in the reversal task. Spatial learning abilities of aged APP+PS1 and NTg were assessed by testing them in the hidden version of the Morris water maze in experiment 4. Collectively, our results indicate that aged APP+PS1 mice have difficulty generalizing previously learned information when presented in novel recombinations. In contrast, young pre-plaque APP+PS1 and NTg controls are able to generalize the learned information without difficulty. As seen in other studies young APP+PS1 mice demonstrate a deficit in reversal learning[19,20,21]. When the same cohort was tested at 14 months in a different odor pair, the reversal deficit was no longer

present[19]. Notably, the same mice were not impaired in a hippocampal-dependent spatial version of the Morris water maze. Based on the human version of the task, we have reason to believe that the mouse version of the transfer generalization task is also hippocampal-dependent. To determine which structures are necessary for the learning and completion of the task, lesion must be performed. Because the human version of the task may be an early detection tool for AD, the mouse task will allow for discriminating of the brain mechanisms necessary. Also, this new cognitive assessment tool for rodents should have great utility in evaluating the effectiveness of drugs and other interventions aimed at halting or even reversing cognitive decline associated with AD.

CHAPTER II

METHODS

Subjects

Experiment 1: C57BL/6J (n=10, 5 months old) female retired breeders from Jax Mice were used for validation of the Transfer Task.

Experiment 2: Three month old female Tg (APPswe, PSEN1dE9) (n=13 to 15) and age-matched non transgenic littermates of B6C3F1/J background strain (n=7) were used. The APP+PS1 double transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and the mutant human presenilin 1 (PS1-dE9). These mice develop beta-amyloid deposits throughout the brain, including the hippocampus, beginning at 6 to 7 months of age and have large amounts of deposits reported by 12 months of age[17]. Animals were first tested at three months of age then again at twelve months of age in order to assess performance over time.

For both experiments, mice were individually housed in the AAALAC-accredited vivarium in the Psychology Building at Texas A&M University in College Station. Mice were maintained on a 12 hour light/dark cycles and climate controlled at 25°C. All testing was conducted during the light cycle and mice in the study were screened daily for health problems. All animal procedures were conducted in accordance with approved institutional animal care procedures and NIH guidelines. All mice were given

at least two weeks with ad lib access to food and water to habituate to the vivarium. After habituation period, mice were food restricted to 85% of their free feeding weight and handled one week prior to testing.

Testing apparatus

The testing box was an open topped Plexiglass black plastic box with two small terracotta pots securely attached to the floor. The stimuli for discrimination were either odors that were applied to the rim of the pots or media that filled the pots and hid the food reward.

