

2005

## Dissecting Out the Contribution of Cognitive, Social, and Physical Activities to Environmental Enrichment's Ability to Protect Alzheimer's Mice Against Cognitive Impairment

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Dissecting Out the Contribution of Cognitive, Social, and Physical Activities to  
Environmental Enrichment's Ability to Protect Alzheimer's Mice Against Cognitive  
Impairment

by

Jennifer R. Cracchiolo

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science  
Department of Arts and Sciences  
College of Biology  
University of South Florida

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Date of Approval:  
July 29, 2005

Keywords: Memory, Radial Arm Water Maze, Behavior, Neurodegenerative Diseases,  $\beta$ -Amyloid

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## Table of Contents

List of Tables .....	iii
List of Figures .....	iv
Abstract .....	v
Alzheimer's Disease Background .....	1
Behavior Associated with Alzheimer's Disease .....	2
Pathology Associated with Alzheimer's Disease .....	5
$\beta$ -Amyloid & Neurofibrillary Tangles .....	5
Neuronal Loss, Synaptic Loss, Brain Atrophy .....	11
Genetics of Alzheimer's Disease .....	14
Amyloid Precursor Protein .....	15
The Presenilins .....	16
Apolipoprotein .....	17
Risks of Alzheimer's Disease .....	17
Diagnosis of Alzheimer's Disease .....	21
Treatment of Alzheimer's disease .....	27
Animal Models of Alzheimer's Disease .....	31
The PDAPP Mouse Model of Alzheimer's Disease .....	32
PDAPP Pathology .....	33
Behavior of PDAPP Model .....	37
APP Swedish Mutation Transgenic Mouse Line .....	41
APPsw Brain Pathology .....	41
APPsw Behavior .....	44
APP Swedish + PS1 Mutation Transgenic Mouse Model .....	49
APPsw +PS1 Pathology .....	49
Behavior of APP+PS1 Mice .....	53
APP Mutant Mice: Imperfect Transgenic Model .....	58
Environmental Enrichment .....	61
Enriched Environment Protection and Treatment: Human Studies .....	61
Effects of Enriched Environment in Rodents .....	65
Cognitive Effects of Enriched Environment in Rodents .....	65

Neurohistologic and Neurochemical Effects of Environmental Enrichment in Rodents .....	69
Effects of Environmental Enrichment in “Alzheimer’s” Transgenic Mice .....	75
Complete Environmental Enrichment versus Its Components .....	82
Specific Aims.....	86
Material and Methods.....	88
Animals & General Protocol.....	88
Enriched Environments.....	89
Behavioral Assessment .....	90
Tissue and Blood Collection.....	94
Corticosterone Quantification.....	95
Extraction of Brain Protein for Sandwich Enzyme-Linked Immunosorbent Assay (ELISA).....	95
Blood Cytokine Levels .....	97
Golgi Cox Analysis.....	98
Statistical Analysis.....	99
Results.....	101
Behavioral Analysis.....	101
Neuropathologic Measures .....	112
Factor Analysis/ Discriminant Function Analysis .....	116
Correlation Analysis .....	122
Discussion.....	126
General Summary .....	126
Behavioral Measures.....	131
Neuropathologic Analysis .....	135
Correlations Analyses between Neuropathology, Blood Measures and Behavior .....	139
Multimetric Analyses (FA and DFA) .....	141
Reference .....	146

## List of Tables

Table 1. Factor loading of behavioral measures, with or without pathologic measures.....	118
Table 2. Summary of discriminant functional analyses of behavioral measures.....	120
Table 3. For all animals, a correlation matrix of behavioral measures and plasma cytokine levels.....	123
Table 4. For all animals, a correlation matrix of behavioral measures and dendritic length/branching.....	125

## List of Figures

Figure 1. General protocol time line for enrichment study.....	89
Figure 2. Y-maze Entries and Percent Spontaneous Alternations.....	103
Figure 3. Circular Platform overall escape latencies.....	104
Figure 4. Morris Water Maze acquisition overall and across 5 two-day blocks.....	105
Figure 5. Morris Water Maze Memory Retention, as indexed by time spend in the former platform-containing quadrant, annulus crossings, and quadrant preference.....	107
Figure 6. Recognition/Identification performance in Platform Recognition testing overall and for each of the 4 days of testing.....	108
Figure 7. RAWM overall and final block escape latencies for Trials 1, 4 and 5.....	110
Figure 8. RAWM escape latencies for final block Trials 4 and 5.....	111
Figure 9. Plasma Corticosterone levels by treatment (housing), genotype, and gender.....	113
Figure 10. Standardized mean signal intensities for 10 plasma cytokines in APP+PS1 and NT mice.....	114
Figure 11. Quantification of Soluble A $\beta$ (1-40 and 1-42) and Insoluble A $\beta$ (1-40 and 1-42) in APP+PS1 mice within different housing environments.....	115
Figure 12. Housing and transgene-dependent changes in dendritic length/branching of neurons from APP+PS1 and NT mice.....	117

Dissecting Out the Contribution of Cognitive, Social, and Physical Activities to  
Environmental Enrichment's Ability to Protect Alzheimer's (transgenic) Mice Against  
Cognitive Impairment

Jennifer R. Cracchiolo

Abstract

Retrospective studies suggest that lifestyle activities may provide protection against Alzheimer's Disease (AD). However, such studies can be inaccurate and prospective longitudinal studies investigating lifestyle protection against AD are both impractical and impossible to control for. Transgenic (Tg+) AD mice offer a model in a well controlled environment for testing the potential for environmental factors to impact AD development. In an initial study, Tg+ and non-transgenic (Tg-) mice were housed in either environmentally enriched (EE) or standard housing (SH) from 2-6 months of age, with a behavioral battery given during the last 5 weeks of housing. In the Morris maze, platform recognition, and radial arm water maze tasks, Tg+/EE mice were completely protected from cognitive impairment present in Tg+/SH mice and comparable to control Tg-/SH mice in cognitive performance. The current study utilized the same cognitive-based behavioral battery and multivariate statistical analysis to investigate the protective effects of "complete" environment enrichment (EE) versus several of its components (physical activity, social interactions) in AD transgenic mice. The AD transgenic mice

utilized develop beta-amyloid (A $\beta$ ) deposition and cognitive impairment by 6-7 months of age. Similar to our initial study, results show that “complete” EE (physical, social, and cognitive activities) from 2 to 8 months of age completely protected AD transgenic mice from cognitive impairment in tasks representing different cognitive domains – working memory, reference learning, and search/recognition. In strong contrast, Tg+ mice reared in environments that included physical activity and social interaction, or only social interaction, were not protected from cognitive impairment in adulthood – enhanced cognitive activity was required over and above that present in these other environments. Through use of discriminant function analysis, EE and/or NT mice were consistently discriminated from the poorer performing other housing groups. The cognitive benefits observed in EE-housed Tg+ mice occurred without significant changes in cortical A $\beta$  levels, plasma cytokine levels, or plasma corticosterone levels, suggesting involvement of mechanisms independent of these endpoints. However, EE-housed Tg+ mice did have decreased dendritic length of neurons in the parietal cortex (but not hippocampus). Noteworthy is that plasma cytokine levels and hippocampal dendritic length consistently correlated with cognitive measures, suggesting their involvement in underlying mechanisms of cognitive performance. The present work provides the first evidence that “complete” EE (including enhanced cognitive activity) is needed to provide cognitive protection against AD in a Tg+ model of the disease, while the physical and social activity components of EE do not alone lead to protection. These results suggest that humans desiring to gain maximal environmental protection against AD should live a lifestyle high in cognitive, social, and physical activities together.



## **Alzheimer's Disease Background**

With projected life expectancy hypothesized to reach 80 by 2050, emphasis on health of the aging population has become a centralized area of research. With achievement of such great ages comes an increase in health burdens, in particular neurodegenerative disorders. Of these numerous neurodegenerative disorders, Alzheimer's disease (AD) has provoked immense interest in biomedical, media, and public health arenas. Prevalence of AD, which rises linearly with age and accounts for 81% of dementia, affects 10% of all individuals over the age of 65 and 40% over 85 years of age (Evans et al., 1989). Alzheimer's disease has grand implications including an enormous monetary burden on health care systems as well as the loss of livelihood of patients and their family members. Statistics suggest that cases of AD will increase significantly in the future, solidifying the importance of research into viable prevention and treatment options for the aging population.

The first AD patient, Auguste D., described by Alois Alzheimer, displayed what are now considered characteristic symptoms of AD including progressive memory loss, loss of social skills, and a decrease in functional language. While Alzheimer observed the cognitive manifestations and basic pathological features of the disease, it was not until advances in microscopy that hallmark pathological features including neuritic  $\beta$ -amyloid plaques and neurofibrillary tangles were described in detail. In a time course of 2 to 20 years patients first dwindle cognitively while motor and sensory function remain

relatively intact. However, as pathological constituents ravage neuronal systems, phenotypic characteristics of motor diseases begin to appear in the form of irregular gaits and loss of coordination. At present time only postmortem autopsy reveals absolute diagnosis of AD.

The potential effects of interventions to delay the onset of AD are immense. It is estimated that there are approximately 360,000 new case of AD each year. Perhaps more striking is the implication of delaying onset of the disease by 5 years. If research findings successfully can delay onset by 5 years, a reduction of 1.5 million cases after 10 years and 4.04 million cases after 50 years could be witnessed (Brookmeyer et al., 1998). This delay could translate into billions of savings economically.

Enriched lifestyles may offer the medium to provide this delay of onset. Longitudinal studies state that engagement in mental, social, or productive activities was inversely related to incidences of dementia (Wang et al., 2002). The literature would suggest that stimulating mental and physical activities may offer protection from dementia. Research into the mechanism of these cognitive benefits still needs clarification. Future investigation into the “enriched environment phenomenon” may offer quality of life benefits to patients and caretakers as well as a decrease in financial burden for health care systems.

### ***Behavior Associated with Alzheimer’s Disease***

AD is the most common form of dementia and often takes an archetypical clinical course which reflects the underlying expanding neuropathology. The clinical phase of AD, a period of 5-10 years (Locascio et al., 1995), is characterized by short and long term

memory loss, paranoia, delusions, language deficit and cognitive impairment. The clinical sequence of degenerative loss in AD has been described as the progression of functional loss of capacity. The stages of this degeneration in many ways mirror the normal maturation of human development (Reisberg et al., 1999).

Mild cognitive impairment (MCI) has been described as a transition state between typical aging and dementia. Ten to fifteen percent of patients diagnosed with MCI convert to AD annually (Petersen et al., 2001). When diagnosing MCI, important criteria include the memory complaint being corroborated by an informant and second, the memory impairment is appropriate when age and education is considered. MCI may be useful in identifying individuals functioning at normal levels who are at risk for later developing AD. Such patients display normal activities of daily living and normal general cognitive function, however, complain of a short-term memory (Petersen et al., 1997). MCI has been shown to exclusively involve short-term memory impairment (Shankle et al., 2005). While many studies have shown increased rates of progression of AD with individuals that display MCI, there have been conflicting conclusions regarding why not all individuals identified with MCI progress to AD. One hypothesis suggested by Petersen et al. 1997 is that diagnosed MCI patient's behavior is more heterogeneous than thought. Patients diagnosed with MCI encompasses a group of individuals that may have a progressive form of MCI, leading to dementia, while others may not progress, simply remaining stagnate within the criteria for MCI. Petersen et al. (2001) find that such heterogeneity requires a sub-classification. Sub-groups may include amnesic MCI, MCI with slight memory impairment in multiple domains, or MCI with impairment of a single

non-memory domain. Subdividing MCI may result in a more reliable future diagnosis to patients displaying such symptoms.

In the early stages of AD, a significant impairment of working (short-term) and memory is the outstanding clinical feature (Forstl & Kurz, 1999). While memory of the patient's early years and long term memory remains relatively intact, working memory impairments displayed in early stages of AD lead to difficulties in daily activities. The loss of a patient's ability to plan, judge, and organize leads to a decrease in the patient's ability to perform household chores. Upon casual inspection language may still appear to be intact, however, communication begins to deteriorate due to an attenuated vocabulary. Other impairments may be observed in object naming and semantic memory (Chobor et al., 1990). Frequently early AD patients will display difficulty with driving due to the loss of spatial orientation. Early, non-cognitive disturbances appear to be variable and mild. AD patients often show signs of depression and apathy (Forstl & Kurz, 1999). In early stages of AD motor function remains relatively intact.

The moderate stage of AD brings a further deterioration of working memory, forcing the patient to "live in the past (Beatty et al., 1988)." Language, logical reasoning, long term memory planning, reading and organizing significantly decline in this stage. Sequencing becomes impaired, leading to loss of the ability to feed and dress oneself. Many AD patients develop prosopagnosia making once familiar faces foreign. Restlessness, aggression, disorientation, and incontinence are also often found in the moderate stage of the illness. Psychiatric symptoms include hallucination, wandering and hoarding (Devanand et al., 1997). As motor function declines at this moderate AD stage, risk of falls increases due to hesitant gait and stooped posture (Forstl & Kurz, 1999).

Most all cognitive functions are severely impaired in the late stages of AD (Forstl & Kurz, 1999). Long term memory, present in mild and moderate stages of AD, dissipates in the final stages of the illness. Language is reduced to single phrases or words. Many patients are bedridden and enter a vegetative state. Pneumonia followed by myocardial infarction and septicaemia are the most frequent cause of death in AD (Forstl & Kurz, 1999)

### ***Pathology Associated with Alzheimer's Disease***

Many major pathological processes contribute to the widespread brain destruction presented clinically in AD. These processes comprise the two hallmark pathological features of beta-amyloid plaques ( $A\beta$ ) and neurofibrillary tangles (NFT), as well as the resultant processes of inflammation, synaptic loss, neuronal cell loss, and brain atrophy. Each of these components contributes to and interrelates with each other in a maze of cause and effect that is still being elucidated (Rosenthal & Khotianov, 2003).

### ***$\beta$ -Amyloid & Neurofibrillary Tangles***

The “ $\beta$ -amyloid hypothesis” of AD is supported by a substantial amount of research confirming the significance detriment  $A\beta$  plaques have on neurons as well as the beneficial effects seen upon prevention and clearance of these plaques (Loo et al., 1993, Morgan et al., 2000). Loo et al. (1993) reported that apoptosis is induced by  $A\beta$  in cultured central nervous system neurons while in an animal model of AD, Morgan et al. (2000) found that by decreasing formation of plaques with an  $A\beta$  vaccine, memory loss is

prevented. A $\beta$  is generated from the abnormal cleavage of a much larger transmembrane protein called amyloid precursor protein (APP) discussed later. The product of this abnormal cleavage by  $\beta$ - and  $\gamma$ -secretase results in A $\beta$  formation within the cytoplasm which is taken up vesicles and released into the extracellular space (Busciglio et al., 1993). Once released, star-shaped compact masses of A $\beta$  plaques begin to form (Selkoe, 2001). Such plaques are often associated with glia cells. Microglia are found within and adjacent to the central amyloid core while the astrocytes surround the outside of the plaque. The time course for the production of the detrimental, dense core A $\beta$  plaques is unknown however it is assumed that the clinical phase of AD is preceded by a 15–30 year period of time in which continuous deposition of A $\beta$  plaques takes place. Plaques in the AD brain accumulate in the parietal, temporal cortex, and most notably in the hippocampus.

Immunohistochemical stains have revealed that the dense A $\beta$  plaques described above are not the sole A $\beta$  species found in the AD brain. Such staining using antibodies show a far more extensive number of A $\beta$  deposits than originally observed with congophilic stains. Yamaguchi et al. (1988) described these diffuse type of senile plaques not stained by congo red in the brains of AD type dementia patients.

Examination of the A $\beta$  peptide revealed that the A $\beta$  fragment either ends at amino acid 40 or 42. Fragments ending in A $\beta$ 42 and lacking in A $\beta$  40 comprise the diffuse plaques. In contrast, the compact variety contains primarily A $\beta$  40 (Iwatsubo et al., 1995). It has been hypothesized that the diffuse, “preamyloid,” A $\beta$  42 plaque is a precursor to the compact dense plaques seen later in the AD brain (Selkoe, 2001). Examining normal

aged patients as well as transgenic animal models of AD provides evidence for this theory. The brains of aged, cognitively normal humans often display diffuse amyloid deposition however often lack extensive compact barnacle like plaques found in AD (Wang et al., 1999). A second support for this theory is that transgenic animals over expressing mutant APP first develop diffuse plaque before the later development of compact plaques. These observations suggest a preclinical pathology that may be a possible therapeutic target to alter the course of AD progression before behavioral symptoms appear.

A $\beta$  is not confined to the neuronal extracellular space. Plaques are also found in the vasculature of the AD patient. Cerebral amyloid angiopathy (CAA) is defined as deposition of a congophilic material in meningeal and cerebral arteries and arterioles within the brain. While CAA is commonly present in the aged brain, 98% of AD patient's brains exhibit vascular A $\beta$  plaques. CAA is formed when smooth muscle cells synthesize A $\beta$ -40 intracellularly, which aggregates extracellularly into fibrils that then induces smooth muscle degeneration, making the tissues susceptible to hemorrhage. Small infarcts in AD patients may have limited impact on the global cognitive decline observed clinically in moderate to late AD. However they may influence early stages by promoting an earlier development of dementia. A second concern of compromised vasoregulation caused by CAA is the cascade of events followed by a decrease in blood flow. Vasoconstriction causing in a decrease in blood flow will result in capillary damage, therefore impeding glucose transport. A decrease in the brains primary source of energy translates to decrease metabolism and dysfunction of neurons. It has been suggested that beneficial cognitive effects observed from administration of vasodilators

and blood thinners such as such as ginkgo biloba and NSAIDs, respectively, may work through reversing this mechanism (Jellinger, 2002).

The presence of A $\beta$  results in an inflammatory cascade. In an attempt to clear the diffuse plaques, a robust microglia activation takes place. Microglia cells make an effort to clear debris and cellular damage associated with AD. However, in the process they release free radicals and cytokines, the latter of which activates astrocytes (McGeer & McGeer, 1995). The primary effect of microglia cells is phagocytosis and release of cytokines. Cytokine release attracts reactive astrocytes. Microglia cells also release dangerous free radicals resulting in oxidative damage to surrounding tissue. Both the astrocyte's generation of reactive oxygen species (Abramov et al., 2004) and the microglia release of free radicals contribute to an oxidative imbalance. Astrocytes also secrete inflammatory proteins, found in plaques, such as APOE and ACT (Abraham et al., 1988). These inflammatory proteins encourage the conversion of A $\beta$  from the soluble to aggregated species (Ma et al., 1994). An increase in compact A $\beta$  results in a mechanism of neuronal and synaptic loss (Yankner et al., 1995). The result of this inflammatory cascade is a primary source of cognitive impairment observed in the clinical symptoms of AD behavior.

Braak and Braak (1991a) have described three stages in the development of amyloid deposition. The first development of plaques is seen in the basal neocortex and the poorly myelinated temporal areas such as the perirhinal and ecto-rhinal cortex. A second stage is revealed when the plaques begin to branch out into adjoining neocortical areas as well as the hippocampus. In the final stage, dense deposits decorate all areas of the cortex and extend deep into the inner and outer pyramidal cell layers of the



hippocampal CA1 region. Prevalence of the final stage increases with age, however presence of early stages can be observed in young adults. At a late point a maximum density of A $\beta$  plaques is reached, however gray matter still remains relatively free of plaques (Braak & Braak, 1991a).

Neurofibrillary tangles (NFT) are a second pathologic feature found in AD. The category of NFTs encompass both the NFTs which develop within the nerve cell body as well as the neuropil threads (NT) which are found in the distal portions of dendritic processes (Braak & Braak, 1986). In the cortex, NFTs and NTs are selective for pyramidal cells (Braak & Braak, 1991a). These tangles result when the multifunctional tau protein is hyperphosphorylated. Tau proteins are a member of the microtubule-associated proteins (Weingarten et al., 1974). While these proteins are found mainly in the brain, they are seen in trace amounts in the periphery. The tau protein is involved in the assembly and stabilization of microtubules; however when this normal occurring protein is hyperphosphorylated, dimers of these phospho-tau units form insoluble paired helical filament structures that impair axonal transport, and ultimately lead to neuronal death. This cascade of events leads to loss of signal across the axon and neuronal death. The kinase involved in the abnormal phosphorylation is unknown, however, possible players include Cdk-5 and Gsk-3 $\beta$  which are overly active in AD (Cruz & Tsai, 2004, Maccioni et al., 2001). Tangle bearing neurons are found in large numbers in the frontal, temporal, and parietal cortex as well as in the hippocampus and amygdale (Selkoe, 2000).

Braak and Braak 1991a describe the evolution of NFT's as a "gradual development of brain destruction which begins in a few limbic areas of the cerebral cortex, which then spreads in a predictable, nonrandom manner across the hippocampus,

the neocortex, and a number of sub cortical nuclei.” In a pre-lesions AD brain, void of both A $\beta$  plaques and NFTs, abnormally phosphorylated tau protein is observed in the transentorhinal cortex (Braak and Braak, 1991a). As neurons develop NFTs in the transentorhinal cortex, criteria for stages I and II of AD neuropathological changes are met. It is important to note that at this stage brain changes are not significant enough to produce clinical behavioral symptoms. As NFT formation progresses into stages III and IV, there is striking neurodegeneration with in both the entorhinal and transentorhinal regions. While the neocortex remains intact there are mild changes in the hippocampus. It is this stage that first cognitive and personality changes are observed. It is important to note that it is not the destruction of the entorhinal and transentorhinal cortices alone which produce the behavioral changes, but instead it is loss of communication between the neocortex and the hippocampus. Personality changes can be attributed to disruption of the limbic circuit, also seen in stages III and IV. In end stages of AD, NFTs can be observed decorating virtually all subdivisions of the cerebral cortex. Braak and Braak 1991a make particular note of “severe destruction of neocortical association areas.”

The complex interaction between A $\beta$  and NFT formation is still being elucidated. NFTs are not solely found in AD. Other neurodegenerative diseases lacking A $\beta$  pathology have NFTs present that are indistinguishable from those found in the AD brain. While these two hallmark pathological features can occur independently, Gotz et al., (2001) described a cause and effect interaction between the two. Gotz et al., (2001) observed that injection of A $\beta$ 42 fibrils into the somatosensory cortex and hippocampus of 5- to 6-month-old P301L tau transgenic mice caused a fivefold increase in the numbers of NFTs in cell bodies within the amygdala, from where neurons project to the injection

sites. This data would suggest that presence of A $\beta$  induces NFT formation or at least accelerates its production. Recent work by Oddo et al., (2004) showed that A $\beta$  immunotherapy leads to clearance of both A $\beta$  plaques as well as hyperphosphorylated tau aggregates. The clearance of the two hallmark lesions were cleared in a hierarchal fashion with the clearance of A $\beta$  plaques preceding the clearance of hyperphosphorylated tau. Upon return of the lesions, the A $\beta$  plaques returned before the tau associated pathology (Oddo, 2004). This data would provide evidence in support for the “amyloid cascade hypotheses” stating that “accumulation of A $\beta$  in the brain is the primary influence driving AD pathogenesis, and that the rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between A $\beta$  production and A $\beta$  clearance (Hardy, 2002).”

#### *Neuronal Loss, Synaptic Loss, Brain Atrophy*

Neuronal loss in the entorhinal cortex, hippocampus, frontal, parietal and temporal cortices has been observed in AD brains. Neurons in layer II of the entorhinal cortex and the CA1 region of the hippocampus are particularly vulnerable to the AD process (Mattson, 2004). The pattern of neuronal death in AD is similar, to but different from, the pattern seen in typical aging (West et al., 1994, Fukutani et al., 1995).

Degenerative changes found in AD in the CA2, CA3, CA4 and presubiculum overlap changes observed in normal aging however, neuronal loss seen in entorhinal cortex, CA1, and para and post subiculum, which are generally preserved in the aged hippocampus, die in AD (Fukutani et al., 1995). This would suggest that AD is not simply an exaggerated aging process. Neuronal death in AD is said to occur in a spatio-

temporal pattern (Mattson, 2004). Neuronal death occurs over a long period of time, suggesting that neurons drop off in small numbers over the course of the lengthy disease process. The spatio-temporal pattern combined with the relatively small number of neurons dying at any one time would suggest that apoptosis is involved in the neuronal death present in AD. In contrast to apoptosis, necrosis of neuron occurs in large number over a short period of time. Due to the long pre-clinical and clinical phase and of the disease, death by apoptosis is a likely mechanism. Further evidence includes altered expression of apoptosis-related genes such as Bcl-2 family members, prostate apoptosis response-4 (par-4), DNA response genes, and p53, within neurons associated with plaques in AD brains (Mattson, 2000) . Other evidence for apoptosis in AD includes the presence of APP mutations, A $\beta$ , and formation of NFTs are all possible trigger of the programmed cell death cascade (Chan et al., 2002 , Mattson, 2004). While apoptosis may play a significant role in neuronal death, a combination of both necrosis and apoptosis most likely contributes to the profile of the AD brain.

Synaptic loss is established as a reliable neurobiological correlate of the cognitive deficits associated with AD (DeKosky & Scheff, 1990). Synapses are extremely vulnerable in AD due to there high content of disease-related protein, APP and Presenilins, as well as their high metabolic and oxidative loads (Mattson, 2004). Emerging evidence suggests that synapses are not functioning optimally even before structural deterioration. While synaptic loss is a consequence of neuronal death, Coleman and Yao (2003) have shown evidence that compromised synapses are observed in living intact neurons in the AD brain. Specifically the function of synapses appears to be compromised. Coleman and colleagues hypothesis impaired synaptic function may be

due disruption in synaptic vesicle trafficking. Their recent work shows a reduced expression of genes related to synaptic vesicle trafficking in AD (Coleman & Yao, 2003). Early memory loss in AD patients has been hypothesized to be a synapse dysfunction caused by soluble A $\beta$  oligomers (Lacor et al., 2004). A $\beta$  oligomers, present in AD brains as well as transgenic mouse models of AD, rapidly block LTP (Chen et al., 2000; Walsh et al., 2002). The hypothesis that the AD brain contains functionally altered, however, structurally sound synapses offers hope that early impairment found in AD may be reversible with new therapeutics targeting this synapse dysfunction.

Calcium plays a fundamental role in learning and memory and is also involved in neuronal and synaptic health and function. Disruption to calcium homeostasis is closely involved in the neuronal death observed in AD (Verkhratsky and Toescu, 2003). Bathing neurons in excess calcium induces neurotoxicity. Improper regulation of intracellular calcium ions is associated with A $\beta$  (Mattson, 1997) and NFT's (Saito et al., 1993). A $\beta$ 's oxidative stress characteristics have the ability to impair calcium pumps and increase influx through voltage-dependent Ca<sup>++</sup> channels while NFT containing neurons show high amounts of calcium as well as hyperactivity of calcium-dependent proteases (Mattson, 2004). The disruption in calcium, leading to neuronal and synaptic loss in AD, is the targeted mechanism by which a new class of NMDA receptor antagonist exploits.

Cerebral atrophy is observed in the AD brain at autopsy as well as through imaging techniques. Widening sulci and dwindling gyri comprise the gross anatomical features of the AD brain. Ventricular dilation is also observed. Annual rates of global brain atrophy in AD patients is about 2–3% compared with 0.2–0.5% in healthy controls

(Fox & Schott, 2004). Pennanen et al. (2004) observed brain volume changes in the hippocampus and entorhinal cortex of patients with MCI and early stages of AD. “Volumes of the hippocampus and entorhinal cortex were significantly reduced in the following order: control > MCI > AD (Pennanen , 2004).” In the final stages of the disease, A $\beta$  and NFT’s combined with synaptic and neuronal loss can be accountable for a substantial loss in total brain volume.

### *Genetics of Alzheimer’s Disease*

Ten percent of cases of Alzheimer’s disease suggest a familial mode of transmission (Selkoe, 2001). The other 90% of cases have been described as non-familial or sporadic cases. Through phenotypic analysis, using identified AD related genes, the majority of cases appear in the absence of a genetic link. Many investigators hypothesize that a larger percentage of cases will be linked to genetic predisposition as interrelated genes are found. Phenotypically, familial and sporadic cases of AD are often indistinguishable (Selkoe, 2001). Often the autosomal dominant form of AD will result in an early age of onset, however progresses as does the late onset sporadic form.

The genes contributing to the 10 percent of familial cases have added a great deal to the understanding of AD. Mutations in 4 genes have been affirmed to increase risk of AD. These include mutations in the amyloid precursor proteins (APP), presenilins 1 and 2 genes (PS1 and PS2) as well as alteration in the apolipoprotein E alleles (ApoE).

### *Amyloid Precursor Protein*

The finding that both AD and Down Syndrome brains shared A $\beta$  plaques with an identical sequence narrowed the search for a gene involved in familial AD to chromosome 21 (Glennner & Wong, 1984). These findings combined with the later cloning of the APP gene (Tanzi et al., 1987) set the stage for a new era in AD research. APP is a transmembrane polypeptide that is translated in the ER and then posttranslationally modified through a secretory pathway (Selkoe, 2001). The N-terminal moiety of APP is projected towards the extracellular domain while the C-terminal projects into the cytoplasm (Neve et al., 2000). Three major isoforms exist including 695, 751, and 770. APP is processed by cellular proteases known as  $\alpha$ ,  $\beta$  and  $\gamma$  secretases. The benign cleavage by  $\alpha$ -secretase at 688 results in the release of a large soluble fragment into the extracellular space and in turn prevents the formation of A $\beta$ . In contrast, the  $\beta$ -secretase cuts at residue 671 releasing an ectodomain derivative and exposing residue 1 of the A $\beta$  peptide. Previous to cleavage by  $\beta$ -secretase a constitutive cleavage at 711 or 713 by  $\gamma$ -secretase results in the formation of Ab40 or Ab42 respectively. These detrimental A $\beta$  forming cleavages increase with age and or a genetic mutation (discussed below).

The missense mutations of the APP gene are rare causes of FAD. While rare, these mutations represent the first findings of a genetic cause of AD. Mutations of the

APP gene are strategically located near known cleavage sites indicating their effect on proteolytic processing of AD. Families that carry APP missense mutations have AD onset before 65 and as early as 30 (Selkoe, 2001). Mullan et al. (1992) identified a double mutation at codons 670 and 671 in exon 16 which co-segregated with AD in two large early-onset AD families from Sweden. Base pair transversion from G to T and A to C results in amino acid substitutions of Lys to Asn and Met to Leu at 670 and 671 respectively. These mutations located at the N-terminus of the peptide increase the cutting of  $\beta$ -secretase resulting in the generation of more A $\beta$ 40 and A $\beta$ 42. Mutations at the C-terminus end of APP selectively increase A $\beta$ 42. The APP 717 mutation is an example of a single missense mutation found at the C-terminus. This mutation replaces a Val for a Leu and results in the increase of  $\gamma$ -secretase. An increase in  $\gamma$ -secretase increases release of A $\beta$  peptide from the larger APP protein, resulting in an increase in production of A $\beta$  1-42.

