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Effects of Hyperoxia in Alzheimer's Transgenic Mice

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Effects of Hyperoxia in Alzheimer's Transgenic Mice

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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Department of Biology
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Hyperoxia Treatment Triggers Cognitive Impairment in Alzheimer's Transgenic Mice

April A Cox

ABSTRACT

An association between major surgery in the elderly and precipitation of Alzheimer's disease (AD) has been reported. Hyperoxia (100%) oxygen is commonly administered after surgery to increase the oxygen content of blood. However, hyperoxia is a potent cerebral vasoconstrictor and generator of free radicals, as is β -amyloid ($A\beta$). This study was aimed at examining behavioral, neuropathological, and neurochemical effects of hyperoxia treatments in APP^{sw} transgenic mice (Tg⁺), which have elevated brain $A\beta$ levels by 3-4 months of age but are not yet cognitively-impaired. At 3 months of age, Tg⁺ mice were pre-tested in the radial arm water maze (RAWM) task of working memory and found to be unimpaired. At 4.5 months of age, half of the Tg⁺ mice received the first of 3 equally-spaced hyperoxia sessions (3 hrs each) given over the ensuing 3 months. The other half of the Tg⁺ mice were exposed to compressed air during these 3 sessions. RAWM testing performed immediately following the final gas session at 7.5 months of age revealed significant working memory impairment in Tg⁺ mice exposed to hyperoxia. The Tg⁺ group that was exposed to placebo treatment showed a trend towards impairment, however, was not significantly different from the non-transgenic group. Hyperoxia-induced memory impairment in Tg⁺ mice did not involve changes in brain $A\beta$ deposition, degenerative cell numbers in hippocampus, neocortical lipid peroxidation, or hippocampal levels of APP, ApoE, COX-2, or GFAP. The combination of excess $A\beta$ and hyperoxia could have induced greater oxidative stress and cerebral vasoconstriction than either one alone, resulting in a pathologic cerebral

hypoperfusion that triggered subsequent cognitive impairment. These results suggest that humans genetically pre-disposed to AD and those with increased brain A β levels have increased risk of developing cognitive impairment following hyperoxia treatment and cast doubt on the wide spread use of hyperoxia in aged individuals at risk for developing AD.

Alzheimer's Disease Background

Alzheimer's disease can be described as a "disease of the brain that results in an illness of the mind" (Martin et al., 2002). The term Alzheimer's Disease (AD), first discovered by Alois Alzheimer in 1906, is marked by severe memory loss as well as an array of both psychological and physical symptoms. The primary risk factor for developing AD is age. As humans progressively have longer life spans it is not, therefore unexpected to also see a dramatic rise in the prevalence of the disease. In the year 2000 there were approximately 4.5 million diagnosed persons with AD in the US, by the year 2050 it is estimated that there will be 13.2 million persons with AD (Herbert et al., 2003).

AD has been under study for over a century, yet, there is still a profound lack of understanding as to the exact molecular mechanisms underlying the disease. Research has led to the identification of genetic mutations responsible for the early onset form of AD. These mutations have provided a great deal of insight into some of the underlying causes of the disease as well as provide researchers with various animal models. While there are currently several different treatments designed to slow the progression of the disease, none of the current treatments have been able to provide a cure for this debilitating disease. Hopefully, with further research and a better understanding of the disease a cure will soon be in sight.

Behavioral Characterization

AD has become synonymous with the general public as a disease primarily marked by memory loss. However, the disease encompasses an array of behavioral

symptoms such as paranoia, confusion, disorientation, loss of mental competence, and difficulty in understanding language (Martin et al., 2002). The symptoms of AD are highly dynamic. Symptoms emerge and evolve at different rates and at different stages of the disease. While there is a large degree of variation in the symptoms an individual might be faced with, the disease does follow a predictable sequence of progression.

Currently, it is commonly believed that pathological changes associated with AD begin many years prior to the manifestation of clinical symptoms, this time is termed the latent period. The earliest observable behavioral symptom is an impairment in short-term (working) memory. Short-term memory is defined as the memory used to recall information that persists for only a few minutes after being exposed to the memory encoding stimuli. This early loss of memory coincides with studies that suggest the hippocampus and entorhinal cortex are the first brain areas to be affected by the neurofibrillary pathology, areas that are believed to be important in new memory formation (Braak & Braak, 1991). A patient who only suffers from short-term (working) memory impairment and who retains the ability to perform everyday tasks can be classified as being in the pre-dementia stage (Nestor et al., 2004). This stage has also been termed mild cognitive impairment (MCI). The underlying concept of MCI is that it represents the earliest perceptible stage of AD (Nestor et al., 2004). Thus, a patient with a diagnosis of MCI would be considered at high risk for the development of AD. However, currently due to a lack of homogeneity in the clinical testing and evaluation of MCI, a diagnosis does not fully correlate with an eventual development of AD. There is an obvious need for researchers to more precisely define this stage of AD as it has been

suggested that the MCI stage might be an optimal time to intervene with drug treatment (Voisin et al., 2003).

The time course of progression of MCI to AD is extremely variable. As mentioned earlier the first symptom to appear is a deficit in short-term memory that can begin at least 5 years prior to disease diagnosis. Deficits in semantic memory (ability to recognize words, faces, and objects), attentional processes, and executive functions slowly begin to emerge in a patient who is in the pre-diagnosis stage (Nestor et al., 2004). When these deficits become widespread and severe enough to interfere with a patient's normal life, and the criteria for a diagnosis of AD are met as set by various groups (for example, American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders), a patient would be then officially diagnosed with early stage AD (Nestor, 2004). Neuropsychiatric symptoms also begin to emerge during the early and moderate stages of AD. The most common symptoms include depression, apathy, and irritability (Hart et al., 2003). Also, but less frequently patients experience hallucinations, elation, and disinhibition (Hart et al., 2003). A diagnosis of moderate stage of AD is normally given to a patient whose symptoms have progressively worsened and who also starts to suffer from language deficits and a long term memory loss. The time frame of progression varies among individuals with some patients progressing from early to end stage AD in only a few years and others living with the diagnosis for 10-20 years. The late or advanced stage of AD is marked by a very severe loss of memory and cognitive functions, and a severe loss of motor skills. In end stage AD a patient would be in a semi-vegetative bedridden state, death would most likely result from illness.

Pathology

The complexity of the underlying pathological mechanisms causing AD can be attested to the fact that after nearly 100 years of research into the disease there still is not a complete understanding. Currently, the pathological hallmarks of the disease include: neuritic plaques, neurofibrillary tangles (NFT's), loss of cholinergic function, widespread neuronal loss and synaptic modifications in the cortex, hippocampus and other brain areas responsible for memory and cognition (Parihar et al., 2004). There is much debate over the exact relationship among these pathologies. It has been firmly established that neuritic plaque formation precedes neurofibrillary formation in cortical areas (Vickers et al., 2000). Also, it has been established that pathologies begin developing as far as twenty years before the onset of clinical symptoms during the latent period.

One of the first changes the brain would undergo during the latent period is a reduction in viable acetylcholine receptors (Kihara et al., 2004). More specifically, the M1, M2 and M4 subtypes of muscarinic acetylcholine receptors have all been reported to be decreased in AD (Volpicelli et al., 2004). The functioning of the M1 receptors subtype is thought to be down-regulated in AD and treatment with M1 receptor agonists has shown positive results (Fisher et al., 2000) The loss of activity in the cholinergic system is a target point for many pharmaceutical therapies. Second to a loss of acetylcholine receptors, the brain begins to develop and accumulate β -amyloid ($A\beta$) plaques. The plaques consist of extracellular deposits of $A\beta$ arranged in a star-shaped mass with a compact core. The $A\beta$ that comprises the plaque is a byproduct that results from cleavage events to the larger parent protein; amyloid precursor protein (APP).

APP is a transmembrane protein that is found in the endoplasmic reticulum and is posttranslationally modified through the secretory pathway to yield several different

peptides (Selkoe, 2001). The major secreted derivative of APP is α -APP_s a result of cleavage by α -secretase 12 amino acids away from the NH₂ terminal (Selkoe, 2001). There have been a number of suggestions made as to the exact functioning of the α -APP_s in a normal physiological state. Researchers have proposed that the α -APP_s peptide may act as an autocrine factor or as a neurotrophic factor. The deletion of the APP gene does not confer early mortality or high morbidity suggesting that other homologous proteins may assume whatever role the α -APP_s plays (Selkoe, 2001).

In an alternate toxic form of APP processing, the toxic A β protein can be formed. First, the enzyme β -secretase cleaves the APP protein at the N-terminus amino acid 671. Then a second enzyme, γ -secretase cuts the remaining larger fragment of the APP protein at amino acid 711 or 713 to produce the A β ₄₀ or A β ₄₂ fragment (Tamagno et al., 2002). The A β fragments then begin to aggregate together to form first into dimers and oligomeric isoforms. Then as aggregation continues the fragments form diffuse plaques and then finally compact plaques. Activated glial cells such as microglia and astrocytes will surround the amyloid plaques in response to their formation and produce toxins such as inflammatory cytokines that lead to further neurodegeneration (Mattson et al., 2004). There is also some evidence that activated microglia can clear A β deposits (Jantzen et al., 2002). A β ₁₋₄₀ is the major species produced, however, the A β ₁₋₄₂ is less soluble and aggregates more readily (Veurink et al., 2003). These plaques are found most notably in the hippocampus and the association cortices. Another key protein, apolipoprotein E (apoE) plays a vital role in the formation of neuritic plaques. While the exact mechanism is still unknown, studies done with transgenic mice have found that the presence of apoE is necessary in the formation of plaques (Marques et al., 2004). Current research is

focused on the role of proteolytic fragments in the formation of plaques. The exact time course for the formation of a neuritic plaque is unknown. However, it is widely accepted that the process occurs rather slowly.

The accumulated A β 42 in plaques then begins to release diffusible oligomers of the A β 42 and protofibrils that are thought to have adverse effects on neighboring cells (Selkoe, 2001). Dendritic loss, neuronal death, and dystrophic neurites are often found within and surrounding the plaques. This cellular damage is thought to be a result of the increased oxidative stress seen around plaques. Studies have shown that A β can induce the formation of oxygen dependent free radicals that lead to lipid and protein oxidation (Veurink et al., 2003). The formation of free radicals centered around A β plaques results in areas of increased oxidative stress.

The term oxidative stress is a general term used to describe the level of oxidative damage being caused by oxidants, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), to the surrounding cells and tissues (Emerit et al., 2004). Oxidative stress occurs when the production of oxidants exceeds the antioxidant capabilities of the cell (Maccioni et al., 2001). ROS, such as the superoxide (O_2^-) free radical are formed as normal byproducts of metabolic reactions, such as the energy generating reactions that occur in the mitochondria. Oxygen is the primary source of free radicals in aerobic organisms, and exposure to hyperoxia (>21% Oxygen) increases the production of free radicals (Reiter et al., 1998). The role that oxidative stress plays in the pathogenesis of AD has been widely explored. The AD brain has been found to over-produce ROS and NOS, both of which are implicated in cellular damage and apoptosis (Maccioni et al., 2001). Studies have found that the AD brain has increases in lipid

peroxidation, decreases in polyunsaturated fatty acids, increases in 4-hydroxynonenal (an aldehyde product of lipid peroxidation), and increases in protein and DNA oxidation; all of which are evidence of oxidative stress (Markesbery et al., 1997). Oxidative stress plays a pivotal role in disrupting cellular functions such as, ion transport, calcium mobilization, and excitotoxicity and inducing apoptosis in neurons, astrocytes and microglia (Emerit et al., 2004). Excitotoxicity, the process by which excess glutamate over-activates glutamate receptors resulting in intracellular calcium overload with subsequent neural cell death, is seen in the AD brain and is closely linked with oxidative stress (Facheris et al., 2004). The exact source of the increased oxidative stress seen in the AD brain is not yet fully known. However, one study suggested that it may result from the mitochondrial dysfunction induced by the presence of A β , and by glial activation (Emerit et al., 2004). It is widely accepted that oxidative stress is an early event in AD and most likely plays an active role in the pathogenesis of the disease (Practico et al, 2002).