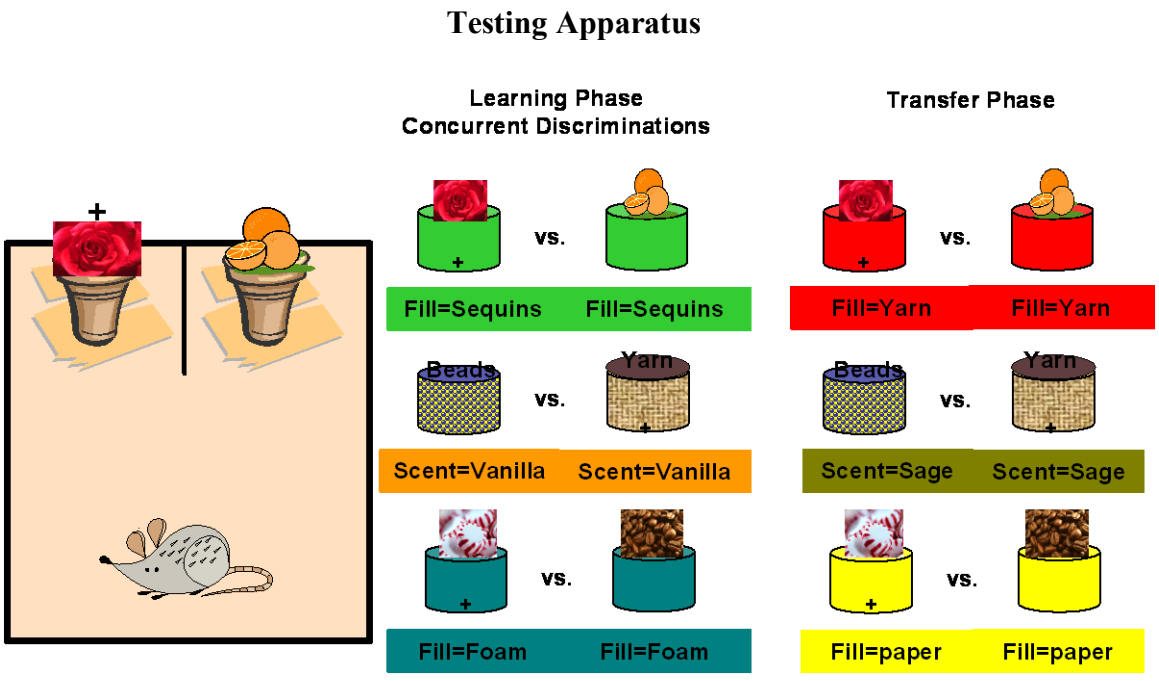

Figure 1. Testing apparatus. The middle column shows the matching odor/media pairs used during the learning phase of the task in which three different pairs were learned. The last column on the right shows the transfer phase in which the irrelevant dimension of the task was changed.

Transfer generalization task

Shaping: Shaping took place in the box described above and consisted of training the mice to dig in the two terracotta pots filled with mix media which obscured a chocolate food pellet. On each trial the mouse was placed in the start zone and allowed to explore both pots. Mice were considered shaped to dig when they could retrieve both rewards in less than 3 minutes in 12 consecutive trials. Mice were tested the day after completing shaping.

Initial discrimination: For the initial discrimination, one of the two pots is seeded with a chocolate reward with either the odor or the digging media as the relevant stimuli. Several crushed chocolate pellets are added to the tops of each pot to disguise the reward of the chocolate pellet. Also to help disguise the reward, another chocolate pellet is placed underneath the pot without the reward where the mouse cannot access the pellet. For the first four trials of every new discrimination problem, mice were allowed to dig in both pots until they obtained the reward. On these trials, only their first choice was scored. On trials thereafter, mice were only allowed to dig in one pot and were removed from the testing apparatus after the one dig. A dig in a pot was scored if a mouse displaced the digging media with either its paws or nose. The positive and negative pairs of discrimination and the sequence of the discriminations were randomized across mice. Once six consecutive choices of the correct pot were made, mice were considered to have learned the discrimination and allowed to move on to the next discrimination

problem. Both number of trials and number of errors to criterion are recorded and used as measures of performance.

Concurrent discrimination/learning phase: In the concurrent discrimination phase, mice are presented with three pairs of odor/digging media combinations. Within each pair the pots differ in odor or digging media but not both. Mice are considered to have reached criterion when they have achieved six consecutive digs where each pair is presented twice. Both number of trials and number of errors to criterion are recorded and used as measures of performance.

Transfer phase: Immediately after the concurrent discrimination, mice begin the transfer phase. In the transfer phase, the irrelevant dimension of the task is changed and 30 trials are presented pseudo-randomly. This design allows an opportunity to assess the ability of the mouse to generalize the predictive value of the positive odor/digging media to food in a new context. The percent of correct responses is used to assess performance.

Odor threshold testing

Anosmia has been shown to emerge as a consequence of chronological age and in Alzheimer's disease[22], therefore, mice were assed for their ability to detect and respond to decreasing concentrations of odorants. Odor threshold testing was performed in the same apparatus as in Figure 1. First, mice discriminated against full strength sandal wood and mineral oil applied directly to the rim of the terracotta pots. The food reward

was in the bottom of the sandal wood pot and mice were required to reach criterion before moving to the next step. After reaching criterion, the mice were tested with a series of discriminations on which the odor was systematically diluted versus mineral oil alone. Mice were given 16 trials at each dilution and the percent error is compared.

Reversal testing

Reversal testing is performed in the same apparatus as Figure 1. Mice are tested with the odor pair hazelnut and peppermint and mixed media is used. First mice have to reach criterion when one of the odors is the positive stimulus (eg. Hazelnut is positive). After reaching criterion, the positive and negative stimuli are switched and mice are required to reach criterion on the new discrimination (eg. Peppermint is now positive). The order that the pairs are presented is randomized across mice and mice are considered to have reached criterion when they have achieved six consecutive digs. Both number of trials and number of errors to criterion are recorded and used as measures of performance.

Water maze

The spatial version of the Morris water maze is a standard task used to assess hippocampal/medial temporal lobe function in rodents. Data from assessments of aging and AD models have had varied results with many studies finding subtle or no deficits on the task. To compare the sensitivity of the transfer task to the Morris water maze, the APP+PS1 and aged-matched NTg mice were tested in a hidden version of the Morris water maze.