### *The Presenilins*

Due to the genetically heterogeneous nature of AD, the finding of other autosomal dominant mutations resulting in FAD was a clear objective. Missense mutations of the presenilins provide the most common cause of autosomal dominant cases of AD to date (Selkoe, 2001). The presenilin mutations selectively increase  $\gamma$ -secretase cleavage of C99 and C83, yielding peptides ending in A $\beta$ 42. When examining families harboring these mutations, an approximately two-fold increase of A $\beta$ 42 was observed (Scheuner et al., 1996). Currently two genes of interest make up the presenilin family. The PS1 missense mutations, found on chromosome 14, have been linked to



families with clinical onset of AD in their 40s and 50s and as early as 30s. A possible 75 missense mutations have been found on the PS1 gene (Hardy, 1997). A homologous PS2 gene linked to AD pathogenesis was found in a series of German families originally from the Volga valley in Russia (Levy-Lahad, 1995). The 3 mutations identified on the PS2 gene result in AD with a later and more variable age of onset than mutations in PS1 (Hutton, 1997)

### *Apolipoprotein*

While mutations of the APP gene and the presenilins make up a small percentage of AD cases, the presences of the Apolipoprotein E allele (ApoE) represents a major genetic predisposition to AD. ApoE, located on chromosome 21, exists in three variants: ApoE2, ApoE3 and ApoE4. ApoE is involved in the transport of cholesterol and is important in local circuits of lipids turnover (Veurink et al., 2003). The presence of 1 or 2 copies of the ApoE4 species increase the risk of AD (to be discussed later) and decrease age of onset. In contrast to APP and PS mutations, ApoE represents a genetic player of AD not inherited in an autosomal dominant fashion. Presence of the ApoE allele is a genetic factor linked to late-onset AD cases. Thus the presence of ApoE 4 precipitates the disorder primarily in subjects in their 60s and 70s (Selkoe, 2001).

### ***Risks of Alzheimer 's Disease***

There are several generally accepted risk factors of AD, while there are many others still under investigation. Unmodifiable risk factors include genetic mutations (discussed above), family history, and being a female. Head injury, diet, and education

levels are examples of potentially modifiable risk factors. Rising age is the principle risk factor in the development of AD. Of the population aged 65 years and older, the proportion of individuals with AD roughly doubles with each additional 10 years of age. By 85, over 50 % of the population has developed AD ( NIH, 1995). This increased risk with age peaks at 90 and then proceeds to drop off. First degree relatives of sporadic AD patients, despite no FAD association, have a lifetime risk increased by 3 or 4 fold (Liddell et al., 2001). Females are also twice as likely to develop AD as males (Jorm et al. 1987).

Another major unmodifiable risk factor in the development of AD is inheritance of one or two copies of the ApoE4 gene. Possession of ApoE 4 alleles increases risk of developing AD in a dose dependant fashion. Individuals with two copies have earlier onset than those possessing one allele (Corder et al., 1993). While presence of the ApoE4 allele is not diagnostic of AD, their presence accounts for 50% of early-onset AD cases and 20% of late-onset cases (Rosenthal & Khotianov, 2003). The presence of ApoE4 alone causes increase risk in the white population while both ApoE 2 and 4 increase the risk in African Americans (Maestre et al., 1995). While ApoE genotyping is commercially available, it offers little advantage as a prognostic indicator. Females carrying the ApoE 4 allele have a 45% probability of developing AD by age 73, while male have only a 25 % probability of developing AD (Breitner et al.,1999).

The mechanism by which presence of ApoE4 may be increasing risk of AD is still in need of clarification; however several hypotheses have been explored. Gearing et al. (1996) showed that the extent of A $\beta$  deposition positively correlates with the number of ApoE4 alleles. ApoE4 has also been shown to increase the size and density of A $\beta$  plaques

(Yamaguchi et al., 2001). Many suggest that these findings are due to ApoE's involvement in the clearance of soluble A $\beta$  from the brain. A lack of clearance may promote the aggregation of A $\beta$  peptide therefore increasing insoluble A $\beta$  form. The ApoE isoforms have differential binding to A $\beta$  (ApoE2>ApoE3>ApoE4) (Yang et al., 1997). This would suggest that risk may be due to the absence of the high affinity binding ApoE2 form rather than the presence of the ApoE4 form. Further evidence for this hypothesis is ApoE's association with antioxidant activity. In a step down paradigm, ApoE2 is the most effective antioxidant while ApoE4 is the least effective (Miyata & Smith, 1996). Carefully designed in vitro and in vivo experiments are still needed to ultimately clarify ApoE's role in the increase risk of developing AD.

There are also a number of potentially modifiable risk factors of AD. Head trauma has been linked to an increase risk of developing AD. After a significant trauma to the human brain, both A $\beta$  (Roberts et al., 1994) and tau pathology (Smith et al., 2003) appear even in young patients (Emmerling et al., 2000). Levels of A $\beta$  in the CSF are also elevated after injury. Repeated mild traumas have been shown to accelerate A $\beta$  production and induce cognitive impairment (Geddes et al., 1999). This correlation between head trauma and increase in A $\beta$  offers a mechanism by which head trauma increase risk of developing AD (Jellinger, 2004).

Many findings support that environmental factors (including diet), are important variables in increasing or decreasing the risk of AD. People of Japanese origin living in the United States have a 6.24% chance of developing dementia, while their counterparts living in the Japanese homeland have a less than 2% risk (Graves et al., 1996). A possible reason for this discrepancy has been attributed to differences in diet. Two lines

of research connect the high levels of total cholesterol with increase risk of AD. The first direct correlation is an association between elevated midlife total cholesterol levels and late-life Alzheimer disease (Notkola et al., 1998). A second point of indirect evidence is work showing a reduced rate of the disease in people who used statins to reduce their blood total cholesterol level (Jick et al., 2000). These findings combined with ApoE's involvement in cholesterol transport makes for a causal web between environmental and genetic interaction. Kalmijn et al. (1997) suggested that a diet high in saturated fat increases the risk of dementia, whereas fish consumption may decreased this risk. Along with an increase in fish (omega-3 fatty acids) consumption of other dietary supplements have also been correlated with a decrease in risk of AD. Reactive oxygen species are associated with neuronal damage in AD, making the addition of antioxidants into the diet a possible risk reducer. Results from studies trying to link increase in antioxidant vitamin consumption and risk of developing AD are inconsistent. A study in 5395 people age 55 years and older found that dietary intake of vitamins E and C, but not supplement intake, was associated with a low risk of AD (Engelhart et al., 2002). Morris et al. (1998) showed that use of higher-dose vitamin E and vitamin C supplements may lower the risk of AD. In contrast, another 4 year study involving 980 individuals age 65 years and older did not find any associations between dietary, supplement, or total intake of carotenes, vitamin E, or vitamin C with a low risk of AD (Luchsinger et al., 2003). Due to the relative safety of dietary and supplemental vitamins, the addition of these vitamins may be seen as a benign measure, not proven, in trials of primary prevention.

Another medical risk factor associated with AD is raised blood pressure.

Kivipelto et al. (2001) colleagues examined midlife vascular risk factors and AD. This

study showed high systolic blood pressure in midlife was a significant risk for AD in later life. In contrast, high diastolic pressure in midlife did not translate into an increase risk. While diastolic pressure, in this study appeared to be less of a risk factor, previous studies observed an increased risk with elevated diastolic pressure (Skoog et al., 1996; Launer et al., 2000). Kivipelto et al (2001) noted “our data should not be interpreted as discounting the potential risk of Alzheimer's disease related to raised diastolic blood pressure but rather as emphasizing the importance of raised systolic blood pressure, even in people with normal diastolic blood pressure.” Similar to the indirect support for decreased cholesterol with use of statins, discussed above, patients treated with antihypertensive drugs may be at decreased risk for dementia (Forette et al., 1998).

A plethora of research has been conducted on education levels and risk of developing AD. Several studies have reported an increased risk of dementia and Alzheimer's disease among less-educated persons (Schmand et al., 1997; Evans et al., 1997; Katzman, 1993). Due to the intimate relationship between education and the cognitive component of environmental enrichment, levels of education and risk of AD will be discussed in detail in a later section.

### ***Diagnosis of Alzheimer's Disease***

While no cure is currently available, early diagnosis allows for patients to take advantage of current pharmaceutical treatments as well as prepare for future care while the patient is still cognoscente. A definitive diagnosis of AD can not be made until autopsy, however using a combination of neurological exams, laboratory and genetic tests and brain imaging techniques 90% accuracy of diagnosis can be reached.

Neurological exams (NEs) enable clinicians to evaluate a patient's cognitive status, emotional, psychological, motor and sensory function. Evaluation of these measures provides information about the presence and progression of the disease. Firstly (NEs) offer a relatively sensitive diagnostic tool for AD. NEs also distinguish AD from other neurological illnesses. After initial diagnosis, periodical NEs offer a way in which to track the disease's progression. NEs also offers a way in which to customize treatments. Due to the variability in symptoms associated with AD, NEs offer a systematic description of symptoms which can then be addressed on an individual basis.

The Mini Mental State Exam (MMSE) is a widely used NE used to identify dementia. Folstein, the developer of the MMSE, described it as "practical method for grading the cognitive state" (Folstein et al, 1975). The test includes questions which evaluate orientation, memory, attention, calculation ability, language, and writing. There is a maximum score of 30 attainable, while a patient with AD will score a 26 or below. It is important to note that the education of the patient should be taken into account when using the MMSE as an indicator of dementia. Once a score below 26 is obtained and a diagnosis of dementia is given, laboratory tests (discussed below) need to be performed to rule out other common causes of dementia. There are many other NEs available, including tests which measure cognitive symptoms such as clock draw test and the Blessed test. Tests for non-cognitive symptoms include Behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD) and the Neuropsychiatric Inventory (NPI).

Brain imaging through radiological techniques are used as diagnostic tools of AD. This diagnostic area can be broken up into structural radiography and functional

radiography. Structural radiography implements the use of MRIs, and CT scans to identify gross anatomical changes due to AD or other forms of dementia. Functional radiography, fMRI's and PET scans, can detect focal metabolic changes in the brain. Both of these subcategories can be useful additions in the differential diagnosis of AD.

Magnetic resonance imaging (MRI) allows for volumetric measurements of anatomically relevant structure in the AD brain. As discussed above, the entorhinal cortex and the hippocampus show early neuropathological changes with the onset of AD. Numerous studies have shown MRI-based volumetric measurements of the entorhinal cortex and the hippocampus to be reliable diagnostic tools of early AD (Huesgen., et al 1993; Juottonen et al., 1998). Pennanen et al. (2004) have found MRI imaging to be advantageous in the classification of patients with mild cognitive impairment (MCI), early AD and cognitively normal elderly subjects. MRIs showed volumes of the hippocampus and entorhinal were significantly reduced in the following order: control > MCI > AD. Computerized tomography (CT) scans are a second tool of structural radiography. While the CT scan has less resolution than a MRI, they are readily available and can contribute as a component of AD diagnostics. Like MRIs, CT scans give the ability to detect, in vivo, anatomical changes including cerebral atrophy and ventricular enlargement. Condefer et al., (2004) examined the CT's impacted on diagnosis of dementia. The data collected showed that the addition of a CT scan in the diagnostic process of AD may be expected to impact diagnosis and treatment of dementia in 10-15% of cases. The MRI and CT brain imaging techniques are not sufficient alone to diagnose AD and continue to be a source to differentiate AD from other causes of dementia.

Functional brain imaging techniques offer the ability to indirectly measure neuronal activity in individual patients. Positron Emission Tomography (PET) and functional magnetic resonance imaging (fMRI) are two methods implemented in functional brain imaging. The most common methodology of PET imaging used in the characterization of AD is the measurement of cerebral metabolic rate using glucose analogue 18-fluorodeoxyglucose (FDG). After intravenous delivery, FDG is taken up by cells via a glucose transporter and becomes trapped after phosphorylation by hexokinase (Lee et al., 2003). The resultant PET image reveals the distribution of FDG uptake, which is a measure of cerebral metabolic rate in regions of interest. Individuals with a dementing illness other than AD often exhibit different abnormal metabolic patterns, thus making it possible to differentiate AD from other dementias. Bilateral temporo-parital hypometabolism is a consistently found pattern reported on PET scans in the AD brain (Lee et al., 2003). Nestor et al. (2004) found patients suspected of having AD to show dysfunction in temporo-parieto-occipital cortices. As the disease progresses, PET scans show metabolism in sensory and motor areas to remain relatively intact; however, severe AD patients often show frontal lobe hypo-metabolism (Lee et al., 2003). Hoffman et al., (2000) confirmed that bilateral temporo-parietal hypo-metabolism highly correlates with the pathological diagnosis of AD, making PET-FDG a likely diagnostic tool. A second novel use of PET scanning currently being examined as a diagnostic tool of AD, is the imaging of A $\beta$  plaques in vivo. Klunk et al., (2004) implemented a tracer called Pittsburgh Compound-B (PIB) to visualize amyloid deposition. The PIB is a neutral benzothiazole that binds to A $\beta$  and crosses the blood brain barrier. This compound also readily clears from the brain. PET scanning with PIB in AD patients decorated



association cortex region, a region commonly linked to high A $\beta$  deposition in the AD brain. This new technique could offer advancements in diagnosis as well as improved monitoring during clinical trials of new AD pharmaceuticals.

Like the PET scan fMRI offers a method to indirectly measure functional changes associated with neuronal activity. The most common fMRI methodology used is termed the blood oxygen level-dependent method (BOLD) (Lee et al., 2003). This method measures signal change associated with oxygenation in cerebral blood vessels. Changes in neuronal activity results in a decreased oxygenated to deoxygenated blood ratio. The presence of oxygenated blood results in a constant signal while deoxygenated blood results in a loss of signal. When a cognitive task is performed, a surplus of oxygenated blood results in a brighter signal. fMRIs have several benefits in comparison to PET scans and therefore are the preferred method in functional radiography AD diagnostic techniques. Primarily fMRI does not involve the invasive injection of radioactive dye. This benefit allows for repeated procedure during the course of the disease with no danger to the patient. A second benefit is that fMRI may be performed on any clinical MRI scanner and is therefore accessible to many patients. Numerous approaches using fMRI have emerged in the study of AD diagnostics. One approach examines fMRIs conducted during cognitive tasks that are performed easily by non-AD individuals, but which are more challenging for patients at risk of developing AD. When comparing fMRIs of control individuals and asymptomatic ApoE4 carriers, Brookheimer et al., (2000) observed that patterns of brain activation during tasks requiring memory differed depending on the genetic risk (presence of ApoE4 allele). fMRIs may help to identify patients with neurochemical changes in the early stages of AD by studying

signal reactivity to pharmacological challenge. The data collected showed that impaired cholinergic systems can be identified with fMRI (Goekoop et al., 2004), making this imaging technique a viable tool of early detection.

Many other causes of dementia can mimic AD. A standard laboratory workup is conducted to rule out nutritional deficiencies, infection, metabolic disorders, and drug effects as possible culprits of dementia. While these laboratory tests rule out AD there are other tests under investigation as diagnostic tools of AD. Cerebrospinal fluid tests for the presence of proteins associated with AD pathology. This extremely invasive procedure measures total tau, phosphorylated tau and A $\beta$ 42. A patient with AD would show high levels of tau protein and phosphorylated tau, however a low level of A $\beta$ 42 (Blennow & Vanmechelen, 2003). Tau protein represents neuronal damage while phosphorylated tau specifically reflects the phosphorylation state of tau, which correlates to the formation of tangles. A $\beta$ 42 levels are decreased, due in part to trapping of brain A $\beta$  into plaques, as well as other unknown reasons (Blennow & Vanmechelen, 2003). Serum levels of iron binding protein p97 are another possible biochemical marker involved in AD. Kennard et al. (1996) provided evidence that the soluble form of iron binding protein p97 is found in elevated amounts in the serum of AD patients. While still in its infancy, this test represents the first blood test, aside from genetic testing, that may have potential for identifying subjects afflicted with AD.

A final diagnostic tool to be discussed is the controversial genetic testing option for patient with a possible predisposition to AD. As discussed above many mutations of the APP, PS1, PS2 have been found and linked to genetic causation of AD. Presence of ApoE4 has also been linked with increase prevalence of AD. Many clinicians raise the

question if testing for these abnormalities meet the criteria for a diagnostic test. van der Cammen et al. (2004) states “In general, a medical test should be useful in such a way that it raises diagnostic certainty and has positive implications for the treatment and well-being of the individual patient.” Many have questioned if genetic testing of AD meets these criteria. The test for ApoE, commercially available, is the most important genetic determinate for late-onset AD. Numerous studies have constantly found ApoE genotyping alone does not have sufficient sensitivity in diagnosing susceptibility to developing AD (Mayeux et al., 1998; Slioter et al., 1996). When causal genes such as APP, PS1 and PS2 were examined in early-onset AD cases, less than 10% of cases were linked to relevant AD genes, leaving 90% of cases with no association (van der Cammen et al., 2004). While genetic testing in the research world may offer insight into the molecular mechanism of AD and allows for the generation of useful transgenic animal models, its place as a clinical diagnostic tool of AD is still in need of maturation.

### ***Treatment of Alzheimer’s disease***

Although there is currently no cure for AD, the FDA has approved two classes of drugs that have shown to be safe and effective in the treatment of AD. The first class to be approved include the acetylcholinesterase inhibitors Aricept, Exelon & Reminyl, Cognex. These palliative treatments increase the compromised brain acetylcholine levels found in AD by decreasing the breakdown of acetylcholine by acetylcholinesterase in the synaptic cleft. While this class of drugs does not slow the progression of AD, it does provide cognitive benefits for patient in the early stages of AD (Sonkusare et al., 2005). As the disease progresses the acetylcholinesterase inhibitors class of drugs loss

efficiency. The window of effectiveness is typically 6 months to 2 years (Delagarza et al., 2003).

A second class of drugs recently approved for moderate to severe AD are NMDA- receptor blockers. While intact NMDA function correlated with cognition and learning, over-stimulation of glutamatergic system may result in neuronal damage. An over-excited glutamatergic system will result in an abundance of intracellular calcium, resulting in a toxic environment for neurons. Memantine (Namenda) is an uncompetitive NMDA-receptor antagonist that has been show to be effective in the treatment of moderate to severe AD (Reisberg et al., 2003). The blocking of NMDA-receptors leads to suppression of over-excited glutamatergic systems and therefore removes neuronal calcium overload. Implementing this mechanism, Memantine treatment reduced clinical deterioration in moderate to severe AD (Reisberg et al., 2003). In severely demented patients, treatment with memantine improved function, decreased care dependence and decreased behavioral symptoms associated with severe AD (Winblad & Poritis, 1999). It has been suggested that a combination therapy including an acetylcholinesterase inhibitors and memantine may offer the most benefits to AD patients.

Many alternative non-FDA approved compounds have emerged as possible treatments of AD. Nonsteroidal antiinflammatory drugs (NSAIDs) are the most widely used drugs for management of pain, fever, and inflammation (Gasparini et al., 2004). There mechanism of action is largely attributed to the inhibition of cyclooxygenase (COX 1-2), leading to the suppression of prostaglandins synthesis. Epidemiological studies have shown that prolonged use of NSAIDs leads to a decrease risk of developing AD (Zandi et al., 2002; 2004). While NSAIDs as a prevention have shown positive results

NSAIDs as treatments are faced with mediocre beneficial findings and controversial mechanisms of action. Aisen et al. (2003) found no beneficial cognitive effects of treatment with a Cox-2 inhibitor (rofecoxib) or a non-selective NSAID (naproxen) in patients with mild to moderate AD. Reines et al. (2004) provide further support for this finding by showing “Rofecoxib had no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study.” Some suggest NSAIDs work by managing the chronic inflammation associated with AD pathogenesis, while others suggest a mechanism through which NSAIDs alter the activity of  $\gamma$  secretase and therefore alter the production of A $\beta$  (Lleó et al., 2004). Recently the National Institute on Aging halted the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT), in part because of the recall of cox-2 inhibitor rofecoxib (Vioxx) due to increased risk of cardiovascular events and health concerns surrounding other NSAIDs including celecoxib (Celebrex), and naproxen (Aleve).

Antioxidants have also been noted as possible treatments of AD. As discussed above, the amyloid cascades associated with an increase in oxidative stress. Antioxidants such as alpha-tocopherol (vitamin E) and Ginkgo Biloba have been investigated as possible treatments for AD. Vitamin E (2000 IU/day) has been shown to slow the progression of AD by about 7 months when taken over a two year period of time (Berman & Brodaty, 2004). Ginkgo Biloba has been shown to increase  $\alpha$  waves in AD patients as well as stabilize and improve cognitive function (Maurer et al., 1997). Larger clinical trials are needed to provide further support for these antioxidants as treatments for AD.

Aside from treating the cognitive symptoms of AD, psychiatric and behavioral disturbances associated with AD are often treated with FDA-approved anti-psychotic and anti-depression drugs. Risperidone (Risperdal), a serotonin-dopamine antagonist, has been shown to significantly improve symptoms of psychosis and aggressive behavior in patients with severe dementia (Katz et al., 1999). Selective serotonin reuptake inhibitors and benzodiazepines have also been used in treating depression, insomnia and anxiety in patients with AD.

New treatments for AD seeking FDA approval are on the horizon. Research into developing drugs which stop progression of AD rather than treat symptoms are needed in the clinical arena. Compounds that inhibit the enzymes responsible for abnormal APP cleavage are under heavy investigation. Vaccinations against A $\beta$  have been met with problems during phase 2 clinical trials, however, passive rather than active A $\beta$  vaccination appears promising. A new therapeutic currently involved in phase III clinical trials has been shown to have potential as a new therapeutic for AD. Alzheimed is expected to act on two levels: firstly to prevent and stop the formation A $\beta$  fibrils in the brain and secondly to inhibit the inflammatory response associated with A $\beta$  build-up in AD (Geerts, 2004). The developments of transgenic animal models have catalyzed the development of new therapeutics and will continue to be a medium for which novel treatments can be developed.

### **Animal Models of Alzheimer's Disease**

Genetically altered mice offer a tool to define in vivo functions that have been observed in vitro. The ability to model a disease in a transgenic mouse enables researchers to study mechanisms of disease progression, examine possible preventions and devise viable treatments. In order for the creation of a transgenic animal to become feasible, genes responsible for the disease must be identified through linkage studies. Once identified, the generation of a transgenic mouse model can proceed.

Generating a transgenic mouse model begins with the selection of a transgene (Picciotto & Wickman, 1998). A transgene is defined as the segment of DNA that is transcribed into a mouse model. The classical transgenic construction requires a segment of DNA to be transcribed then subcloned down-stream of a neuron-specific promoter. The promoter dictates the level of expression, tissue specificity and temporal pattern of the gene involved. The gene and promoter are then excised from the plasmid, resulting in the isolated transgene. Multiple copies of the isolated segment are injected into the pronucleus of a single fertilized egg and then implanted into a foster mother. At time of injection into the pronucleus, the gene is either integrated or degraded. Those eggs that integrate the gene become viable offspring, and carry the transgene in their gametes, go on to comprise the transgenic fonder mouse. Once the fonder line is screened for number of transgenes integrated, sight of integration, and the transgene's

interaction with neighboring endogenous genes, the founder line goes on to generate a colony of transgenic mice.

An important element in the creation of a transgenic mouse model is the background strain of the embryos chosen. A blend of cognitive, motivational, and sensorimotor elements are needed to construct a transgenic animal that can be implemented in behavior. A strain which is cognitively superior may overshadow a behaviorally detrimental transgene. When modeling the pathology of a disease, strain background plays an equally important role. Mice expressing the APP gene, generated on a C57BL/6 background, presents with A $\beta$  plaques and corresponding behavioral impairment (Picciotto & Wickman, 1998). In contrast, when the APP transgene is inserted into an embryo of a mouse with a FVB background, plaques are absent (Picciotto & Wickman, 1998). Transgenic mice with hybrid strain backgrounds offer a more practical model that demonstrates pathology and behavior which closely mimics the disease modeled.

### ***The PDAPP Mouse Model of Alzheimer's Disease***

The PDAPP mouse model of AD is one of several models implementing the missense mutations of the APP gene. These mutations have been linked to early onset familial AD. PDAPP mice were generated using the platelet-derived growth factor directing the human APP gene encoding the 717 mutation (Games et al., 1995). The mice were derived from several hybrid backgrounds, resulting in a mixed strain of C57BL/6 +DBA+ Swiss-Webster. Games et al. (1995) confirmed the successful insertion of the altered human APP gene and showed the PDAPP mouse had a 10 fold



overexpression of APP independent of endogenous mouse APP levels. This expression was observed in multiple tissues and most notably in the brain. In contrast to previously generated transgenic models containing human non-mutated APP, the PDAPP model exhibited A $\beta$ -production & deposition. The success in generating a transgenic model with substantial A $\beta$  deposition may have been due to numerous factors. Primarily, the higher levels of the human APP expression may have led to the generation of reproducible A $\beta$  pathology. Secondly, as discussed above, the promoter drives the amount of expression and tissue specificity. Implementing the platelet-derived growth factor promoter allowed for targeted expression preferentially in neurons of the cortex, hippocampus, hypothalamus and cerebellum.

#### *PDAPP Pathology*

The A $\beta$  peptides observed in the PDAPP model ranged from diffuse soluble forms to compact plaques stainable with thioflavin S, Bielschowsky silver stain and Congo red. The outskirts of the plaques were intimately surrounded with reactive astrocytes, replicating plaques found in the brains of AD patients. Also closely related to the AD brain, A $\beta$  plaques were associated with dystrophic neurites. A final observation noted by Games et al. (1995) was the reduced synaptic and dendritic density in the hippocampus of PDAPP mice.

Games et al. (1995) characterized the progression of plaque development in the heterozygous PDAPP mice. At 4 months no plaques were present and transgenic animals were indistinguishable from nontransgenic littermates. By 6-9 months plaques began to appear in the hippocampus, corpus callosum and cerebral cortex. Animals greater than 9

months showed “numerous” dense deposits. This progression closely mimics progression in the AD patient.

Masliah et al. (1996), Irizarry et al. (1997), Dodart et al. (2000a), and Chen et al (2000) went on to further analyze the neurodegenerative pathology in the PDAPP model. Using the same line discussed above by Games et al. (1995), an extensive analysis of pathology was done through use of electron microscopy by Masliah et al. (1996). Masliah et al. (1996) described the extracellular plaques observed in PDAPP mice to be “strikingly similar to those observed in AD brains.” Confirming previous findings by Games et al. (1995), Masliah et al. (1996) described dense neuritic plaques in frontal cortex and hippocampus. Associated with these plaques were dystrophic neurites and reactive astrocytes. Further analysis of this same line of PDAPP mice by Irizarry et al. (1997) described levels of APP expression, regional pattern of deposition, and neuron counts in relevant regions. Across all 3 time points investigated (4, 11 and 18 months), APP expression remained stagnant. Despite the absence of difference in expression across time, progression of A $\beta$  deposition in a regionally specific manner was observed. At 8 months of age A $\beta$  deposition was isolated to the cingulate cortex. By 12 months, A $\beta$  spread into surrounding CA1 of the hippocampus and entorhinal cortex. At 18 months, a substantial increase in A $\beta$  was observed in the order of 20-50%. Implementing stereological techniques, neurons in the CA1, cingulate and entorhinal cortex were counted. In sharp contrast to human AD brains, no neuronal loss was observed in any region at any time point in the PDAPP mice. This lack of neuronal death in PDAPP mice strongly disputes theories that A $\beta$  plaques directly cause neuronal death by neurotoxic mechanisms. Irizarry et al. (1997) point out that absence of neuronal death may

have been due to substandard methodological protocols not identifying small subpopulations of neuronal death. A second hypothesis suggested that greater time of exposure to toxic A $\beta$  is needed to produce neuronal death. Later, Dodart et al. (2000a) provided work supporting previous findings of “age-dependent region specific” deposition of A $\beta$  in heterozygous PDAPP transgenic mice. Additional data of a direct comparison between homozygous and heterozygous PDAPP mice enabled the analysis of dose dependent expression of APP on the resulting A $\beta$  pathology. At a 10-12 month time point, a three-four fold greater total number of plaques in homozygous vs heterozygous animals was seen. While this impressive distinction was observed in cortical regions, less separation was observed in the hippocampus. In contrast to early work by Games et al. (1995), Dodart et al. (2000a) observe first mature A $\beta$  deposits in 3-4 old month animals. These plaques were exclusively located in the CA1 region of the hippocampus, the medial part of the cingulated cortex, and corpus callosum at the level of the dorsal hippocampus. This contradiction to Games et al. (1995)’s findings of “no obvious pathology detected at 3-4 months,” may be due to extending pathological examination past “obvious” pathological changes to more obscure region specific changes. At 6-7 month and 10-12 month time points, while Dodart et al. (2000a) provides a more extensive exploration of region specific distribution of pathology, the findings closely mimic previous abbreviated findings by Games et al. (1995). In behaviorally tested animals, Chen et al. (2000) describe relatively low levels of deposition in the hippocampus of 9 month old animals with an increase in density at the 16 and 22 month time points. The findings at the 9 month time point are difficult to compare to previous work (Games et al., 1995; Masliah et al., 1996; Irizarry et al., 1997) due to the possible

confounding variable of behavioral testing. However, Dodart et al. (2000a) shows “numerous mature deposits” at 6-7 months in similarly behaviorally tested PDAPP mice. This difference may be due to Dodart et al.’s counting of plaques (definition of “numerous plaques” unknown) versus Chen et al. (2000) analysis of plaque density. In summary of A $\beta$  associated pathology in PDAPP transgenic model, these mice exhibit regional and age-dependent A $\beta$  deposition, independent of a constant over expression of APP, as well as lack of neuronal loss associated with the AD brain.

The earliest change in the AD brain which correlates with cognitive function is synaptic loss. Alterations in synapses in the PDAPP model have also been observed. Dodart et al. (2000a) observed an increase in synaptic density in young 3-4 month old animals, however, saw a decrease in mice older than 6 months. The decrease in later life was suggested to be related to the neurotoxic effects of A $\beta$ . The increase early in life was observed in the absence of A $\beta$  plaques and correlated with APP expression. This would suggest a beneficial effect of overexpression of APP. APP expression has been linked to neurite outgrowth and synaptotrophic properties (Morimoto et al., 1998; Wallace et al., 1997). Larson et al. (1999) studied synaptic transmission and long term potentiation in hippocampal slices from young (4-5 months) and old (27-29 months) PDAPP mice. As discussed above, young PDAPP mice have high expression of APP but lack plaques. Aged animals have the same level of high APP expression; however, have a high burden of A $\beta$  plaques. In vivo electrophysiological testing revealed that young PDAPP mice had enhanced paired-pulse facilitation, a significant decrease in maximal size of field EPSPs, and an increase in decay rate of LTPs. These results suggest synaptic changes occur independent of plaque deposition. To state synaptic changes are completely independent

of A $\beta$  is misleading due to the absence of a measurement of soluble A $\beta$  forms in this work. In the old animals, the most striking difference observed was that maximal synaptic field potentials were greatly reduced. In contrast to young animals, aged PDAPP mice showed significantly reduced paired-pulse facilitation. Despite a decrease in the number of functional synapses, LTP mechanisms were reported to be intact in these aged animals. Larson et al. (1999) concluded PDAPP mice have altered synaptic communication preceding presence of plaques. As well, disturbance in synaptic transmission in the presence of plaques are not linked to altered LTP.