The damage caused by A β accumulation and oxidative stress to neighboring cells triggers an inflammatory response. One of the first inflammatory responses seen is activated microglia and their cytokine release (Selkoe, 2001). Specifically, microglia exposed to A β secrete pro-inflammatory cytokines interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor α (TNF α) (Tuppo et al., 2005). The release of these cytokines in turn attracts astrocytes which in turn release the additional inflammatory proteins ACT and ApoE (Selkoe, 2001). Astrocytes clustered around A β plaques have been shown to secrete chemokines, cytokines, and reactive oxygen species that may contribute to the neuronal damage seen surrounding A β plaques (Johnstone et al., 1999).

In addition to secreting cytokines and stimulating astrocytes, microglia also appear to play a role in A β clearance. Activated microglia have been shown to actively phagocytize and clear A β away from deposition sites (Rogers et al., 2001).

The inflammatory response seen in the brain after the accumulation of A β plaques is thought to play a pivotal role in the cascade of pathological changes seen in the AD brain, including accentuation of NFT formation. This idea has been coined the amyloid cascade hypothesis and has been overwhelmingly the most popular current concept for the explanation of AD. However, there are researchers who challenge the amyloid hypothesis.

One of the biggest pieces of evidence that researchers who oppose the amyloid theory use is the concept that A β levels have little predictive value for the cognitive state of the patient (Lee et al., 2004). They also use the premise that studies used to formulate the amyloid theory were done in cell culture models and that they are not good indicators of *in vivo* processes. As an alternate theory researchers have been suggesting for years that A β does not function as the disease-causing pathogenic agent, but rather A β plays a protective role in the brain (Lee et al., 2004). A recent study found that A β functions as an endogenous regulator of neuronal activity (Esteban et al., 2004). There has been growing evidence that points towards the antioxidant capabilities of A β as demonstrated by its ability to prevent lipoprotein oxidation in the CSF (Kontush et al., 2001). A β 's ability to bind and chelate Cu is the most likely mechanism through which it reduces oxidative stress (Atwood et al., 2003). These findings are in accord with earlier work that has found A β plaque load to be inversely correlated with oxidative stress (Nunomura et al., 2001). Thus, oxidative stress triggers A β generation in an attempt to decrease the

oxidative stress load (Atwood et al., 2003). Individuals with high A β plaque loads that remain cognitively normal may be regarded as showing a healthy response to the oxidative stress encountered during ageing. Researchers in favor of viewing A β as a side effect, rather than causative agent of the disease also acknowledge the toxic properties of the protein. They propose that at high enough oxidative stress burdens A β begins to accumulate and results in uncontrollable plaque growth with the subsequent toxic effects mentioned above (Atwood et al., 2003). This theory, in sharp contrast to the amyloid cascade hypothesis, calls into question the wide spread use of therapeutic strategies aimed at reducing A β loads.

NFTs, the second hallmark lesion of AD, are intracellular aggregates composed of the microtubule associated tau protein (Dickson et al., 2004). NFT's form as a result of hyperphosphorylation of the tau protein at several different residues. The phosphorylation of the tau protein leads to a dissociation from the microtubules and eventual aggregation into paired helical filaments (Rosenberg et al., 2000). These tangles can be found in numerous brain regions including: hippocampus, parahippocampal gyrus, amygdala, frontal, temporal and occipital association cortices (Selkoe, 2001). NFTs are also found in numerous other neurodegenerative disorders (Kuf's disease, subacute sclerosing panencephalitis, etc), and in the brains of aged non-demented individuals (Gomez-Ramos et al., 1998). There is evidence that suggests A β plays a causative role in the formation of neurofibrillary tangles (NFTs). However, the exact relationship is still uncertain. The fact that NFTs can arise in the absence of beta-amyloid deposition suggests that tangles can form as a result of a variety of neuronal conditions.

Currently, there are many different theories as to the exact etiopathology that induces the formation of NFT's in AD. One of these theories of NFT formation has arisen by examining the brains from pre-diagnosed AD patients. Researchers have found that the pathologies associated with A β plaques very closely resembles structural damage to the axons of neurons (Vickers et al., 2000). The structural damage to the axons caused by the accumulation of A β early in the disease may trigger an inflammatory response in the brain that becomes prolonged and results in the formation of NFT's (Vickers et al., 2000). The degree of cognitive impairment in an AD patient correlates more closely with the amount and distribution of NFT's than with the amount of A β deposited in the brain (Bennett et al., 2004). This finding puts an increased emphasis on the role that NFT's play in the progression of AD.

During the course of AD progression a patient would also expect to see a substantial decrease in brain volume. This global volume loss is due to many factors; death of neurons, gyri shrinking, widened sulci, and loss of synapses.

Genetics

Alzheimer's disease can be subdivided into two main groups: familial and sporadic. Familial AD (FAD) accounts for approximately 10% of all cases and is characterized by known genetic mutations and a much earlier age of onset. Symptoms can arise in patients with FAD as early as 40 years of age but generally occur no later than 60 years of age. Phenotypically, familial AD is almost indistinguishable from sporadic AD, except for the fact that some forms of familial AD have a more rapid progression than the sporadic forms. Sporadic AD accounts for approximately 90% of all cases and is distinguished by a late age of onset. Sporadic AD also has an associated

genetic mutation that has been discovered, the ApoE4 allele, that will be discussed in detail later. This mutation does not definitively confer development of AD but persons carrying the gene are considered to be at a much higher risk. Age of onset varies greatly in the sporadic form of AD, ranging from 65-90 years old. Once a person reaches 90 their chances of developing AD drops dramatically.

The first genetic mutation that was discovered in the familial form of AD was a mutation in the gene coding for APP (Selkoe, 2001). There are 16 known pathogenic mutations and 4 nonpathogenic mutations in the APP gene (Zekanowski et al., 2004). Mutations result in changes in the peptide sequence located next to the β -secretase and γ -secretase cleavage sites. These changes in sequence, although subtle, are thought to have a major affect on the proteolytic cleavage of the protein. The double Swedish mutation (K670N/M671L), a mutation isolated from a family from Sweden, affects β -secretase activity and results in elevated levels of $A\beta_{40}$ and $A\beta_{42}$ (Zekanowski et al., 2004). The London mutation affects the activity of γ -secretase, resulting in elevated levels of both $A\beta_{40}$ and $A\beta_{42}$, with the greatest increase seen in $A\beta_{42}$. In general, families carrying AD pathogenic mutations of APP develop symptoms in their fifties. APP mutations only account for 4-6% of the familial form of AD (Zekanowski, 2004). These mutations have helped provide more evidence for the amyloid cascade hypothesis.

APP gene dosage has proven to be another key piece of evidence for researchers supporting the amyloid hypothesis. The effects of the location of the APP gene on allele 21 is clearly seen in patients with down's syndrome. An individual with trisomy 21 can expect to see identical neuropathology as compared with AD due to the increased gene

expression seen with an extra copy of the APP gene. Individuals identified with this mutation often have AD onset during their 50's (Selkoe, 2001).

The most common genetic mutation resulting in FAD are those found in presenilin 1 genes (PS1) and presenilin 2 genes (PS2) (Zekanowski et al., 2004). While the exact functions of the PS1 and PS2 genes are not clear, there is growing evidence that suggests they both may serve as the active sites of the γ -secretase complex (Zekanowski et al., 2004). The mutations directly affect APP processing, resulting in increased amounts of $A\beta_{42}$, and providing even more evidence for the amyloid cascade hypothesis. Currently, there have been more than 100 mutations identified in the PS1 gene and only 9 in the PS2, with nearly all being missense mutations located around the transmembrane domains of the proteins (Zekanowski et al., 2004). Animal models of APP mutations crossed with animals models carrying the PS1 mutation yield animals with dramatically increased levels of $A\beta$ plaques. The fact that presenilin mutations result in increased $A\beta$ levels further supports the amyloid cascade hypothesis.

Diagnosis

The tools and techniques used in the diagnosis of AD are advancing as fast as technology and knowledge of the disease permits. For detecting the early form of AD, a test must be able to accurately discriminate between pathological processes and normal ageing, as well as other neurological disorders that may cause memory loss (Nestor, 2004). The diagnosis of AD is commonly based on the criteria put forward by the National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (Cummings, 2004). According to the NINCDS-ADRDA there are three levels of diagnosis: definite (clinical

diagnosis with histological confirmation), probable (typical clinical syndrome without histological confirmation) or possible (atypical clinical syndrome but no alternative diagnosis apparent without histological confirmation) (Cummings, 2004).

A patient who initially presents themselves with dementia as a candidate for early AD would first go through a battery of tests ruling out other dementia causing diseases such as syphilis, AIDS, inflammatory diseases or exposure to dementia-inducing toxins (Cummings, 2004). After ruling out other causes of dementia, a patient would most likely begin cognitive testing. The most common cognitive test given is the Mini-Mental State Exam (MMSE). Psychological testing is required for a clinical diagnosis of AD and can help the physician determine the progression of the disease. Up until very recently, psychological testing alone was considered adequate to provide enough evidence for a diagnosis of AD. Psychological testing does have its weaknesses. One weakness is the high variability in performance seen in the normal human population, which can result in test scores not indicative of cognitive status. The tests themselves can be biased, and norms are established from pools of people not necessarily indicative of the population at large (Zamrini et al., 2004). Other approaches using biological markers or neuroimaging eliminate the pitfalls seen in psychological screening of AD patients.

The most researched biological markers used to diagnose AD are A β proteins and levels of total and phosphorylated tau. A β is generated in all individuals from normal APP metabolism and is secreted in the extracellular space allowing for its detection in both the cerebral spinal fluid (CSF) and blood plasma (Seubert et al., 2002). The majority of studies have found a decrease in the amount of A β_{42} in the CSF of AD

patients (Sobow et al., 2004). However, the use of decreased levels of $A\beta_{42}$ is not a definitive diagnosis of AD alone since decreased levels of $A\beta_{42}$ have also been found in patients with depression and other dementias (Andreasen et al., 2001). Numerous studies have found increased amounts of both normal and phosphorylated tau in the CSF of AD patients (Sobow et al., 2004). The combination of analyzing the ratio of normal tau to $A\beta_{42}$ has proven to be the most effective tool in using biological markers in the diagnosis of AD (Gomez-Tortosa et al., 2003). The accuracy of using these biological markers alone in diagnosing AD is widely questioned. However, the analysis of biological markers can greatly help in an AD diagnosis when paired with other diagnostic techniques such as brain imaging.

A landmark announcement by Medicare in June, 2004 stated that it will be providing coverage for brain scans in order to help confirm diagnosis of AD. Scientists have known for years that many different imaging techniques including positron emission tomography (PET), magnetic resonance imaging (MRI) and computer tomography (CT) scans can help tremendously in confirming AD. Also, due to the fact that AD begins decades prior to psychological symptoms, imaging studies may provide doctors with a tool for diagnosing AD in the very beginning stages. Neuroimaging can provide doctors with not only preclinical disease state, but also rate of disease progression if imaging studies are done in series (Zamrini et al, 2004). The implications of being able to diagnose a patient 20 years prior to disease onset are astounding in that it opens the door for possible prevention therapeutics to be utilized.

Both structural and functional neuroimaging techniques offer insight into disease progress and diagnosis. Structural imaging uncovers anatomical changes that are

occurring in the brain prior or during disease pathogenesis. Imaging studies recently have shown that atrophy in the medial temporal lobe shows very high predicted cognitive decline (Fox et al., 2004). Longitudinal studies have shown that the severity of AD correlated with rates of hippocampal volume loss (Zamrini et al., 2004). Neuroimaging of structural changes that occur not only give researchers insight into the disease pathogenesis, but also may provide a tool that can accurately assess an individual's risk for developing AD.

Functional imaging may offer an even more accurate way to diagnose AD. The most common way researchers measure cerebral activity is through Positron Emission Tomography (PET). PET scanning works by injecting the patient with a small amount of tracer drug that attaches itself to a variety of energy sources, most often 2-deoxyglucose (2DG). The 2DG, tagged with a positron emitting isotope, will accumulate in areas that are more active than others. The tracer isotope emits positrons, that collide with electrons nearby, resulting in gamma rays that are picked up by sensors and mapped onto different brain regions showing areas of high activity, and also more importantly in the case of diagnosing AD, areas of low activity. A patient suspected of having AD will show a dysfunction or reduced brain activity (low 2DG levels) in temporo-parieto-occipital cortices on a PET scan (Nestor et al., 2004).