The water maze consisted of a 4 foot diameter tank filled with water clouded by nontoxic paint. The tank was surrounded by black curtains affixed with white geometric cues. The tank was divided into four quadrants, each with a platform position equidistant from the center to the wall. During the spatial reference memory assessment, a retractable escape platform was located in the southwest quadrant of the maze and submerged 1.2 cm below the water's surface. During cue training, the tank was filled to 1cm below a black visible platform. Each mouse's swim was tracked and analyzed using a computer-based video tracking system. Before every trial, a black beaker was used to carefully place the mice in the water facing the wall of the tank. Throughout the experiment, mice that failed to reach the platform after the designated amount of time were guided to it with a wand.

Cue training was used to assess visual acuity and motor ability of the mice to escape to the platform independent of their spatial learning ability. During cue training, the platform and start positions were varied on each trial. Training consisted of 2 days, in which mice were given 60 seconds per trial to find the visible escape platform. After find the platform or being guided there by an experimenter, mice remained on the platform for 30 seconds before being removed from the tank. At the conclusion of each trail, mice were returned to a holding cage, which is placed on a heating pad for approximately 10 minutes between trails.

Beginning on the day after cue training was completed, mice received 6 consecutive days of training to a hidden, stationary platform to assess spatial reference memory. During each trail, mice were given 60 seconds to search for the hidden platform, followed by 30 second post-trial period in which they were allowed to remain on the platform. Mice completed four trials per day. The eighth and the twenty fourth trials were a probe trail during which the escape platform was retracted to the bottom of the tank for the first 30 seconds of the 60 seconds trial. As in cue training, mice were given a 10 minute inter-trial rest interval between trials for both training and the two probe trials. The start position varied on each trial.

The water maze data was analyzed using a computer-based video tracking system; Water 2020, developed by HVS Image. To further assess performance and to eliminate confounds, the HVS system was programmed such that is the subjects' speed decreased to below 0.05 m/sec, performance was recorded for an analysis of "floating" and time spent in the outer 10 percent of the tank was recorded for an analysis of "thigmotaxis". Swim speed was also analyzed across cue training and training trials. Training trial performance was analyzed using pat length, the total distance traveled from the start position to the platform. A two factor repeated measures ANOVA was used to evaluate differences across training days. To analyze performance on the probe trials, percent time in quadrant was compared between exposed groups using a two-factor repeated measure ANOVA.

CHAPTER III

RESULTS

Experiment 1

The goal of Experiment 1 was to confirm that the mice could learn a series of concurrent discriminations and could perceive and respond to the recombination of stimuli in the transfer phase of the task. C57BL/B6 mice (n=10) were trained on a series of 3 concurrent discrimination problems. Immediately after reaching criterion performance, half of the mice (n=5) received 30 trials in which the irrelevant dimension not predictive of food was altered (i.e., a novel recombination of stimuli, "test group") and half of the mice (n=5) received 30 trials that were identical to those learned initially (i.e., no recombination, "control group"). As shown in Fig. 2, both groups learned the concurrent discriminations comparably (errors to criterion: $F(1, 8) = 00$, $p = 0$ – identical means). However, during the transfer phase, those subjects that had the irrelevant dimension changed performed significantly worse than the mice that received discrimination problems identical to those presented in the concurrent discrimination phase ($F(1, 8) = 8.73$, $p = 0.02$). These data demonstrate that mice are sensitive to alterations in the non-predictive stimulus dimension, suggesting that similar cognitive processes mediate performance in the mouse and human versions of the transfer generalization task.