In 2000, Dodart et al. noted neuroanatomical abnormalities that had been overlooked in previous studies discussed above. Dodart et al. (2000) described “rather marked hippocampal atrophy as early as 3 months of age. A 20 to 40 percent decrease that did not progress with age was observed. The early presence and lack of progression of this neuroanatomical abnormality suggests that overexpression of APP may effect neurodevelopment.

#### *Behavior of PDAPP Model*

The pathological changes found in the PDAPP mice translated into cognitively-based behavioral changes. In sensorimotor tasks, PDAPP mice performed identical to nontransgenic mice at both early and late time points (Dodart, 1999; Nilsson, 2004). Dodart et al. (1999) found no motor impairments at 3, 6, or 9 months in a motor-based radial maze. Motor activity in the radial maze was recorded and an increase in freezing behavior was noted in the PDAPP mice. This stereotypical behavior was only observed when the task was novel and soon ceased after the first testing session. Nilsson et al.

(2004) confirmed intact sensory-motor ability of PDAPP mice by showing no differences in open field task, balance beam, string agility and Y-maze entries compared to Tg- mice. The findings that PDAPP mice were not impaired in sensorimotor tasks provide evidence that any impairments in cognitive functions are not due to physical, motivational, or exploratory behavioral differences.

While the sensory-motor tasks performed at numerous time points extracted no differences in PDAPP mice from wild type mice at numerous time points, cognitive based tasks reveal impairment that in several studies correlated with the pathological abnormalities described above. Both Dodart et al. (1999, 2000b) and Chen et al. (2000) analyzed recognition memory in PDAPP mice cross-sectionally in an object-recognition task. Dodart et al. (1999) found no recognition impairment in young three month old animals. However, at 6 months, homozygous animals showed impairment while heterozygous animals remained cognitively intact. By 9 months both homozygous and heterozygous PDAPP mice had significantly less recognition index than wild type mice. In sharp contrast, Chen et al. (2000) found no impairment in object-recognition tasks in heterozygous PDAPP mice at 6-9, 13-15 and 18-21 month time points. These different findings could be attributed to task protocol differences. Chen et al. (2000) tested animals for 5 days with a 5 minute habituation period while Dodart et al. (1999) administered a single day of testing with a single 50 minute habituation one day prior to testing. Analysis of 5 days of testing may have diluted observable working memory impairments that may have been present in the first day of testing.

In the same animals analyzed pathologically by Dodart et al. (2000a) (discussed above), the Radial Arm Maze (RAM) was implemented, as a test of working memory.

The RAM is a more challenging task than object-recognition, as well as includes a reference memory component (which was not present in the object-recognition task). Dodart et al. (1999) found working memory impairment in the RAM task in both homozygous and heterozygous mice at 3, 6 and 10 month time points. Due to the hippocampal-dependent nature of this task it is hard to decipher if the early impairment observed at 3 months, in the absence significant plaque formation, was due to AD related pathologies or the contribution of the hippocampal atrophy observed in this transgenic line at this time. In the reference memory component of the RAM task, homozygous and heterozygous mice made significantly more reference memory errors than wild type at all three time points. While the defects in objection recognition task (discussed above) appeared to follow the A $\beta$  deposition time course, both working memory and reference memory impairment observed in the RAM appear to be present, at least at the 3 month time point, independent of A $\beta$  deposition.

Chen et al (2000) later implemented a new water maze training protocol which forced the memory retrieval function to select for a most recently encoded platform location. Testing PDAPP mice at 6-9, 13-15, and 18-21 months in this new “training-to-criterion” uncover two key findings. The first of the findings included an age-dependent deficit in trials to reach criterion. The 6-9 month old mice reached criterion in a number of trails that were non-significant when compared to the wild type animals. The 13-15 and 18-21 month old animals took significantly more trials to reach criterion when compared to the age matched wild type mice. The second finding is age independent. Despite the age differences in the PDAPP animals, all PDAPP mice were significantly impaired in finding the first platform location in a series. In comparing the age

dependent and independent impairments of PDAPP mice with A $\beta$  burden using a correlation analysis, Chen et al. (2000) found there to be a significant, age-related, inverse correlation between learning capacity and plaque burden. It is important to note that all animals (including the low pathology bearing young animals) were included in the A $\beta$  correlation analysis, which may have elevated the relationship between pathology and behavior.

In an extensive cognitive examination of PDAPP mice as well as other transgenic mice lacking ApoE or overexpressing human ACT, Nilsson et al. (2004) found no impairment in both Morris Water Maze (MWM) or Radial Arm Water Maze (RAWM) at the 2 month time point in PDAPP mice. The finding that these mice were intact in both a spatial memory retention task (MWM) and a working memory task (RAWM) may indicate that the hippocampal atrophy, which correlated with poor performance in a spatial discrimination task (Dodart et al. 2000), was no longer present after numerous generations of crossbreeding. At a later time point, Nilsson et al. (2004) found cognitive impairment associated with both diffuse and compact A $\beta$  deposition in the hippocampus. At 18 months, PDAPP mice were found to be impaired in spatial learning tasks including both a long term memory (Morris Water Maze) and working memory task (RAWM). The working memory impairment observed in the RAWM correlated with both diffuse plaques (6E10 staining) and mature compact plaques (congo red staining). This correlation suggests a cause and effect relationship between extent of deposition and cognitive dysfunction.



### ***APP Swedish Mutation Transgenic Mouse Line***

The APP<sup>sw</sup> (Tg2576) mouse model of AD represents a widely analyzed and implemented model of AD. These transgenic mice overexpress the 695-amino acid isoform of human amyloid precursor protein (APP) with a double missense mutation at 670 and 671. These mutations mimic those found in a large Swedish family which exhibit early onset AD. The transgene is expressed in C57B6/SJL mice and is controlled by the hamster prion protein promoter.

### ***APP<sup>sw</sup> Brain Pathology***

The Tg2576 APP mouse expresses the APP transgenic at unchanged levels between 2 and 14 months (Hsiao et al., 1996). Irizarry et al.'s (1997) observations agreed with, as well as added, a regional specificity to APP expression. Results from transgene expression revealed APP is expressed predominately in the neuronal layers of the hippocampal formation and in lesser amounts in cortical regions (Irizarry et al. 1997).

Despite constant expression of the APP transgene, formation of A $\beta$  is observed in an age specific manner. Hsiao et al.'s (1996) measurements of A $\beta$ 40 and 42 concentrations increased drastically until 13 months of age. Compact classic senile plaques were observed by 11-13 months in the frontal, temporal, entorhinal cortex, hippocampus, presubiculum, subiculum and cerebellum (Hsiao et al. 1996). The observation of plaques in the cerebellum is incongruent with the absence of APP

expression in the cerebellum. At 16 months Irizarry et al. (1997) observed age-related A $\beta$  deposition in the cingulate cortex, entorhinal cortex, dentate gyrus and the CA1. In very old, high burdened 23 month old mice, brain A $\beta$  inversely correlated with levels of A $\beta$  in CSF (Kawarabayashi, 2001).

Also associated with the Tg2576 transgenic mouse is oxidative stress and impaired synaptic plasticity. Smith et al. (1998) describe a “tight association” between oxidative stress and A $\beta$ . Tissues from 13-27 month old animals exhibited a global increase in oxidative stress (Smith et al., 1998). This finding makes the Tg2576 mouse a valuable model in the development of antioxidant therapy for AD. Chapman et al. (1999) analyzed the synaptic communication in 12-15 month old mice Tg2576 mice. While short term potentiation was intact, the extremely memory dependent long term potentiation (LTP) was impaired (Chapman et al., 1999). A group of slices from young 2-8 month old animals were also analyzed. Chapman et al. (1999) found no impairment in any synaptic plasticity measures in young animals, indicating that defects observed in old animals were related to presence of A $\beta$  (Chapman et al., 1999). In contradiction with the Chapman et al.’s (1999) work, Fitzjohn et al. (2001) found age-related impairment in synaptic transmission; however, they observed no impairment in LTP at both 12 and 18 months of age. Synaptic transmission was restored with the addition of a glutamate receptor antagonist, kynurenate, in slices from mice at 12 months; however, slices from the 18 month old time point were unable to be rescued (Fitzjohn et al. (2001). This finding is of particular importance because it provides evidence for the discrepancies between the Chapman et al. (1999) study and the Fitzjohn et al. (2001) study. The former

study used kynurenate treated slices to measure synaptic transmission and LTP, while the latter study showed that kynurenate rescues synaptic transmission impairment.

While the Tg2576 mouse mimics AD pathology in many ways, it lacks the neuronal loss observed in the AD brain. Irizarry et al. (1997) assessed A $\beta$  neurotoxicity by evaluating neuronal loss in the Tg2576 mouse. At 16 months Tg2576 mice showed no significant difference in neuron cell count in the CA1 region when compared to nontransgenic mice. While neuronal loss is absent in this model, at this time point evidence of axonal changes and gliosis are apparent (Irizarry, et al. 1997). This finding of insignificant neuronal loss indicates A $\beta$  deposits are not acutely neurotoxic in the mouse brain (Irizarry, et al. 1997).

A subclass of the APPsw mice, the APP23 mutant mouse exhibits some pathologic characteristics not found in the Tg2576 mouse. The first widely documented finding is presence of cerebrovascular amyloid in the APP23 mouse. Calhoun et al. (1999) described a mouse, at 14-21 months of age, that in addition to having the commonly observed amyloid plaques also shows accumulation of A $\beta$  in vasculature. A second characteristic observed, however not as widely duplicated, is presence of neuronal loss. In 1998, Calhoun et al. published work showing the 14-18 month old APP23 mouse exhibited neuronal loss in the CA1 region. Neocortex was also analyzed and despite thinning of pyramidal cell layers, no quantitative evidence of neuronal loss was observed.

### *APPsw Behavior*

The pathological characteristics discussed above often translate into behavioral impairment in the APPsw mouse. Correlations have been made between cognition and A $\beta$  deposition, synaptic plasticity and synaptophysin immunoreactivity.

Activity levels, sensory-motor measures and anxiety levels have been measured in the Tg2576 mouse. King & Arendash (2002) found the Tg2576 mice to have increased activity in the open field task only at the 3 month time point. This increase in activity at 3 months was absent at later time points suggesting an A $\beta$  independent mechanism. In an analysis of sensorimotor abilities (tested by open field task, balance beam task, string agility task and Y-maze entries), the Tg2576 mouse showed balance beam impairment at 3, 14 and 19 months (King & Arendash, 2002). This sensorimotor impairment was confirmed in an observation of impairment at 14 and 19 months in the string agility task (King & Arendash, 2002). Despite numerous generations of Tg2576 breedings and slight differences in background strain, early impairment in the balance beam was also observed at 5 and 6.5 months in this transgenic line (Arendash et al., 2004). In a test of anxiety the Tg2576 mouse showed no overt hyperanxiety behaviors in the elevated plus maze throughout life (King & Arendash, 2002; Arendash, 2004). In contradiction to this finding, Lalonde et al. (2003) describe hyperactivity in 17 month old Tg2576 mice in an open field “speed of movement toward center” measure. This impairment was short

lived, only lasting 2 out of 3 days of testing and can not be justly compared to findings of the former work due to the differences in the two tasks measuring anxiety.

The Tg2576 transgenic mouse shows robust cognitive impairment across a broad range of cognitive domains. Hsiao et al. (1996) tested the Tg2576 mouse in a spontaneous spatial alternation task (Y-maze) at 2 and 10 months. At 2 months both transgenic and non-transgenic mice tended to alternate their choices similarly; however, at 10 months a decrease in alternations in Tg<sup>+</sup> mice was observed. Impairment in Y-maze alternation was observed as early as 3 months as well as at 5, 8.5 and 19 months of age in Tg2576 mice (King & Arendash, 2002; Arendash, 2004). In the circular platform, a task of spatial learning/memory, transgenic Tg2576 mice exhibited no impairment at 3, 9, 14 and 19 months (King & Arendash, 2002). However, in 7 month old animals, when learned cues were placed 180 degrees from their original location (e.g. reversal learning), the transgenic mice were unable to adapt and were found to be significantly impaired (Pompl, 1999). This finding showed that the Tg2576 mouse was not impaired in learning, however was impaired in the reversal learning aspect of the task. This task relies on the animals innate aversion toward unlikable stimulus, however it is often difficult to measure each animals different aversion to the stimulus making results from the circular platform task somewhat challenging to interpreted.

In a sensitive task of spatial memory, Hsiao et al. (1996) found 9-10 month old Tg2576 mice to be cognitively impaired in Morris Water Maze (MWM) when compared to age-matched controls. This impairment was absent in non-A $\beta$  burdened Tg<sup>+</sup> mice at 2 months of age, indicating an association between A $\beta$  burden and cognitive impairment. At 6 months Tg<sup>+</sup> mice were marginally impaired, differing only in escape latency on the

last day of testing. These findings show a nice progression of impairment which mirrors increased A $\beta$  load. Similar age-related impairments were demonstrated by Chapman et al. (1999) and Westerman et al. (2002). Both groups observed a lack of cognitive impairment in young Tg2576, with emerging impairment in (MWM) later in life. In a retention trial of MWM, Westerman et al. (2002) observed a total quadrant preference for the goal arm in a subpopulation of young (4-5) Tg2576 mice, however spatial reference memory deteriorated in 6-11 month old mice. Examination of escape latencies in MWM acquisition showed age-related impairment in Tg2576 mice that was not observed until middle age (12-18 months). Very old 20-25 month old mice were significantly impaired in both MWM acquisition and retention when compared to Tg- littermates, showing no ability to acquire or retain any spatial information. Noteworthy is that a group of mice labeled “performance-incompetent mice”, were eliminated, which generated a cohort of mice in which sensorimotor performance deficits could be factored out of the interpretation of behavioral data. King and Arendash et al. (2002) found that in 19 month old Tg+ mice, higher levels of synaptophysin immunostaining was correlated with impaired spatial reference memory as tested by the MWM task. Interestingly, 19 month old transgenic mice had significantly higher levels of synaptophysin immunostaining than age-matched Tg- mice. King & Arendash (2002) suggest compensatory changes in synaptic morphology and staining of dystrophic neurites associated with A $\beta$  deposition. Taken collectively these findings would suggest that increased hippocampal synaptophysin levels are a manifestation of pathophysiological synaptic processing.

In contradiction to the findings of Westerman et al. (2002), King & Arendash (2002) showed no impairment through 19 months in the MWM. Neither MWM acquisition nor retention was able to separate Tg2576 mice from non-transgenics, suggest intact long term and spatial memory in significantly aged Tg+ mice. While these findings are consistent with the findings by Holcomb et al. (1999) who also showed no impairment in Tg2576 mice through 9 months, they are not consistent with findings by Hsiao et al. (1996), Chapman et al. (1999), and Westerman et al. (2002). Procedural differences may account for these differences. Other points of consideration are behavioral changes due to numerous generations of inbreeding, as well as the point that King & Arendash (2002) eliminated animals which were non-performers; if included such animals may have contributed to a cognitively impaired effect. Recently Arendash et al. (2004) observed impairment in MWM in APPsw mice at 5, 6.5 and 8.5 months. These results are surprisingly different from their early research showing no impairment through 19 months. Several factors could contribute to these discrepancies. The most likely explanation is changes in background strain. Evidence for this is provided by work from Savonenko et al. (2003), who showed that expression of APP at 3-fold times over endogenous levels was not enough to induce cognitive impairment in 24-26 month old transgenic mice that were on a full C57BL/6J background. The unimpaired APPsw mice had a more homogenous C57 background (an excellent performing background) in King & Arendash (2002), while their impaired counterparts in the later study (Arendash et al. 2004) had a mixed background. A second possibility is that through crossbreeding over several generations, the Arendash APPsw transgene line became behaviorally more sensitive to mutant APP expression, which would result in earlier impairment.

Recent work by Arendash et al. (2004) showed that APPsw mice at 6.5 months of age had significantly impaired working memory, as measured by the Radial Arm Water Maze (RAWM) task. Compared to non-transgenic mice, APP transgenic mice made significantly more errors during block 2, Trial 4 (final acquisition trial), as well as made significantly more working memory errors on Trial 5 of the last 3 blocks of testing. These recent findings are not consistent with earlier work (Morgan et al. 2000) showing that APPsw mice were unimpaired in the RAWM at 5-7 months of age. Arendash et al., (2004) suggest that several years of breeding have apparently resulted in the APP line becoming more behaviorally sensitive to mutant APP expression and/or the process of A $\beta$  deposition at an earlier age.

Taking 15 measures from 9 different activity, anxiety sensorimotor, and cognitive tasks, Arendash et al. (2004) used discriminant function analysis to separate APPsw mice from non-transgenic mice based on behavior. These findings show that APP mice are impaired globally over multiple behavioral measures.

The APP23 transgenic mouse has many similarities, behaviorally, to that of the closely generated Tg2576. As observed in Tg2576 mice, APP23 mice exhibit no anxiety compared to the non-transgenic mice. However, unlike the former model, APP23 mice were not impaired on any motor coordination task (Lalonde et al. 2002). In MWM, the APP23 mice were impaired as early as 3 months as well as at 6, and 24 months (Lalonde et al. 2002; Dumont et al. 2004).



### ***APP Swedish + PS1 Mutation Transgenic Mouse Model***

An apparent continuation of the of the transgenic mouse lines was the creation of a mouse expressing human PS1. This model lacked impressive A $\beta$  pathology despite an increase in A $\beta$  1-42/-1-40 ratio (Duff, 1996). However, co-expressing human PS1 and APP<sub>sw</sub> mutations lead to a transgenic mouse with impressive pathology observed months earlier than the single Tg2576.

### ***APP<sub>sw</sub> +PS1 Pathology***

The addition of mutant PS1 through crossbreeding with the APP<sub>sw</sub> mouse has been shown to accelerate amyloid pathology in the brains of transgenic mice (Borchelt et al, 1997; Holcomb, 1998). Borchelt et al. (1996) described a 50% increase in the ratio of Ab42/40 in double transgenic (APP<sub>sw</sub> + PS1) mice when compared to singles at a 2-3 month time point. This finding translates to an acceleration of A $\beta$  deposition in the double transgenic mice. In a direct comparison between single and double transgenic mice, single transgenics were plaque free until 18 months of age. In contrast, APP<sub>sw</sub> +PS1 mice exhibited plaques as early as 9 months (Borchelt et al, 1997). This finding that the addition of the PS1 mutation dramatically accelerates the rate of A $\beta$  deposition demonstrates PS1 acting in a synergistic relationship with the mutated APP gene. The Borchelt mouse line is slightly different from a more widely used APP<sub>sw</sub>+PS1 mouse, which exhibits plaques much earlier. In the more widely implemented APP<sub>sw</sub> +PS1

mouse, Holcomb et al. (1998) showed that in 6-16 week double transgenic mice there was a 41% increase in transgenic human A $\beta$  1-42. This was quite different from the unchanged A $\beta$  40 and 42 levels observed in the 6-16 week single APPsw mice. In the double transgenic mice, levels of A $\beta$  40 and 42 increased in 12-16 week mice and showed further increase at 24-32 weeks of age. This increase was not observed for the most part in the single transgenic mice. Histological analysis showed that 13-16 week old double transgenic mice had compact plaques, measured by thioflavin S staining, that were absent in single transgenic littermates. Thioflavin S staining increased substantially in the doubly transgenic brains of 24-32 week old mice. This work was continued by Gordon et al. (2002), whom describe a regional specificity of deposition in a time course manner. At 6 months of age, doubly transgenic mice had multiple deposits particularly in the frontal, entorhinal cortices and hippocampus. Through 9, 12 and 16 month, the number of deposits increased and began to infringe on the striatum, thalamus and brain stem.

Closely associated with the dense plaques were dystrophic neuritis, reactive astrocytes, and microglia activation (Borchelt et al, 1997; Gordon et al, 2002). Gordon et al. (2002) observed A $\beta$  deposits invested with dystrophic neurites as early as 6 months. Using GFAP immunostaining, Gordon et al. (2002) measured astrocyte reactivity. At 3 months, enhanced GFAP staining was restricted to the hippocampus. This would suggest the hippocampus plaques were the most mature and therefore where the A $\beta$  pathology had begun. As animals aged, increased GFAP density in the striatum, and cerebral cortex was observed. In a direct comparison of single and doubly transgenic mice, as would be intuitive, the high burden doubly transgenic mice had significantly more GFAP staining

than less-burdened single APP<sup>sw</sup> mice. Using MHC-II immunostaining techniques, Gordon et al. (2002) showed increased microglia activation with increased age in APP<sup>sw</sup> + PS1 mice. Consistence with work previously reported, activated microglia and astrocytes increased synchronously with A $\beta$  burden, and were closely associated with plaques (Matsuoka, et al. 2001) Taken together, the time course of A $\beta$  deposition, the characteristics of the A $\beta$  plaque, and the inflammatory response which they instigate, the doubly transgenic APP<sup>sw</sup> + PS1 mouse closely resembles A $\beta$  pathology found in the AD brain.

While the doubly transgenic mice develop significant A $\beta$  burdens, no neuron loss was initially seen with the APP + PS1 transgenic mouse line (Takeuchi et al, 2000). However, a recent study by Sadowski et al., (2004) showed significant neuronal loss in the CA1 region of 22-23 month old APP+PS1 mice. While there is no global neuron loss in this model (such as the extensive cortical and hippocampal loss seen in AD), many findings suggest a lack of neuronal health in the APP<sup>sw</sup> +PS1 model. Changes in glucose metabolism (Sadowski, et al, 2004), dendrites, dendritic spines (Moolman et al, 2004), reorganization of cholinergic terminals (Wong et al. 1999) and resultant impairments in synaptic plasticity (Dickey, et al. 2003) have all been documented. In an assessment of brain metabolism, Sadowski et al. (2004) used <sup>14</sup>C-2DG and showed no impairment at 2 months. However, at 22 months the brain glucose utilization (BGU) index in the hippocampus of APP+PS1 mice was decrease by 26.6%. This defect correlated with spatial memory deficits (Sadowski et al, 2004). Impairment in glucose utilization is also observed in the human AD brain, which is associated with early cognitive impairment (De Santi et al, 2001).

Along with impairment in neuronal metabolism, APP+PS1 mice also have alterations in dendrites and dendritic spines. At 11 months, APP+PS1 had significantly less spines and total dendrite area in the hippocampus (Moolman et al, 2004). Other characteristics observed included swollen bulbous dystrophic neurites. Moolman et al. (2004) went on to characterize human AD hippocampal tissue and compare the findings in APP + PS1 mice. Both quantitatively and qualitatively similarities were observed. Moolman et al. (2004) reported that “images of neurons from the AD brain were remarkably similar to those from the 11 month-old APP+PS1 mice.” Also similar was the 50% loss of dendritic spines in the AD brain and transgenic mice, when compared to controls. As well, cholinergic neurons are affected by the introduced mutant APP<sup>sw</sup> and PS1 transgenes. Wong et al. (1999) found there to be reorganization of cholinergic terminals in the cortex and hippocampus of double transgenic mice. These mice had prominent cholinergic synaptic deficits, as measured by staining of vesicular acetylcholine transporter (VACHT) boutons (Wong et al, 1999). As one would anticipate the deficits observed in synapses translated into impairment in LTP, Trinchese et al. (2004) observed abnormal LTP as early as 3 months that paralleled plaque appearance. In 17-18 month APP+PS1 mice, Dickey et al. (2003) showed selectively reduced expression of synaptic plasticity-related genes including Arc, Zif268, NR2B and GluR1.

In summary, APP + PS1 first show A $\beta$  deposits at 4-6 months of age, which increase with age. This increase occurs despite a constant APP expression level throughout life. Similar to the AD brain, plaques are associated with dystrophic neurites, reactive astrocytes, and microglia activation. While there is no global neuron loss, such

as the extensive cortical and hippocampal loss seen in AD, neuronal loss is observed in the CA1 region of very old 22-23 month old APP + PS1 mice.

### *Behavior of APP+PS1 Mice*

Just as in the single transgenic APP (Tg2576) mice, the double transgenic mice have been widely behaviorally characterized. These mice often show behavioral correlations with their AD- like pathology described above. In sensorimotor tasks, double transgenic mice are often not significantly different from non-transgenic counterparts; however there are selective tasks and time points where the double transgenic mice show impairment. In a task of open field activity, double transgenic mice were shown to have increased line crosses (activity) at 15-17 months, but not at 5-7 months (Arendash et al., 2001b). This increased activity at 15-17 months was not reproducible in a later study (Jensen et al, 2005). In contradiction with the above work (Arendash et al., 2001b), decreased line crossing was observed at 4.5 to 6 months (Jensen et al. 2005), but not 15-16.5 months. Liu et al. (2002) observed a decrease in activity in doubly transgenic mice at 7 months. Other testing in open field has shown no significant differences in activity (Roach et al, 2004). The variability in findings across these studies in open field activity may indicate that activity levels are extremely sensitive to background strains. While all animals discussed have the addition of the APP and PS1 transgene, multiple generations of inbreeding may cause differences in activity across numerous studies.

In balance beam, double transgenic mice showed early impairment at 5-7 months and late impairment at 15-17 months (Arendash et al, 2001a; Arendash et al 2001b).

After numerous generations of breedings, impairment in balance beam was no longer apparent at 4.5-6 or 15-16.5 months of age, although strong trends were present (Jensen et al. 2005). It could be argued that animals lacking sensory motor impairment at any time point are a better model to test cognition by eliminating any sensorimotor impairment that would potentially confound any observed cognitive effect. However, no correlations between activity, balance beam and cognition were observed by the Arendash laboratory, indicating that changes in activity or impaired balance beam performance do not deleteriously affect cognition. Arendash et al. (2001) showed no effects of transgenicity in the elevated plus maze (test of anxiety) at 5-7 and 15-17 months. In contrast, Jensen et al. (2005) describe an increase in anxiety at 15-16.5 months. Though this finding was observed in one of 3 measures of anxiety (% time in open arms), it is this measure that is most linked to anxiety (Jensen et al., 2005).

Similarly to single APP mice, double transgenic mice are impaired cognitively over a multitude of cognitive domains. While one would anticipate the increased A $\beta$  burden earlier in the double transgenic mice would translate into earlier cognitive impairment, this is often not observed. In 3 month old animals, Holcomb et al. (1998) showed significant differences in Y- maze alternations between single and double transgenic mice when compared to non-transgenic. Both single and double transgenic were equally impaired when compared to non-transgenics. Later, in 3 and 6 month old animals, Holcomb et al. (1999) showed equally significant differences in Y- maze alternations between single and double transgenic mice when compared to non-transgenic. This impairment in Y-maze was only present in the double transgenic mice at the 9 month time-point. However, at no time point were the single transgenic mice

(lacking compact A $\beta$ ) significantly from the double transgenic mice (Holcomb et al, 1998; Holcomb et al, 1999). This work uncovers impairment not associated with compact plaque deposition because APP<sup>sw</sup> mice lack A $\beta$  deposits at all test points evaluated, leading to the hypothesis that early soluble (oligomeric) forms of A $\beta$  may be the primary cause of impairment in this task. Later work showed no transgenic impairment in Y-maze at any time point in the doubly transgenic mouse line (Arendash et al., 2001a; Arendash et al., 2001b; Jensen et al., 2005). The authors suggest that the Y-maze task is relatively insensitive to mutant APP-transgenic associated cognitive impairment.

In an early behavioral characterization of the APP + PS1 mouse Holcomb et al. (1999) observed no significant impairment at 3, 6 and 9 months in MWM, a task of spatial reference learning and memory. While there was no impairment in this study, a substantial body of literature shows that APP+PS1 mice become impaired in MWM (Arendash et al., 2001; Liu et al., 2002; Trinchese et al., 2004; Jensen et al., 2005). At 5-7 months, double transgenic mice showed no impairment over 10 days of testing, however by 15-17 months spatial learning impairment was observed (Arendash et al. 2001a). Arendash et al. (2001a) also observed no effects of transgenicity on the percent of time spent in the former platform-containing quadrant at either 5-7 or 15-17 months. In more recent work by Jensen et al. (2005) from the same lab, and using the 10 day MWM protocol, double transgenic mice were significantly impaired at both 4.5-6 and 15-16.5 time points in acquisition. Probe trial testing for reference memory indicated that 4.5-6 and 15-16.5 month old mice non-transgenic mice showed an exclusive quadrant preference for the platform containing quadrant, but not APP+PS1 mice at the same ages.

These early acquisitional and memory retention impairments seen by Jensen et al. (2005) are in contradiction to previously mentioned work by the same lab (Arendash et al. 2001a). Since both studies used the same water maze protocol, variation in protocol is a moot point in this comparison. A likely reason for the observed early impairment involves the numerous generations of cross breeding between studies. This resulted in the APP+PS1 mice becoming more behaviorally sensitive to mutant APP expression and/or the process of A $\beta$  aggregation. Thus, APP<sup>sw</sup> + PS1 mice appear to have become more impaired earlier as seen by Jensen et al. (2005). Similar to Jensen et al (2005), Trinchese et al. (2004) noted no impairment in MWM until 6 months. It should be noted that differences in water maze protocols occurred. The Arendash protocol included 4 trails for 10 days of acquisition while Trinchese only tested in 3 trials, for 3 days 3 times a day.

Changes in behavioral sensitivity are also observed when analyzing results of the platform recognition task. In this task of recognition and identification, Arendash et al. (2001a) observed no differences between non-transgenic mice and APP+PS1 mice at 5-7 or 15-17 months of age. In contrast, Jensen et al. (2005), saw no impairment at 4.5-6 months, but did observe impairment at the 15-16.5 month time point. This impairment is further evidence of increased behavioral sensitivity after numerous generations of crossbreeding.

An early symptom of AD is the loss of working memory. Using a task of spatial working memory (Radial Arm Water Maze RAWM), doubly transgenic mice 15-16 months were observed to be impaired in the final block of testing on the final memory retention trial (trial 5) (Gordon et al., 2001; Morgan et al., 2000; Arendash et al., 2001a).



Similarly to findings in formerly discussed cognitive tasks, Y-maze and Morris water maze, the doubly transgenic mice were not significantly more impaired than single transgenic mice (Morgan et al., 2000). This observation provides more evidence that the higher burden found in the APP + PS1 mice does not translate into intensified impairment. Gordon et al, (2001) also showed that the working memory sensitive RAWM correlated with A $\beta$  deposition in the frontal cortex and the hippocampus. It is important to note that young mice free of A $\beta$  burden (5-6 months) did not show impairment in RAWM in early studies (Arendash et al., 2001a). In this initial work, APP+ PS1 mice showed impairment only in the T5 memory retention trial, with no impairment in T4 (Gordon et al., 2001; Arendash et al., 2001a). In more recent work, Jensen et al. (2005) also describes a somewhat earlier and more profound working memory impairment than had been reported by the same group (Arendash, 2001a). Jensen et al. (2005) observed RAWM impairment not only at the later 15-16.5 time-point, but also months at 4.5-6 months. At both 4.5-6 & 15-16.5 month time-points, doubly transgenic mice showed robust impairment on T4 and T5 across all 3 blocks. This early RAWM impairment, taken collectively with earlier impairment in the MWM task, shows that, for the Arendash lab's results, numerous generations of crossbreeding lead these transgenic mice to become more behaviorally sensitive to mutant APP expression and/or the process of A $\beta$  aggregation. In another study from the same group, RAWM working memory impairments were observed during T4 in very old 18 month APP<sup>sw</sup> + PS1 animals (Austin et al., 2003), although no impairment was observed on T5 in 18 month old animals. Finally, Trinchese et al. (2004) observed another characteristic seen in AD patients. At 2 months, doubly transgenic mice were not impaired in working (RAWM) or

long term memory (Morris water maze). At 3-4 months, the mice still exhibited intact long term memory, however they showed impairment in RAWM working memory. By 6-7 months Trinchese et al. (2004) showed that the double transgenic mice show impairment in long term and working memory tasks. While this impairment profile includes earlier impairment than that of former work, progression of memory impairment closely mimics the early impairment of working memory followed by later impairment in long term memory seen in AD patients.