Another interesting imaging technique that has emerged in the last year is a technique using a novel amyloid-imaging PET tracer, called Pittsburgh Compound-B (PIB) (Klunk et al., 2004). A study done recently involved tracing amyloid deposition in 16 patients showed that PIB retention was increased primarily in the association cortex, an area known to contain large amyloid load in AD patients (Klunk et al., 2004). This

new imaging technique may provide researchers with a tool to quantify amyloid load in living patients. Also, the technique has the ability to monitor the effects of drugs designed to limit amyloid deposition.

In addition to PET scanning, another technique, Single-Photon Emission Computed Tomography (SPECT) is also used in functional imaging studies. The basic idea behind SPECT scanning is the same to PET scanning. Only SPECT scanning utilizes a radioactive compound that is injected into the patient. Regions with higher activity show an increased signal that is mapped onto brain regions. SPECT scans have also helped to provide more evidence for the argument that MCI is an antecedent to AD. A study using SPECT scanning techniques revealed that patients diagnosed with MCI who showed a hypoperfusion in the posterior cingulate cortex later converted to AD (Nestor et al., 2004). Findings such as these are greatly improving the accuracy of AD diagnosis.

Scanning techniques offer a promising tool for a more accurate, earlier detection of AD. However, due to the nature of the disease, it is still essential to perform cognitive testing. Brain imaging techniques, alongside cognitive testing and biological marker analysis can provide doctors with a powerful tool in the diagnosis of AD.

Risk Factors

The only known genetic inheritance that is considered to be a risk factor for developing sporadic AD is the $\epsilon 4$ allele of the apolipoprotein gene. Apolipoprotein (ApoE) has three alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. ApoE is involved in a number of functions including cholesterol transport, dendritic growth, neuronal repair and it has possible anti-inflammatory functions (Lahiri et al., 2004). Individuals who are homozygous for the

ApoE4 allele of the protein would be considered at a higher risk for the eventual development of AD and account for 10-15% of AD cases (Zekanowski et al., 2004). However, only one third of people who inherit these alleles develop AD illustrating further the complexity of factors involved with the disease. Inheritance of the ϵ 4 allele of ApoE results in increased amyloid accumulation, neurotoxicity, and increased oxidative stress (Lahiri et al., 2004). While this is the only widely documented genetic inheritance associated with an increased occurrence of sporadic AD, researchers believe that most likely mutations in as many as 50 other genes could act as risk factors (Zekanowski et al., 2004). Although these genes have yet to be discovered, the fact that first degree relatives of sporadic AD patients are considered to be at a higher risk helps provide evidence that genetics may play a larger role than what is currently known.

The primary risk factor for AD is aging, which is unavoidable. Interestingly, an individual's risk for developing AD will peak at around the age of 90 and then decline in the mid 90's. Even though an individual has no way of controlling the risk factor of aging, there are many other risk factors that fall within a person's ability to control. Environmental factors including lifestyle choices have been investigated using identical twin studies. Using twins that are discordant for AD, researchers have examined varying environmental factors that may have played a role in the development of the disease. One such study found that a higher level of schooling was correlated with a decreased risk of developing AD (Raiha et al., 1998). Also, researchers from the same twin study found that a reduced risk was associated with ambidextrousness, and an increased risk was associated with both marriage and widowhood (Raiha et al., 1998).

Vascular risk factors, such as hypertension, have also been shown to be associated with AD (Skoog et al., 2003). In order for hypertension to be considered a risk factor for AD, an individual would have experienced it during mid life, decades prior to developing AD. Cholesterol levels have been examined as possible risk factors. Studies have shown that individuals with higher total cholesterol levels had nearly tripled their risk in developing AD (Wellington et al., 2004). One risk factor that can be controlled is an individual's diet. A diet high in vitamins C, B6, E, B12 and folate have been associated with a lower risk of AD (Luchsinger et al., 2004). Studies have reported that diets low in fish and cereals are associated with an increased risk for the development of AD (Grant et al., 1999). The wide array of risk factors seen to play a role in AD, is further testament to the complex nature of the disease in terms of both its development and progression.

Treatments

While a cure has not yet been discovered for AD, many treatments are available that have been proven to lessen symptoms. The treatments can be broken down into two broad categories: drug therapies and alternative treatments. Drug therapies include; antioxidants, cholinesterase inhibitors, NMDA receptor antagonists (Memantine), anti-inflammatory agents, neuropsychiatric drugs, herbal supplements, and statins. Also, there are several drug therapies currently under clinical investigation including the anti-amyloid drug Alzhemed, and estrogen replacement therapies. Alternative treatments include psychotherapy, music therapy, exercise, and other environmentally stimulating activities.

Alpha-tocopherol (vitamin E) has been investigated as a potential therapeutic for AD due to its antioxidant capabilities. Vitamin E's primary function is to defend tissues

against oxidative damage by reducing lipid peroxidation in membranes (Conte et al., 2004). It has been shown to successfully cross the blood brain barrier and significantly reduce brain lipid peroxidation rates (Conte et al., 2004). In the Tg2576 mouse model for AD, vitamin E has been shown to reduce A β loads (Sung et al., 2004). The increased oxidative stress seen in AD due to deposition of A β and resultant neuroinflammation is thought to play a central role in the disease. By administering a potent antioxidant such as vitamin E, the disease progress may be retarded. Several epidemiological studies have pointed towards the protective effects that vitamin E plays in AD, and current clinical practice is in favor of using it as a treatment for AD (Berman et al., 2004).

Cholinesterase inhibitors have become the standard of treatment for patients with AD (Cummings, 2004). The brain of an AD patient is marked by an extreme decrease in cholinergic activity. A cholinesterase inhibitor works to prevent the enzyme responsible for breaking down acetylcholine, thus, increasing the amount available for neuronal signaling. Four cholinesterase inhibitors are currently FDA approved in the treatment of AD: tacrine, donepezil, rivastigmine, and galantamine (Cummings, 2004). Of the four, tacrine (Cognex) is rarely used due to its potential liver toxicity (Delagarza et al., 2003). Clinical trials have shown that treatment with cholinesterase inhibitors delays nursing home placement and stabilizes or improves cognitive function (Delagarza et al., 2003). The effects of cholinesterase inhibitors are however, modest. The drugs improve cognitive function in mild dementia only and cannot prevent or slow the disease progression (Sonkusare et al., 2005). Studies have indicated that treatment with these drugs will delay further cognitive deterioration by about one year (Delagarza et al., 2003).

The FDA has also approved another drug, Memantine, for the treatment of AD. Memantine is a partial NMDA receptor antagonist. NMDA-receptor-mediated glutamate excitotoxicity plays a pivotal role in the neuronal death seen as a result of A β deposition (Sonkusare, 2005). By blocking the function of NMDA receptors, the drug prohibits the toxic effects of the receptors that are seen in AD. Memantine has been approved for the treatment of moderate to severe AD, and has been found to improve cognitive, social and physical impairments.

Antiflammatory drugs (NSAIDs) have been considered as a possible therapeutic in AD. The inflammation seen around neuritic plaques is thought to play a role in the subsequent neuronal damage seen surrounding the plaques (Delagarza et al., 2003). By reducing the inflammatory response, the amount of neuronal damage could also be limited. Large clinical trials have not yet been able to show an improvement in patients undergoing anti-inflammatory drug therapies (Imbimbo et al., 2004). Current research has shown the ability of selective anti-inflammatory drugs to decrease production of A β (Imbimbo et al., 2004). New research is underway to develop new NSAID analogues capable of providing both anti-inflammatory protection as well as anti-amyloid activity.

Patients suffering from AD often experience psychiatric and behavioral disturbances. In order to alleviate these symptoms on both the patient and caregiver, it has become fairly common to administer a variety of drugs used to alleviate the individual symptoms of the patient. Anti-psychotic drugs such as risperidone are commonly used in patients experiencing psychosis (Weiser et al., 2002). Selective serotonin reuptake inhibitors (SSRIs) are used in patients experiencing depression. Also, mood stabilizers and sedatives are used to help stabilize behavior in AD patients. These

drugs are all used to manage the symptoms of AD only and do not confer any protective or therapeutic effects to the disease itself.

Ginkgo biloba has been the most popular herbal supplement used in both the prevention and treatment of AD. Studies have shown that AD patients treated with ginkgo biloba have had modest improvements as compared with control groups (Cummings, 2004). The exact function that ginkgo biloba serves is not yet clear, however, it is thought that it confers its benefits through antioxidant and/or vasodilatory properties. Another herbal supplement, huperzine A is also used in the treatment of AD (Zangara et al., 2003). Huperzine A serves as a cholinesterase inhibitor, it crosses the blood brain barrier easily, and has a prolonged biological half-life (Jiang et al., 2003). Although it is not yet FDA approved in the United States for treatment of AD, other countries such as China have been using this supplement for a number of years.

Cholesterol lowering drugs (statins) have been shown to have protective effects against AD. Recent evidence suggests that cholesterol metabolism modulates A β production and that drugs designed to inhibit cholesterol metabolism may be beneficial in the treatment of AD (Wolozin et al., 2004). There have been several studies that have reported preliminary evidence that chronic treatment with statins is linked to a significantly decreased risk of developing AD (Wolozin et al., 2000).

There are numerous current clinical trials investigating various drug therapies currently. A current phase III trial being conducted at the National Institutes of Health (NIH) involves the new drug Alzemed, a drug designed to prevent the aggregation of A β and lower oxidative stress (Neurochem, Inc. 2004). Another phase III trial at the NIH

is using a neurotrophic agent Cerebrolysin that has been found to decrease A β and protect synaptic terminals in a rodent model of AD (Rockenstein et al., 2003).

In addition to the numerous pharmaceutical therapies available for AD patients there are also several non-pharmacological treatments commonly used. Caregivers have found that by enriching the patients environment through adding activities such as light exercise or by allowing them to listen to music or view videotapes of family members that their neuropsychiatric symptoms are greatly alleviated (Cummings, 2004). While these treatments are designed to lessen the severity of symptoms during the progression of the disease, a recent study points towards the therapeutic potential that environmental enrichment can have. A study using a mouse model of AD found that long-term environmental enrichment greatly improved cognitive function in animals that otherwise are cognitively impaired (Arendash et al., 2004). This study suggests that environmental enrichment can provide therapeutic cognitive benefits to patients with AD.

Animal Models

Transgenic Mice

Animal models have proven to be very useful in the research of many diseases such as Parkinson's, diabetes, and ALS. The creation of an animal model allows researchers to test potential drug therapies as well as study in further detail the pathologies underlying the disease. The designs and creations of animal models are being modified as further technology and knowledge of diseases advances. While the genes inserted or methods of gene insertion may change, the basic concept underlying the creation of an animal model remains the same.

In order to create an animal model for a disease with a known genetic mutation the gene encoding the mutation must be first identified and then cloned. Next, embryonic stem cells are removed from the donor mother and the cloned gene of interest is inserted into the embryonic stem cells in a random insertion through the use of a vector. The cells that have incorporated the mutated gene are then selected and reinserted into the embryo. The embryo is reinjected into the mother, and the resultant offspring will be a chimeric animal. A chimeric animal contains both normal cells and cells containing the genetic insertion or transgene. The chimeric animal will then be crossed with a wild-type mouse to create a heterozygote transgenic animal that can be bred with another heterozygote animal to create a homozygous transgenic animal. The resultant animal will be a transgenic animal expressing the gene product inserted into its genome with hopes of mimicking the human disease. An important element of designing a transgenic animal is choosing an appropriate promoter. Promoters determine the level, tissue specificity, and temporal pattern of the transgene being expressed (Picciotto & Wickman, 1998).