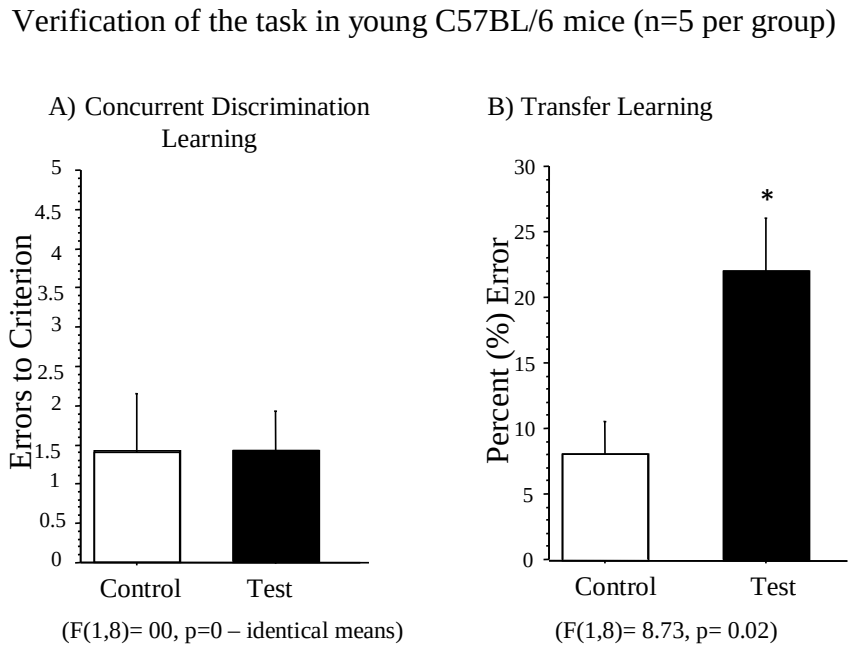

Figure 2. Verification of the task in young C57BL/6 mice(n=5 per group). Errors to criterion in the concurrent learning phase (A) and percent error in the transfer phase (B) of the mouse version of the transfer generalization task. Panel (A) shows groups of C57BL/6J mice (n=5 each) counterbalanced by performance into "control" and "test" groups such that no differences between groups were evident in the learning of concurrent discriminations. Panel (B) shows a significant increase in errors in the "test" group that received a change in the irrelevant stimulus during the transfer phase as compared to the control group that received identical discrimination problems to those presented during the learning phase. See text for statistical analysis.

Experiment 2

The goal of experiment 2 was to determine if aged APP+PS1 mice were impaired on the transfer phase of the transfer generalization task relative to aged-matched controls (n=7) as would be expected if the mouse task is sensitive to age-related pathological changes associated with pathological aging.

Concurrent learning phase

A one factor ANOVA (genotype) revealed no difference of genotype during the final concurrent discrimination learning using errors to criterion as the performance measure (Figure 3(A); $F(1,17)= 0.118$, $p=0.735$).

Transfer generalization task

APP+PS1 mice were significantly impaired relative to aged-matched control mice in their ability to perform the discrimination problems when the irrelevant stimulus was changed during the transfer phase of the task (Figure 3(B), main effect of genotype ;$F (1, 17) = 12.161$, $p= 0.003$).

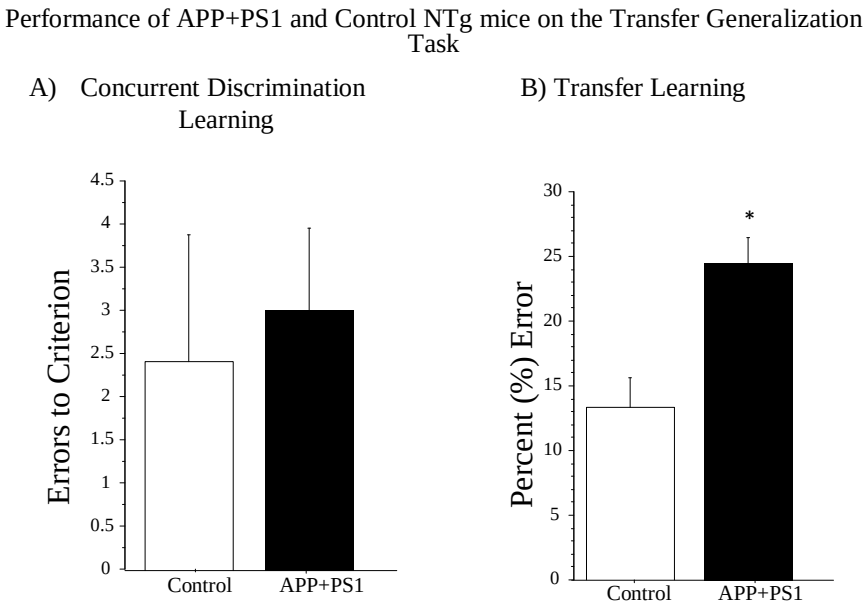

Figure 3. Performance of APP+PS1 and control NTg mice on the Transfer Generalization Task. (A) shows errors to criterion in the concurrent discrimination learning phase and (B) shows percent error in the transfer phase of the task. Note that while there is no difference in performance between control and APP+PS1 in number of errors while learning the initial discriminations, APP+PS1 mice were significantly impaired relative to the NTg age-matched controls on the transfer phase of the task. See text for statistical analyses.

Experiment 3

A second cohort of mice was tested longitudinally in the transfer generalization task and reversal learning. Three month-old APP+PS1 mice tested in the Transfer Generalization Task did not commit more errors than NTg when learning the concurrent discriminations (figure 4(A); $F(1,15)= 0.001$, $p= 0.98$). Furthermore, as seen in figure 4(B), young APP+Ps1 performed comparably to the NTg in the transfer phase of the task ($F(1,15)= 0.001$, $p= 0.98$).