In summary, the APP+PS1 transgenic mice from the Arendash colony first show reference memory and working memory impairment at 5-6 months. Jensen et al. (2005) showed this impairment in Morris Water Maze Acquisition/ Retention and RAWM. This impairment is also observed in 15-16.5 month old animals. Present at 15-16.5 months, that was absent in young mice, is an identification/strategy switching impairment, as measured by platform recognition (Jensen et al., 2005). While increased activity has been observed in APP+PS1 mice at 5-6 months of age, no changes in sensorimotor function or anxiety have been observed at this time point (Jensen et al. 2005).

### ***APP Mutant Mice: Imperfect Transgenic Model***

While there are many characteristics of transgenic mouse models that closely emulate characteristics of AD, APP transgenic mice are not a perfect model of the disease progression, pathology or behavior. The behavioral characteristics of AD are broad and often inconsistent between patients. The complex layered behavior of the disease cannot be broken down into specific non-overlapping categories such as exploration, anxiety, long-term and short-term memory. Despite these complications, cognitive impairment

involving spatial working memory and reference memory impairment is nicely reproduced in APP transgenic lines. Trinchese et al. (2004) showed the progression of typical AD-like cognitive impairment in APP+PS1 mice, first showing working memory impairment and later reference memory impairment. The age-related platform recognition impairment observed in APP + PS1 mice by Jensen et al. (2005) nicely mimics the strategy switching impairment observed in AD patients. APP transgenics also show the characteristic anxiety often observed in people with AD (Jensen et al., 2005). There are some behavioral characteristics of AD that can not be reproduced in transgenic mice including, hallucinations, depression, and paranoia. While not perfect, behavioral testing of transgenic mice to analysis therapeutics and mechanisms is a noteworthy tool which has been paramount in exploring the disease.

As for pathology, while at first glance the APP transgenic mice do appear to closely mimic AD brain pathology there are some notable differences. The first significant difference is the lack of overt neuron loss in the transgenic mice. Despite high concentration of the neurotoxic A $\beta$  in the mouse brain, this does not translate into the neuronal loss observed in AD brains. This could be due to A $\beta$  being less toxic in the mouse brain when compared to human brains or the short life-span of the mice relative to humans. Lack of neuronal loss could also be due to the much decreased inflammatory response A $\beta$  has in the mouse brain. Evidence for this hypothesis was observed by Schwab et al. (2004) in a direct comparison of lesions in the neocortex and hippocampus in elderly APP23 transgenic mice and lesions from the AD brain. Despite similar staining for A $\beta$  protein, positive ApoE staining, and comparable levels of reactive astrocytes, a largely reduced level of microglia activation was observed in aged

transgenics. In contrast to lesions of the AD brain, Tg<sup>+</sup> mice had weakly activated microglia, which expressed low levels of complement receptor activation. Schwab et al. suggest that this weak immune response in Tg<sup>+</sup> mice compared to the very strong immune response in human AD could be why A $\beta$  vaccination in mice was useful and resulted in the clearance of A $\beta$ , whereas in AD, stimulation to an already strongly activated immune system had grave results. Differences in microglia activation make it difficult to anticipate if immune-activating therapies that result in positive effects in mouse models, will result in similar effects in humans. Another distinct difference in APP transgenic mice observed by Schwab et al. (2004) was the absence of NFTs in the Tg<sup>+</sup> mice. In the AD brain, A $\beta$  deposits were surrounded with the presence of ghost tangles, while the neurons around the A $\beta$  deposits in the Tg<sup>+</sup> mice appeared to just be displaced (Schwab et al. 2004). The A $\beta$  hypothesis theorizes that presence of A $\beta$  induces production of NFTs. APP transgenic mice are free of NFTs despite high plaque burden. Overall, it is hard to decipher if the mechanism leading to impairment in transgenic mice is the same as causes of impairment in human AD. While flaws of the APP models are present, the development of mice with “AD like” behavior and pathology has been monumental in advancing AD research.

## **Environmental Enrichment**

### *Enriched Environment Protection and Treatment: Human Studies*

A large body of literature suggests that life experiences including education, occupation, physical activity and social interactions may provide protection against dementia later in life. These findings would suggest environmental factors could impact the development of AD. Cognitive stimulation in the forms of educational attainment, occupations, participation in non-occupational cognitive activities, “environmental complexity,” “favorable life experiences,” and leisure activities have been examined in association with decreased AD risk. Much of this work is the product of retrospective case-control studies. Low education attainment has been associated with an increase risk of AD (Stern et al., 1994, Ott et al., 1995, Letenneur et al., 1999). Why education is protective against AD is not clear. Possible explanations include that education provides a cognitive reserve, while others suggest that higher levels of education enables patients to simply performed better on neurological tests. Some have suggested that low education results in earlier onset of AD (Friedland et al., 1993). Stern et al. (1994) found that low levels of education resulted in an increase risk of AD. However, they were unable to decipher if lower levels of risk, associated with higher education, was due to “decreasing ease of clinical detection or by imparting a reserve that delays the onset of clinical manifestations.” In a 5 year longitudinal study Letenneur et al. (1999) supported

the above work, showing low educational attainment is associated with higher risk of AD. While many have found this correlation, several studies have failed to confirm this association. After adjusting for age and examining subtypes of dementia, Cobb et al. (1995) found no association between risk of AD and education. While education is participated in during early-life, work by Friedland et al. (2001) would suggest mid-life cognitive activity is associated with lower risk of AD. Occupational attainment falls into the category of mid-life cognitive stimulation. Both Stern et al. (1994) and Smyth et al. (2004) observed delay of AD development if subjects had held mentally stimulating occupations. Other mid-life activities, such as social and leisure activities, physical activity and cognitive stimulation have also been investigated and found to be associated with decrease risk of AD (Scarmeas et al., 2001; Wang et al., 2002). Verghese et al. (2003) showed that individuals that participated in leisure activities such as reading, playing board games, playing a musical instrument and dancing had a decrease risk for developing AD. Work by Schooler & Mulatu (2001) suggests that carrying out substantively complex tasks late in life still has the capacity to improve intellectual functioning. Moreover, physical activity performed in mid-life has also been linked to a decrease in AD (Friedland et al., 2001; Churchill et al., 2002). As a component of the “Nun Study,” Wilson et al. (2002) provided evidence that cognitively stimulating activities participated later in life was associated with decreased risk of AD. A large group of non-demented clergy was followed annually for up to 7 years. The data collected showed that participation in cognitively stimulating activities was associated with a decrease of global cognitive impairment, working memory impairment as well as perceptual speed impairment (Wilson et al., 2002). Taken together, these findings

suggest that lifestyle in both early and mid-life may influence the development of AD. More work is needed to show if each component of enriched lifestyles (social, physical, cognitive activity) are contributing equally to the effects observed or if one weighs more heavily as a protector. Such a dissection would, however, be most difficult to achieve in human studies.

A substantial body of literature has shown EE as a protective entity. This has led to the investigation of EE as a possible treatment option. Many treatment studies focus on the potential of “cognitive rehabilitative intervention” as a treatment for AD. Ball et al. (2002) showed that interventions conducted in small group settings lasting 60-72 minutes over a 5-6 week period of time resulted in improvement in “targeted cognitive abilities” in older adults. Quayhagen et al. (1995) extended these findings to AD patients by showing improved overall cognitive/memory function following cognitive rehabilitative intervention. Weekly cognitive sessions were performed by caretakers. Those in the treatment group initially showed improvement in behavior as well as memory. However, this benefit declined to baseline after 9 months. In contrast, the placebo group declined in cognitive test scores over the course of the clinical trial. While relatively short lived, the 9 month treatment benefit observed closely competes with current pharmaceutical treatments that are often effective up to one year. It is also important to note that intervention was only administered in a short term (5-12 week) bi-weekly manner, suggesting an even greater potential of cognitive rehabilitative intervention in a chronic and more rigorous framework. In more recent work Davis et al. (2001) and Farina et al. (2002) observed modest cognitive benefits from cognitive rehabilitative intervention. For Davis et al. (2001), cognitive intervention included 5

weeks of training in face-name associations, spaced retrieval, and cognitive stimulation. When patients were analyzed post- cognitive intervention, there was improvement in tasks which were included in cognitive intervention sections (face-name associations, spaced retrieval). However, these benefits did not translate into improved overall neuropsychological testing or improved quality of life. Similarly unimpressive cognitive benefits after cognitive intervention were observed by Farina et al. (2002). Two types of cognitive intervention were tested in this study. Test subjects were given “procedural memory intervention” or training of “partially spared cognitive functions.” In a test of “functional living skills,” both groups showed improvement. However, only the group given “procedural memory intervention” showed improvement on the Attentional Matrices and Verbal Fluency for Letters tasks. More recent work by Loewenstein et al. (2004) showed that mildly impaired AD patients that were enrolled in a cognitive rehabilitation (CR) program maintained performance on specific cognitive and functional tasks. CR training consisted of two 45-minute training sessions twice per week for 14 weeks. Similar to other studies, Davis et al. (2001), performed CR training in specific tasks (face-name association tasks, object recall training, functional tasks) and observed benefits within these trained tasks. As discussed above, these more recent studies also involved acute and sparse cognitive intervention that may not be as beneficial as a long-term intensity therapy. More work involving chronic administration of cognitive intervention is needed in order to better gauge the potential of cognitive intervention as a viable treatment option for AD.



### *Effects of Enriched Environment in Rodents*

The effects of EE in rodent are often compared between standard group housed rodents and rodents living in larger cages containing toys, wheels and tunnels. Most work is done with animals living in the augmented cage (Kempermann et al., 1997) while other studies have acute exposure for a few hours a day (Frick et al., 2003). Some studies enhance their EE by administering intra-weekly cognitive sessions in which animals are taken from their cages and exposed to new environments (Arendash et al. 2004; Teather et al. 2002). Many studies will add an “impoverished” test group including singly housed mouse in standard cages (Mohammed et al., 1990). Do to the complexity of the enriched environments, it is hard to piece out if complete enrichment (cognitive, physical, and social stimulation) or if one or several components of EE are leading to the changes observed.

### *Cognitive Effects of Enriched Environment in Rodents*

While there is a limited amount of work involving EE and cognitive benefits in AD transgenic mice, a substantial body of literature offers insight into the effects of EE on cognition in wild type mice. While it is unknown if the cognitive benefits often observed in wild type mice will translate into beneficial effects in AD transgenic mice, the work done in typical rodents offers an exploitable resource to predict possible effects of EE in AD transgenic mice.

The effects of EE have been tested in young, middle and aged rodents. Furthermore, studies have also tested effects of enriched environment on genetically manipulated, dietary altered, and lesioned rodents. Locomotor activity is among many of the behaviors explored in animals exposed to EE. Van Waas and Soffie (1996) tested if EE changed activity levels in rats. In a y-maze task, despite age, young (4 months) and old mice (22 months) reared in an EE did not have increases in “arms visited.” This work was corroborated by Wolfer et al. (2004), who found no significant differences in enriched “adult” mice versus standard housed mice in an open field task. This absence of differences in activity in enriched animals suggests that differences in performance in exploration and cognitive tasks are not due to underlying differences in activity. EE mice also show no differences in anxiety levels, as measured by the elevated plus maze (Tsai et al. 2003).

Hippocampal forms of learning and memory have been shown to be widely affected by EE ( Kempermann et al., 1997, 1998, 2002; Wincur et al. 1999; Teather et al. 2002; Frick et al. 2003; Arnaiz et al. 2004). Kempermann et al. (1997, 1998) have constantly showed that mice exposed to an EE from weaning to adulthood ( 2, 6, and 18 months) perform superior in Morris Water Maze when compared to standard housed mice. Work by Teather et al. (2002) showed 6 month old rats living in an EE had superior performance in hippocampal dependent tasks (standard water maze when compared to “restricted” housed (RC) rats. In contrast, EE mice and RC rats were identical on tasks that were hippocampal independent (Teather et al. 2002). Frick et al. (2003) showed that EE reduces age-related impairment in spatial memory. In the Morris Water Maze (MWM) task, 18 month old mice living in an EE 25-29 days prior to testing

were not impaired while those in standard housing showed impairment in both acquisition and retention portions of the MWM. It is important to note that no treatment differences were observed in the cued version of the MWM, showing that EE is not simply improving vision of the aged mice. Equally noteworthy, after statistical separation of males and females, both benefited from EE. This would suggest that both males and female could potentially benefit from age-related cognitive protection provided by enrichment. Kempermann et al. (2002) showed that EE late in life benefited aged mice. In this study mice entered EE at 10 months and were observed to be cognitively superior in MWM when compared to SH mice at 20 months. Benefits in rats were also observed by Arnaiz et al. (2004). This work showed that rats at 27 months of age (Arnaiz et al., 2004) benefited in MWM from life time EE. In contrast to this work, Wolfer et al. (2004) observe no differences in MWM between “adult mice” living in an EE when compared to standard house mice. This discrepancy could be due to the age of animals tested. No definition of adult mice was given, raising the possibility that the animals tested were too young to have any impairment to protect against. The work of Wolfer et al. (2004) would suggest that EE may not be useful for augmenting baseline cognition. Duration and starting time of EE on cognition was investigated by Williams et al. (2001) and Kobayashi et al. (2002). Williams et al. (2001) tested mice at numerous time points in the MWM after exposure to enrichment in early life (35 to 94 days of age) or later in life (100 to 159 days of age). Three rounds of (MWM) testing were included over the extent of the study. A baseline test, followed by a first and second test period was conducted. This extensive testing can lead to over testing and therefore mask impairments or cognitive benefits from EE. While the authors suggest that EE at the

earlier period of time offered benefits in MWM performance, these benefits were modest at best. No difference amongst any of the groups (individually housed, group housed, enrichment early, enrichment late) was observed at the first test point. In the second test period, differences were observed only between individually housed mice and all other groups. This lack of robust differences in the EE mice could be due to the 3 rounds of 5 day MWM testing. Kobayashi et al. (2002) tested if long-term exposure offered greater benefits than short-term exposure. In the cognitive based Hebb-Williams Maze task, rats reared in an EE from weaning until 25 months benefited more from EE than those given short term (3 months) of EE right before testing (e.g. EE between 22-25 months of age) . However, both long term and short term EE benefited 15 month old mice equally. The authors concluded that short-term enrichment has potential to improve aging animals; however, long-term enrichment is superior in protecting cognition in aged animals. This work offers data that would suggest living an enriched life may lead to successful aging, as well as showing that while not as impressive as long-term EE, short-term enrichment later in life may also be beneficial.

Until this point, experiments conducted with EE as the sole variable have been discussed; however, EE has been explored in the presence of other variables. Winocur and Greenwood (1999) showed high dietary fat translated into negative cognitive effects that can be overcome with EE. Kalmijn et al (1997) showed that high fat intake leads to increased risk of dementia in humans. Taken together, these studies suggest EE may reduce risk of AD by reversing mechanisms of cognitive impairment that fat intake exacerbates. Traumatic brain injury and ischemic stroke often result in cognitive impairment. Wagner et al. (2002) and Dahlqvist et al. (2004) showed positive cognitive

effects after EE treatment in male rats after traumatic brain injury and rats after focal cerebral ischemia, respectively. Neurotransmitter abnormalities are associated with and targeted by pharmaceuticals in AD. Degroot et al. (2005) found increased hippocampal acetylcholine (ACh) efflux when actively manipulating a novel object. These effluxes lead to an enhancement in cognition when tested in a radial arm maze. This would suggest that if EE works primarily through a mechanism of increased ACh, its therapeutic value for late stages of AD may not be significant due to the substantial loss of cholinergic neurons in the AD brain. This possibility appears remote, however, in view of multiple other neurochemical, histologic, and genetic mechanisms that are impacted by EE, as will be discussed in the next section. Overall, EE has been shown effective in healthily aging, as well as in reversing effects from non-healthy insults such as high fat diets and traumatic brain injury.

#### *Neurohistologic and Neurochemical Effects of Environmental Enrichment in Rodents*

A plethora of changes in neurohistology and neurochemistry have been observed in rodents exposed to EE. One widely documented change is increase neurogenesis. Contrary to past thought, the brain has the capacity to produce new nerve cells in adulthood. Two areas have been shown to give rise to new neurons. These areas are the subventricular zone of the anterior lateral ventricles (which give rise to cells that become neurons in the olfactory bulb) and the subgranular zone in the dentate gyrus (which generates new granule cell neurons in the hippocampus). EE has been shown to induce hippocampal but not olfactory bulb neurogenesis in rodents (Brown et al., 2003). This

selective neurogenesis by EE in the hippocampus, an area incongruently destroyed in AD, makes it a noteworthy area of exploration in treatment of AD.

In 1997 Kempermann et al. showed more hippocampal neurons in adult rodents living in an enriched environment. This work was consistently reproduced in Kempermann et al., 1998a, 1998b, 1999, 2002 and Nilsson et al., 1999. Implementing bromodeoxyuridine (BrdU) (which is incorporated into dividing cells and their progeny) and stereology techniques, Kempermann et al. (1997) was able to measure a 57% percent increase in BrdU labeled cells and a significant increase of 15% in volume of the granule cell layer in 3-4 month old mice that had lived in EE for 40 days. Positive BrdU staining could indicate increase neuronal proliferation or simply increase survival of neurons. To explore these options Kempermann et al. (1997) sacrificed mice one day after BrdU injections and 4 weeks after injection. In the group sacrificed one day after injection, no significant differences were observed in EE mice when compared to control mice (Kempermann et al., 1997, 1998a). However, in animals sacrificed 4 weeks after injections, a significant increase in labeled cells was observed. Take together these findings show that EE alone does not increase proliferation of neurons, but rather is increasing their survival. This survival may be impart due to the inhibition of spontaneous apoptosis observed in rats reared in EE (Young, 1999). Young et al. (1999) described a 45% reduction in apoptotic death in the rat hippocampus. This EE-induced prevention of apoptosis could be a mechanism by which EE is improving survival of new neurons. It is important to note that increased survival with no changes in proliferation is background strain dependent. In contrast to work above performed on behaviorally superior C57BL/6 mice, 129/SvJ mice [(which have significantly less hippocampal

neurogenesis and do not perform well in learned tasks (Kempermann et al, 1997b)] had increased survival and proliferation of neurons (Kempermann et al. 1998b). This is different from previous studies using C57BL mice, which reported only increased survival, not proliferation. Along with strain background dependence (Kempermann et al., 1998b), EE-induced neurogenesis is also dependent on temporal variance.

Kempermann & Gage (1999) found that animals exposed to EE and then removed from EE had twice as many proliferating cells in the dentate gyrus when compared to standard housed and long-term exposed animals. Kempermann and Gage (1999) hypothesize that this increase of neurogenesis in short-term enrichment could be due to early stimulation preserving neurogenic potential, combined with novelty (changing environments), both of which contributed to the EE-induced neurogenesis. While novelty may be contributing to the short-term enrichment effect, both groups had exposure to EE early in life, making this hypothesis that short-term enrichment effects were due to early life exposure somewhat weak.

The work discussed thus far has been done in adult animals. However, does EE have the potential to induce neurogenesis in aged mice? Kempermann et al. (2002) observed a five-fold induction of dentate gyrus neurons in 20 month old mice. Animals were placed in enrichment or standard housing at 10 months old and then maintained for 10 additional months. At first analysis, no significant differences were observed in BrdU staining. However, when neuronal phenotype was taken into account, it was observed that the control group had increased astrocytes in a compensatory manner. When looking just at BrdU staining, which overlapped with NeuN staining for neurons, a 5-fold increase in neurogenesis of EE mice emerged. This work shows that beginning EE in

middle age may be just as beneficial as starting early. This work also highlights the ability of EE's benefits to extend into late life.

The work above exemplifies the power of environmental enrichment in inducing neurogenesis in adult and aged wild type mice, but what are the implications of EE induced neurogenesis in AD? Haughey et al. (2002) showed disruption of neurogenesis by A $\beta$  in the dentate gyrus of AD transgenic models. In 12-14 month old APP mice Haughey et al. (2002) described a reduction in neural progenitor cell when compared to age matched controls. Twelve days after a final Brdu injection there was a greater decrease, 55%, in APP mutant mice compared to the 25% decrease in control mice (Haughey et al., 2002). In contrast to what is observed in transgenic models, Jin et al. (2003) described an increase in neurogenesis at autopsy in the dentate gyrus of AD brains when compared to brains of individuals without neurological disorders. The authors suggest a compensatory theme which occurs without cognitive benefit. This brings into question if increased neurogenesis, through EE, will translate into benefits in AD patients. Jin et al. (2003) suggest possible reasons for the limited repair capacity of increased neurogenesis in AD. Authors note the small amount of dentate gyrus region-limited neurogenesis is not enough to compensate for the mass destruction present in the AD brain. A second point is that the microenvironment of AD brains may be too toxic for new neurons, thus keeping them from becoming fully functional, mature neurons that integrant into surviving brain circuitry. While these obstacles are present, the evidence that the AD brain has the capacity to maintain neurogenesis offers the possibility that measures which increase neurogenesis, such as EE, may have therapeutic value in AD.



While neurogenesis has been the most widely documented effect of EE, other pathological changes are observed after exposure to an enriched environment. Synaptic loss correlates with cognitive deficits associated with AD ( DeKosky, 1990). Mice 27-28 months that were exposed to EE for 3 hours a day for 14 days prior to behavioral testing showed increase synaptophysin levels combined with cognitive benefits (Frick & Fernandez, 2002). While increased synaptic area was associated with improved cognition, no correlation analysis was performed. Increased synaptophysin, indicative of synaptic area, was increased in the frontalparietal cortex and the hippocampus. Further evidenced of increased synaptic area induced by EE was observed by Saito et al. (1994). Saito et al. (1994) showed a decrease in synaptic content in aged mice that was prevented if reared in an EE. Noteworthy for AD is the finding that mice with an ApoE4 transgene do not show EE-induced synaptogenesis (Levi et al, 2003). This may suggest that presence of ApoE4 blocks some effects of EE exposure. While synaptophysin is a measure of synaptic area, changes in dendritic morphology offer another measure of neuronal health and synaptic plasticity. Using golgi-cox morphological analysis, Faherty et al. (2003) found increases in dendritic length in the CA1 region and the dentate gyrus of mice reared in an EE until 4-5 months of age. Spine density was analyzed by Comery et al. (1995). These investigators found a 30 percent increase in spine density within the corpus striatum of 2 month old rats exposed to EE, compared to individually reared littermates. More recently in 3 month old deer mice, Turner et al. (2003) observed increases in average number of spines in layer V pyramidal neurons of the motor cortex and in medium spiny neurons of the dorsolateral striatum. No differences were observed in the granule cells of the dentate gyrus.

EE has been shown to change growth factor levels in the brain ( Pham, et al., 1999; Ickes et al., 2000). Work by Pham et al. (1999) showed that NGF levels and NGF receptors are both increased in 14 month old rats housed in an EE. These increases occurred in the hippocampus, visual and entorhinal cortices after 12 months of EE and were linked to improvement in spatial learning, although no correlation analysis was run. Along with increases in NGF, Ickes et al. (2000) observed regional increases in brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) levels in 14 month old rats that had lived in EE from weaning. Both BDNF and NGF were increased in the very AD relevant hippocampal formation. In addition to changes at the protein level, it is important to note that EE has been shown to effect gene expression. Genes linked to neuronal structure, neuronal plasticity, and transmission have been linked to EE (Rampon et al., 2000, Keyvani et al. 2004, Pinaud et al. 2004). Pinaud et al. (2001) showed rats exposed to EE 1 hour a day for 3 weeks had marked up-regulation of arc in the cerebral cortex. This gene, associated with plasticity, was also up-regulated in the CA1, CA2, and CA3 hippocampal subfields. Rampon et al. (2000) analyzed various time exposures to EE and changes in gene expression. Four month old mice were exposed to EE for 3 hours, 6 hours, 2 days or 14 days. Early gene expression changes were measured in a comparison of mice exposed to EE for 3 or 6 hours. Seventy-seven percent of genes were consistently altered at both 3 and 6 hours. Some up-regulated genes included DNA methyltransferase (maintenance of DNA during replication), myelin gene expression factor and estrogen-responsive finger protein (transcription factors). Genes that were down regulated included caspase-6 (pro-apoptotic) and proyl oligopeptidase (regulated degradation of neuropeptides). For animals that were in EE for

2 and 14 days, a different late gene expression was observed. For these time points, changes in genes involved in neuronal transmission and structure were the common theme. X-box binding protein (involved in the cAMP pathway) and postsynaptic density 95 (involved in NMDA receptor anchoring) are two example of up-regulated gene expression at the 2 and 14 day time points.

EE has also been shown to effect stress-related organs and hormones. Despite beneficial effects of EE, Moncek et al (2004)'s work would suggest EE to be a chronic stress environment. Changes indicative of stress, such as increased adrenal weight and increased stress hormones such as corticosterone and adrenocorticotrophic hormone (ACTH), were observed in EE mice. In contrast, Belz et al. (2003) showed a decrease in base line stress hormones and a decrease in stress hormone response after mild stress induction in EE mice. The differences in these studies can be attributed to differences in housing protocol. In the study that found increases in stress, 10 male rats were group housed in an enriched environment. In contrast, Belz et al. (2003) measured stress responses in singly housed mice with toys augmenting their home cage. The increased stress measurement found in the Moncek et al. (2004) paper could be the result of a hierarchy competition in the EE cages. In summary, EE has been shown to effect measures at the gene to protein level, which often translate into morphological and behavior benefits.

#### *Effects of Environmental Enrichment in "Alzheimer's" Transgenic Mice*

While limited, some work involving the effects of EE on "Alzheimer's" transgenic mice has been done. The literature thus far is inconstant from a pathological

standpoint and sparse behaviorally. The current literature can be broken down into EE as a protective entity and EE as a treatment. For protective studies, mice are either exposed or reared in an EE from weaning while treatment studies involve putting animals together after behavioral impairment and pathology is present.

Jankowsky et al. (2003) offered the first work in preventative EE and AD. APP/PS1 females were used and emphasis was placed on EE effects on A $\beta$ . Mice were kept in an EE until 8.5 months of age at which time they were sacrificed. The methods state that animals from different cages were combined midway through the study and new young animals were added as older animals were removed. Despite 64 animals used, results were most impressive in 3 sibling pairs, which were highlighted in the results. For these 3 sibling pairs, Jankowsky et al. (2003) found increases in A $\beta$  burden. Results were more profound in hippocampus than cortex in numerous measures. In a measurement of aggregated A $\beta$  by size-exclusion filter trap assay, EE mice had a 30% increase in A $\beta$  in the hippocampus with only a 16 % increase in the cortex. Total A $\beta$ , measured by ELSIA, showed a 52% increase in the hippocampus versus a 31 % increase in the cortex. These results came as a surprise, taking into account the numerous positive effects of EE that have been documented. Rather than highlighting possible mechanisms by which EE exacerbates A $\beta$  deposition, it may be more appropriate to discuss why this EE protocol exacerbated A $\beta$  deposition. The paradigm described above may have led to a more stressful than enriching environment for the mice measured. The adding and subtracting of animals throughout the study removed a stable social base that could be considered crucial to the EE effect. This lack of a stable social environment may have also added stress. While not as extreme among females, a social hierarchy is still present and an

always changing hierarchy may have added to this stress. Also it is important to note that the majority of the results showing an increase in A $\beta$  were seen in 6 out of 64 mice.

In direct contradiction to Jankowsky et al. (2003), recent work by Lazarov et al. (2005) showed that EE reduces A $\beta$  pathology in transgenic mice. Similarly to the protection protocol of Jankowsky et al. (2003), APP/PS1 transgenic animals entered EE or SH at weaning and were exposed to an EE for 5 months. Unlike the former study which used females, only males were used in the latter study. Probing with 3D6 antibodies, EE-housed mice had significantly less amyloid burden in the hippocampus and the cortex. This theme continued for abundance and size of A $\beta$  deposits using thioflavine S staining. Reduction of non-deposited A $\beta$  levels was also measured. SH mice has significantly more detergent soluble and formic acid soluble A $\beta$  40 and 42. Taken together, these result show a global decrease in A $\beta$  pathology that spans various species of A $\beta$ .

Lazarov et al. (2005) also included an analysis of gene expression in EE mice. DNA microarray analysis revealed selective upregulation in levels of transcripts encoded by genes associated with learning and memory, vasculogenesis, neurogenesis, cell survival pathways, A $\beta$  sequestration, and prostaglandin synthesis. The upregulation of these genes appear to be constant with effects observed in wild type mice. The 11.2-44.3-fold increase in A $\beta$  sequestering genes may be responsible for the robust decrease of A $\beta$  in many forms. It is also important to note that the physical activity component of EE was dissected and analyzed in this paper. Results of this subsection will be highlighted below. Overall Lazarov et al. (2005) showed a robust decrease in brain A $\beta$  levels

coupled with positive upregulation of genes that may have lead to the decreases in A $\beta$  in transgenic mice.

Work by Jankowsky et al. (2005) is the first to show that EE has the potential to protect against cognitive impairment in a transgenic model of AD. Mice expressing APP and/or PS1 were placed in EE at 2 months of age and tested cognitively at 8 months of age. Mice were tested in standard Morris Water Maze (MWM), repeated reversal MWM, and radial arm water maze (RAWM). Analysis of behavioral data demonstrated that mice with APP alone or in combination with PS1, which lived in a standard house, were impaired in hippocampal-dependent learning and memory. In MWM acquisition, both the APP and APP+PS1 mice significantly benefited from enrichment when compared to standard housed mice. Compared with standard housed mice of the same genotype, both APP and APP+PS1 in enrichment housing mice swam significantly shorter distances in MWM acquisition. While an improvement was observed, enrichment did not enable the APP+PS1 transgenic mice to perform as well as the enriched non-transgenics or the PS1 mice in MWM acquisition. MWM retention benefits were only observed in APP single transgenic mice. Time spend in the goal quadrant for MWM retention was significantly increased in APP mice living in an EE. In contrast, an improvement in retention was not observed for APP+PS1 mice living in EE. Double transgenic EE mice spent significantly less time in the goal quadrant than all other genotypes given EE.