The process of creating the AD specific transgenic animals is modified slightly from the process described above. The fertilized egg is removed from the pregnant mother and the transgene, in cDNA form, is introduced into the single-cell mouse embryo through pronuclear injection (Picciotto & Wickman, 1998). The transgene injected embryo will be re-implanted into a pseudopregnant mother. The inserted DNA will become integrated into the mouse's genome through a random insertion event. Most often multiple copies of the gene are integrated into the genome. The copy number can have an affect on the transcriptional level of the gene. However, the site of integration seems to have a larger effect on transcription. Assuming the insertion event occurred at

the one cell stage all of the cells that compose the resultant offspring should contain the transgene. If the insertion is done in an embryo that has already undergone multiple rounds of cell division, then a chimeric animal will be produced.

Researchers involved in the field of AD have been successful in creating several different mouse models of AD including the PDAPP, Psw (APP23), and the APPsw(Tg2576) + PS1 models. All of these animal models utilize the genetic mutations discovered in FAD in the APP and Presenilin genes. The mouse models currently used have proven to be very beneficial to researchers in helping to elucidate further the disease mechanisms involved in AD. There is, however, much criticism centered on the fact that the mouse models for AD are incomplete models that do not mirror perfectly the disease as seen in humans.

PDAPP Model

One of the first mouse models developed for AD is the PDAPP model. The animal model was generated by a single substitution in the APP gene associated with familial AD (P717V) being driven by a platelet-derived growth factor (PDGF)- β promoter (Games et al, 1995). The PDAPP model expresses high levels of human mutant APP that result in the formation of neuritic plaques, synaptic loss, astrocytosis and microgliosis (Games et al, 1995). Plaque formation is seen between 6-9 months of age, and is found in the hippocampus, corpus callosum and cerebral cortex (Games et al, 1995). Also, it has been reported that age-dependent changes in synaptic densities has been seen in this animal model (Dodart et al, 2000). This animal model has failed to exhibit any neuronal loss in the entorhinal cortex, CA1 hippocampal subfield or cingulate cortex through 18 months of age (Irizarry et al, 1997). Synaptic transmission

has been studied in the PDAPP model, and one study reported that there is abnormal neurotransmission in the hippocampal circuits prior to A β deposition (Giacchino et al, 2000). The generation of the PDAPP mouse model has shown that the overproduction of the mutant APP gene is sufficient to cause plaque formation with subsequent activation of astro-glial cells that is similar to what is seen in AD (Masliah et al, 1996).

The PDAPP model also exhibits numerous behavioral impairments associated with AD like behavior. A study reported that the model shows deficits in the radial arm maze task (an eight-arm dry radial maze designed to test spatial discrimination) as early as 3 months of age (Dodart et al, 1999). The cognitive deficits in spatial learning may be due to hippocampal atrophy and modifications in synaptic density (Dodart et al, 2000). Impairments in an object recognition task (tested by analyzing a rodents' propensity to explore a novel object as opposed to a familiar one in an open field) are seen at 6 months of age in homozygous animals, and in 9-10 months of age in heterozygous animals (Dodart et al, 1999). Another study testing PDAPP animals across a full test battery of cognitive and sensorimotor tasks (at an early and late time point) reported that at 2 months there were no cognitive deficits in the PDAPP model (Nilsson et al, 2004). At the late time point (16 months), however, testing revealed an impairment in the final block of Morris water maze acquisition and in overall radial arm water maze performance (Nilsson et al, 2004). It is noteworthy that the mixed background of mice in the Nilsson et al. (2004) study resulted in elimination of hippocampal atrophy present in earlier studies (Dodart et al., 1999). Studies have also been done examining the effect of amyloid plaque formation on behavior. One study reported that in the modified water maze (the escape platform is moved across 5 successive locations and animals are given 8

trials a day designed to test working memory) that both age-related and age-independent working memory impairments are seen in the PDAPP model (Chen et al, 2000). This study also reported that the impairments in this modified water-maze correlated with an age-related increase in plaque burden (Chen et al, 2000). An additional more recent study has also reported deficits in spatial learning in the circular maze task in both young (3-5 months) and aged (20-26 months) PDAPP animals (Huitron-Resendiz et al, 2002). The authors suggest that glucose hypometabolism, hippocampal atrophy, and age-related increases in A β deposition may be the cause of the impairments seen. A study focusing on changes in emotionality in this mouse model found abnormalities in fear (tested by analyzing posture patterns in a fear conditioning paradigm) and exploratory activity (tested by analyzing motor patterns in the fear conditioning paradigm) in 11 month old females (Gerlai et al, 2002). Changes in emotionality are prominent effects of AD, and it is therefore, important to have an animal model that incorporates not only pathological and cognitive changes but also emotional changes that mimic AD.

P_{sw}(APP23) & APP_{sw} Models

The “Swedish Mutation” was discovered in the gene encoding the APP protein in a Swedish family with a familial form of AD. The mutation has been used in the development of two animal models: the P_{sw}(APP23) and the APP_{sw}(Tg2576). Both of these animal models contain the same transgene with a double mutation (K670N-M671L) found in the 695 amino acid long human APP gene (Mullan et al, 1995). These two AD animal models differ in the promoters used to drive expression of the mutant APP and the insertion sites of the transgene (both insertions are random events). These differences result in two animal models showing unique pathologies.

The APP_{sw} (Tg2576) mouse model for AD contains the 695 amino acid long human APP gene encoding the double mutation found in a Swedish form of familial AD (K670N-M671L) driven by a hamster prion protein promoter (Irizarry et al, 1997). The Tg2576 animal model exhibits soluble A β formation at 6 months of age and plaque formation by 11-13 months of age accompanied by neuritic dystrophy and activated astrocytes and microglia (Irizarry et al, 1997). Plaques are found predominately in the cortical and limbic regions of the brain. This model expresses the mutant APP only in neurons, presumably due to the actions of the prion promoter (Hsiao et al, 1995). Glial-mediated inflammatory response has been studied in detail in the Tg2576 model. It has been found that the inflammatory cytokine interleukin-1 β and the tumor necrosis factor α are localized to A β plaques (Benzing et al, 1999). Also this animal model has been shown to express increased glial fibrillary acidic protein, a protein found to be increased in the AD brain (Lim et al, 2000). This model does not have any neuronal death in the CA1 region of the hippocampus, a region that undergoes profound loss of neurons in human AD (West, 1994). The behavioral deficits seen in this animal model maybe attributed to impairments in synaptic plasticity (Chapman et al, 1999).

Sensorimotor analysis of the Tg2576 model has yielded conflicting results. One study reported deficiencies in the balance beam and string tests at 3-, 14-, and 19-month old animals (King & Arendash, 2002). Another study also found impairments in balance beam at 5 and 6.5 months of age (Arendash et al, 2004). In contradiction to these findings, a study by Lalonde et al (2003) reported that between 15-20 months of age (average age 17 months), the Tg2576 model did not display any deficits on the balance beam and rotorod test. The discrepancy may be due to differences in equipment used to

test the animals or, less likely; it may be due to differences in the strain background of the mice used. The Tg2576 model has also been shown to exhibit increased activity in the open field test at 17 months of age (Lalonde et al, 2003). Similar findings regarding activity level have been reported. A study done reported that at 3 months of age there was a significant increase in activity in the open field task, and overall increased activity through 19 months of age (King & Arendash, 2002). In disagreement to these findings of increased activity a study done on 5 month old mice reported no differences in activity/exploratory activity (Arendash et al, 2004). Changes in anxiety have also been reported. A study done using Tg2576 animals at 17 months of age reported finding reduced anxiety in the elevated plus maze (Lalonde et al, 2003). A study done in younger mice, 5 months of age, reported no differences in anxiety in the elevated plus maze (Arendash et al, 2004). The seeming discrepancies found in the Tg2576 model may be attributed to a number of variables including differences in background strain, alterations in procedures used during testing, and use of animals at various ages.

There have also been conflicting reports regarding the cognitive abilities of the Tg2576 model. The Y-maze task for spontaneous alternation has been utilized by several researchers in characterizing the Tg2576 model. One study reported that at 3 and 19 months of age Tg2576 mice had significantly reduced spontaneous alternations; however at 9 and 14 months of age there was a modest impairment only (King & Arendash, 2002). The authors suggest that the Y-maze task may be relatively insensitive to cognitive impairment associated with the transgene, possibly accounting for the differences in ages at which researchers report finding impairments. Another study tested spontaneous alternation at 3 and 10 months of age and reported that only at 10 months of age was

there significantly less spontaneous alternations in transgenics compared to non-transgenic controls (Hsiao et al, 1996). A study done on 17 month old Tg2576 mice reported impaired spontaneous alternation (Lalonde et al, 2003). In agreement with these results another study tested spontaneous alternation at 5 and 8.5 months and reported that overall there was a significant impairment (Arendash et al, 2004). Regardless of the exact time point in which impairments are seen in the Y-maze, impairments are consistently found in the performance of the Tg2576 mouse model for this test of cognitive function.

The circular platform (a spatial reference memory task) is also used to test cognitive function in rodent models. A study done in 7 month old Tg2576 mice revealed an impairment in circular platform “reversal learning” (modified methodology in which animals must relearn a new escape location) (Pompl et al, 1999). A study done on 3, 9, 14, and 19 month old Tg2576 mice found no significant decreases in performance in the standard circular platform task (7 days of testing omitting the “reversal learning” phase) (Arendash, 2002). A more recent study using the standard protocol for circular platform testing also reported no impairment at 6 months of age (Arendash et al, 2004).

The Morris water maze is another task that is commonly used by researchers and is designed to test reference/spatial learning and memory. A study was done on 2-, 6-, 9-, 10, and 12-15 month Tg2576 mice investigating performance in the Morris water maze (Hsiao et al, 1996). The 12-15 month old mice were tested in a follow up study that retested a subset of the original 2 and 6 month old mice. The study revealed that at the 2 and 6 month time points there was not a significant difference between the transgenic and non-transgenic matched controls in the Morris water maze task. At the 9- to 10 month

age, the escape latency of transgenic mice during acquisitional testing was significantly increased; during the probe trial, the transgenic animals had a significantly reduced number of center crossings (Hsiao et al, 1996). The 12-15 month old mice showed impaired latencies after the 5th trial block and on probe trials. Another study was done using the Morris water maze on 3, 9, 14 and 19 month old Tg2576 mice. Compared to non-transgenic controls, Tg2576 mice were unimpaired collectively and at separate time points in both the learning and memory retention phase of testing (King & Arendash, 2002). A recent study was done on 5.5 month old Tg2576 mice and found impairment in escape latency (time taken to find the submerged escape platform) across all 9 days of testing, the earliest time point at which impairment has been observed (Arendash et al, 2004). This study also reported an impairment in the memory retention trial (final probe trial day 10) and an overall impairment over all three probe trials. The apparent inconsistent Morris water maze results in the later two studies from the same laboratory (King & Arendash, 2002; Arendash et al., 2004) may be due to differences in the strain background. The colony's crossbreeding over several years has apparently resulted in both their Tg2576 and APP/PS1 lines becoming behaviorally more sensitive to mutant APP expression and/or the process of A β deposition at an earlier age.

The Tg2576 model has also been evaluated in the radial arm water maze (RAWM), an excellent working memory task showing a high degree of sensitivity to A β deposition in numerous AD transgenic lines (Arendash et al, 2001). At 6.5-7 months Tg2576 mice have been reported to show working memory impairments as assessed through Trials 4 and 5 of the RAWM (Arendash et al, 2004). This study also revealed especially prominent impairments in trial 5 of the RAWM, designed to mimic

“registration-recall” testing of AD patients, providing added evidence for the working memory impairment seen. It is widely reported that cognitive impairments in the Tg2576 model precedes the appearance of A β plaque formation at around 9-11 months, suggesting that the soluble form may be causing the early phase of cognitive impairment (King et al, 1999).

Also preceding plaque formation is evidence of increased oxidative stress in the form of lipid peroxidation. A study done examining the urine, plasma, and brain tissues for the lipid peroxidation by product 8,12-iso-iPF_{2 α} -VI, found that at 8 months of age all of the samples showed increased lipid peroxidation (Pratico et al, 2001). Another study examining the oxidative stress markers NHE and HO-1 also found that the Tg2576 model exhibits oxidative damage associated with A β accumulation (Smith et al, 1998).