At 6 moths, the same cohort of APP+PS1 and control mice presented comparable learning in a simple odor pair discrimination without an irrelevant feature (figure 4(C); $F(1,15)=2.09$, $p>0.05$). However, in agreement with previously reported data in Tg2576 mice, young APP+PS1 mice were impaired when learning the reversal discrimination, performing worse than controls (figure 4(C); $F(1,15)= 6.34$, $p<0.04$).

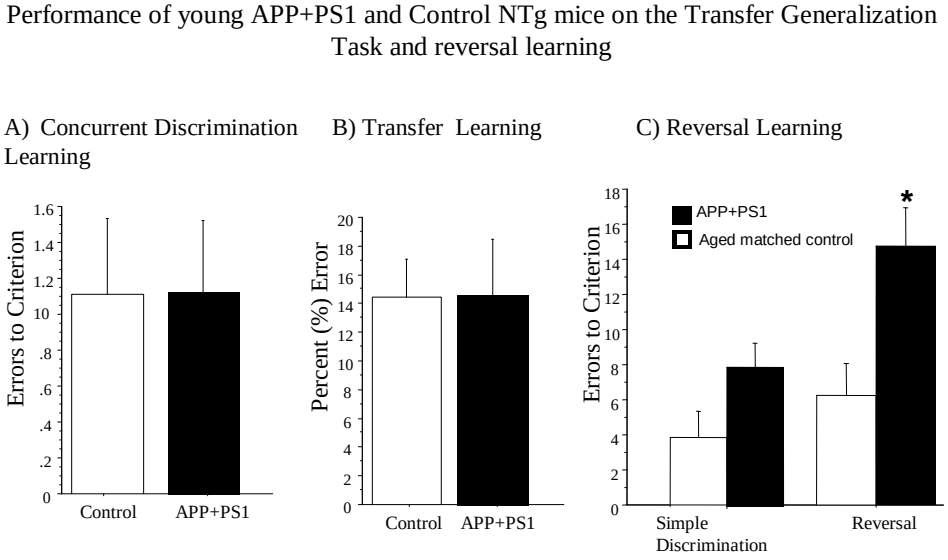

Figure 4. Performance of young APP+PS1 and control NTg mice on the Transfer Generalization Task and reversal learning. Performance of young (3 mon) APP+PS1 (N=8) and age-matched NTg (N=9) on the Transfer Generalization task and reversal learning. (A) shows errors to criterion in the concurrent discrimination learning and (B) shows percent error in the transfer phase of the task. No difference in performance between APP+PS1 and control mice was observed in either phase of the task. (C) However, in agreement with previously reported data in Tg2576 mice, reversal learning deficits were observed at this early age in APP+PS1 mice compared to the NTg mice. See text for statistical analyses.

Reversal learning was tested again in the same cohort at 14 to 15 months. A repeated measures ANOVA revealed that there was no difference in errors to criterion due to genotype on the learning of the new media and odor pairs ($F(1, 15) = 1.33$, $p>0.05$). There was also no difference in performance between genotypes (demonstrated by no difference in errors to criterion) in the reversal learning of the pairs ($F(1, 15) = 0.196$, $p>0.05$).

The second cohort of APP+PS1 and NTg mice was retested on the transfer generalization task at 13 months of age. In agreement with the first study, 13 month old APP+PS1 mice learned the concurrent discriminations on par with age-matched NTg control mice (Figure 5 (A); $F(1,15)= 0.018$, $p=0.89$) but the transgenic mice were considerably impaired on transfer phase of the task (Figure 5(B); $F(1,15)= 4.693$, $p=0.047$)..