Similar results were observed in the repeated-reversal version of MWM and the RAWM. APP and APP+PS1 mice given EE swam shorter distances in repeated reversal MWM and made fewer errors in the RAWM than standard housed mice of the same genotype. This was shown by their ability to decrease “average distance traveled” in the

MWM repeated reversal task to levels comparable to standard housed non-transgenic mice. In the repeated-reversal version of the MWM, both enriched APP and enriched APP+PS1 did not decrease their latency as quickly as non-transgenic and PS1 enriched mice. Noteworthy for both the MWM repeated reversal task and RAWM task is that, following EE exposure, both APP and APP+PS1 mice overcame a genotype effect and were not significantly different from standard housed or even enriched non-transgenic mice. This suggests that APP mutant mice (APP and APP+PS1) benefited more from EE than PS1 or non-transgenic mice. This improvement in cognition was accompanied by increases in A $\beta$  levels. This finding is consistent with earlier work by Jankowsky et al. (2004) showing EE leads to increased levels of A $\beta$ . In contrast to the earlier protocol used by Jankowsky et al. (2004), mice were maintained in a consistent social environment throughout the study. While not clear, Jankowsky et al. (2005) suggest increased synaptic activity leading to increases in A $\beta$  production as a mechanism by which A $\beta$  levels are elevated with EE. Taking together, the review by van Praag (2000) showing enhanced synaptic strength and connectivity in EE mice and the findings by Kamenetz et al. (2003) that increased synaptic activity enhances APP processing by BACE1, the mechanism offered by Jankowsky et al (2005) appears reasonable. Their results would suggest that EE can strongly modulate A $\beta$ 's impact on the brain and make it a less relevant impairing factor.

While there has been some work showing pathological changes in AD Tg+ mice reared in EE, there is limited literature showing if the pathological changes translate into cognitive protection. Costa et al. (2005) found that PDAPP+PS1 mice placed in a “complete” EE (including social, physical and cognitive stimulation) from weaning are

protected from the cognitive deficits observed at 7.5 months in PDAPP+PS1 transgenic mice. In the platform recognition task, a measurement of object recognition and identification, EE mice were identical to the non-transgenic mice and were completely protected from the cognitive impairment observed in their standard housed (SH) litter mates. Equally impressive was the protection in working memory impairment, measured by the RAWM task. For overall latency on trial 4 and trial 5 of the RAWM, SH Tg+ mice were significantly impaired. In contrast the Tg+ mice that had lived in an EE were indistinguishable from non-transgenic mice and significantly better than Tg+ SH mice.

In order to decipher a mechanism through which cognitive protection was achieved, AD pathology and gene micro-array was done by Costa et al. (2005). In order to separate out any effects that could have been due to the intensive behavioral test battery used, a second cohort of mice that did not undergo behavioral testing was also analyzed. Two measures of A $\beta$  pathology was performed, including measurement of both diffuse and compact A $\beta$  deposition. Diffuse A $\beta$  immunoreactivity, using the 6E10 monoclonal antibody, revealed that there was no difference in total A $\beta$  load between Tg+/EE and Tg+/SH mice in either cerebral cortex or hippocampus for the “non-behaviorally tested” group. Though not significant, a trend towards decreases in A $\beta$  deposition was observed. This same trend was seen for compact plaque deposits, as measured by Thioflavin S staining. Importantly, Costa et al. (2005) did observe that “behaviorally-tested” EE mice had significantly lower A $\beta$  deposition than those mice that underwent only EE. This shows the EE alone was not enough to decrease A $\beta$ ; however EE and behavioral testing (itself a form of EE) did significantly decrease A $\beta$  levels, suggesting an additive effect.



In a micro-array analysis performed in Costa et al. (2005), roughly 70 genes showed statistically significant changes by a factor of 2.0 or more, in response to EE. Some of the more robust changes that have implications in the AD process included up-regulation of A $\beta$  sequestering genes, and neuroprotective genes, as well as down-regulation of genes involved in memory impairment. For example, insulin-like growth factor (*IGF-2*), which has been shown to play a neuroprotective role against A $\beta$ , was up-regulated in EE mice by 2.5 fold. A 10-fold increase in transthyretin (*TTR*), an A $\beta$ -binding protein shown to sequester A $\beta$  and inhibit amyloid formation, was also observed in the EE mice. Furthermore, phosphodiesterase 4 $\beta$  was down-regulated 2.2 fold and the cholecystokinin  $\beta$  receptor was down-regulated 3.2 fold, both of which when experimentally inhibited, are known to improve memory. Overall Costa et al. (submitted) have shown that “complete” EE has the potential to protect PDAPP + PS1 mice from cognitive impairment through mechanisms involving reductions in A $\beta$  deposition and beneficial changes in gene expression.

EE as a treatment in transgenic mice has not been widely researched. However in a single study, Arendash et al. (2004) showed improved cognition in aged transgenic mice despite stable levels of A $\beta$ . Moderately A $\beta$ -burdened APP<sup>sw</sup> mice entered EE at 16 months of age and lived in EE until 22 months at sacrifice. At 20 months, animals were taken through multiple behavioral tasks, testing a wide range of cognitive domains. In Morris Water Maze (MWM) acquisition and retention EE mice outperformed SH mice. In platform recognition task, which requires mice to switch from a reference learning/memory strategy to an identify/recognize strategy, EE mice easily transitioned

while the SH mice lagged behind in their effort to convert. Discriminant function analysis using 8 behavioral measures was able to separate the EE mice from the SH mice revealing significantly better overall performance.

Despite these behavioral benefits observed in the EE treated mice, no differences in A $\beta$  were observed. In both the parietal cortex and hippocampus no differences were seen in diffuse or compact plaques measured by 6E10 staining and Thioflavin S, respectively. A cognitive benefit without a large decrease in brain A $\beta$  deposition lead the authors to the possibility that an A $\beta$ -independent mechanism is driving the positive treatment effects of EE.

### ***Complete Environmental Enrichment versus Its Components***

As discussed, the effects of EE are wide ranging and are in some case quite robust. However, are these effects due to the complete EE experience or are there components of enrichment (social, physical, cognitive) that contribute more than others. While challenging to separate out the components of EE, some have attempted to and have observed EE component dependent results.

Recent work observed that walking alone was associated with a reduced risk of dementia (Abbott, et al., 2004). This prospective cohort study assessed the distance walked per day for 2 years of 2257 men aged 71-93. Men that walked the least had a 1.8 fold increase of dementia. This work would suggest that physical activity as modest as walking 2 miles a day could be enough physical activity and enrichment to decrease the risk of dementia. It is important to note that prospective studies such as these are often challenging to control. It is also a potential confound that those who walk more probably

also lead healthy lives in other domains of life such as diet. Exercise has been tested as a treatment for AD (Mahendra & Arkin, 2003; Teri et al. 2003). Patients that were enrolled in an exercise and caregiver training program had improved neurological exams (Teri et al. 2003). These improvements lead to a decrease in institutionalizing combined with less behavioral disturbances. Improvement with physical activity was also observed by Mahendra & Arkin (2003) whom described a comprehensive cognitive-linguistic intervention program for mild to moderate AD. Patients that participated in the 4 year program maintained or improved performance on numerous measures. While these studies offer some evidence of increased physical activity being beneficial as a prevention and treatment of AD, these studies did not look at the physical effects independently - they were paired with other activities such as supervised volunteer work and behavioral management techniques.

In rodent studies, physical activity has been show to enhance genes involved in brain health (metabolic, anti-aging, immunity genes) and plasticity (neurotrophic factor trafficking and vesicle recycling) (Cotman & Berchtold, 2002). Increase BDNF levels (Johnson & Mitchell, 2003), and enhance neurogenesis (van Praag et al., 1999; Kitamura et al, 2003; Ehninger & Kempermann, 2003) were also observed. Recent work by Lazarov et al. (2005) found that EE mice with the highest physical activity had the greatest reduction in A $\beta$ . It is hard to decipher if the animals with less A $\beta$  were more active due to less impairment or if the physical activity lead to the decrease in A $\beta$ . In a transgenic model (TgCRND8) of AD, Adlard et al. (2005) showed that physical activity decreased amyloid load. Five month old transgenic mice reared in an EE had a 38% decrease in A $\beta$  in the frontal cortex and a 40% decrease in A $\beta$  in the hippocampus when

compared to transgenic standard-housed mice. This change in A $\beta$  was not associated with differences in APP expression, or  $\alpha$ ,  $\beta$ ,  $\gamma$ -secretase expression. Overall, physical activity appears to have positive effects on brain health and could be a leading contributor to EE effects observed in humans and rodents.

Social activity is another component of EE that has been explored. An epidemiological study in humans showed that socially-oriented activities may protect against dementia (Wang et al., 2002). While social activity appeared to decrease risk, a connection between physical activities and decreased risk of dementia was not observed. However, mentally stimulating activities did appear to correlate with decreased risk of AD. Work in rodents has not shown social interaction alone as a benefit variable. Early work by Rosenzweig et al. (1978) showed that social grouping alone could not account for cerebral effects of EE. Male rats were exposed to several types of environments, some including social interaction and others lacking social interaction. After living 30 days in their designated environments, animals with social interaction were not different in brain region weight, RNA and DNA contents, or acetylcholinesterase activity from singly housed rats. Social interaction alone has been shown to not be effective in improving MWM performance in adult mice (Williams et al., 2001). This contradiction between humans and rodents could be due the hierarchy stress present in mice that is absent in human social activities; alternatively other unknown variables may have contributed to the benefit against dementia seen with social activity in humans.

van Praag et al. (2000) states that voluntary exercise and EE have “notably similar results” while social interactions alone cannot elicit the effects observed in EE. At this point it appears that no single variable can account for the changes observed in EE mice.

Ehninger et al. (2003) showed that EE increased proliferation of astrocytes in layer 1 of the motor cortex, while voluntary wheel running caused an induction in proliferation of microglia in superficial cortical layers. This would suggest that different components of EE could be eliciting different anatomical and electrophysiological changes resulting in a global behavioral benefit. van Praag et al. (1999) showed that both complete EE and voluntary wheel running enhanced the survival of newborn neurons in the dentate gyrus. Taking into account this work, it is not unreasonable to suggest that isolated elements of EE may result in similar anatomical and physiological changes by a common pathway (van Praag et al., 2000). Direct comparisons of several morphological and biochemical measures, as well as comparisons in a wide spectrum of behavioral tasks are needed to draw definite conclusions regarding the contributions that individual components of EE have.

## **Specific Aims**

Costa et al. (2005) has shown that “complete” environmental enrichment protects against behavioral impairment in PDAPP+PS1 transgenic mice, however it is unknown which component(s) of complete EE are responsible for that protection and what mechanisms are involved therein. Therefore, the specific aims of this thesis are:

**Specific Aim 1: To identify the contribution of each component (cognitive, social, physical) of “complete” environmental enrichment that lead to protective effects on cognitive performance in AD transgenic mice.** This will be achieved through implementation of our AD Tg<sup>+</sup> mice in a controlled longitudinal investigation of the components of environmental enrichment.

**Specific Aim 2: To elucidate the mechanisms through which environmental enrichment, and its various components, protect against cognitive impairment in AD transgenic mice.** Brain  $\beta$ -Amyloid levels, plasma cytokines levels, dendritic branching and spine density (all implicated to be involved with the protective ability of environmental enrichment) will be analyzed in order to provide a mechanisms by which enrichment (and its component activities) may be providing cognitive protection in AD Tg<sup>+</sup> mice.

**Specific Aim 3: To clarify the stress associated with living in a “complete” enriched environment as well as living in variable housing environments that contribute to “complete” enrichment.** With stress levels potentially confounding behavioral and pathological effects of enrichment (and its component activities), corticosterone levels will be measured.

**Specific Aim 4: To determine the inter-relationships between behavioral, neurohistologic, and biochemical measures taken from the same animals.** This unique, controlled study enables the analysis of interactions between behavioral and pathological findings within the same animal. Through correlation analysis, identification of these interactions could aid in the clarification of the mechanisms whereby enrichment benefits cognitive performance in AD transgenic mice.

## Material and Methods

### *Animals & General Protocol*

All mice contained a mixed background of 56.25% C57B6, 12.5% B6D2F1, 18.75 % SJL, and 12.5% SW. Mice were generated from a cross between male mice, heterozygous for the mutant APPK670N, M671L gene, and mutant PS1 (6.2 line) females. Mice were initially genotyped at the time of weaning and then had a confirmatory genotyping at 4 months of age. A total of 38 mice were used in three genotypic categories: non-transgenics, APP/PS1 double transgenic mice, and APP single transgenic mice. Mice were randomly divided into the various housing groups, where they lived through testing. *Ad libitum* access to rodent chow & water was provided, with mice maintained on a 14/10 light/dark cycle.

Figure 1 presents a timeline for this study's procedures. Beginning at 6 weeks of age, transgenic mice and non-transgenic litter mates were moved from standard social cages to one of the following housing environments: an impoverished environment (n=8), a social housed environment (n=9), a physical activity environment (n=5), or into a "complete" enrichment environment (n=6). At 6 months into their respective housing conditions (and at 7.5-8.5 months of age), all mice were tested in a 5-week behavioral battery, while still living in their designated housing. Following completion of the behavioral battery at 8.5-9.5 months of age, all animals were euthanized and brains of the APP+PS1 mice and non-transgenic controls were removed for histopathology and



neurochemistry. All procedures used were reviewed and approved by the USF Institutional Animal Care and Use Committee (IACUC).

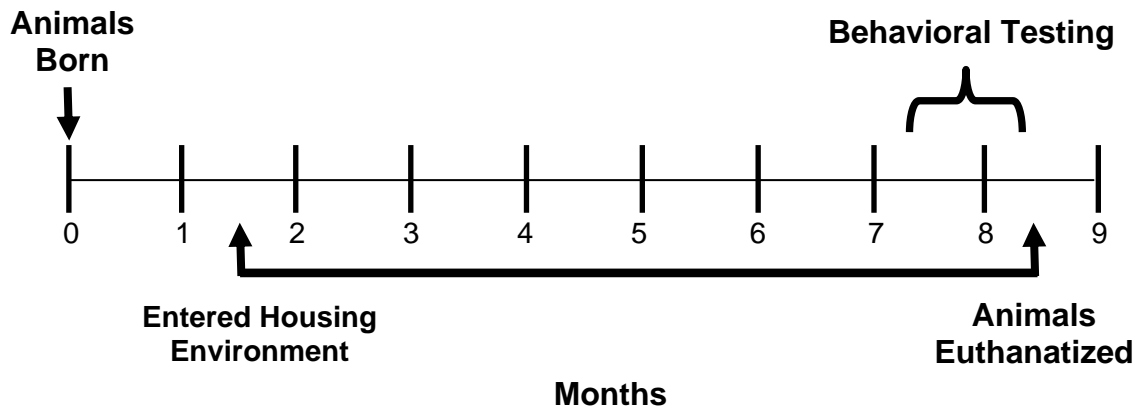


Figure 1. General protocol time line for enrichment study.

### *Enriched Environments*

At 6 weeks of age, transgenic mice were placed into one of five test environments. In a step-wise ascension towards the “complete” enrichment paradigm, transgenic mice were put into an impoverished environment (n= 8; 6 APP+PS1 and 2 APP), a social housed environment (n=8; 4 APP+PS1 and 4 APP ), a physical activity environment (n=5; 5 APP+PS1), or into a “complete” enrichment environment (n=6; 6 APP+PS1). Animals of the same gender, however of different genotypes, were housed together. Both groups of transgenic mice (APP+PS1 and APP) in all conditions performed identically, so their behavioral data were combined for statistical analysis. However, because APP+PS1 and APP mice were significantly different pathologically at euthanasia (e.g., no A $\beta$  plaques burdens in APP mice) the APP mice were eliminated from neuropathological and neurochemical analysis. A group of socially housed non-

transgenic mice were included as a standard housed control (n=12). Animals living in an “impoverished environment” were housed individually and had access to only food and water within their standard mouse cage (6.5”wide, 10.5 ” long, 5.5 ”high). Socially housed mice lived in standard mouse cages with other mice (2 - 4 mice/cage) of the same gender, thus constituting a “social activity” group. Another group of socially housed mice had both a larger rat cage and access to running wheels. This “physical activity” group had cages that were 7” wide, 11” long, and 5” high, with each cage equipped with 2 running wheels. “Complete” enrichment mice had social, physical and cognitive stimulation. A 110 liter Sterilite container (19”wide, 32 ” long, 13.5 ”high), with an inner “CrittterTrailTWO” rodent house, was used as housing for the “Complete” enrichment group. Housing for this group (5-7 mice/ cage) also contained running wheels and toys in the courtyard surrounding the rodent house; these items were changed weekly for novelty. “Complete” enrichment mice were also placed in novel, complex environments for at least 1 hour 3 times a week over the course of the study. Mice lived in their designated environment from 6 weeks of age until euthanasia at about 9 months of age. All cages contained an igloo and neslets.

### *Behavioral Assessment*

While still living in their selected housing environment, mice were tested in Y-maze, standard water maze, circular platform, platform recognition, and radial arm water maze tasks, in that order. All behavioral testing was conducted during the light cycle.

Y-maze. In this single day task, each animal was placed in a walled Y-maze for a single 5 minute trial to test both general activity (entries) and basic mnemonic processing

(percent alternations). Each of the 3 Y-maze arms was 21 by 4 cms with 40 cm high walls. The total number of arm entries and the sequence of arm choices were recorded. Basic mnemonic function was measured as percentage of spontaneous alternation (ratio of arm choices differing from the previous two choices divided by the total number of entries). For example, the sequence of arm entries (2,3,1,3,2,1,2,3) has six alternation opportunities (total entries-2) and the percent alternation would be 67%.

Standard Water Maze. A 100-cm circular pool was divided into 4 equal quadrants by drawing lines on the bottom of the pool. Quadrant 2 (goal quadrant) contained a clear, 9-cm diameter submerged platform, 1.5 cms below the water. Surrounding the pool were visual cues, which were placed proximal and distal to the pools edge. Visual/spatial cues consisted of large, brightly colored 2D and 3D objects, including a beach ball, poster, and inflatable pool toys. During testing, the pool water was maintained at 23-27 °C. For each of the 10 days of acquisition, mice were given 4 trials. For each of the four successive 60-sec trials per day, mice were started from a different quadrant; the same quadrant start pattern was used across all 10 days of acquisition. Latency to find the platform (maximum of 60s) was recorded, and the average latency of the 4 trials was calculated for use in statistical evaluation. Once the mouse found the platform, it was allowed to stay for 30s. If after 60s the mouse did not find the platform, it was gently lead to the platform and given a 30s stay. After 10 days of acquisition testing, memory retention was evaluated in a single 60s probe trial the following day. For this trial, the submerged platform was removed and animals were released from the quadrant directly opposite the goal quadrant. This single trial was video recorded and the percent time spent in each quadrant, as well as the number of annulus crossings, was analyzed.

Circular Platform Task. The circular platform maze consisted of a 69-cm circular platform with 16 holes equally placed around the periphery of the walled maze. Surrounding the maze were two different colored shower curtains that had 2-dimensional visual cues attached to them for use by the mice in navigating the maze. One of the holes contained an escape box that remained in the same location for all 8 days of testing. Animals were encouraged to find the escape by adding aversive stimuli of bright lights and fan wind to the maze. The aversive stimuli included two 150-W flood lamps hung 76cm above the platform and one high-speed fan 15 cm above the platform. During a single 5 minute daily trial, both the number of errors (head pokes into non-escape holes) and latency to find the escape hole (up to 300 sec.) were recorded. Although the escape hole remained the same for any given animal over 8 days of testing, the escape box was relocated after each animal's trial to one of 3 other hole locations to control for olfactory cues. The maze surface was cleaned with dilute vinegar after each animal for added olfactory cue control.

Platform Recognition. The platform recognition task measures the ability to search for and identify/recognize a variably-placed elevated platform. It requires animals to switch strategies and ignore the spatial cues present around a circular pool, which was the same pool used in earlier Morris water maze testing. A visible platform, 9-cm in diameter with an attached 10 x 40 cm ensign, was placed into the same 100-cm pool in which standard water maze was conducted. The visible platform was elevated 0.8 cm above the water's surface. For 4 days of testing, animals were placed in the pool at the same start position for each of 4 trials, with the platform being moved to a different one of the 4 quadrants for each trial. The latency to ascend the platform was recorded (60s

maximum) and the daily 4 trials were averaged. A 30s stay was given when the mice found the platform. Mice that did not find the platform within the 60s were gently guided to the platform by the experimenter and allowed to stay for 30s.

Radial Arm Water Maze. Spatial working memory was assessed in a “win-stay” version of the radial arm water maze (RAWM) task. In the same 100-cm pool utilized for Morris Water Maze and Platform Recognition testing, an aluminum insert was introduced in order to divide the pool into 6 equally spaced swim arms (30.5 cm length x 19 cm width) radiating from a central circular swim area (40 cm diameter). The insert extended 5 cm above the surface of the water, allowing the mice to easily view surrounding visual cues, which were generously placed outside of the pool. Visual/spatial cues consisted of large brightly colored 2D and 3D objects, including a beach ball, poster, and inflatable pool toys. During testing, the pool water was maintained at 23-27 °C. In one of the arms, a transparent 9cm submerged escape platform was placed 1.5cm below the water near the wall end. Each mouse was given five 1 minute trials per day for nine days. The last of the four consecutive acquisition trials (Trial 4, T4) and a 30 minute delayed retention trial (Trial 5, T5) are indices of working memory. On any given day, the escape platform location was placed at the end of one of the 6 arms, with the platform moved to a different arm in a semi-random fashion for each day of testing. In contrast to the stationary platform of standard water maze, moving the escape platform forced the animal to learn a new platform location daily, therefore evaluating working memory. On each day, different start arms for each of the five daily trials were selected from the remaining five swim arms in a semi-random sequence that involved all five arms. For any given trial, the mouse was placed into that trial’s start arm, facing the center swim

area, and given 60s to find the platform. When the mouse made an incorrect choice, it was gently pulled back to that trial's start arm and an error was recorded. An error was also recorded if the mouse failed to make a choice in 20s (in which case it was returned to that trials start arm), or if the animal entered the platform-containing arm, but failed to locate the platform. A 30s stay was given once the mouse had found the platform. If the mouse did not find the platform within a 60s trial, it was guided by the experimenter to the platform, allowed to stay for 30s, and was assigned a latency of 60s. For animals that did not make at least 3 choices an error value of 5.6 was assigned as a penalty. This number was calculated by averaging errors for all animals that did not locate the platform for Block 1 (day1-day3) on Trial 1 (T1). Both errors (incorrect arm choices) and escape latency were recorded for each daily trial.

#### *Tissue and Blood Collection*

Following the final day of behavioral testing, a blood sample (.5 mL) was taken from the submandibular vein, plasma was separated, and stored at -80°C for later analysis of corticosterone levels. Two days after plasma collection, animals were deeply anesthetized with pentobarbital and a second blood sample was collected intra-cardially, and stored at -80°C for later analysis of cytokines. Animals were then perfused with 100ml of 0.9% saline. Post mortem brains were immediately removed and bisected sagittally. The left hemisphere was placed in 4% paraformaldehyde over night and then transferred to a graded series of sucrose solutions (10%, 20%, and finally 30%) wherein tissues remained until histologic sectioning. The right hemisphere was chilled in cold saline and then dissected into 4 major areas: 1) a 2-3mm thick coronal slice through the

posterior cortex/hippocampus, 2) striatum, 3) anterior cortex, and 4) cerebellum. The posterior cortex/hippocampus slice was drop fixed in 10% neutral buffered formalin for later Golgi Cox staining. The remaining brain regions were transferred into individual 1.5 ml Eppendorf tubes, immediately frozen on dry ice, and stored at -80°C for later neurochemical analysis.

#### *Corticosterone Quantification*

Corticosterone (CS) levels were measured using a radioimmunoassay (RIA). Serum levels of CS were determined using a double-antibody RIA kit purchased from ICN Biomedicals (Costa Mesa, CA). Samples from all mice were assayed in duplicate. Approximately 5ng/ml was the minimum detectable concentration.

#### *Extraction of Brain Protein for Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)*

A 5% sucrose homogenate (wet weight of tissue/ volume) from frozen mouse anterior cortex was prepared and extracted as described by Schmidt et al. (2004). This procedure began with the weighing of each tissue in an empty microcentrifuge tube. Once weighed, 2mls of tissue homogenization buffer (THB) per 100 mg of tissue was added to the sample. Immediately prior to homogenization, a protease inhibitor cocktail (20% of wet tissue weight to volume ratio) was added to prevent degradation of proteins. While the sample was on ice, the calculated amount of THB + inhibitors was added to the sample and fully homogenized. After homogenization, samples were frozen and kept at -80°C until Diethyl Amine (DEA) and Formic Acid (FA) extraction was done.

DEA extraction was used to separate soluble A $\beta$  from the brain homogenate. First, 100  $\mu$ L of 5 % homogenate was mixed with 0.4% DEA (diluted in 100mM NaCl) on ice. The mixture was then transferred into a thick-walled polycarbonate tube and centrifuged at 100,000 x g for 1 hour at 4°C. After centrifuging, 170 $\mu$ l of supernatant was removed and added to a second tube containing 17  $\mu$ l of 0.5M Tris Base, pH 6.8 (1  $\mu$ l per 10 $\mu$ l of supernatant). After briefly vortexing, samples were frozen on dry ice and stored at -80°C. For the FA extraction of insoluble A $\beta$ , 100 $\mu$ l of THB was added to the pellet from the DEA extraction to return the mixture to the original volume. Following the addition of 220 $\mu$ l of 95% FA, pellets were sonicated for 1 minute on ice. The mixture was centrifuged at 100,000 x g for 1 hour at 4°C. After centrifugation, 52.5  $\mu$ l of the intermediate phase of the FA extracted mixture was added to 1 ml of FA neutralization solution. Samples were vortexed, immediately frozen on dry ice, and stored at -80°C.

#### *A $\beta$ Sandwich ELSIA*

Sandwich ELSIA kits for A $\beta$  1-40 and 1-42 analysis were purchased from Signet Laboratories and the instructions provided were followed. Briefly, for the 1-40 kit, a standard curve was generated with the highest point set at 2000 ng/ml and the lowest point set at 0 ng/ml. Before loading the 96 well plate, tissue samples were diluted in provided wash/sample diluent (FA 1:500, DEA 1:200). Either diluted tissue or standard curve samples were loaded in duplicate onto the 96 well plate. The sample and the standard curve wells were allowed to incubate overnight at 4-8°C. After incubation, plates were washed, diluted primary antibody was added, and incubated for 2 hours. The plates were then washed again and the secondary antibody- HRP complex was added for



2 hours. Wells were again washed, orthophenylenediamine dihydrochloride (OPD) substrate solution was added, and incubated in a dark room for 45 minutes. Stop solution was added and the optical density was read at 490nm. For the A $\beta$  1-42 ELISA, all steps were the same as for A $\beta$  1-40, except that samples were diluted to 1:100 for DEA extracted samples. To account for background, the average optical densities of the 0 ng/ml wells on the standard curve were subtracted across all 96 wells. After generation of the standard curve, optical densities were corrected to reflect protein to wet brain concentration.

#### *Blood Cytokine Levels*

Relative cytokine levels were determined through the use of a custom Mouse Cytokine Antibody Array purchased from RayBiotech Incorporated. Ten separate anti-mouse-cytokine antibodies were provided on membranes. Cytokines analyzed included; IL1- $\alpha$ , IL1 $\beta$ , IL-2, IL-4, IL-6, IL-10, IL-12, IL-12 (p70), TNF $\alpha$ , IFN $\gamma$ , and GM-CSF. Membranes were first treated with blocking buffer and then incubated with 1 ml of diluted (1:10) plasma for 1 hour. Samples were decanted and 1x secondary biotinylated antibodies were added and incubated for one hour. A second two hour incubation was conducted with diluted (1:1000) labeled-streptavidin, completing the conjugated secondary antibody complex. The final detection step involved incubation for 1 min. with provided detection buffers. Using Fujifilm AR x-ray film, signals from membranes were detected and developed using back-lit photography. For duplicated samples, analysis of signal mean intensities minus background signal intensity were determined using Kodak 1D Image Analysis Software. In order to account for the large variability in

signal intensities among the various cytokines, signals were standardized on a zero to one scale based on minimum and maximum mean intensity readings for each cytokine.

Standardized values were then used to compare cytokine levels between animals.

### *Golgi Cox Analysis*

The 2-3 mm thick hippocampus/posterior cortex slices that had been stored in 10% neutral buffered formalin were stained *en bloc* using the Rapid Golgi Method (Valverde, 1993). Briefly, blocks of tissue were immersed in a mixture of osmium tetroxide and potassium dichromate for 5-6 days. After 5-6 days, tissues was then subsequently immersed in a silver nitrate solution for 36-38 hours. Tissue blocks were then dehydrated and embedded in nitrocellulose. Stained blocks were cut on a sliding microtome at 120 $\mu$ m, cleared in alpha-terpineol, rinsed in xylene, and cover-slipped under Permount. For analysis of dendritic branching, camera lucida drawings of basilar dendritic arbors from randomly selected neurons were generated. For both parietal cortex Layer V and hippocampus CA1 region, six neurons were drawn per animal and were then subsequently quantified for the amount and distribution of dendritic arbors. Quantification of dendritic distribution was accomplished by the using Sholl analysis, Method of Concentric Circles (Sholl, 1953).

Neuronal dendritic spine densities were also analyzed. Dendritic spines were counted directly on a Zeiss research microscope using 100x long-working distance oil-immersion objectives. Both the terminal tips of layer V pyramidal cells and hippocampal CA1 pyramidal cells were used for the spine analysis. Spines were counted on 3-5

terminal tip segments per neuron, 30 microns in length, from 6 neurons per brain (neurons were different from the 6 used in dendritic branching analysis).

### *Statistical Analysis*

Behavioral Analysis. Behavioral performance was statistically evaluated to determine any group difference based on housing condition or transgenicity. For both of the single day tasks ( Y-maze and water maze retention) one-way ANOVAs were used. For multi-day tasks (standard water maze acquisition, circular platform, platform recognition and RAWM) both one-way ANOVAs and two-way repeated measure ANOVAs were performed. Prior to analysis, MWM data was broken down into five-2 day blocks and the RAWM data was divided into three-3 day blocks, to aid in data presentation and analysis. After ANOVA analysis, *post hoc* pair-by-pair differences between groups (planned comparisons) were resolved using the Fisher LSD test. All group comparisons were considered significant at  $P < 0.05$ . While few in number, any outliers or non-performers (e.g. repeated circulars, consistent floaters) were eliminated from behavioral statistical analysis.

Histological/Biochemical Analysis. Pathological data analysis including both histological and biochemical portions of the experiment were performed using ANOVA. Statistical analysis of the spine counts and dendritic branching were carried out using ANOVA with a post-hoc tukey test. In order to test if relationships were present between pathologic, biochemical, and behavioral measures, correlation analysis was performed using the Systat analytical software package. Correlation values shown are the results of a pair, uncorrected correlation analysis.

FA and DFAs: To group behavioral, biochemical, and histologic measures by common factors, Factor Analyses (FA) were performed using Systat software. Regardless of genotype or housing, FA used all collected data to relate measures into individual factors. In an initial FA, all 15 behavioral measures were included. This enabled the determination of how different behavioral tasks related to one another, as well as how performance of one task might predict performance in other tasks. Follow-up FA's included not only the 15 behavioral measures, but also the histologic and biochemical measures as well. These FA's extracted inter-relations within behavioral, histologic and biochemical data. To determine if the 5 experimental groups (NT, IMP, SH, PA, and EE) were distinguishable from one another based on the behavioral measures set, DFA was performed using Systat software. DFA was performed using all 15 measures as well as with only the behavioral measures that loaded in factor 1. Both a direct entry and stepwise-forward DFA was conducted in each case. The direct entry method uses all measures available, while the step-wise forward method selects measures based on their variance contribution and adds them in a step-wise fashion to best discriminate between groups. All of the above DFA's were then repeated with inclusion of histologic and biochemical measures.