The primary pathological difference between the APP23 and Tg2576 mouse model is found in the vasculature. The APP23 model exhibits deposition of A β in the cerebral vasculature (CAA) that is remarkably similar to that observed in human AD (Calhoun et al, 1999). The CAA found in the APP23 model is associated with local neuronal death, dysfunctional synapses, activation of microglia and microhemorrhage (Calhoun et al, 1999). The APP23 model can also serve as a mouse model of cerebral amyloid angiopathy. The accumulation of A β in the arterioles and capillaries leads to the death of vascular smooth muscle cells, aneurismal vasodilatation, and a weakening of the vessel wall that can lead to rupture and severe hemorrhage (Winkler et al, 2001). CAA is one of the prominent features of AD thus, making the APP23 animal model a valuable tool in the study of AD (Mueggler et al, 2004).

The APP23 mouse model displays impaired spatial learning abilities at 2 years of age in the Morris water maze, and hyperactivity assessed through the open field task (Dumont et al, 2004). Learning and memory deficits in the APP23 model, as examined in the Morris water maze, have been reported as early as 3-months of age (Van Dam et al, 2003). An additional study found an impairment in latency and swim distance beginning at 3 months of age and persisting through 18 months (Kelly et al, 2003). A study looking at 16 month old female APP23 mice found an impairment of spatial learning in the Morris water maze (Lalonde et al, 2002). Studies have also reported disturbed activity patterns similar to the disturbances in circadian rhythms seen in AD patients (Van Dam et al, 2003).

APP_{sw} + PS1 Models

Given the knowledge that mutations in the presenilin 1 (PS1) and presenilin 2 (PS2) gene results in the familial form of AD, the creation of an animal model expressing these mutations was created. As discussed earlier the missense mutations in PS1 and PS2 alters APP processing resulting in the formation of the toxic A β 1-42 (Borchelt et al, 1997). Single transgenic animals expressing the PS1 mutant transgene are not seen to produce significant amounts of A β pathology, however, there is a drastic increase in the ratio of A β 1-42/ A β 1-40 levels (Duff et al, 1996). By crossing the PS1 (M146L) animal model with the Tg2576 mouse, a double transgenic animal (APP/PS1) is produced that exhibits earlier and more pronounced AD like pathology (Borchelt et al, 1997; Holcomb et al, 1998).

A β deposition in the APP/PS1 is seen as early as 12 weeks in the cortex and hippocampus, far earlier than what is seen in the Tg2576 model (Holcomb et al, 1998).

By 12 months of age there is a 20 fold increase in A β deposition in the frontal cortex and a 40 fold increase in A β deposition in the CA1 region of the hippocampus as compared with the Tg2576 model (Takeuchi et al, 2000). The diffuse A β plaque burden (detected through immunostaining with 4G8) peaks at around 1 year of age, however, the fibrillar form of A β (detected through thioflavin s staining) increases through 2.5 years of age, the latest time point tested (Matsuoka et al, 2001). The A β plaque formation activates astrocytes and microglia. The numbers of activated astrocytes and microglia increase in parallel with A β plaque loads (Matsuoko et al, 2001). This robust A β deposition alongside activated astrocytes and microglia in the APP/PS1 model has resulted in the model becoming very popular currently amongst researchers. Recently, it has been reported that the APP/PS1 model exhibits abnormal LTP at 3 months of age, and impairments in basal synaptic transmission (BST) at 6 months of age (Trinchese et al, 2004). Both LTP and BST are underlying processes that occur during memory consolidation and abnormalities in their functioning result in memory deficits. Another study also looking at LTP and synapse function used microarray and quantitative RT-PCR to profile synaptic plasticity genes in the APP/PS1 model. These researchers found reduced mRNA expression of several genes necessary for LTP (Zif268, NR2B, GluR1, etc.) (Dickey et al, 2003). Neuronal morphology in the APP/PS1 model has also been studied using labeling techniques. It is reported that at 11 months of age, the APP/PS1 model displayed swollen bulbous dystrophic neurites alongside significantly reduced spine numbers and reduced total spine area (Moolman et al, 2004). A decrease in the density of cholinergic synapses in the frontal cortex and a decrease in the size of cholinergic synapses in the frontal cortex and hippocampus has been reported at 8 months

of age (Wong et al, 1999). While the exact cause of the synaptic dysfunction is not yet known, it is quite evident that the APP/PS1 model exhibits dysfunctional synaptic function as evidenced by multiple studies. In aged (22 months) APP/PS1 mice, researchers have found a reduced level of glucose utilization in the hippocampus accompanied by a 35.8% dropout of neurons in the CA1 region (Sadowski et al, 2004). The report of neuronal dropout is novel. Prior to this study no findings of neuronal death in the APP/PS1 model had been reported (Takeuchi et al, 2000).

Behavioral testing of the APP/PS1 model has been done including testing in sensorimotor, anxiety and cognitive tasks. Sensorimotor testing has revealed impairments in this mouse model. An impairment in sensorimotor function assessed by the balance beam task has been found to emerge as early as 5 months of age and persist through 16 months of age (Arendash et al, 2001). In contrast to this study a study reported that no sensorimotor function impairment was found in 4.5-6 and 15-16.5 month old animals in the balance beam task (Jensen et al, 2005). The string agility test (a task measuring sensorimotor ability) revealed no differences at 5-7 months of age, and impairments were found at 15-17 months of age as compared to non-transgenic controls (Arendash et al, 2001).

Anxiety and activity testing have also been performed on this animal model. The Y-maze alternation task is also used to determine an activity index through measuring the total number of arm entries. In this task 3-3.5 month old APP/PS1 mice had increased activity as compared with singly transgenic or non-transgenic littermates (Holcomb et al, 1998). Another study found similar results reporting that in 5-7 and 15-17 month old APP/PS1 mice there was a significant increase in arm entries (Arendash et al, 2001).

Open field testing (a task measuring activity and exploratory levels), has revealed increased activity levels at 15-17 months of age (Arendash et al, 2001). A study done on 8 and 22 month old APP/PS1 mice showed no significant changes in activity levels tested in an open-field task (Sadowski et al, 2004). Open field testing has been shown to reveal decreased activity (measured through horizontal locomotor movements) in 7 month old APP/PS1 mice (Liu et al, 2002). However, in contrast, a study has reported that at 4.5-6 months of age APP/PS1 mice had increased open field activity (Jensen et al, 2005). Anxiety testing on the elevated plus maze has revealed increased anxiety in animals 15-16.5 months of age (Jensen et al, 2005). Overall, the majority of studies (Holcomb et al., 1998; Arendash et al., 2001; Jensen et al., 2005) report increased activity in the APP/PS1 model. This increase in activity begins to emerge as early as 3 months of age and is still apparent at 15-17 months of age. The studies that reported either no significant changes in activity (Sadowski et al., 2004) or decreases in activity (Liu et al., 2002) both used photoactometers to assess movement. The use of a photoactometer is a more sensitive technique as it utilizes interruptions in photobeams capable of sensing horizontal and vertical motion to assess movement; whereas the open field testing employed in the other studies mentioned above utilized the number of line crossings (horizontal movement) in the open field as the only measure of activity. Thus, the conflicting results may be due to differences in equipment used.

Cognitive testing including tasks such as the Y-maze alternation task, Morris water maze, and radial arm water maze (RAWM) have shown conflicting results. The Y-maze task of general memory function, revealed an impairment in spontaneous alternations in APP/PS1 mice at 3-3.5 months (Holcomb et al, 1998). However, another

study has reported no differences in Y-maze performance at 5-7 months of age (Arendash et al, 2001). A recent study reported finding no differences in spontaneous alternation in the Y-maze task at both 4.5-6 months and at 15-16.5 months (Jensen et al., 2005). As mentioned prior the incongruity of findings in the Y-maze task for spontaneous alternations may be due to the non-specificity of the task to the impairments conferred by the APP/PS1 genes.

Spatial memory and learning assessed in the Morris water maze revealed no differences in escape latency, time spent in goal arm, or number of platform crossings in animals at 9 months of age (Holcomb et al, 1999). However, a longitudinal study testing at 4.5-6 months and again at 15-16.5 months found a significant impairment in escape latencies at both time points (Jensen et al, 2005). The Jensen et al., 2005 study also reported that in the probe trial at 4.5-6 months there was a significant reduction in annulus crossings as compared with the non-transgenic group; and at 15-16.5 months there was no difference in performance. Working memory has been tested in the radial arm water maze task in the APP/PS1 model at several different ages. A longitudinal study using 5-7 month old APP/PS1 found no differences in the RAWM and at 15-17 months reported that the APP/PS1 animals made significantly more errors during trial 5, the memory retention trial in the RAWM (Arendash et al., 2001). An impairment in working memory during radial arm water maze (RAWM) testing has been found in the APP/PS1 model at 16 months of age, as measured by the number of errors animals made during trial 5 of the task (Austin et al., 2003). Two recent studies have reported impairments in working memory at a much earlier age. A study done on 6-8 month old APP/PS1 mice reported finding significantly more working memory errors in the

APP/PS1 animals vs. the non-transgenic controls in the RAWM (Trinchese et al, 2004). An even more recent study reported that APP/PS1 animals at 4.5-6 months of age made significantly more working memory errors in the RAWM task, revealing cognitive deficits at a much earlier age than previously reported (Jensen et al, 2005). Interestingly, the same transgenic colony was utilized in both Arendash et al., (2001) and Jensen et al. (2005) for RAWM testing. The much earlier impairments in working memory found by Jensen et al., 2005 may be attributable to the fact that the colony utilized during the test battery had been cross-breed over several generations and the resultant transgenic mice may have become more behaviorally sensitive to mutant APP expression and/or the process of A β deposition.

Using the most recent and thorough behavioral evaluation of this model by Jensen et al (2005), an overview of the APP/PS1 model can be formed. The APP/PS1 model was reported by Jensen et al, (2005) to be cognitively impaired at 4.5-6 months of age in the RAWM task (assessed through number of errors, impairment seen overall and in last block), showing impairments in working memory. From this data it is reasonable to assume that any treatment/study that aimed at utilizing animals that are trending towards impairment should begin around 3 months of age. Jensen et al., (2005) reported that at 4.5-6 months of age this model had a significant impairment in Morris water maze acquisition and retention. These findings show an impairment in working and spatial reference memory in the APP/PS1 model at this early time point. Jensen et al, (2005) reported that at the late 15-16.5 month time point an additional cognitive impairment was found in the platform recognition task. Thus, in the APP/PS1 model, one could expect to find a significant cognitive impairment in working, reference and long-term memory to

emerge at 4.5-6 months of age (tested by either the Morris water maze or RAWM) and expect the impairment to persist through 15-16.5 months.

Transgenic animal models have provided researchers with a powerful tool in the study of AD. Animal models are, however, an incomplete model of AD due to their lacking of the complete set of pathologies seen in AD. All of the mutant transgenic APP lines (PDAPP, APP23, Tg2576, APP/PS1) completely lack the formation of NFT's (Loeffler, 2004). As discussed prior, NFT's are one of the pathological hallmarks of AD. It is, therefore, desirable that an animal model would form NFT's. The APP mouse model also differs from AD in regards to their immune and inflammatory response. The APP transgenic mouse displays only weakly activated microglia expressing low levels of complement factors that were gathered only around the periphery of plaques (Schwab, 2004). This is in stark comparison with the human response which involves microglia found within the core of the plaques that are highly activated and express high levels of complement factors (Schwab, 2004). The reason for this diminished immune and inflammatory response is unknown, although it has been hypothesized that it is due to low recognition of mouse complement factors to human A β (Webster, 1997). The plaques themselves are more fibrillar in state, with fibrils radiating out from a dense core, whereas human AD exhibits plaques in a more homogenous state (Schwab, 2004). The A β deposits in the APP23 model are soluble in SDS-containing buffer, however, human AD deposits are insoluble (Schwab, 2004). These differences may be due to post-translational modification differences in the mouse model. The more fibrillar and toxic nature of the animal model plaques has not been definitively shown to result in increased neuronal death. One study reported finding neuronal loss in a limited region around A β

plaques in the Tg2576 model (Tomidokoro, 2001) A study using 16 month old Tg2576 mice found no neuronal loss (Stein, 2002). The debate is centered on whether there is “limited” neuronal loss; however, it is apparent that the animal model does not display the profound neuronal losses seen in AD. The mutant APP transgenic animal model is therefore only a limited model for AD. The lack of a robust immune and inflammatory response may suggest that this animal model be limited to studies involving the prevention of A β accumulation (Schwab, 2004).