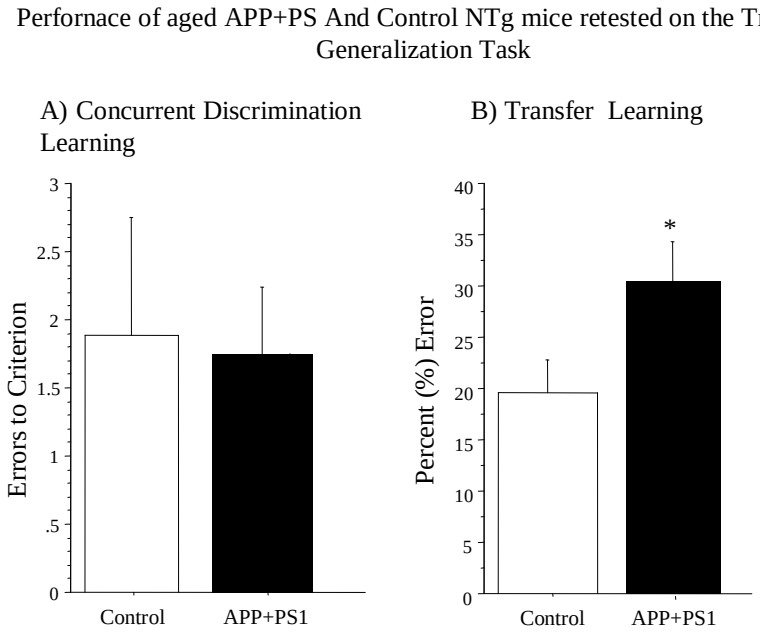

Figure 5. Performance of aged APP+PS1 and control NTg mice retested on the Transfer Generalization Task. (A) shows errors to criterion in the learning/concurrent discrimination phase and (B) shows percent error in the transfer phase of the task. There is no difference in performance between control and APP+PS1 in number of errors in the learning of concurrent discriminations. However, APP-PS1 mice were significantly impaired relative to the NTg age-matched controls on the transfer phase of the task. Note, the deficits in reversal learning observed at 3 months of age were not present at 12 months (data not shown; see text for statistical analyses).

Odor detection threshold testing

To ensure a decreased ability to detect odors the APP+PS1 mice was not a factor in the transfer deficit observed, following the transfer generalization testing, mice were trained on one additional olfactory discrimination problem. Figure 5 (A) shows both groups learned the simple discrimination problem comparably and that there was not a difference due to age (errors to criterion, $F(1,30)= 0.708$, $p= 0.4067$) or genotype (errors

to criterion, F(1,30) = 0.201, p= 0.6571); also, no interaction was observed between age and genotype (trials to criterion, F (1,30)= 0.996, p= 0.3262; errors to criterion, F(1,30)= 0.772, p= 0.3867). Moreover, figure 5(B) shows that the odor threshold did not differ as all groups performed similarly as concentrations of the odorant were decreased (age: F(1,30)= 0.38, p= 0.5424; genotype: F(1,30)= 0.220, p=0.6426; age versus genotype interaction: F(1,30)= 0.057, p= 0.8122). Thus, no significant difference between odor detection abilities was observed between the two groups.

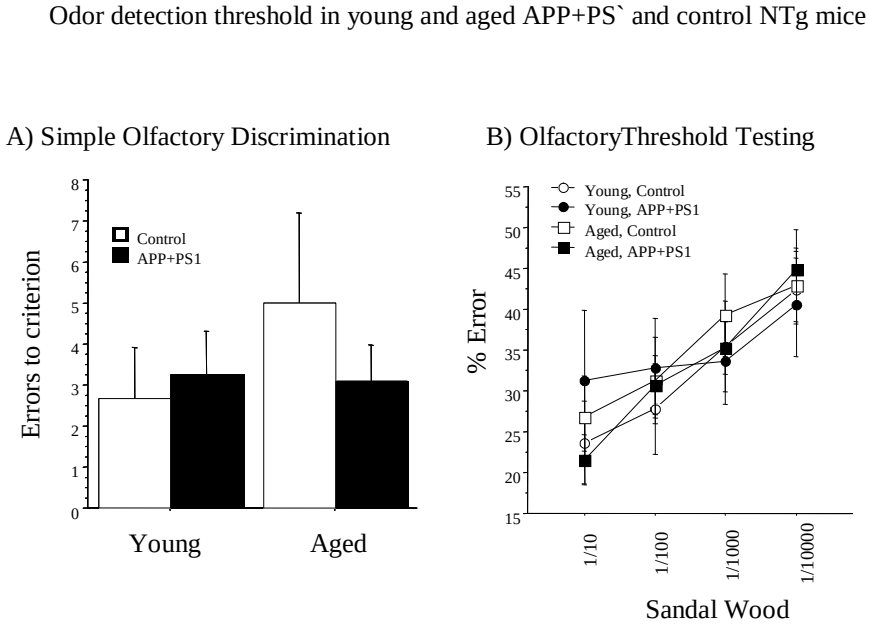

Figure 6. Odor detection threshold in young and aged APP+PS1 and control NTg mice. Odor detection threshold in 3 month (n=8 APP+PS1, n= 9 NTg control) and 12 month (n=7 APP+PS1, n=12 NTg control) APP+PS1 and NTg age-matched mice (performed after training in the Transfer Generalization task). Errors to criterion to learn a novel odor discrimination pair is shown in (A) and the percent error of responses made at decreasing dilutions of the odorant is shown in (B). As expected, all groups' ability to detect the odors decreased with diminishing concentrations of the odorants nearing chance performance at a 1:1000 dilution of that used during training. However, no differences in age or transgene were observed in the ability to detect odorants. See text for statistical analyses.