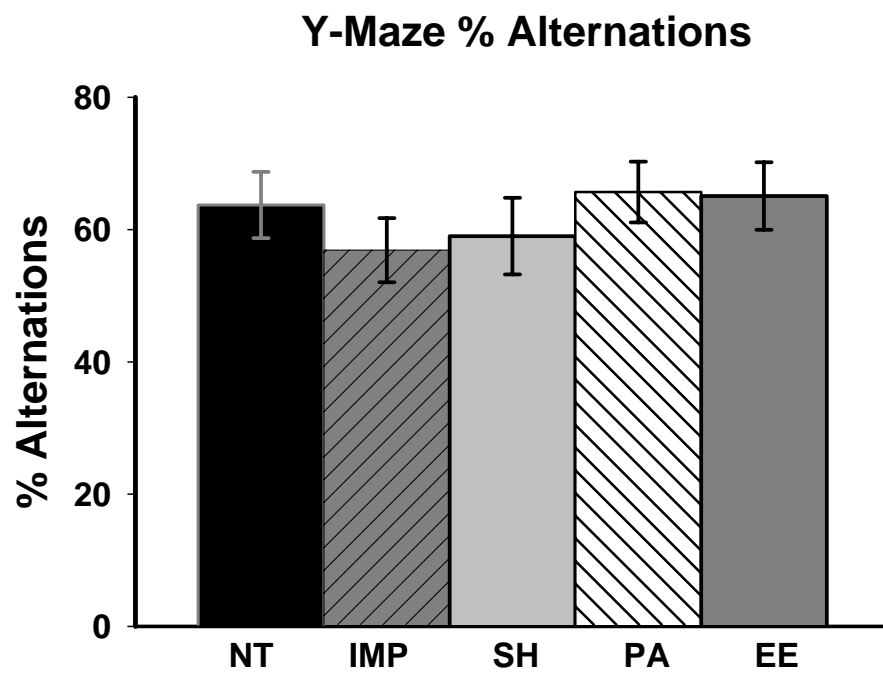
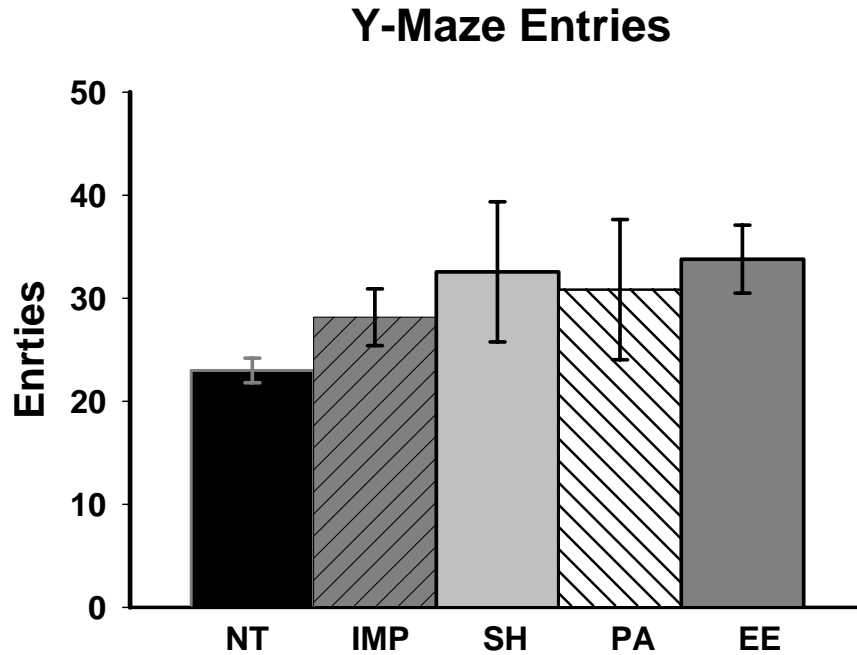
## Results

### *Behavioral Analysis*

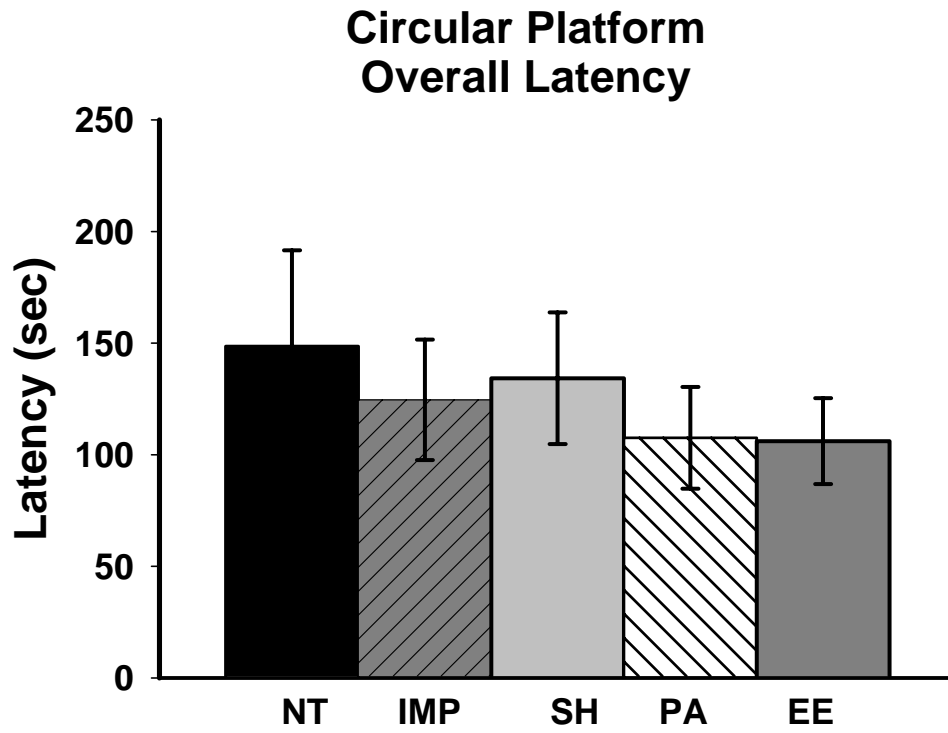
Of the five cognitive-based tasks evaluated, the Y-maze and circular platform tasks revealed no impairment in control Tg+/SH mice when compared to NT mice, and thus no protective effect of environmental enrichment (EE) could be present. In Y-maze testing for spontaneous alternations behavior, no group differences in percent alternation or arm entries were evident (Fig. 2). As well, no group differences were evident in escape latencies over 8 days of circular platform testing for spatial reference memory (Fig. 3). Animals in all groups collectively improved their performance during circular platform testing, as shown by overall reduced escape latencies across days [ $F(7, 238) = 2.50, p < 0.02$ ].

In Morris water maze (MWM) acquisition, escape latency data was divided into five 2 day blocks to facilitate statistical analysis and presentation (Fig. 4). Over all 5 blocks of testing, EE mice exhibited significantly lower escape latencies in comparison to both IMP and PA mice (Fig. 4A), although no effect of transgenicity was evident between NT and control SH mice. Evaluating acquisition across individual blocks revealed that EE mice had significantly lower escape latencies on Block 2 compared to all other groups (Fig. 4B), indicating an ability of EE mice to improve their learning of this task faster than other groups. For all animals collectively across the 5 blocks of testing, there was an overall learning effect [ $F(4,32) = 16.80; p < 0.0001$ ]. As well, a

blocks by treatment interaction was present [ $F(4,128) = 1.92$ ;  $P < 0.025$ ], apparently due to the worsened performance of NT mice during the last two blocks of testing (Fig. 4B).

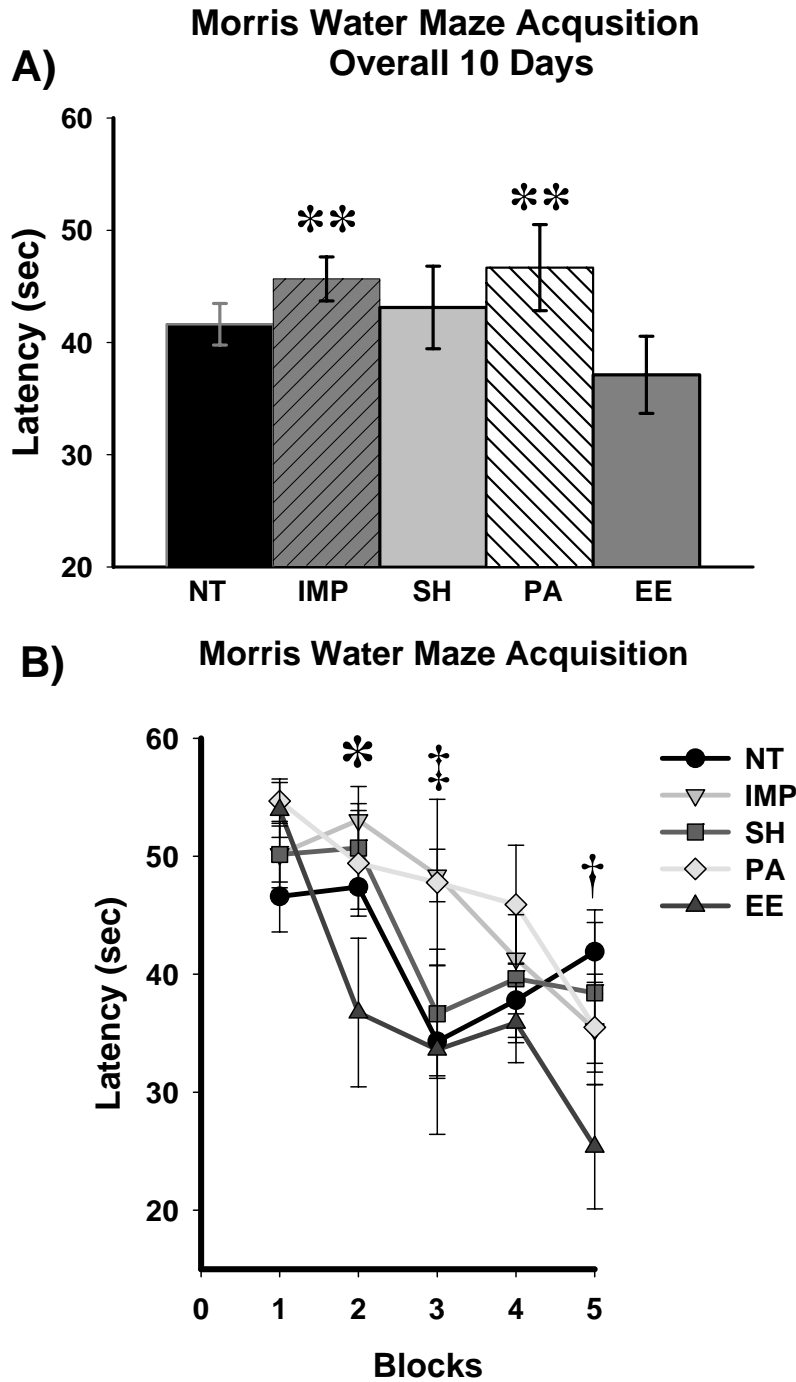


**Figure 2. Y-maze Entries and Percent Spontaneous Alternations.** No group differences were noted for either entries or percent alternation in Y-maze performance. Abbreviations: NT = non-transgenic socially housed mice, IMP = transgenic impoverish housed mice, SH= transgenic socially housed mice, PA= transgenic physical activity mice, EE= transgenic “complete” environment enrichment.



**Figure 3. Circular Platform overall escape latencies.** No group differences were evident over 8 days of testing. Abbreviations: as in Figure 2.

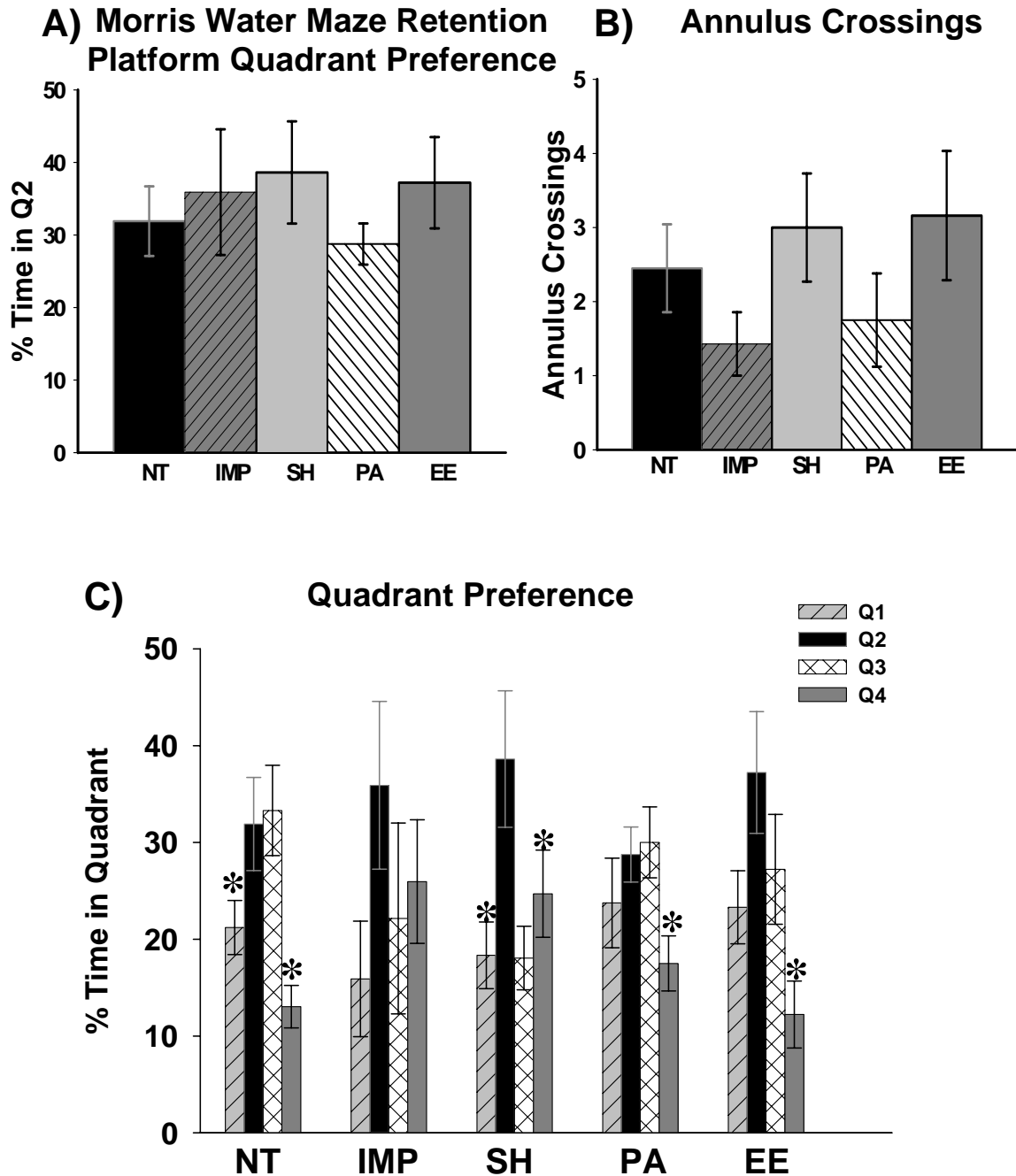




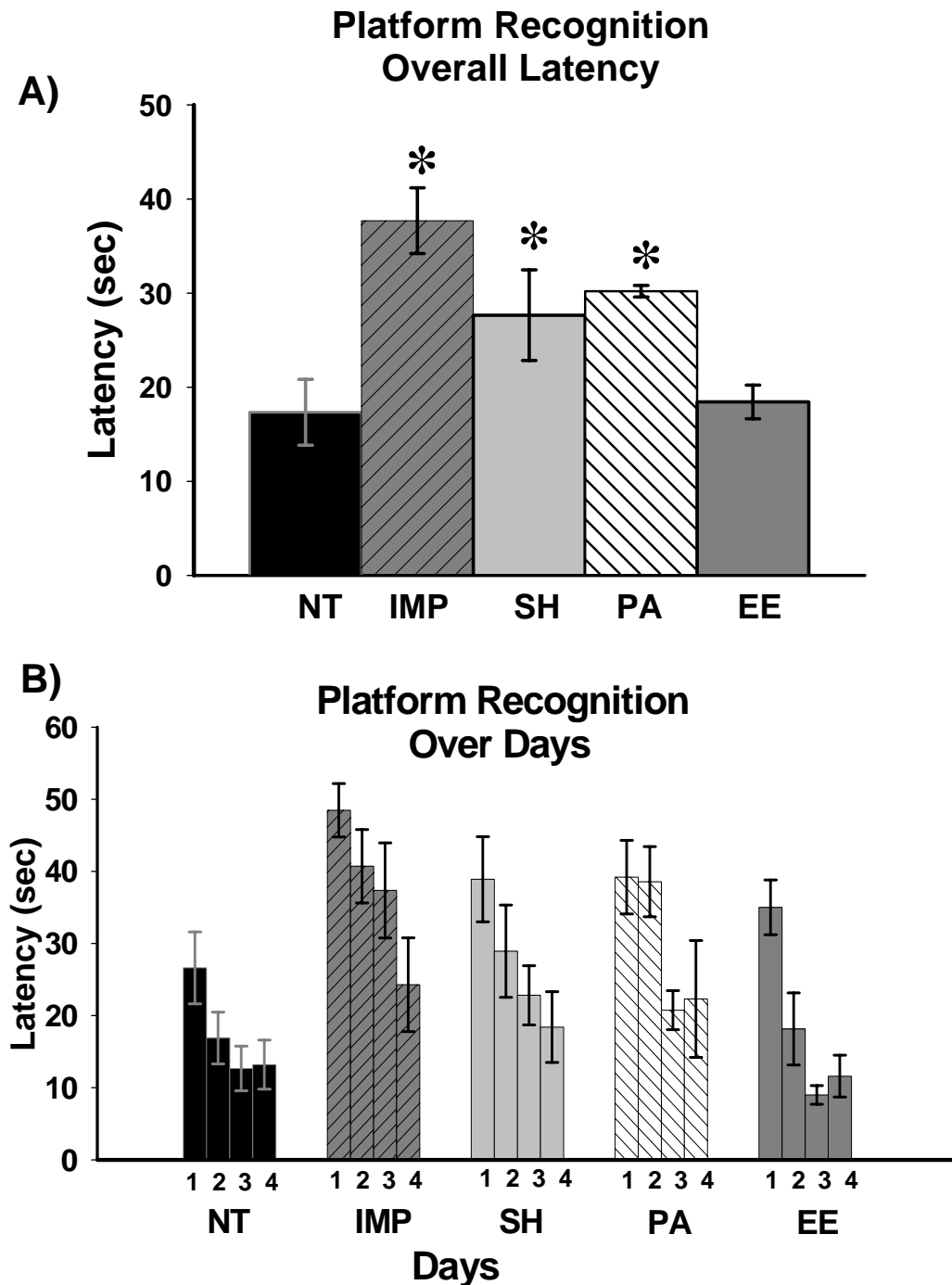
**Figure 4. Morris Water Maze acquisition overall (A) and across 5 two-day blocks (B).** EE mice showed better spatial learning than several other Tg+ groups overall and were able to improve their performance sooner than all other groups. \* = EE mice significantly lower latencies vs. all other groups ( $p < 0.05$  or higher level of significance). ‡ = EE and NT significantly lower latencies from IMP ( $p < 0.05$ ). † = EE mice significantly lower latencies than NT ( $p < 0.02$ ). \*\* = EE significantly lower latencies than IMP and PA ( $p \leq 0.05$ ). Abbreviations: as in Figure 2.

Results from the MWM probe trial are presented in Fig. 5. No housing group spent significantly more time searching in the quadrant formerly containing the submerged platform (Q2) than any other group (Fig 5A). As well, there were no group differences in annulus crossings (Fig 5B). While none of the 5 groups showed an exclusive quadrant preference for the former platform-containing quadrant (Q2), both NT and SH groups exhibited a partial quadrant preference for Q2 (Fig. 5C). In contrast, PA, IMP and EE mice showed no quadrant preference (e.g., only one or no quadrants significantly less in % time vs. Q2). Thus, across 3 indices of reference memory in Morris maze testing, EE mice did not perform significantly better than other Tg+ groups.

In platform recognition testing, an overall groups effects was present across all 4 days of testing [ $F(4,28) = 5.04$ ;  $p < 0.005$ ]. *Post hoc* planned comparisons of overall escape latencies revealed that IMP, SH, and PA groups were impaired overall vs. NT controls, whereas EE mice performed identical to NT controls (Fig. 6A). Analysis of performance on individual days indicated that both NT and EE groups quickly reduced their escape latencies across the 4 days of testing (Fig. 6B). This rapid reduction in escape latency was not observe for IMP, SH and PA mice, indicating that EE mice were much better at changing from the spatial (cued) strategy of the MWM to the recognition/identification strategy of platform recognition. By Day 4, however, there were no group differences in escape latency, indicating that all housing groups were able to eventually reduce their escape latencies to levels comparable to NT controls. Indeed, all animals collectively improved their performance across the 4 days of testing, as evidenced by a strong overall effect of training [ $P(3,84)=25.91$ ;  $p < 0.00001$ ].



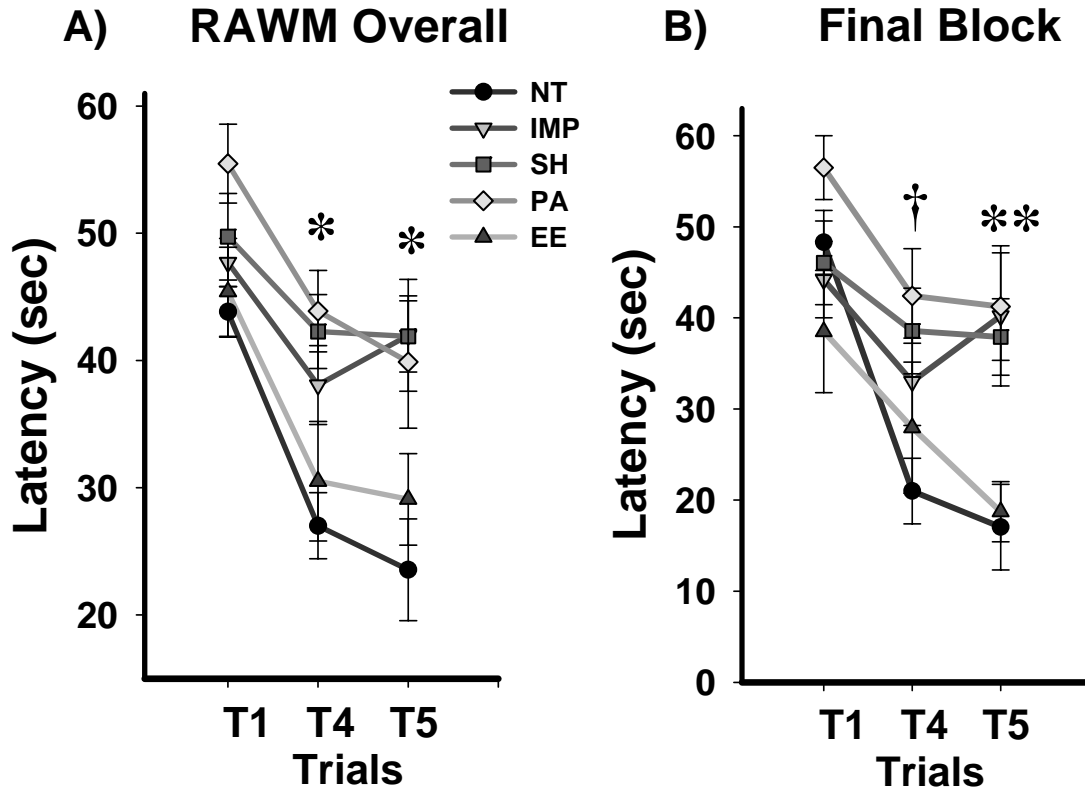
**Figure 5. Morris Water Maze Memory Retention, as indexed by time spend in the former platform-containing quadrant (A), annulus crossings (B), and quadrant preference (C).** No group differences were observed for percent time spent in the former platform-containing quadrant (Q2) or annulus crossings. For percent time spent in individual quadrants, NT and SH groups showed a partial quadrant preference. \* = significantly percent time compared to Q2 ( $p < 0.05$ ). Abbreviations: as in Figure 2.



**Figure 6. Recognition/Identification performance in Platform Recognition testing overall (A) and for each of the 4 days of testing (B).** Over all 4 days of testing (A), all Tg+ groups except EE mice were significantly poorer in performance compared to NT controls. For individual test days, NT mice and EE mice rapidly reduce their escape latency, while IMP, SH, and PA mice have a slowed reduction in latency. \*= significantly higher escape latencies than NT ( $p < 0.05$  or higher level of significance). Abbreviations: as in Figure 2.

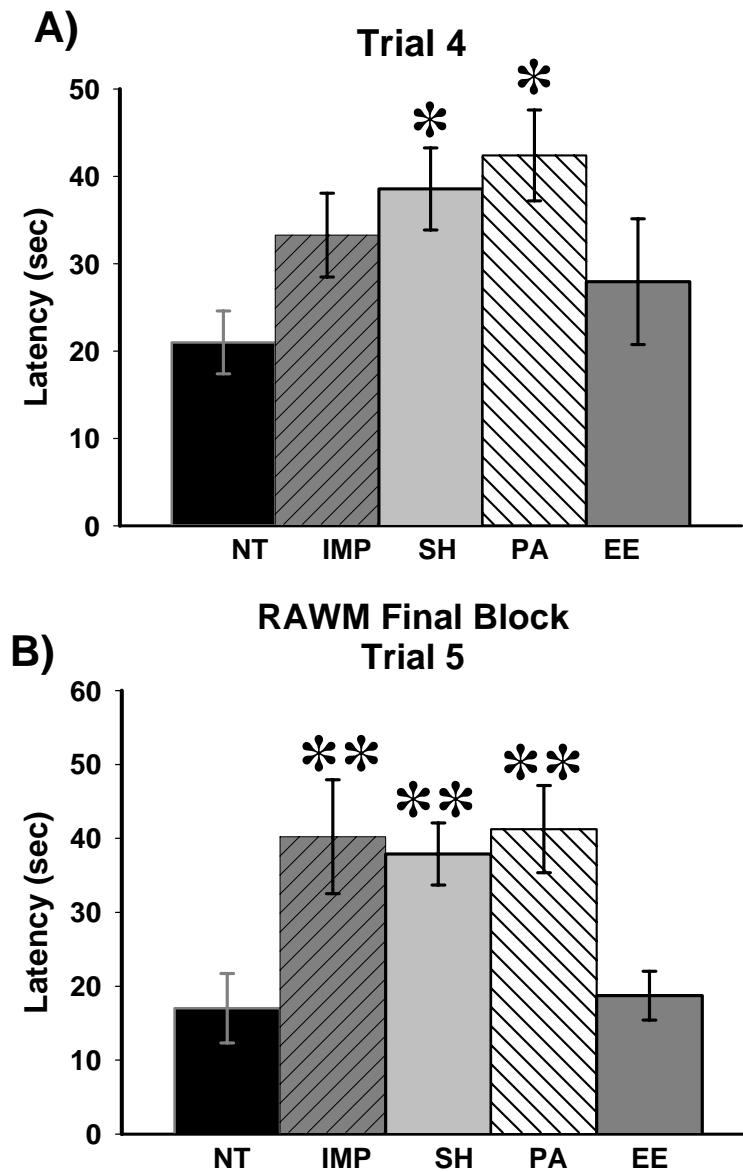
These results show that “complete” enrichment is needed to protect Tg<sup>+</sup> mice against impaired ability to switch from a spatial to a recognition/identification strategy and that physical activity or social interaction are not enough to provide this protection.

Figures 7 and 8 present escape latencies in RAWM testing. Data were evaluated across three 3-day blocks for T1 (randomized initial trial), T4 (final acquisition trial) and T5 (delayed retention trial); T4 and T5 are indices of working memory. An overall groups effect was present for both T4 [ $F(4,28)=4.91$ ;  $p<0.005$ ] and T5 [ $F(4,28)=4.89$ ;  $p<0.005$ ]. *Post hoc* analysis of overall T4 and T5 latencies revealed that IMP, SH, and PA groups were significantly impaired vs. NT controls, whereas performance of EE mice was not different from NT controls and significantly better than the SH transgenic control group (Fig. 7A). By the final block of testing, complete separation of EE and NT mice from all other groups was observed (Fig. 7B). On T5 of this final block, NT control mice had significantly lower escape latencies than IMP, SH, and PA mice ( $P<0.01$ ). In sharp contrast, EE mice were identical to NT mice and significantly better than all other Tg<sup>+</sup> groups ( $P<0.025$ ). Figure 8 echoes the final block findings shown in Figure 7, highlighting the indistinguishable escape latencies between NT and EE mice for T4 of the final block, as well as the complete separation of NT and EE mice from all other Tg<sup>+</sup> groups (IMP, SH, PA) in T5. These RAWM data underscore findings from platform recognition in showing that “complete” EE protects against cognitive (working memory) impairment while physical activity and social housing alone are not protective.



**Figure 7. RAWM overall and final block escape latencies for Trials 1, 4 and 5.** In overall working memory performance (A), NT mice had significantly lower escape latencies than IMP, PA, and SH on both overall T4 and T5. In contrast, EE mice were indistinguishable from the NT group. On the final block of testing (B), NT mice and EE mice had significantly lower escape latencies than all other groups (IMP, SH, PA) for T5. \* = significant difference between NT and IMP, SH, and PA ( $p < 0.02$  or higher level of significance). † = NT significantly difference from PA and SH ( $p < 0.02$ ). \*\* = Both NT and EE significantly different from IMP, SH and PA ( $p < 0.025$  or higher level of significance). Abbreviations: as in Figure 2.

## RAWM Final Block



**Figure 8. RAWM escape latencies for final block Trials 4 and 5.** During T4, NT mice achieved significantly lower escape latencies when compared to SH and PA mice, while EE mice were no different from NT controls. During T5, both NT and EE groups had significantly lower latencies than all other groups (IMP, SH, PA). \*= significantly different from NT group at  $p < 0.02$ . \*\*= significantly different from both NT and EE at  $p < 0.025$  or higher level of significance. Abbreviations: as in Figure 2.

### *Neuropathologic Measures*

On the day following completion of behavioral testing, a blood sample was collected to measure plasma corticosterone levels of mice living in different housing environments. Statistical analysis revealed no differences in corticosterone levels among animals in the different housing environments (Fig. 9A) and no overall genotype effect (Fig. 9B). The finding that different housing of the four transgenic groups did not effect corticosterone levels suggests that stress did not play a role in the differences in cognitive performance observed among these groups. In contrast, male mice collectively had significantly lower levels of corticosterone levels when compared to females [ $F(1,37)=8.44$ ;  $p < 0.01$ ]. Levels were almost 3-fold lower in male mice vs. females (Fig. 9C).