There are also limitations in the mouse model regarding behavior. Ideally, an animal model for a disease mimics that found in humans, but due to the highly psychologically-based nature of AD it is impossible to create a complete mouse model. One of the first striking deficits in using a mouse model is the inability to test verbal skills. Also, it is very difficult to test an animal model for any of the psychoses experiences by AD patients such as hallucinations, depression, and apathy. While there is extensive memory testing in animal models, it is impossible to assess semantic memory. Most behavioral studies use mazes with varying escapes and measures the ability of an animal to learn the escape, thus giving the researcher insight into the animals’ short and long-term spatial memory skills. Semantic memory, ability to recognize people, places, etc. is difficult to measure in an animal model. Often an AD patient will forget a loved ones’ face or be unable to recognize voices of their loved ones. This type of deficit in semantic memory is readily apparent in AD. However, animal testing is limited to testing only object recognition in memory tasks or identification of an escape platform. Nonetheless, animal models have proven to be highly useful in AD

research. It is however, important to understand the limitations that are faced when using animal models in AD research.

Hyperoxia

Behavior

It is estimated that the human brain consumes approximately 20-30% of total energy usage for the entire body and is considered to be the most metabolically active organ (Roland, 1993). Oxygen is regularly consumed by brain cells during normal cellular respiration (Benton et al, 1996). Given this knowledge it is reasonable to assume that varying oxygen saturation levels (hyperoxia or hypoxia) would affect cognitive functioning as well as other metabolic parameters. Researchers have been investigating the effects of administering hyperoxia (oxygen level above 20.9 %) treatments in humans for several years.

A recent study done by Andersson et al (2002) found no differences in cognitive performance after subjects (48 participants with a mean age of 21.1 ± 6.49 years) inhaled 100% oxygen for 1 minute prior to the start of testing. The cognitive testing given during this study was designed to assess working memory, prospective memory, attention and long-term memory. This study also examined physical changes that occurred during the treatment. They found that individuals who underwent the hyperoxia treatment had significantly higher oxygen blood saturation, lasting for 1 minute post treatment (Andersson et al, 2002). Heart rate was not affected by the treatment. This study is in contradiction to several others (Moss et al, 1998; Scholey et al, 1998;1999), who have found significant cognitive improvements following treatment with hyperoxia.

An older study done by Moss et al (1998) reported a significant improvement in immediate and delayed word recall in test subjects (20 participants aged 21-48 years) exposed to 100% oxygen for a duration of 1 minute or 3 minutes prior to the start of testing. This study used 4 different schedules of hyperoxia treatment; 30 seconds, 1 minute, 3 minutes, and constant oxygen administration throughout the test session. The constant oxygen schedule did not show improvements in the cognitive tasks. The researchers speculated that there is a point in which an enhancing factor becomes deleterious. Working memory did not show any enhancement in any of the hyperoxia treatment groups. The researchers suggest that the placement of the working memory task at the end of the test battery may account for their finding. Overall, the authors reported that transient administration (1 and 3 minute exposures) of 100% oxygen improved attention, vigilance and long term memory. They hypothesized that the transient increase in oxygen saturation may result in a global upgrade in metabolites that enhance cognition.

Two studies done by Scholey et al (1998 & 1999) also reported cognitive improvements after exposure to hyperoxia. The study done in 1998 (20 participants with a mean age of 21 years) found enhanced word recall in test subjects exposed to 100% oxygen 5 minutes prior to, immediately before or immediately after word presentation. This study used multiple schedules of treatment given 5 or 10 minutes prior to word presentation, or 5 or 10 minutes after word presentation. The study showed that subjects given oxygen 10 minutes before or 10 minutes after word presentation did not show any improvements. The cognitive testing was limited to word recall. The researchers examined oxygen saturation and reported that the effects of the hyperoxia treatment on

saturation were apparent immediately before, during, or after word presentation where word recall was enhanced. This supports their hypothesis that the additional blood oxygen resulting from the hyperoxia treatments is utilized through the neural mechanisms responsible for memory formation. The study done in 1999 by Sholey et al. had similar findings. Subjects (34 participants with a mean age of 21 years) were exposed to hyperoxia for 1 minute and then tested for reaction time and word recall. The subjects who received the hyperoxia treatments recalled more words and had faster reaction times. Alongside cognitive testing, oxygen saturation was also measured. A significant increase in blood oxygen saturation was seen in the subjects exposed to the hyperoxia that persisted from the gas administration phase through the word presentation phase of the experiment. This finding, in conjunction with the improvements seen in word recall, led the authors to conclude that the elevated blood oxygen is utilized by task-sensitive neural substrates during period of cognitive processing. In all of the studies listed above it is important to note that they utilized an acute administration of oxygen. The effects of longer-term oxygen administration (for a period of several hours rather than minutes) on cognitive performance could, therefore, have drastically different results. It is also important to note that hyperoxia has not been found to have deleterious effects on the cognitive function of healthy individuals. A study done by Prior & Chander (1982) found that hyperoxia exposure (12 hour treatment) post-surgery to elderly patients did not show any deleterious cognitive effects. It is important to note that cognitive testing was performed shortly after hyperoxia exposure (not months thereafter).

There has only been limited research done on the behavioral effects of hyperoxia in rodent models. A study done by Fukui et al (2001) examined learning and memory in

a rat model for oxidative stress. The study involved three different ages of rats; 3 months, 15 months, and 25 months. An additional group of animals were fed a vitamin-E deficient diet for 9 weeks prior to testing. The animals were given time to learn the Morris water-maze task prior to exposure to hyperoxia. After the animals had adequately learned the task they were exposed to 100% oxygen for a lengthy 48 hours prior to the memory retention phase of Morris water-maze testing. Performing retention trials daily for up to 14 days after hyperoxia exposure, they claim that not all of the animals could relearn the task suggesting that the damage done during the treatment was long lasting. The methods used to conclude that the animals could not relearn the task were somewhat confusing. By performing repeated probe trials the researchers were actually assessing “memory-extinction” (long-term memory) rather than the animals’ ability to relearn. Also the study lacked a full explanation of the statistical measures used in their behavioral analysis. However incomplete this study may be, these findings do point towards the toxic nature of long-term exposure to hyperoxia in a rodent model

Pathological and Physiological Effects of Hyperoxia

Hyperoxia is commonly used in hospitals to treat asthma, chronic obstructive pulmonary disease (COPD), premature babies, patients with severe head injury, etc (Pagano & Barazzone-Argiroffo 2003). The use of hyperoxia in severe head injury has been controversial. A study done by Tolia, et al (2004) revealed benefits of using hyperoxia in patients with head injuries. Using patients with severe head injuries exposed to 100% oxygen for 24 hours the researchers examined five cerebral metabolic markers. They found increased glucose levels, decreased glutamate and lactate levels, and reduced intracranial pressure in the group exposed to hyperoxia. Another study done

by Magnoni, et al (2003) also found decreases in lactate. However, they reported no differences in glucose and glutamate levels. A decrease in lactate has also been reported by Schaffranietz, et al (2000) in patients exposed to hyperoxia. These differences may be attributed to the fact that the patients used in the Magnoni, et al (2003) study only received 3 hours of hyperoxia. Of course, treatments with hyperoxia also increase a patients' oxygen saturation making it beneficial to anyone suffering from a disease in which low blood oxygen saturation is a symptom (Kergoat & Faucher, 1999).

While hyperoxia does have some limited benefits there is growing evidence that the treatment has many harmful side effects resulting in permanent damage. One of the most profound and widely documented effects of hyperoxia is found in the vasculature. Hyperoxia is a potent cerebral vasoconstrictor (Ouattara et al, 2004). A study done in humans using MRI to analyze the influence of hyperoxia on cerebral blood flow reported that during hyperoxia, patients had diminished regional cerebral blood flow in all regions except in the parietal and left frontal gray matter (Kolbitsch et al, 2002). The exact mechanisms underlying oxygen induced vasoconstriction are still under investigation. The enzyme super-oxide dismutase (SOD) alongside the signaling molecule nitric oxide (NO) is thought to regulate vasoreactivity. SOD catalyzes the dismutation of superoxide radical (O_2^-) to hydrogen peroxide. Approximately 5% of inspired oxygen is converted to the dangerous superoxide radical (Fridovich, 1983). The SOD class of enzymes plays an important role in antioxidant defense mechanisms. SOD enzymes are implicated in diseases involving oxidative stress such as AD. NO is a diffusible gas that is found in neurons, macrophages and epithelial tissues. In epithelial tissue it functions to relax smooth muscle in the arteriole walls resulting in vasodilation. A study by Johnson (2001)

has shown that O_2^- reacts with NO to form the toxic peroxynitrite ($ONOO^-$).

Peroxynitrite is a potent oxidant that can react with DNA, proteins, and lipids leading to cellular damage. Johnson (2001) also suggested that increased levels of brain $ONOO^-$ can further worsen the damage caused by the overactive microglia, further advancing the progression of AD. A study done by Demchenko, et al (2002) investigated the role of SOD in oxygen-induced cerebral vasoconstriction. The study revealed that SOD promotes cerebral vasodilation by scavenging free O_2^- in a normal state system. During hyperoxia, they found that the effects of brain NO were decreased due to the increased O_2^- production, resulting in vasoconstriction. Thus, SOD works by regulating the availability of O_2^- to act as a vasoconstrictor by degrading the radical, allowing for NO induced vasodilation. In a study done by Park, et al (2005) the effects of NADPH oxidase-derived ROS and $A\beta$ were investigated in regards to cerebrovascular dysfunction. The authors also came to the conclusion that the effects of ROS mediating cerebrovascular dysfunction involve reduced bioavailability of the vasodilator nitric oxide. A study done by Sjoberg, et al (1999) quantified the vasoconstriction seen in the CNS during hyperoxia in a pig model for hyperoxaemia (condition in which the arterial partial pressure of oxygen exceeds 80 mm Hg). They reported that after 25 minutes of administration of 70% oxygen they found an 11% reduction in capillary blood flow, alongside a significant increase in cerebrocortical tissue oxygenation. This decrease of blood flow may be a protective mechanism. By reducing the brains exposure to the toxic high levels of oxygen that are generated during hyperoxia, the brain may be reducing the possible damage incurred.

As mentioned prior, the AD brain contains elevated levels of A β . The increased brain A β levels seen in the APP_{sw} mouse model may play a contributory role towards their increased susceptibility to brain ischemic injury (Xu et al, 1998). Alongside these findings it has also been reported in a study by Iadecola, et al. (1999) that APP₇₁₇ transgenic mice have an alteration in cerebrovascular regulation; specifically, there is a reduced vasodilatory response to acetylcholine and an enhanced response to vasoconstrictors. This suggests that the impairment seen in cerebrovascular responsiveness results in vasculature more prone to vasoconstriction. Several additional studies have been done further investigating the effects of A β on endothelial dysfunction. An *in vitro* study was done by Thomas, et al (1997) revealed that A β increased cerebral vasoconstriction, and decreased vasodilation. The authors also reported that the A β induced endothelial cell damage, apparently caused by reactive oxygen radicals produced by A β . Also, the authors suggest that the vascular damage done by A β may be an early event in AD. Another *in vitro* study done by Crawford, et al (1998) further supports the finding that A β enhances vasoconstriction in rat aortae. Specifically, the authors looked at the effects of A β ₁₋₄₀ & A β ₁₋₄₂ on vasoconstriction. They found that A β ₁₋₄₀ had a more profound vasoconstrictive effect than A β ₁₋₄₂. Interestingly, the authors also reported that the endothelium is not required for vasoactivity. A β -induced vasoactivity is seen immediately after exposure to solubilized A β , and the lack of requirement of the endothelium suggest that the imbalance between NO and O₂⁻ works alongside A β to further increase vasoconstriction. These authors also suggested that chronic vasoconstriction would result in subclinical ischemia that would in turn stimulate increased A β formation around the vasculature. *In vivo* studies have also been done

providing additional evidence that A β serves as a vasoconstrictor. A study done by Arendash, et al (1999) revealed that spontaneously hypotensive rats infused with A β_{1-40} experienced substantial increases in mean arterial blood pressure (MAP). Hypertension has been recognized as a risk factor for AD (Kokmen, et al, 1991), and longitudinal studies have shown that elevated blood pressure is associated with development of the disease 10-15 years later (Skoog, et al, 1996). The authors suggest that the disease process itself may induce the onset of hypertension during the 10-15 years before clinical onset. Also the author suggests that the A β -induced vasoconstriction demonstrated in the rat model may play a contributory role to the AD process in humans. Another study done by Suo, et al. (1998) reported that rats infused with A β_{1-40} had decreased cerebral blood flow and increased cerebro-vascular resistance. This study found that A β specifically affected cerebral vasculature, suggesting that the cerebral hypoperfusion that is observed in early AD may be the result of A β induced vasoconstriction. Consistent with the findings in murine models, human studies using PET have also revealed that in the AD brain there is reduced vascular activity to vasodilatory stimuli (Mentis et al, 1996; Warkentin & Passant, 1997).