Experiment 4

Following olfactory discrimination threshold testing, mice were trained in visible (cued) and hidden platform versions of the water maze. As shown in Fig. 6(A), APP+PS1 and NTg age-matched control mice showed comparable path length to reach the visible platform across days ($F(1, 18) = 0.083$, $p=0.776$) and there was no interaction between genotype and day ($F(1, 18) = 0.158$, $p=0.70$). Fig. 6(B) shows path length across training days 1 through 6. A repeated measures ANOVA revealed that both groups improved performance across training ($F(5, 90) = 2.921$, $p=0.02$) but there was no main effect of genotype ($F(1, 18) = 0.169$, $p= 0.69$) and no interaction between day and genotype ($F(5, 90) = 0.563$, $p=0.73$). Probe trial performance is show in Fig. 6(C). Percent time in target quadrant significantly increased for both groups from the early probe trial on day 2 and the last probe trial on day 6 ($F(1, 18) = 12.164$, $p=0.003$) but no main effect of genotype ($F(1, 18) = 0.547$, $p=0.47$) nor interaction between genotype and probe trial ($F(1, 18) = 0.260$, $p=0.62$) were observed.

Finally, in Fig. 7, note that no main effect of genotype was found on swim speed during the first trial of cue training ($F(1, 18) = 1.349$, $p=0.26$) nor on the first hidden platform training trial ($F(1,18)= 2.022$, $p=0.17$), two measures not confounded by learning.

Cued and spatial reference memory performance of APP+PS1 and control NTg mice assessed in the Morris Water Maze

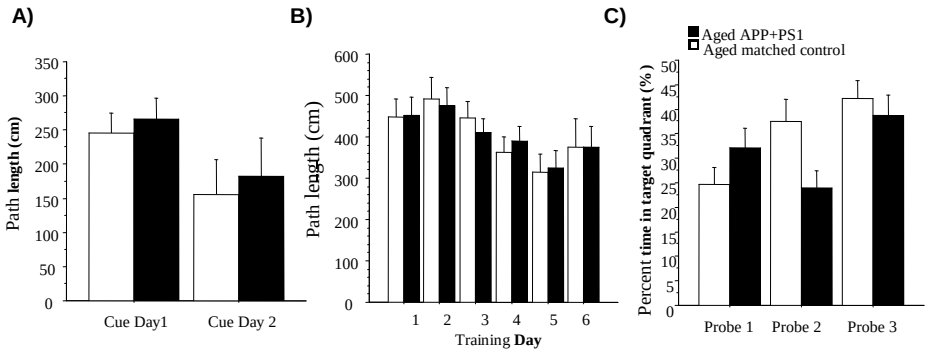

Figure 7. Cued and spatial reference memory performance of APP+PS1 and control NTg mice assessed in the Morris water maze. Panel (A) shows that both groups were able to find a visible platform comparably, demonstrating a lack of sensorimotor or motivational differences between groups. Panel (B) shows that the path from the start position to the stationary hidden platform decreased across the 6 training days for both groups although there was no main effect nor interaction of genotype. These training trial data were confirmed by probe trial data shown in panel (C). Note that mice from both groups show a significant spatial bias for the quadrant where the platform had been during training on the second probe trial but again, no difference was observed between APP+PS1 mice and NTg age-matched controls. See text for statistical analyses.

CHAPTER IV

SUMMARY AND CONCLUSIONS

We have developed a translational memory task that seems to be sensitive to detect memory impairment at early stages of hippocampal dysfunction. There are at least two ways to learn each discrimination problem in both the human and the rodent versions of the transfer generalization task. Using the rodent task as an example, the first is to simply learn the correct mappings from pots to responses (the pot that smells like rose containing sequins is preferential to the pot that smell like citrus containing sequins, etc.). The second is to modify stimulus representations to emphasize relevant information and de-emphasize irrelevant information (rose odor is preferential to citrus odor, regardless of digging media). Based on the evidence from the human task[7], the latter strategy appears to be closely tied to hippocampal function.