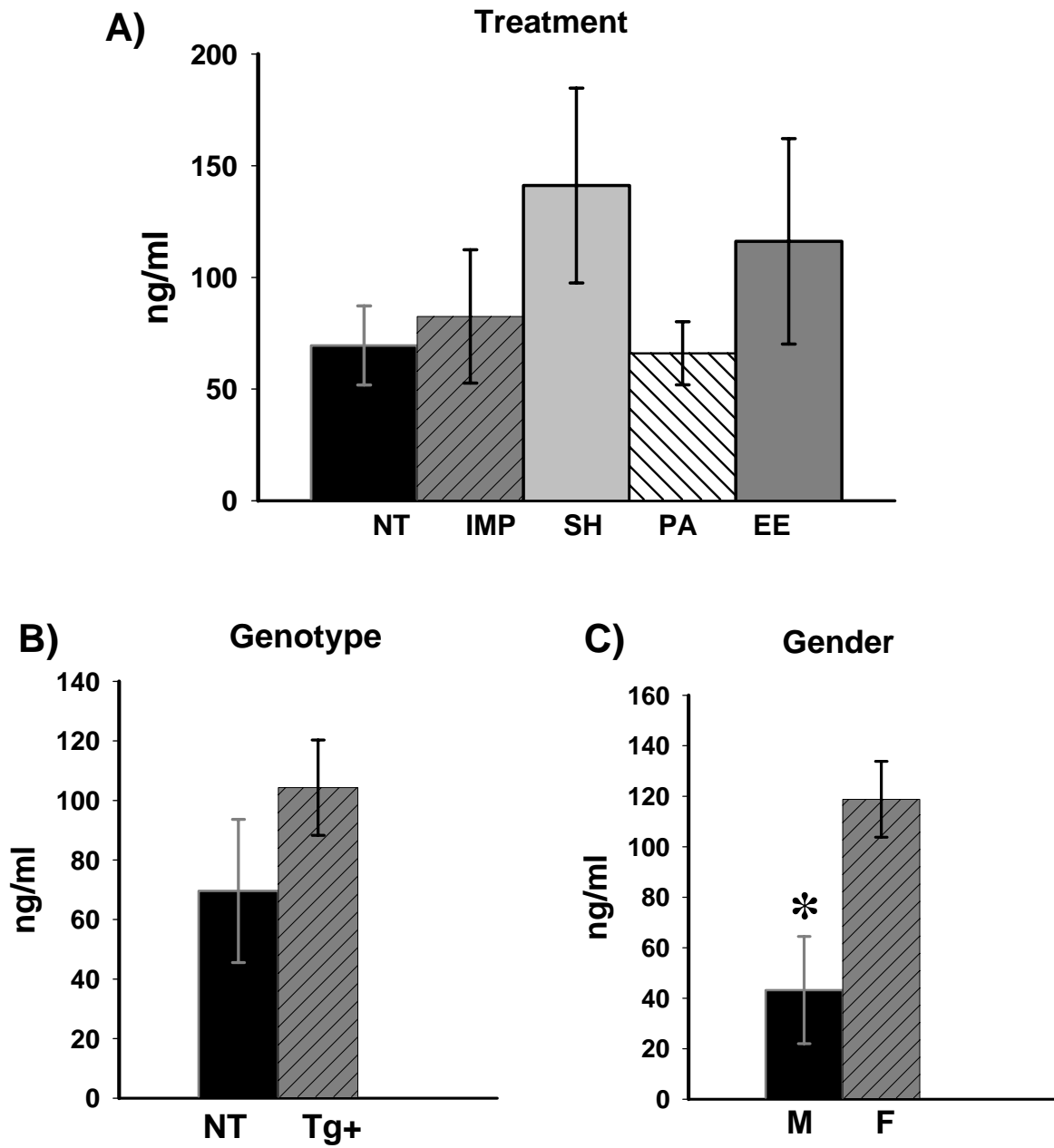
At euthanasia, 3 days following behavioral testing (and approximately 6.5 months into enrichment), a second blood sample was collected from APP+PS1 and NT mice for measurement of plasma cytokine levels. As shown in Fig. 10, no significant differences were observed among the housing groups for any of the 10 pro- and anti-inflammatory cytokines measured. Analysis by genotype revealed no differences in any of these cytokines between APP+PS1 mice collectively and NT mice (data not shown).

For APP+PS1 in each housing environment, A $\beta$  analysis by ELISA was performed on anterior cortex tissues. As shown in Fig.11, no significant group differences were observed in A $\beta$  1-40 or 1-42 levels for either soluble or insoluble species.

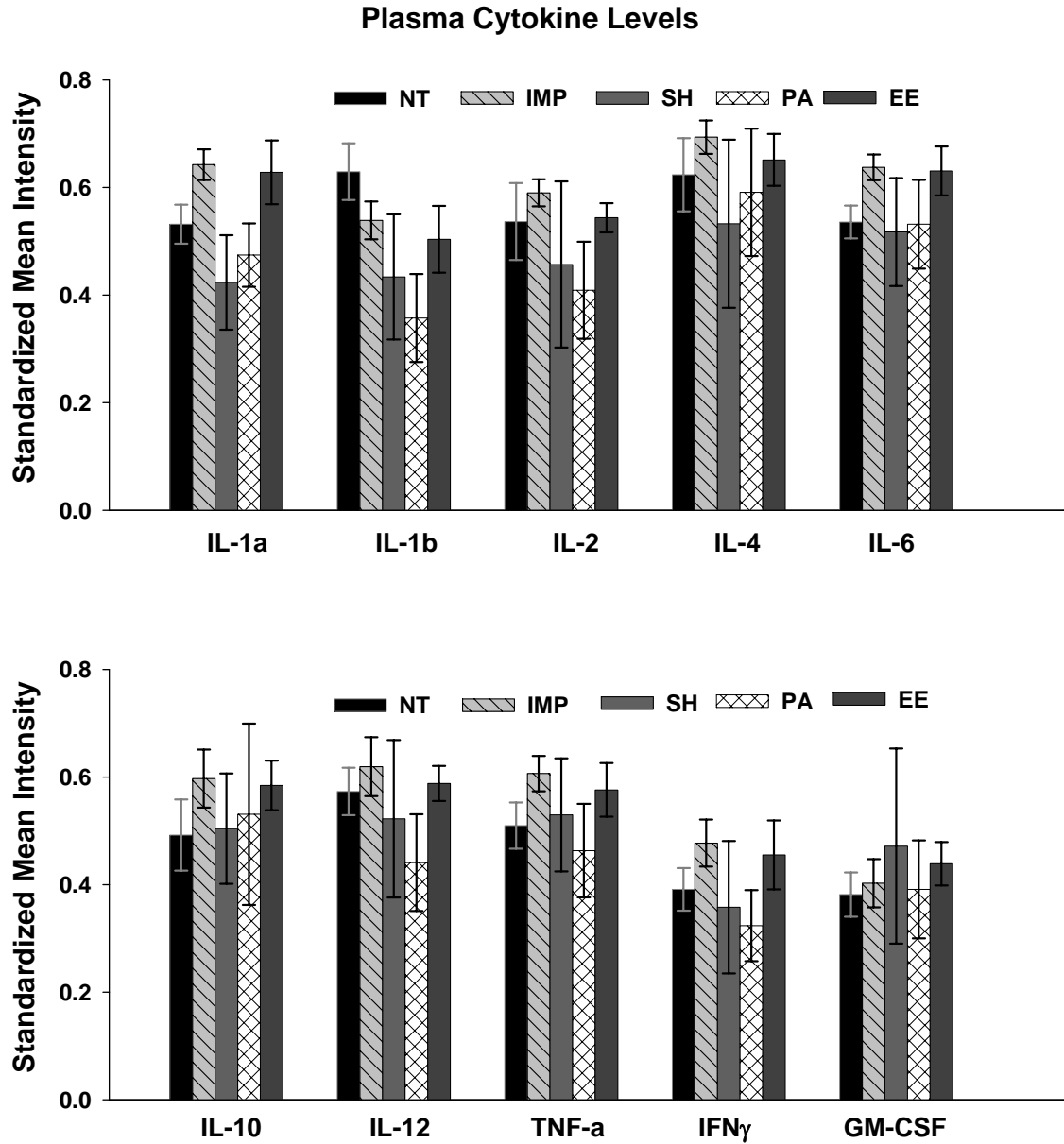
Housing and transgene-dependent changes in dendritic length/branching of neurons from APP+PS1 and NT mice were analyzed by Golgi staining. For neurons in



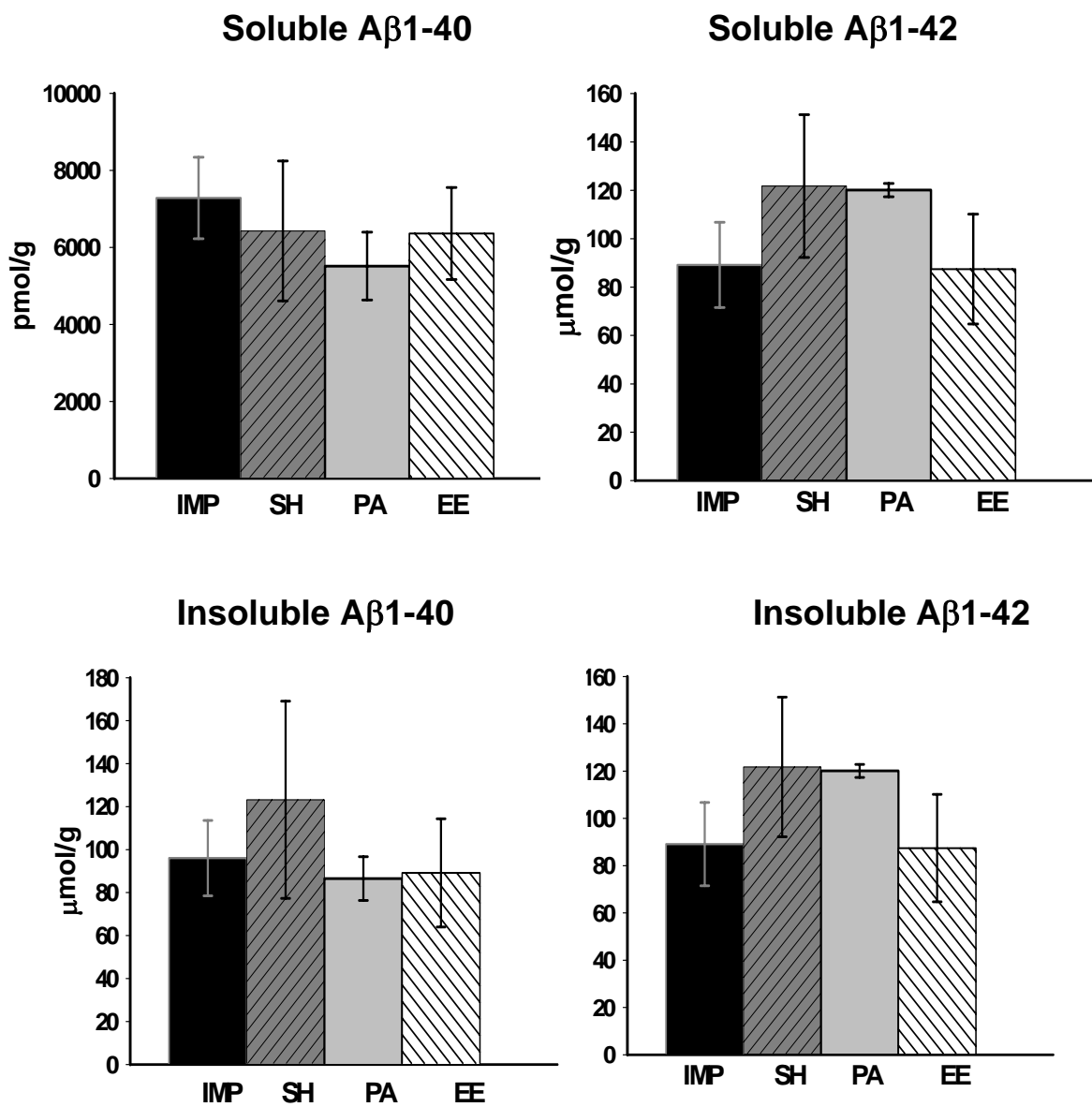
## Plasma Corticosterone Levels



**Figure 9. Plasma Corticosterone levels by A) treatment (housing), B) genotype, and C) gender.** No treatment or transgenic differences were observed for corticosterone levels. In contrast, male mice had significantly lower levels of corticosterone than female mice. \*= significantly lower levels of corticosterone ( $p < 0.01$ ). Abbreviations: as in Fig. 2



**Figure 10. Standardized mean signal intensities for 10 plasma cytokines in APP+PS1 and NT mice.** No group (housing) differences were observed for any cytokine measured. Abbreviations: as in Fig. 2



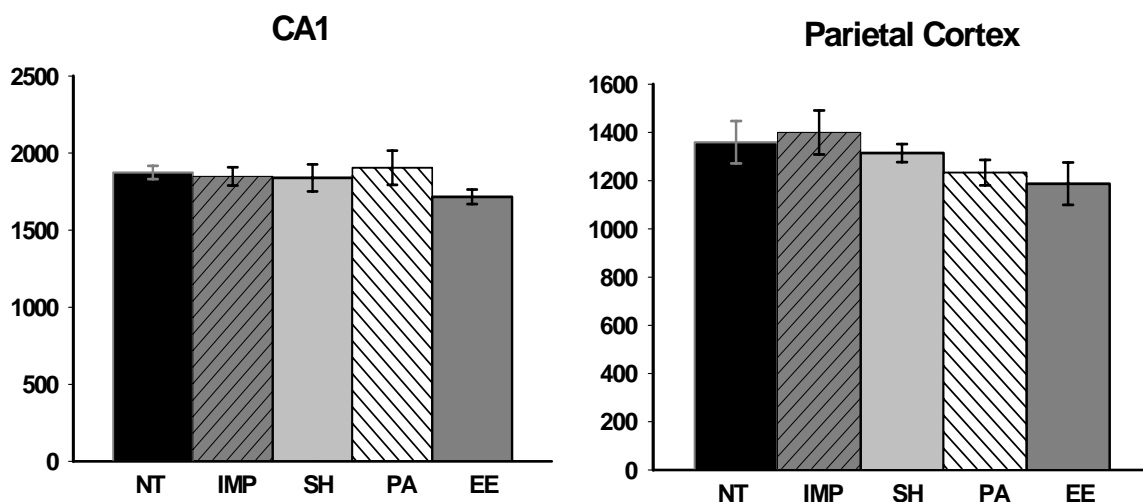
**Figure 11. Quantification of Soluble Aβ (1-40 and 1-42) and Insoluble Aβ (1-40 and 1-42) in APP+PS1 mice within different housing environments.** No group differences between housing groups were noted for either soluble or insoluble 1-40 or 1-42 species. Abbreviations: as in Fig. 2

both the hippocampal CA 1 region and overlying parietal cortex, there were no differences in total dendritic length or dendritic branching between NT and transgenic SH control groups (Fig. 12). Thus, APP+PS1 control mice had a dendritic arbor similar to NT mice. There also was no effect of housing on total dendritic length of neurons in the CA1 region or parietal cortex (Fig. 12, upper), as well as for dendritic branching in the CA1 region (Fig. 12, lower). Although there also was generally no effect of housing on dendritic branching in the parietal cortex, EE mice did have significantly less dendritic branching of neurons in parietal cortex compared to both NT and IMP groups (Fig. 12, lower).

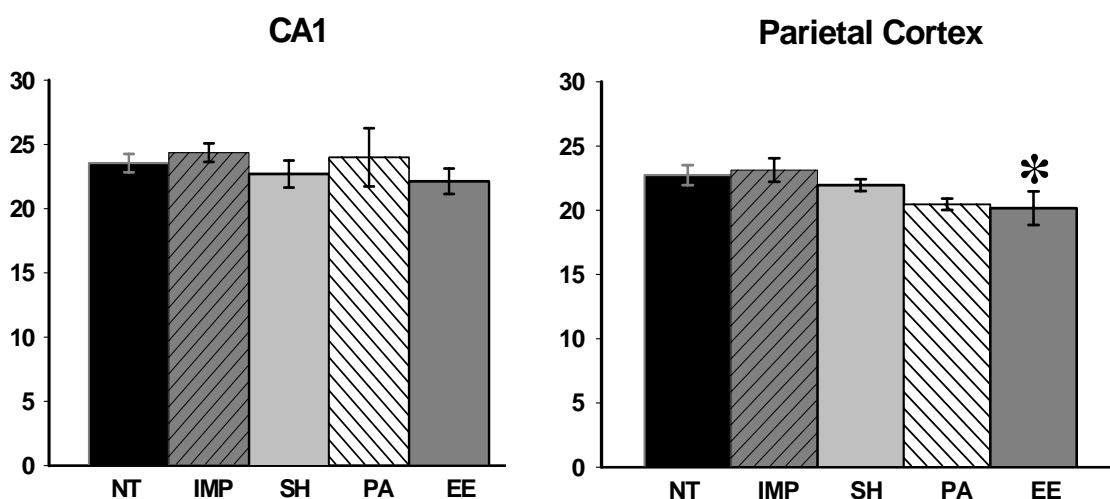
#### ***Factor Analysis/ Discriminant Function Analysis***

FA of behavioral measures with and without neuropathology/neurochemistry measures was performed to determine the underlying relationships between behavioral tasks and pathology (Table 1). Including all NT and Tg+ mice, FA involving all 15 behavioral measures resulted in 12 of those measures loading on four principle factors. Collectively, these four factors accounted for over 65% of the total variance. A measure was considered “significant” for loading on a factor if its component loading value exceeded 0.600 for that factor. All measures for RAWM and platform recognition loaded heavily under factor 1, which provided more variance (32.5%) than any of the other three factors. An activity measure, Y-maze entries, loaded under Factor 2 along with circular platform final latency. The remaining measures (two circular platform measures, Morris water maze retention, and Y-maze alternations) were distributed between the remaining two factors (Table 1).

## Total Branching Length ( $\mu\text{m}$ )



## Total Branching Points



**Figure 12. Housing and transgene-dependent changes in dendritic length/branching of neurons from APP+PS1 and NT mice.** No group differences were observed for either the hippocampal CA 1 region and overlying parietal cortex for dendritic length or dendritic branching between NT and transgenic SH control groups. EE mice did have significantly less dendritic branching of neurons in parietal cortex compared to both NT and IMP groups. \*= significantly less dendritic branching vs. NT and IMP ( $p < 0.05$ ). Abbreviations: as in Fig. 2

**Table 1.** Factor loadings of behavioral measures, with and without pathologic measures<sup>a</sup>

Factor	Behavioral Measures (Both Tg+ and NT)	Behavioral and Pathological Measures (Only Tg+)
1	<b>(32.55)</b> RM-T5-Fin RM-T5 RM-T4-Fin RM-T4 PR-Fin PR-Avg	<b>(29.94)</b> RM-T4 RM-T5 RM-T5-Fin WM-Ret PCTBP RM-T4-Fin CA1TBP CA1DL PR-Avg PCDL
2	<b>(15.47)</b> YM-Ent CPL-Fin	<b>(16.85)</b> YM-Ent CPE-Avg CPL-Avg INSOL-40
3	<b>(10.51)</b> No Significant Loadings	<b>(14.10)</b> YM-Alt CORT CPE-Fin
4	<b>(9.85)</b> CPE-Fin WM-Ret	<b>(13.71)</b> SOL-40 SOL-42 CPL-Fin
5	<b>(8.26)</b> CPE-Fin YM-Alt	<b>(9.51)</b> CORT
6		<b>(7.02)</b> INSOL-42

<sup>a</sup>Percent of total variance explained by a given factor is indicated in bold type within parentheses.

**SOL-40**, soluble A $\beta$  1–40; **INSOL-40**, insoluble A $\beta$  1–40; **SOL-42**, soluble A $\beta$  1–42; **CORT**, levels of corticosterone; **CA1-DL**, total dendritic length within CA1 region; **CA1-TBP**, total branching points within the CA1; **PC-DL**, total dendritic length within parietal cortex; **PC-TBP**, total branching points within parietal cortex; **YM-Ent**, Y-maze entries; **YM-Alt**, Y-Maze % alternations; **WM-Fin**, water maze latency on last day; **WM-Avg**, water maze latency over all days; **WM-Ret**, water maze % time spent in Q2 during probe trial; **CPE-Fin**, circular platform errors on last day; **CPE-Avg**, circular platform errors over all days; **CPL-Fin**, circular platform latency on last day; **CPL-Fin**, circular platform latency over all days; **PR-Fin**, platform recognition latency on final day; **PR-Avg**, platform recognition latency over all days; **RM-T4-Fin**, RAWM latency for trial 4 of final block; **RM-T5-Fin**, RAWM latency for trial 5 of final block; **RM-T4-Avg**, RAWM latency over all blocks for trial 4; **RM-T5-Avg**, RAWM latency over all blocks for trial 5.

When the 8 pathological measures (4 A $\beta$  deposition measures, 4 dendritic measures) and corticosterone levels were included in FA involving only Tg+ mice, all four measures of dendritic length/dendritic branching loaded in factor 1 (Table 1). Similar to the FA without pathologic measures, RAWM and platform recognition measures also loaded in factor 1. In addition, SWM retention also loaded on factor 1. The loading of neuro-morphologic and behavioral measures together indicates an underlying relationship between the two, which will be further elucidated in the Correlation Analysis section below. The 4 A $\beta$  deposition measures loaded either alone or in factors somewhat independent of behavioral measures. Plasma corticosterone levels loaded alone in factor 5, as well as with one circular platform measure and Y-maze alternations in factor 3. In a final FA that involved behavioral measures and plasma cytokine levels in all APP+PS1 mice, 4 factors results (data not shown). Interestingly, factor 1 loaded all 10 cytokines and both Morris maze acquisition measures, while factor 2 contained all RAWM and PR measures

DFA was utilized to determine if behavioral performance of the five housing groups (NT, IMP, SH, PA and EE) or the three main housing groups (NT, SH, and EE) could be distinguished from one another (Table 2). Two DFA methods were utilized: the “direct entry” method (which includes all behavioral measures evaluated) and the “stepwise-forward” method (which selects behavioral measures from the total group of measures based on their contribution to the variance). For both the 5 and the 3 group analyses, direct entry DFAs could not discriminate between the housing groups based on their behavioral performance. In sharp contrast, a stepwise-forward DFA could

**Table 2.** Summary of discriminant functional analyses of behavioral measures.

Measures	# of Groups	Direct Entry Method	Stepwise-forward Method	
			Significance	Measures Retained
All 15	5	N.S.	p<0.005 NT vs IMP, SH, PA	RM-T5 WM-Avg WM-Fin WM-Ret
All 15	3	N.S.	p<0.01 NT, EE vs SH	RM-T5-Fin WM-Fin
Factor 1 (6 cognitive measures)	5	N.S.	p<0.05 EE vs IMP, SH, PA	RM-T5-Fin
Factor 1 (6 cognitive measures)	3	N.S.	p= 0.07 EE vs SH	RM-T5-Fin

p- values are from Wilks's  $\lambda$ . N.S, not significant; all other abbreviations defined in Table 1.



effectively discriminate NT from IMP, SH, and PA groups ( $p < 0.005$ ), but not the NT and EE groups. Four behavioral measures (including RAWM overall T5 latency and all 3 Morris water maze measures) were retained as providing maximal discrimination. When only 3 groups were included, stepwise-forward DFA distinguished NT and EE from the SH group ( $p < 0.01$ ). Two measures (one from RAWM and one from MWM) provided maximal discrimination.

Additional DFAs were performed utilizing only behavioral measures that had loaded on factor 1 in FA (see Table 1). For all five groups or the main three groups, the direct entry method was again unsuccessful in discriminating between groups (Table 2). In contrast, stepwise-forward DFA's nicely discriminated between housing groups. With all five groups included, the stepwise-forward method separated EE mice from IMP, SH and PA groups ( $p < 0.01$ ), but could not distinguish EE from NT mice (Table 2). When stepwise-forward DFA was repeated using only the three main groups, EE and SH groups were nearly separated ( $p = 0.07$ ), but not EE and SH groups. Trial 5 latency on the final block of RAWM testing was the sole measure retained for of these stepwise-forward DFAs.

Thus, for all behavioral measures included, or with inclusion of the cognitive measures in factor 1, the four stepwise-forward DFA's were able to discriminate the comparably-performing NT and EE groups from the poorer performing IMP, SH, and PA groups.

### *Correlation Analysis*

Behavior vs. Plasma Cytokine Levels. Table 3 shows a correlation matrix between plasma levels of the 10 cytokines measured and the 15 behavior measures for all Tg+ mice collectively. A total of 18 correlations were present, 14 of which involved the three Morris water maze measures. Three correlations were evident between plasma cytokines levels and Y-maze alternation percentage. Correlations between behavior and plasma levels of both TNF- $\alpha$  and IFN $\gamma$  were most prevalent. For all 18 correlations, higher plasma cytokine levels were associated with better cognitive performance. A very similar pattern of correlations was present when all animals (NT and Tg+) were included in the correlation analysis.

Behavior vs. Plasma Corticosterone Levels. With all animals (NT and Tg+) included, no correlations were present between any of the 15 behavioral measures and plasma corticosterone levels. Similarly, for all Tg+ mice or individual Tg+ mouse housing groups, no correlations were evident.

Behavior vs. Cortical A $\beta$  Levels. Correlation analyses were performed between the 15 behavioral measures and the 4 cortical A $\beta$  measures (A $\beta$ 1-40 and A $\beta$ 1-42, both soluble and insoluble forms) for all APP+PS1 mice collectively. Only one correlation was significant: higher Y-maze alternations (better performance) was inversely correlated with soluble A $\beta$ (1-40) levels [ $r = -0.517$ ;  $p < 0.05$ ]. Even when correlation analysis was performed for each housing group separately, few and inconsistent correlations were present between behavioral measures and cortical A $\beta$  levels. These correlations involved

**Table 3. For all animals, a correlation matrix of behavioral measures and plasma cytokine levels.**

		YM-Alt	YM-E	WM-Fin	WM-OA	WM-Ret	CPE-Fin	CPE-Avg	CPL-Fin	CPL-Avg	PR-Fin	PR-OA	RM-T4-Fin	RM-T5-Fin	RM-T4-Avg	RM-T5-Avg
<b>TNF-<math>\alpha</math></b>	r	0.408	-0.143	-0.548	-0.564	0.520	0.110	-0.301	0.270	0.219	-0.404	-0.037	-0.231	-0.315	-0.356	-0.191
	p	0.583	0.583	0.015	0.012	0.047	0.652	0.210	0.263	0.368	0.087	0.116	0.342	0.189	0.134	0.434
<b>IFN-<math>\gamma</math></b>	r	0.589	-0.288	-0.469	-0.394	0.704	0.108	-0.273	0.384	0.379	-0.383	-0.345	-0.226	-0.366	-0.344	-0.247
	p	0.013	0.262	0.043	0.095	0.003	0.660	0.258	0.105	0.110	0.105	0.149	0.352	0.159	0.149	0.307
<b>IL-1<math>\alpha</math></b>	r	0.422	-0.256	-0.542	-0.521	0.460	0.165	-0.162	0.241	0.253	-0.383	-0.269	-0.274	-0.378	-0.485	-0.272
	p	0.091	0.321	0.017	0.022	0.085	0.501	0.507	0.319	0.296	0.106	0.266	0.256	0.111	0.035	0.261
<b>IL-1<math>\beta</math></b>	r	0.385	-0.008	-0.362	-0.453	0.417	-0.078	-0.423	0.216	0.096	-0.193	-0.002	-0.288	-0.414	-0.397	-0.197
	p	0.975	0.975	0.128	0.051	0.122	0.751	0.071	0.375	0.697	0.428	0.993	0.233	0.078	0.092	0.419
<b>IL-2</b>	r	-0.027	-0.082	-0.424	-0.633	0.076	0.199	-0.177	0.077	-0.029	-0.368	-0.147	-0.248	-0.352	-0.402	-0.173
	p	0.755	0.755	0.070	0.004	0.882	0.414	0.469	0.753	0.905	0.122	0.548	0.305	0.139	0.088	0.478
<b>IL-4</b>	r	0.328	-0.180	-0.404	-0.439	0.471	-0.102	-0.204	0.201	0.321	-0.395	-0.199	-0.241	-0.266	-0.414	-0.289
	p	0.489	0.489	0.086	0.060	0.076	0.678	0.402	0.409	0.180	0.094	0.413	0.319	0.271	0.078	0.230
<b>IL-6</b>	r	0.325	-0.086	-0.494	-0.561	0.416	0.044	-0.330	0.305	0.238	-0.364	-0.261	-0.111	-0.222	-0.333	-0.183
	p	0.744	0.744	0.032	0.013	0.123	0.859	0.168	0.205	0.327	0.126	0.280	0.650	0.362	0.163	0.454
<b>IL-10</b>	r	0.448	-0.106	-0.500	-0.543	0.498	-0.007	-0.198	0.385	0.349	-0.249	-0.174	-0.187	-0.312	-0.327	-0.249
	p	0.072	0.685	0.029	0.016	0.059	0.977	0.416	0.103	0.143	0.305	0.477	0.443	0.193	0.172	0.303
<b>IL-12</b>	r	0.503	-0.185	-0.421	-0.434	0.603	0.103	-0.323	0.414	0.355	-0.355	-0.272	-0.152	-0.380	-0.388	-0.336
	p	0.040	0.478	0.073	0.064	0.017	0.673	0.177	0.078	0.135	0.136	0.259	0.534	0.108	0.101	0.160
<b>GM-CSF</b>	r	0.632	-0.329	-0.273	-0.286	0.503	0.200	-0.086	0.366	0.342	-0.520	-0.421	-0.109	-0.294	-0.195	-0.273
	p	0.007	0.197	0.257	0.236	0.056	0.411	0.726	0.123	0.151	0.022	0.073	0.657	0.222	0.423	0.258

Positive correlations ( shaded boxes) were noted between many cytokines and both Y-maze and Morris water maze performance. r= Pearson product-moment correlation coefficient. p= probability. All behavioral measure abbreviations are defined in Table 1.

soluble and insoluble A $\beta$  levels to the same extent. For all APP+PS1 mice collectively or for individual housing groups, surprisingly few correlations involved RAWM measures.

*Behavior vs. Dendritic Morphology.* For all APP+PS1 and NT mice combined, correlation analysis between behavior and dendritic morphology revealed strong and consistent correlations between total dendritic length of neurons in the hippocampal CA1 region and both platform recognition and RAWM measures (Table 4). Both platform recognition final day and overall latency, as well as RAWM Trial 4 (final block and over all) and Trial 5 (final block) correlated positively with dendritic length in the CA1 region. Therefore, lesser dendritic length of CA1 neurons was associated with better performance in these tasks. Importantly, 4 of these five correlations were present when only APP+PS1 mice were analyzed, while only one was present when NT mice were analyzed separately. Thus, APP+PS1 mice were driving these correlations involving dendritic morphology in the hippocampal CA1 region. No correlations were evident between behavior and dendritic morphology in the parietal cortex (Table 4).