The pathological effects of hyperoxia in rodent models have been much more extensively studied. A study done by Urano, et al (1997) using a rat model investigated the morphological changes through electron microscopy in the brain associated with exposure to 100% oxygen for 48 hours followed by immediate sacrifice. They found swollen astrocytes around vessels, deformed nerve cell nuclei, swollen mitochondria, and abnormal accumulation of synaptic vesicles in swollen nerve terminals. Also, they reported changes in the plasma membrane including decreased membrane fluidity,

increases in membrane permeability to sucrose, and an increased cholesterol/phospholipids ratio of the membrane. The authors suggest that the increased amount of free radicals generated may damage nerve terminals and peroxidize the plasma membrane.

Several studies have been done examining the cellular and biochemical effects that hyperoxia has on the brain. The effects of hyperoxia on inducible nitric oxide synthase (iNOS) expression has been studied in rat pups by Hoehn, et al (2003). Seven day old rat pups were exposed to >80% oxygen for 24 hours, immediately sacrificed, and then the amount and distribution of iNOS was examined. Biologically, iNOS is an enzyme responsible for synthesizing NO (a vasodilator). The study revealed that animals exposed to hyperoxia had increased total brain iNOS levels. The increased iNOS lead to the formation of peroxynitrite ($\text{NO}^- + \text{O}_2^- \rightarrow \text{ONOO}^-$), a toxic molecule that can cause oxidative cellular damage. The researchers concluded that the increase in iNOS and resultant increase in peroxynitrite may lead to subsequent damage to brain structures. Another study done by Mamdouha, et al (1986) also looked into the effects of hyperoxia on brain structures and found neuronal necrosis. The study utilized a series of different schedules of alternating hypoxia/hyperoxia treatments (100% O₂ for 3 hours) in both young and adult rats that were immediately sacrificed following treatment. The study revealed extensive neuronal karyorrhexis in the subiculum, cingulated cortex, thalamus, and reticular formation in newborn rats exposed to hyperoxia. The more mature and the adult rats did not develop any neuronal karyorrhexis demonstrating their ability to cope with the treatment better. The researchers hypothesized that the selective vulnerability of the immature brain was due to lack of full development of the antioxidant

defense mechanisms seen in the adult animal. Also, the researchers hypothesized that the neuronal necrosis seen in the newborn rats may be the result of lipid peroxidation of the cell membranes due to the antioxidant defense mechanisms becoming overwhelmed. An additional study done by Taglialatela, et al (1998) was done examining effects of antioxidants in rats exposed to hyperoxia. The study used two groups of newborn rats; one control group and one group given Buthionine sulfoximine (BSO) a glutathione synthesis inhibitor. Glutathione is a powerful antioxidant and can provide protection for the mitochondria against oxygen radicals. The animals were exposed to 95% oxygen for 5 days, sacrificed immediately, and measured for nerve growth factor protein (NGF), glutathione, and the extent of apoptosis. The study revealed that hyperoxia decreased the amount of NGF protein, and that the animals treated with both the BSO and hyperoxia had a substantial increase in brain apoptosis. The authors suggest that the oxidative stress of hyperoxia in conjunction with limited glutathione resulted in neuronal damage. Thus, several studies have revealed that in a brain that is limited in its antioxidant capabilities hyperoxia can induce neuronal damage. It is important to note that the above studies (Hoehn et al, 2003; Mamdouha et al, 1986; Taglialatela et al, 1998) sacrificed the animals immediately following the hyperoxia treatment limiting the scope of effects to only the immediate and short-term effects.

Numerous studies examining the effects of hyperoxia in lung tissue have also been conducted. Hyperoxia has been widely documented to cause extensive alveolar cell death through mechanisms still under debate (Pagano & Barazzzone-Argiroffo, 2003). A study done by Buccellato, et al (2004) examined the role of reactive oxygen species (ROS) such as O_2^- in the induction of cell death. The researchers exposed rat epithelial

cells to hyperoxia and found that the treatment resulted in activation of Bax in the mitochondrial membrane with subsequent cytochrome c release and cell death. Bax is a pro-apoptotic protein that is involved in regulating programmed cell death (De Smet et al, 2004). The researchers hypothesized that Bax activation was dependent on the generation of ROS. Hyperoxia has been shown to generate intracellular production of O_2^- and H_2O_2 by the mitochondria (Freeman & Crapo, 1981). Another study done by Pagano, et al (2004) focused on the role of cytochrome c (a cell death promoting factor) in the alveolar cell death seen during hyperoxia in a rat model. Hyperoxia induces high amounts of cytochrome c to be released from the mitochondria into the surrounding cytosol. Their study revealed that by blocking the mitochondria from releasing cytochrome c through administration of cyclosporine A, lung tissue could be protected from damage during hyperoxia treatments. While the exact biological events are not yet fully understood it is apparent that mitochondria play an integral role in hyperoxia induced cell death. It is witnessed by several studies that hyperoxia has an exacerbating effect on antioxidant defense systems. The toxic nature of excess free radicals can result in a multitude of damaging effects such as apoptosis, mitochondrial dysfunction, increases in harmful enzymes, and structural changes.

Linking Hyperoxia and Precipitation of AD

A study done by Shua-Haim, et al (1998) suggested a link between surgery in the elderly and precipitation of AD. The study reported an acute onset of AD post-surgery. The acute onset is in contradiction to the definition by the NINCDS-ADRDA that states the onset of AD is gradual. This suggests that the patients may have been suffering from a “pre-clinical” form of AD prior to the surgery. The surgery including all of the

treatments surrounding surgery resulted in a rapid neurological degeneration and subsequent diagnosis of AD. This study suggests that some aspect of surgery or post-surgical care may act as a risk factor in aged individuals.

Along this line, a study was done by Newman, et al (1995) investigating a number of factors surrounding surgery and their predictive value for cognitive decline. The study revealed that the strongest predictor of cognitive decline post surgery is age. The mean arterial pressure (MAP) was also examined, and the study revealed that in older patients MAP may play a role in post-surgery cognitive decline. Also they looked at the apolipoprotein E- ϵ 4 allele, the gene variant associated with the sporadic form of AD, as a genetic predictor. They found a significant association between the presence of the apolipoprotein E- ϵ 4 allele in patients who had declines in cognitive performance post-surgery. This evidence further point towards the exacerbating effects that surgery has on patients who may be at a higher risk for developing AD.

Surgery involves numerous components including anesthesia, administration of numerous drugs, changes in blood pressure, and possible exposure to hyperoxia post-surgery. Studies trying to tease out one variable as being the causative agent for the cognitive deterioration are difficult as the typical surgery incorporates many complicating factors. Several studies have attempted to isolate the effects of anesthesia. A recent retrospective hospital-based case-control study done by Gasparini, et al (2002) reported finding no association between exposure to anesthesia and development of AD. The study examined 115 AD patients' hospital records for exposure to anesthesia and found that the exposure to anesthesia 1 and 5 years prior to disease onset was not correlated to future development of AD. An older study done by Bohnen, et al (1994) further

supports their findings. In a retrospective, population based, case-control study they looked at the exposure of 208 AD patients and found that it is unlikely that even multiple exposures to anesthesia increases the risk of developing AD. However, the same authors also reported that the age at which an individual was exposed to and extent of anesthesia given may play a more important role. The study investigated the cumulative exposure to anesthesia for more than 40 years prior to onset of AD, and found that the age of AD onset was inversely related to the cumulative exposure to anesthesia before the age of 50. The authors suggest that the onset of AD may be related to exposure to anesthesia at a relatively earlier age.

Blood pressure has also been investigated with conflicting findings. A study done by Moller, et al (1998) reported that hypotension (abnormally low blood pressure) was not a significant risk factor in predicting cognitive dysfunction post-surgery. However, recent work by Dr. T. Monk (personal communication) has suggested that hypotension can be extremely dangerous to a patient during surgery. Dr. Monk conducted a study in which he found that for every minute that a patient's systolic blood pressure dropped below 80 mmHg there was a 4% increase in the chance of death within one year post-surgery. These findings are very alarming, as they point towards the extremely dangerous nature of hypotension during surgery. It also points towards the irreversible effects that hypotension exerts on the body. Hypotension can result in a state of hypoperfusion in the brain. The steep drop in blood pressure results in a decrease of oxygenated blood being available to adequately sustain the metabolic demands of the brain. Patients that exhibit hypotension during surgery are given treatments with hyperoxia post-surgery in order to boost a patient's blood oxygen saturation (J. Robert,

personal communication). He hypothesizes that hypotension alters the bodies inflammatory response. Treatment with anti-inflammatory agents post surgery has been found to reduce the number of deaths seen 1 year post surgery.

Hyperoxia treatments are frequently given to patients post-surgery to increase the oxygen content of blood, but it also acts as a potent cerebrovasoconstrictor resulting in decreased cortical blood flow (Rostrup et al, 95). As a vasoconstrictor, it is plausible that treatments with hyperoxia could also result in a hypoperfusion of the brain.

Hyperoxia exposure alone has not been shown to have deleterious cognitive effects as discussed in several studies above. However, the studies conducted looking at its effects were done on either young individuals or individuals not proven to be at risk for developing AD. The evidence presented above linking surgery and precipitation of AD alongside the knowledge that hyperoxia has the potential to cause a variety of toxic side effects has provoked a study to be conducted investigating hyperoxia as a potential causative agent of cognitive impairment in aged individuals post-surgery, who have subclinical AD.

Specific Aims

Hyperoxia has been proven to generate free radicals (O_2^-) thereby inducing oxidative stress, confer structural damage in nerves, upregulate enzymes responsible for cellular death, peroxidize membranes, and decrease cerebral blood flow. These deleterious effects have been discovered using both human and animal models that had compromised/immature antioxidant defense systems such as those seen in the studies using infant models or in the human studies using patients with traumatic brain injuries.

Oxidative stress is one of the primary pathological mechanisms underlying AD. As mentioned earlier, oxidative stress plays a critical role in the early stages of the disease; perhaps, paving the way for more severe neuro-degeneration (Pratico & Sung, 2004). An increased level of A β (both soluble and insoluble) is also one of the primary pathological mechanisms underlying AD. A β has been shown in the numerous studies mentioned above to act as a vasoconstrictor and as a generator of free radicals. Both A β and hyperoxia are capable of generating superoxide radicals, causing cerebrovascular constriction, thus decreasing cerebral blood flow (brain hypoperfusion). I propose that hyperoxia treatments given to cognitivel- normal APP/PS1 transgenic mice will work in combination with their brain A β to markedly increase production of superoxide radicals, causing oxidative stress damage to brain tissue and result in cerebrovascular constriction with subsequent cerebral hypoperfusion. I propose that the damage done by the increase in oxidative stress and cerebral hypoperfusion will result in cognitive impairments.