First we validated the transfer phase of the task, and confirmed that indeed there was a difference in difficulty level between the transfer and the learning of the concurrent phase. We observed no difference in learning between C57B6 mice that received the "test" condition (i.e., transfer) and those that did not (control). These observations indicate that both groups of animals took about the same number of trials to achieve criterion and the number of errors were also equivalent. However, when the transfer phase of the task was introduced, the mice in the "test" condition performed significantly worse than mice in the "control" condition, demonstrating that the change in the irrelevant stimulus influenced the correct choice even in young subjects. These data

support that by changing the irrelevant stimulus, a secondary cognitive process is occurring (compared to simple recall of discrimination information acquired during the learning phase). They also suggest that different neural substrates may be involved in the acquisition of discrimination problems and the ability to recall this information when contingencies (even ones irrelevant to solving the problem) are altered. We also noted that although young mice in the "test" condition performed significantly worse than mice in "control" condition, the mice still performed well and thus decrements associated with age and/or disease/brain damage should be detectable. These results also suggest that different cognitive processes underlie the discrimination learning and the generalization if this learned information in an altered context (i.e. with the irrelevant dimension changed). Overall, we were able to confirm that mice can successfully learn up to 3 different discriminations simultaneously and when an irrelevant dimension is changed, mice perform significantly worse.

The aged APP+PS1 mice are impaired in the transfer but not in the concurrent discrimination/ learning phase of the task. In our longitudinal study of the transfer generalization task we were able to reproduce the results of our aged cohort and observed that young mice were not impaired in the transfer phase of the task. This result agrees with the prediction from human studies in which the patients with hippocampal atrophy were able to learn the concurrent discriminations as well as non-atrophied peers. Furthermore, the longitudinal study also demonstrates that the transfer generalization task can be used in a within subject, test-retest manner in mice. The second cohort was also

impaired at 6 months in reversal learning. Interestingly, 6 months of age is the reported age in which the AB plaques start appearing in this mouse model. Just as previous studies[19,20], mice that were impaired early in their lifespan were not impaired at a later age (in our study 14 to 15 mon), when AB plaques could be more abundant in the brain. We could speculate that the early impairment is erased later in the lifespan of the mice due to an alternative compensatory mechanism that allows for the mice to acquire reversal learning. Another explanation could be that reversal learning testing is too simplistic and cannot be used in within subject group testing. More studies are necessary to test both hypotheses.

Our water maze assessment results are also in accordance with the human data, which found no impairment of the atrophied individuals on other hippocampal-dependent tasks. The transgenic mice were not impaired in the spatial reference version of the water maze. These data suggest that generalizations involving recombinations of familiar stimuli (transfer learning) may be particularly dependent on the hippocampus (based on human data) and is an early indicator of mild cognitive impairment and Alzheimer's disease, and thus may be more sensitive to detecting impairment than other standard cognitive assessments. We must acknowledge, however, that other brain regions (prefrontal cortex, striatum, basal forebrain) may be necessary for unimpaired performance on the transfer generalization task.

Considering that the deficits seen in the transfer phase are not due to detection impairments (see results from odor detection testing), we believe we have developed and successfully established the parameters of the rodent model of the human transfer task, which is highly predictive of mild hippocampal atrophy seen in non-demented elderly[7] and which is predictive of future cognitive decline[6,7]. Our experiment also provides a better behavioral paradigm for translational research between rodents and humans, resolving a high-priority need for current AD research.

Our next goal is to develop a highly sensitive, largely-automated task that can be used for inexpensive, high-throughput drug screening with transgenic mice. The mouse generalization transfer task, which requires only 3 successive days of shaping and testing, is well-suited for this purpose. There is also a need for confirmation of the neural substrate for the transfer generalizations. Hippocampal lesion studies and pharmacological studies are needed address this question.

Due to the ability of the rodent transfer task to be used for longitudinal experimental designs, it will allow for evaluation of t therapeutic interventions. One of the impediments to translational research has been trying to extend cross-sectional experimental designs used in rodent to the interpretation of longitudinal experimental designs used in human clinical trials. Tasks such as the water maze are not easily adaptable for re-assessment due to the large amount of procedural components of the task that are retained and make the re-test less sensitive to detect differences due to

ceiling effects (a practice effect). The adaptability of the transfer task to a longitudinal design with the use of novel exemplar pairings is an added advantage to this task. Ultimately, as promising agents are identified, longitudinal studies with chronic pharmacological treatments can be conducted with the goal of preventing all cognitive deficits (including those associated with transfer generalizations).

The transfer task sensitively measured robust deficits in cognitive function when deficits are not observed on standard assessments of basal forebrain/ medial temporal lobe function (e.g., a spatial reference version of the Morris water maze task). Thus, in agreement with the human data, the newly developed analogous transfer task for rodents appears more sensitive in detecting cognitive deficits in an animal model of AD.

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