Brain A $\beta$  Levels vs. Dendritic Morphology. For all Tg+ mice collectively, there were no correlations present between brain A $\beta$  levels and dendritic length/branching in either hippocampus or parietal cortex.

**Table 4. For all animals, a correlation matrix of behavioral measures and dendritic length/branching.**

		YM-Alt	YM-E	WM-Fin	WM-OA	WM-Ret	CPE-Fin	CPE-Avg	CPL-Fin	CPL-Avg	PR-Fin	PR-OA	RM-T4-Fin	RM-T5-Fin	RM-T4-Avg	RM-T5-Avg
<b>CA1-DL</b>	r	0.059	0.015	0.099	0.243	-0.157	0.217	0.008	0.122	0.246	0.448	0.475	0.395	0.361	0.362	0.317
	p	0.771	0.942	0.603	0.187	0.445	0.24	0.965	0.513	0.183	0.012	0.007	0.028	0.046	0.045	0.083
<b>CA1-TBP</b>	r	-0.102	0.167	-0.096	-0.206	0.001	0.233	-0.073	-0.012	-0.235	0.032	0.123	0.063	-0.004	-0.016	-0.022
	p	0.614	0.406	0.613	0.266	0.997	0.206	0.695	0.951	0.202	0.865	0.51	0.737	0.982	0.932	0.908
<b>CP-DL</b>	r	-0.237	-0.263	0.059	0.062	0.111	0.256	0.017	0.361	0.246	0.064	0.075	0.011	0.199	0.059	0.083
	p	0.243	0.195	0.761	0.743	0.598	0.172	0.929	0.050	0.190	0.735	0.695	0.953	0.293	0.757	0.663
<b>CP-TBP</b>	r	-0.230	-0.168	0.027	0.111	0.162	0.294	-0.157	0.321	0.172	0.141	0.104	-0.011	0.154	-0.092	-0.044
	p	0.259	0.411	0.891	0.56	0.439	0.115	0.408	0.084	0.364	0.457	0.586	0.953	0.416	0.629	0.816

Positive correlations (bold font in shaded boxes) were noted between dendritic length of neurons in the CA1 region and both platform recognition and RAWM measures.  $r$ = Pearson product-moment correlation coefficient.  $p$ = probability. Abbreviations: CA1-DL, total dendritic length within CA1 region; CA1-TBP, total branching points within the CA1; PC-DL, total dendritic length within parietal cortex; PC-TBP, total branching points within parietal cortex; All behavioral measure abbreviations are defined in Table 1.

## **Discussion**

### *General Summary*

The present study utilized an elaborate cognitive-based behavioral battery and multivariate statistical analysis to investigate the protective effects of “complete” environment enrichment (EE) versus several of its components (physical activity, social interactions) in AD transgenic mice. Our results show that “complete” EE (physical, social, and cognitive activities) entirely protected AD transgenic mice from cognitive impairment in tasks representing different cognitive domains – working memory, reference learning, and search/recognition. In strong contrast, transgenic (Tg+) mice reared in environments that included physical activity and social interaction, or only the addition of social interaction, were not protected from cognitive impairment in adulthood. Noteworthy is that there were never any differences between IMP and SH transgenic groups in any task, indicating that individually-housed mice are not at a disadvantage for cognitive performance and that either IMP OR SH housing is a suitable Tg control. Through use of discriminant function analysis to determine if housing groups could be distinguished from one another based on multiple behavioral measures, EE and/or NT mice were consistently discriminated from the poorer performing other housing groups. Thus, the importance of “complete” EE as the protective paradigm is again underscored. Importantly, EE mice were protected from cognitive impairment through 8.5 months of age, suggesting its potential to prevent or at least delay onset of AD.

The cognitive benefits observed in EE-housed APP+PS1 mutant mice occurred without significant changes in cortical A $\beta$  levels, plasma cytokine levels, or plasma corticosterone levels, suggesting involvement of mechanisms independent of these endpoints. However, the EE-housed mice did have decreased dendritic length of neurons in the parietal cortex (but not hippocampus), suggesting that some extent of dendritic “pruning” may be involved in the cognitive benefits observed. Despite the lack of robust changes in pathologic measures, correlation analysis offered possible underlining mechanisms involved in superior cognitive performance. Although the lack of correlations between behavioral measures and cortical A $\beta$  measures in APP+PS1 mice offers additional evidence of an A $\beta$ -independent mechanism involved in EE’s protective effects, it is important to note that only anterior cortex was analysed. It is possible that measurement of A $\beta$  levels in hippocampal tissue would have elucidated EE-associated changes and more numerous correlations with cognitive performance. As well, plasma cytokine levels and hippocampal dendritic length constantly correlated with cognitive measures, suggesting their involvement in underlying mechanisms of cognitive performance. In factor analysis, moreover, all four dendritic length/branching measures loaded in factor 1, along side key cognitive measures. This finding emphasizes dendritic morphology’s underlying involvement in cognitive performance.

A large body of literature in epidemiological research suggests enriching life experiences (including education, occupation, physical activity and social interactions) may provide protection against dementia later in life (Scarmeas et al., 2001; Friedland et al. 2001; Verghese et al. 2003.) Many such retrospective studies suggest cognitive protection from an enriched life style. In addition, cognitive benefit, as well as a plethora

of neurochemical/neurohistologic changes have also been noted in wild type rodents exposed to an enriched environment (van Praag et al., 2000). Both young adult and aged rodents show cognitive benefit following several months of environment enrichment (Kemperman et al., 1997; 1998; 2002; Teather et al., 2002; Frick and Fernandez, 2003). Morris water maze learning has been the task most used to demonstrate improved cognitive performance in enriched mice and rats, although delayed alternation, visual discrimination, and food preference tasks have also been utilized (Winocur and Greenwood, 1999; Teather et al. 2002; Need et al. 2003). As for neurochemical/neurohistologic changes, neurogenesis within the hippocampal dentate gyrus is increased by environmental enrichment in both young adult and aged mice (Kempermann et al., 1997; 1998; 2002; Van Praag et al., 1999). Additionally, there is evidence that synaptogenesis occurs following enrichment in rodents, as evidence by studies showing 1) greater synaptophysin levels in hippocampus and neocortex (Frick and Fernandez, 2003), 2) an increased number of Golgi-stained dendritic branches and dendritic spines in neocortex/neostriatum (Comery et al., 1995; Turner et al., 2003), and 3) greater length of Golgi-stained dendrites in hippocampus (Faherty et al., 2003). In addition, brain levels of NGF, BDNF, NT-3, and GDNF have been shown to be increased by environmental enrichment in rodents (Young et al., 1999; Ickes et al., 2000; Johnson et al., 2003). Moreover, gene microarray analysis indicates that genes involving synaptogenesis, NMDA receptor function, and neuronal growth are all up-regulated in the brain following environmental enrichment (Rampon et al., 2000). It has been proposed that the interaction between cognitive, social, and physical activities in the “complete” enriched environment may be essential for cognitive benefit as well as the



neurochemical/neurohistologic changes and that no single one of these activities alone leads to these beneficial alterations (van Praag et al., 2000). However, no direct evidence for this premise has appeared in the scientific literature.

In our initial report, we found that complete EE (e.g., physical, social, and cognitive activity combined) improves cognition in *aged* Alzheimer's transgenic (Tg2576) mice despite stable beta-amyloid deposition (Arendash et al. 2004), suggesting a therapeutic value of cognitive stimulation for AD patients that is independent of A $\beta$  deposition. In our more recent submitted work (Costa, 2005), we have shown extensive cognitive *protection* from “complete” EE in another APP mouse model of AD. This improvement was observed without changes in A $\beta$  deposition in non-behaviorally-tested PDAPP+PS1 transgenic mice; however, extensive changes in gene expression were evident. Specifically, while PDAPP+PS1 mice exposed to EE alone showed no significant decreases in A $\beta$ , mice given *both* EE and behavioral testing showed a 50% reduction in brain A $\beta$ . Microarray analysis using hippocampal tissue revealed large EE-induced changes in the expression of genes/proteins related to memory, neuroprotection, and A $\beta$  sequestration (Costa, 2005).

The robust cognitive protection provided by “complete” EE calls into question if the components of EE (physical, social, and cognitive activity) contribute equally to the behavioral benefits observed through such an “enriched” lifestyle. Recent epidemiologic/retrospective studies have tried to separate these components of enrichment and have reported that enhanced physical activity (Friedland et al., 2001; Churchill et al., 2002), and social activity (Wang et al., 2002) are associated with decreased incidence of AD. This has led to the conclusion that physical or social activity

alone can provide substantive protection against AD. However, in the present closely controlled animal model study, no such protection of physical activity or social activity alone was observed in APP mutant transgenic mice. Retrospective human data can be challenging to decipher and/or misleading, due to the many confounding variables inherently present and the inaccuracies associated with recall of life experiences over decades. Indeed, the complete separation of physical or social activity in either a retrospective or longitudinal prospective human study would be impossible to accomplish in that varying degrees of all three components are always present. For example, those individuals that lead a lifestyle involving high physical activity or high social activity may also lead cognitively stimulating lives. In any event, the present work shows the first evidence in an AD transgenic model that “complete” EE is needed to provide cognitive protection against AD, while the physical and social activity components of EE do not alone lead to protection. Thus, it appears that cognitive activity is central to the protection observed.

Our results are in contrast to several animal studies that investigated effects of physical activity and social activity on cognitive function. In both wild type and AD transgenic mice there is literature that suggests physical activity alone can result in cognitive benefit and/or neurochemical/neurohistologic changes. Physical activity has been shown to enhance genes involved in brain health (metabolic, anti-aging, immunity genes) and plasticity (neurotrophic factor trafficking and vesicle recycling) (Cotman & Berchtold, 2002). Increase BDNF levels (Johnson & Mitchell, 2003), and enhance neurogenesis (van Praag et al., 1999; Kitamura et al, 2003; Ehninger & Kempermann, 2003) were also observed in rodents through increased physical activity. Several studies

indicate that physical activity alone can improve Morris water maze performance (Fordyce and Farrar, 1991; Fordyce et al., 1993). Recent work, directly relevant to the current study involved APP mutant mice and found that physical activity was associated with decreases in brain A $\beta$  deposition (?) (Lazarov et al., 2005; Adlard et al., 2005). In both studies, however, the extent of wheel running (physical activity) associated with reduced A $\beta$  and/or cognitive benefit was at the level of a marathon runner, a level of physical activity rarely attainable in humans. The contribution of social activity to EE benefits has not been investigated in controlled studies involving wild-type rodents, much less in transgenic mice. Through its step-wise design of housing conditions, the present study is the first to dissect out the contribution of each of EE's 3 components to the protection afforded against cognitive impairment in AD transgenic mice.

### *Behavioral Measures*

Our past work has shown the RAWM task to be an extremely sensitive test of working memory (Arendash et al., 2001a; Austin et al., 2003; Nilsson et al., 2004; Jensen et al., 2005), one of the earliest and most prominent symptoms of AD. In our laboratory, APP+PS1 mice are impaired in this task by 5-6 months of age (Jensen et al., 2005), but not at 3 months of age (unpublished observations). Consistent with that premise, Tg+ SH control mice in the present study showed impaired RAWM working memory at 8.5 months of age, most notable in the last block and in overall performance across all blocks. EE-housed Tg+ mice, by contrast, were able to reduce their latencies to levels equivalent to NT mice for both working memory Trials 4 and 5 on the final block, as well as over all blocks. This protection is consistent with our earlier work showing protection

of RAWM performance in APP transgenic mice through “complete” EE housing (Costa et al., 2005). Importantly, this working memory protection was not observed in IMP, PA or SH groups. In fact, complete separation between EE and NT groups versus all other groups was observed on Trial 5 of the final block. Our complete EE results are consistent with findings from Jankowsky et al. (2005), who reported superior RAWM performance of Tg+ mice reared in a complete EE versus SH Tg+ mice. Also noteworthy in the present study is that Tg+ mice living in an impoverished environment appear to not be at risk for enhanced cognitive impairment during aging, as shown by their identical RAWM performance to the SH transgenic group. Our RAWM results suggest that “complete” EE may have significant protective potential against the working-memory impairment associated with AD.

Despite the lack of a transgenic effect on Morris maze acquisition when Tg+ SH control mice were compared to NT mice, “complete” EE mice showed superior performance when compared to other transgenic housing groups on both individual block data and overall acquisition. The absence of a transgenic effect may be attributed to the relatively poor performance of NT mice, rather than the superior performance of the transgenic SH mice. We have previously reported acquisitional impairment in the Morris maze as early as 4.5-6 months of age (Jensen et al., 2005). In contrast, the transgenic SH mice in the present study were not significantly different from NT mice at 8.5 months of age. During the last several blocks of testing, escape latencies of NT mice reported in Jensen et al. (2005) were around 15 seconds versus around 40 seconds for NT mice in the current study. These differences could be the result of different strains between colonies used or different F generations between the two colonies. In any event,

EE mice were able to improve their learning acquisition at a faster rate than other groups in the present study. A congruent effect was observed in recent work by Jankowsky et al. (2005), whom showed significantly decreased escape distances in APP+PS1 mice reared in “complete” EE when compared to Tg+ control mice.

In Morris maze reference memory retention, a transgenic effect was absent in all three measures (platform quadrant preference, annulus crossings, and quadrant preference). As well, no group differences were present for zone 2 preference or annulus crossings. NT and SH mice showed partial quadrant preference for the goal quadrant while the other groups did not. Although there was no effect of transgenicity in Morris maze retention, complete EE did not provide benefit to transgenic mice relevant to the other transgenic housing groups (IMP, SH, PA). Similarity, Jankowsky et al. (2005) found that APP+PS1 mice did not benefit from EE during memory retention testing compared to transgenic SH controls.

By performing the platform recognition task after the Morris maze task, mice must switch from the spatial strategy of the Morris maze to a recognition/identification strategy. (Arendash et al. 2001a; Austin et al., 2003; Jensen et al. 2005). Over 4 days of testing, the NT mice and EE mice quickly reduced their escape latencies. In contrast, the IMP, SH, and PA transgenic groups lagged in their effort to convert from a spatial to a recognition strategy. Despite all groups being identical by day 4, the strategy switching ability of NT and EE Tg+ mice was clearly superior to the other groups. In this regard, overall latencies revealed IMP, SH and PA mice to be significantly impaired in comparison to NT mice; however, EE mice were identical to the NT group. It should be noted that EE protected against an “age-related” impairment in platform recognition since

our prior work showed that APP+PS1 transgenic mice are not impaired in this task at 5-6 months of age (Jensen et al., 2005).

The above platform recognition results showing complete strategy-switching protection in EE mice is consistent with our earlier EE study, wherein a different APP transgenic line was used (Costa et al. (2005). Results from both studies again underscore the unprotective nature of the individual enrichment components. Similar to RAWM findings, deleterious cognitive effects were not observed in transgenic mice living in an impoverished environment (IMP) versus SH mice, again showing that either housing condition is an appropriate control for intervention strategies. Widely documented in AD patients is impairment in a variety of attention-related tasks, resulting in an inability to “search” or to shift attention from one item to another (Tales et al., 2004). Our platform recognition results involving such strategy switching could thus be of considerable clinical significance. EE could offer significant prophylactic value for search/identification if introduced early and if all components of enrichment (social, physical and cognitive) are included.

SH transgenic control mice were not impaired in either Y-maze spontaneous alternation or circular platform tasks, negating any potential for EE or its components to provide protection. This work is consistent with our previous findings, which showed limited sensitivity of APP+PS1 transgenic mice to Y-maze basic mnemonic function and circular platform reference memory impairment (Arendash et al., 2001a; Jensen et al., 2005). As anticipated, measures from Y-maze and circular platform did not load with measures in the prime cognitive factor in factor analysis. This finding offers further evidence that measures from these two tasks do not relate well to, and are measuring

something different from, the three water-based tasks (Morris maze, platform recognition, and RAWM).

### *Neuropathologic Analysis*

A $\beta$  Neurochemistry. The robust cognitive benefits observed from environmental enrichment occurred without a significant decrease in soluble or insoluble levels of A $\beta$  in anterior cortex; indeed, there were no differences in cortical A $\beta$  deposition among any of the 4 transgenic housing groups. Thus it appears that cognitive benefits from EE occur in the presence of high levels of brain A $\beta$ . It should be noted, however, that technical problems prevented analysis of A $\beta$  levels in the hippocampus, wherein EE-induced changes may have been more likely to occur. Past literature regarding the effects of protection-based EE on brain A $\beta$  levels are inconsistent. Early work by Jankowsky et al. (2003) showed that EE exacerbated A $\beta$  levels in APP+PS1 mice. This was observed in the hippocampus and/or cortex as measured by histological staining, and A $\beta$  ELISA. These results are consistent with recent work from the same laboratory showing improved cognitive performance with EE is associated with increases in hippocampal A $\beta$  levels, as measured by histologic staining and A $\beta$  ELISA (Jankowsky et al., 2005). By contrast, Lazarov et al. (2005) showed significant decreases in cortex and hippocampal A $\beta$  deposition after housing APP+PS1 mice in EE. Additionally, decreases in brain A $\beta$  levels, measured by A $\beta$  ELISA, were observed in EE mice. In our submitted work (Costa et al.; 2005), no change in A $\beta$  deposition resulted from housing PDAPP+PS1 mice in EE; however, when EE was combined with extensive behavioral

testing (a form of EE itself), decreases in A $\beta$  deposition occurred in both hippocampus and cortex. These differences in findings between the three laboratories may be the result of different strain backgrounds and/or different EE protocols. Interestingly, Lazarov et al. (2005) found that enriched mice exhibiting high wheel running activity had significantly less brain A $\beta$  deposition than those with low activity. Several events, other than wheel running-induced decreases in brain A $\beta$ , may have led to these findings. Firstly, mice were not randomly selected to test for physical activity, leading to the question if animals with less A $\beta$  deposition were simply more active than those with higher A $\beta$  burdens. A second possible explanation is that the wheel to mouse ratio was 1 to 2, leading to establishment of a hierarchy for wheel use. Mice that gained more access to wheels may have been the “bully” animals while those that did not have wheel access were lower in the hierarchy, translating into more stressful living. Despite the incongruent findings in APP transgenic models regarding EE’s effects on brain A $\beta$ , the current work and past studies (Costa et al., 2005; Jankowsky, et al., 2005) show cognitive protection despite steady state and even increases in A $\beta$ , suggesting possible A $\beta$ -independent mechanisms contributing to the EE effect.

Golgi Analysis of Dendritic Morphology. The finding that dendritic branching in hippocampus of wild-type mice was increased by complete EE but not physical activity alone (Faherty et al. 2003) offered a possible mechanism by which EE mice in the present study were cognitively protected, while PA mice were not. However, no group differences in dendritic branching or dendritic length were seen, both in terms of a transgenic effect or a housing effect. Thus, EE did not affect dendritic morphology in either hippocampus or parietal cortex of APP+PS1 mice. Dissimilarly, Moolman et al. (2004) have shown



APP+PS1 mice to have significantly less total dendrite area in hippocampus than NT mice. One major difference between Moolman et al. (2004) and the current study is the confounding variable of behavioral testing. Our extensive behavioral testing over 5 weeks may have standardized all groups and therefore disguised any effects on dendritic morphology due to transgenicity or housing. In our prior study (Costa et al., 2005), which used the same behavioral battery, we observed a genotype effect in that EE induced a decrease in dendritic branching in the cortex; however, no effect of complete EE was on dendritic branching was evident. It is important to note that our prior study involved a different AD transgenic model (PDAPP+PS1 mice) rather than the APP +PS1 mice used in the current study. In any event, no group differences were observed at the dendritic level in the present work. Examination of dendritic spines in hippocampus and cortex may reveal group differences among the 5 groups of the present study, and such an examination is in progress. Along this line, recent work by Leggio et al. (2005) in wild-type mice showed an EE-induced increase in dendritic spines in the parietal cortex, with cognitive benefits in both the Morris water maze and RAWM. Whether or not such dendritic changes can be induced by EE in APP transgenic lines, generating high brain levels of A $\beta$ , will be important to determine. Further work at the synaptic (spine) level may offer a mechanism by which “complete” environmental is providing cognitive protection to AD transgenic mice.

### *Blood Measurements*

Cytokine Analysis. Across all 10 plasma cytokines measured, there were no effects of housing condition or of APP+PS1 transgenicity. These results suggest that

neither housing environment nor the APP transgene induce a global, sustained change in the immune system. Thus, cognitive deficits observed in APP transgenics and the cognitive protection seen in EE mice occurred independently of a change in the systemic immune profile. These findings are consistent with work by Marashi et al. (2004) showing no significant differences in spleen cytokine levels (IL-2, IL-4, IL-10, and IFN- $\gamma$ ) of mice raised in EE. Despite the absence of significant changes in cytokine levels, strong correlations were evident between plasma cytokines and cognitive performance (see next section on correlations). For the present study, it should be noted that cytokine measurements were for plasma only, and may not be indicative of regional brain cytokine profiles. In work submitted for publication, we found no differences in brain cytokine levels between NT and APP+PS1 mice of the same age as animals in the present study, suggesting no overt immunological response in the brain to the mutant APP transgene would be expected in this study's Tg+ mice. The absence of brain cytokine differences in Tg+ mice suggests that these mice may be "immune tolerant" to the presence of human A $\beta$ . This response would be expected for any peptide produced endogenously at high levels throughout life. Future studies must be conducted to analyze brain cytokine levels following EE in Tg+ mice to determine if immunologic changes occurring in the CNS could be contributing to the cognitive protection provided by EE.

Corticosterone Levels. A stressful environment has the potential to mask or exacerbate effects independent of the variables tested. In order to control for this variable, plasma corticosterone levels were measured for the different housing groups following completion of behavioral testing. No group differences were observed, based on either housing or transgenicity. Thus, it is unlikely that different levels of stress were

associated with the four housing environments, therefore eliminating the possibility that stress may have contributed to the differences in cognitive performance between housing groups. In contrast to our findings, Belz et al. (2003) has shown that wild-type mice housed in EE had increased adrenal weight and increased blood stress hormones (such as corticosterone and ACTH), suggesting that EE can be a stressful environment in wild-type mice. Despite no housing differences in corticosterone levels in the present study, gender differences were observed, with male mice having almost 3-fold lower plasma corticosterone levels compared to females. This is consistent with work showing female wild-type mice have significantly higher basal levels of corticosterone than male mice (Finn et al., 2004, Galea et al., 1997).

#### *Correlations Analyses between Neuropathology, Blood Measures and Behavior*

The cognitive benefits of 6 months of “complete” EE occurred in APP transgenic mice without significant decreases in soluble or insoluble levels of brain A $\beta$ , changes in blood cytokines, or changes in dendritic morphology. However, correlation analysis between 15 behavioral measures and pathologic/blood measures revealed some consistent associations, possibly linking the later to the former measures.

Surprisingly, correlation analyses performed between cognitive measures and brain levels of both soluble and insoluble A $\beta$  revealed limited and inconsistent correlations. This is in sharp contrast to the strong correlations we have generally found between cognition and various brain A $\beta$  measures in our earlier work (Arendash et al., 2001a; Gordon et al., 2001; Leighty et al., 2004). This apparent discrepancy may be explained by the measurement of A $\beta$  levels by ELISA in the current study, while our

prior studies involved histologic staining for A $\beta$  burdens. As well, the anterior cortex was analyzed in the present study (due to unavailability of the hippocampus), while hippocampus was most often utilized in our prior studies. Since the hippocampus is critical to working memory- and spatial learning-based tasks such as the RAWM and Morris maze tasks, respectively, closer associations between hippocampal A $\beta$  levels and cognitive performance would have been anticipated. Finally, the various housing conditions themselves (particularly EE) may have neutralized the association between brain A $\beta$  and cognition. The lack of association between A $\beta$  and behavior in the present study adds evidence to the findings of Jankowsky et al. (2005) wherein EE-induced cognitive protection occurred despite increases in brain A $\beta$ , suggesting that EE mitigates cognitive impairment in the presence of amyloid burden.

In contrast to the absence of correlations between A $\beta$  and cognition, consistent correlations were observed for both dendritic morphology and cytokines levels versus cognition. Higher plasma cytokine levels correlated with better cognitive performance. These correlations were limited to Morris water maze and Y-maze alternation measures. Interestingly these tasks were not among those which highlighted the beneficial effects of “complete” EE and the non-protective nature of social and physical interaction. In a prior study involving APP+PS1 mice given adoptively-transferred T cells, we also observed the same positive association between higher levels of plasma cytokines and cognitive performance (unpublished observations). Together, results from these two studies suggest that higher levels of individual cytokines may play a role, or are at least indicative of, improved cognitive performance. Regarding correlations involving dendritic morphology and cognitive performance, dendritic length in the hippocampus

CA1 region (but not parietal cortex) consistently correlated with RAWM and platform recognition measures. Decreased dendritic length was associated with better cognitive performance suggesting a “pruning back” of extraneous branching as a possible mechanism for enhanced cognitive performance. Also noteworthy is that the two strongly correlated tasks (RAWM and platform recognition) were also the tasks in which “complete” EE was most protective. Despite the lack of any significant group differences in any of the dendritic morphology measures, the presence of strong correlations between CA1 dendritic length and key cognitive tasks suggests hippocampal dendritic morphology as an underlying determinant of cognitive performance. It will be important to follow these findings with analysis of dendritic spines from the same animals. Such a study is now ongoing.

#### *Multimetric Analyses (FA and DFA)*

A benefit to utilizing an extensive behavioral battery which tests multiple cognitive domains is the ability to employ higher level statistical analysis to characterize and distinguish between genotype or treatment groups. Using this novel approach in the present study, we were able to determine underlying relationships between behavioral and pathologic measures (via factor analysis; FA), as well as to compare experimental groups across multiple behavioral tasks/measures (via discriminant function analysis; DFA). In past studies we have utilized these advanced statistics to discriminate between the behavioral performance of: 1) mutant APP transgenic lines that vary in their extent of A $\beta$  deposition (Leighty et al., 2004; Nilsson et al., 2004), 2) mutant APPsw vs. mutant Tau transgenic lines (Arendash et al. 2004), 3) A $\beta$  vaccinated mice vs. controls (Jensen et

al. 2005), and 4) environmentally enriched vs. non-enriched APPsw transgenic mice (Arendash et al., 2004). In the present study, we used these sensitive multi-metric statistics to try to distinguish between “complete” enrichment and several of its components.

Factor analysis performed on both behavioral measures alone revealed one primary factor (factor 1) which was comprised of all measures from the RAWM and platform recognition tasks. Other behavioral measures loaded under three lower factors. Noteworthy is that factor 1 was comprised of measures from the two tasks wherein EE’s effects on cognitive performance were most evident. This inclusion of all RAWM and platform recognition measures into the primary factor is very consistent with our prior studies, in which the same loading occurred (Leighty et al., 2004; Jensen et al., 2005). When pathologic measures were included with behavioral measures in FA, all 4 dendritic length/branching measures loaded in factor 1 along with all RAWM and platform recognition measures. This suggests an intimate relationship between dendritic morphology and cognitive performance. These findings underscore the need for further analysis of dendritic structure in the same brains, specifically dendritic spine analysis (which is in progress).

In contrast to the loading of dendritic morphology measures in factor 1 along side key cognitive measures, brain A $\beta$  levels and blood levels of both corticosterone and cytokines loaded in other factors. It is not surprising that A $\beta$  levels did not load in factor 1 since there were few correlations between cognitive performance and brain A $\beta$  levels. In our prior studies wherein strong correlations were present between cognition and brain A $\beta$  deposition, A $\beta$  deposition measures loaded in factor 1 along with key cognitive

measures (Leighty et al., 2004; Jensen et al., 2005). Interestingly, in the FA involving behavioral measures and plasma cytokine levels, factor 1 contained all 10 cytokines along side both Morris maze acquisition measures. In that acquisitional performance in Morris maze was highly correlated with plasma cytokine levels, there clearly is some underlying relationship between performance in this task and blood cytokine levels.

DFA was used to determine if behavioral performance alone could distinguish between the 5 experimental groups of this study. In addition to the DFA on all 5 groups, further DFA analysis involving 3 groups (NT, EE, SH) was performed to clarify any “complete” EE effects that may have been confounded by interactions between all 5 experimental groups. Although the “direct entry” DFA method of using all behavioral measures was largely unsuccessful in discriminating between groups, the “stepwise-forward” DFA method was consistently able to discriminate between groups in either the 5- or the 3-group analysis, irrespectively of whether all behavioral measures were included or whether only factor 1 behavioral measures were included. NT and/or EE groups were always discriminated from all other groups (IMP, SH, PA). For example, utilizing factor 1 measures with all 5 groups included, EE mice were clearly discriminated from IMP, SH, and PA transgenic groups. This was consistent with our analysis of individual measures wherein the EE mice were identical to NT mice in having excellent cognitive performance, while the IMP, SH, and PA groups were impaired in RAWM and platform recognition. The one cognitive measure consistently retained for providing this discrimination was RAWM performance in Trial 5 during the final block of testing. Thus, this measure alone provided maximal discrimination between the groups of this study, although multiple measures and several cognitive domains were

involved in the discrimination when the DFA was initiated with all 15 behavioral measures. Importantly, our stepwise-forward DFA results extend and complement traditional single-measure assessments by indicating that the beneficial effects of “complete” EE occurred across multiple cognitive measures from multiple tasks evaluated as a group. Much like the indexing of multiple behavioral measures/domains of elderly humans or AD patients (Altepetter et al., 1990, Whelihan et al., 1997), DFA of AD transgenic models provides a valuable tool in assessing cognitive performance across various cognitive domains.

In summary, this study clearly shows the effectiveness of “complete” environmental enrichment in protecting cognition AD transgenic mice. In contrast, the physical and social components of enrichment alone did not provide protection, suggesting it is the cognitive or the additive effect of all components that leads to the protective effects of EE. Despite robust behavioral changes, limited changes were observed in the pathologic variables measured. Taken together, A $\beta$  quantification, FA and correlations strongly suggest an A $\beta$  independent mechanism in the current study. However, morphological changes do correlate with behavior and offer a potential underlying mechanism. Although the exact mechanisms whereby “complete enrichment” offers protection while enrichment components do not are unclear, other possible mechanism are under consideration. Firstly, changes in neuronal morphology at the dendritic spine level, resulting in enhanced cognition, have been observed in EE-raised mice (Leggio et al., 2005). This increase in synaptic material, potentially leading to enhanced synaptic transmission, may offer a mechanism by which “complete” EE mice are protected from cognitive impairment. The finding in the present study that decreased



dendritic length correlated with cognitive performance underscores the potential of a morphological dendritic pruning-based mechanism of action. A second prospective mechanism is that “complete” EE is rendering A $\beta$  irrelevant as an impairing factor through increasing neuronal health by enhancing neurotrophic factors and/or enhancing neuro-protective genes (Lazarov et al. 2005; Costa et al., 2005). This mechanism nicely fits with the findings of Jankowsky’s group, who showed that despite increases in A $\beta$  deposition, cognitive protection was present. Over all increases in neuronal health which renders neurons less susceptible to the toxic effects of A $\beta$  as well as enhanced synaptic matter leading to a reserve of synaptic transmission may be working in concert to provide the protective effects of EE. In any event the results of the current study suggest that environment may be a powerful, modifiable variable in the development of AD later in life.

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