In this study I specifically propose to expose Alzheimer's transgenic mice to several hyperoxia treatments and to:

- 1) Assess the cognitive function of the mice before and after hyperoxia treatment in the Radial Arm Water Maze task of working memory
- 2) Measure the amount of oxidative stress incurred through examination of markers of lipid peroxidation in specific brain regions
- 3) Quantify brain A β loads, both diffuse and compact

- 4) Perform neurodegenerative cell staining in specific hippocampal regions
- 5) Perform correlation analyses examining any possible relationships between pathological, neurochemical, and behavioral measures.

Materials and Methods

Animals

A total of 20 mice were utilized during the course of this study. All mice contained a mixed background of 56.25% C57, 12.5% B6, 18.75% SJL, and 25.5% Swiss Webster. The animals genotypically fall into one of three categories; nontransgenic, double transgenic (containing both the APP/PS1 mutations), and single transgenic animals (containing the APP mutation only). All of the mice were bred from a cross between a P (parental generation) heterozygous male mouse carrying the mutant APP_{K670N, M671L} gene with a F1 PS1 (transgenic line 6.2) female mouse to obtain the F2 generation including APP/PS1, APP, PS1, and non-transgenic mice. After weaning, the mice were genotyped and singly housed in cages with rodent chow and water *ad libum*. Mice were maintained in a 10 hour dark 14 hour light cycle, with all behavioral testing done during the light cycle. After the initial round of pre-treatment RAWM testing, transgenic animals were broken into two behaviorally-balanced groups; the hyperoxia group (O₂) and the control group (air). As shown in Table 1, the group of transgenic mice that were exposed to hyperoxia consisted of 3 APP/PS1 mice and 2 single

transgenic APP mice. Pre-treatment performance of the 3 double transgenics and the 2 single transgenic mice was found to be nearly identical, and thus clearly not significant different ($p= 0.7$ for overall T4 and T5). The control group of animals that were exposed to compressed air consisted of 6 APP/PS1 mice, whose behavior was statistically similar to the hyperoxia group (determined by analysis of pre-treatment behavior). A total of 8 non-transgenic mice were used as behavioral controls and were not exposed to either hyperoxia or air treatment. For the biochemical portion of the experiment, the same 8 non-transgenic mice were used as controls (Table 1). Also the same group of 6 APP/PS1 control (air exposed) mice were used as the control group in the biochemical portion of the experiment. The hyperoxia group of animals consisted of 4 APP/PS1 mice. The addition of the extra mouse as compared with the group of 3 APP/PS1 mice used in the behavioral portion is explained due to the fact the fourth mouse was deleted from the behavioral portion of the study. The animal was deleted due to the fact that it was statistically determined to be an outlier after completing the mid-point portion of behavioral testing. The 2 APP animals exposed to hyperoxia were not included in the biochemical portion of the study due to genotype differences.

Genotype & Treatment	Behavior	Biochemical
Non-Transgenics	8	8
APP/PS1 Controls (Air)	6	6
APP/PS1 O2	3	4
APP O2	2	0

Table 1. Animals included in the behavior and biochemical portions of the study.

General Protocol

At 3 months of age, 9 APPsw+PS1, 2 APP transgenic mice and 8 non-transgenic littermates were pre-tested in the radial arm water maze (RAWM) task of working memory for 15 days. The transgenic (Tg+) mice were then divided into two groups balanced in cognitive performance from the RAWM pre-test. One group of Tg+ mice were exposed to 100% oxygen for 3 hours. The remaining Tg+ mice received the same treatment, except that normal air flowed through the gas chamber. The non-transgenic mice were not exposed to any treatment. Beginning three days following gas treatment, all animals were re-tested in the RAWM task for 15 days, with the two groups of Tg+ mice showing no differences in performance. At 5½ and 7 months of age, all animals received a second and third gas treatment, respectively. Final RAWM testing for 9 days was begun three days after the 3rd gas treatment. At the completion of testing, all mice were euthanized and their brains processed for: 1) A β staining with 6E10 antibody and Thioflavin S, 2) degenerative neuronal numbers (acid fuchsin/toulidine blue staining), 3) lipid peroxidation markers (Iso-Furans and 8-IsoProstane), and 4) hippocampal APP, APOE, COX-2, and GFAP levels.

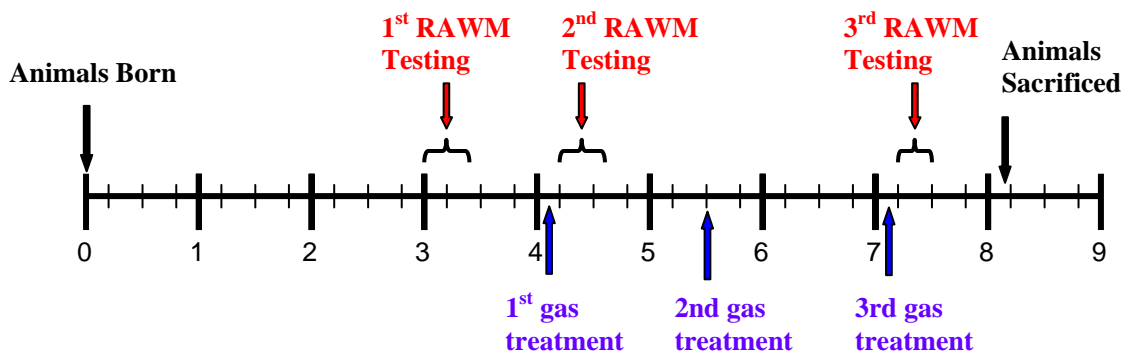


Fig.1. General protocol time line for the hyperoxia study.

Hyperoxia

All animals were food-deprived for 24 hours prior to O₂ gas insult. For gas treatments, a multi-chambered apparatus was constructed of plexiglass with input valves allowing for the flow of 100 % oxygen/compressed air into the chamber. For all gas insults, airflow per minute was regulated by a flow meter and maintained at 1.5x chamber volume. Treatments were conducted under isobaric conditions. Bara-Lyme was used in each chamber to control for excess CO₂. Each gas treatment was administered for 3 hours. The control gas treatments were conducted in the same manner as the hyperoxia with the exception that compressed air (20% O₂) was utilized. All animals received a total of three treatments, administered at approximately 6 week intervals (Fig. 1). The first treatment was given when the animals were 4 months of age. The second treatment was given at 5.5 months of age and the last treatment was given at 7 months of age.

Behavioral Assessment

Spatial working (short-term) memory was assessed in the radial arm water maze task (RAWM). The task is conducted in a 100-cm diameter inflatable pool with an aluminum insert. The aluminum insert creates 6 radial swim arms (30.5 cm length x 19 cm width), with each swim arm radially distributed in the pool from a central circular swim area 40 cm in diameter. The insert extended 5 cm above the surface of the water. The water was maintained at 23-27° Celsius. A transparent escape platform (9 cm diameter) was placed in one of the arms, submerged 1.5 cm below the surface of the water. Around the perimeter of the pool and on an adjacent wall visual/spatial cues were placed. The visual cues consisted of large brightly colored objects distinct in shape. For example, the visual cue located at the end of arm 2 was a suspended beach ball, and the

visual cue at the end of arm 6 was a small closed umbrella. The visual cues were used by animals to orient themselves within the maze and facilitate finding the escape platform. On each day of testing, the animals were given five 1 min. trials. Each trial lasted for 1 minute, with a 30 second delay between trials 1-4 (during which the mouse remains on the platform), and a 30 minute delay between trials 4 and 5. Trials 1-4 are acquisition trials in which the animals are learning the location of the escape platform for that day. The last of four consecutive acquisition trials (trial 4, T4) and the delayed retention trial (trial 5, T5) are indices of working memory. On any given day, the escape platform location is placed at the end of one of the six swim arms. The platform location was moved daily to a different arm in a semi-random fashion. By moving the escape platform, the animal must learn a new location of the platform daily and rely on working memory rather than long-term memory. On each day, different start arms for each of the five daily trials were also selected in a semi-random fashion that incorporated all five arms. At the beginning of any given trial, the mouse was placed into the start arm facing the center of the pool and given 60 sec. to find the platform, with a 30 sec stay. For each trial, the latency (amount of time in sec) and number of errors to find the submerged platform in the goal arm were recorded. An error was recorded when an animal swam into an arm that did not contain the escape platform. Each time an animal made an error, the researcher gently pulled the animal out of the wrong arm and guided the animal back into the start arm. If the animal failed to make an arm choice for 20 sec, or if the animal entered the platform-containing arm but failed to locate the platform, then an error was also recorded and the animal was brought back into the start arm for that trial. An error of 4.4 was given to any animal that did not make at least three choices during a given trial

for the pre-treatment test point. An error of 7.4 was given to any animal that did not make at least three choices during a given trial for the post-treatment test point. The numbers 4.4 and 7.4 were calculated by averaging errors for all animals that did not locate the platform for block 1 (day 1-day 3) on trial 1 (T1). The animals were tested at three different time points: before treatment (15 days), mid-treatment (15 days), and post-treatment (9 days).

Brain Collection and Dissection

Following the third and final behavioral testing in the RAWM, animals were deeply anesthetized with pentobarbital (100mg/kg) and perfused with 100ml of 0.9% saline. Post mortem brains were immediately removed and bisected sagittally. The left hemisphere was fixed in 4% paraformaldehyde. The right hemisphere was chilled in cold saline for 1 minute and then dissected into 5 major brain regions; cerebellum, anterior cortex, striatum, posterior cortex, and hippocampus. The individual brain regions were transferred into individual 1.5 ml Eppendorf tubes and then were quick frozen at -80°C on dry ice for biochemical analyses. The left hemisphere was stored in 4% paraformaldehyde overnight (12 hrs) and then transferred to a graded series of sucrose solutions (stored continually at 4°C) beginning at 10% and finishing at 30%, where tissues remained until sectioning. Tissues were coronally sectioned on a sliding microtome at 25 μ m for A β immunohistochemistry and histology.

Histological Analysis

Acid Fuchsin/Toulidine Blue Staining. Degenerative neurons were stained using a combination of acid fuchsin and toulidine blue staining. Acid fuchsin is an anionic dye that strongly stains the nuclei and cytoplasm of necrotic neurons due to high levels of

proteins rich in arginine and lysine (Kiernan et al., 1998; Victorov et al., 2000). The acidophilia, or strong attraction to acidic dyes, is considered to be a hallmark of neuronal damage and death that may be due specifically to brain ischemia. Toulidine blue is a basic metachromatic stain that stains nuclei dark blue and cytoplasm light blue and serves to intensify staining. Degenerative cells have an irregular atrophied shape and a much darker stain, denoting the acidophilia associated with degeneration. To begin staining, 25 μm coronal sections of brains stored at 4° C in PBS (including hippocampal regions) were mounted on gelatin prepared slides. The slides were brought through a standard rehydration scheme beginning with xylene and progressing from 100% EtOH to 40% EtOH and finishing in two water baths. Rehydrated sections were then dipped in 1% acid fuchsin solution for approximately 35sec. The acid fuchsin stained slides were washed twice in water and then dipped in toluidine blue solution for approximately 45 sec and rewashed in two water baths. After air drying, slides were coverslipped for microscope analysis.

The first analysis performed (semi-quantitatively) scored the degree of cell degeneration in the dentate gyrus associated with the dorsal hippocampus. For any given animal, 5 sections (spaced at least 75 μm apart) were scored using a scale of 0-4 and averaged. In the scale, 0 represents an animal with only 1-2 degenerative cells visible along the superficial granule cell layer, 1 represents an animal with 3-6 sporadic cells along the superficial granule cell layer, 2 represents 7-15 degenerative cells along the superficial layer, 3 represents lines of degenerating cells along the superficial layer, and 4 represents an animal with lines of degenerating cells along the superficial granule cell layer alongside clusters of degenerating cells penetrating deeper layers. Analyses were

conducted blind to genotype and treatment at 20x magnification on a Zeiss MC-63A microscope. Degenerative cell counts were performed in the CA1&2, CA3, and CA4 regions of the dorsal hippocampus. All counts were done on 5 representative sections (spaced at least 75 μm apart) per animal and averaged.

6E-10 & Thioflavin S staining. Staining with the 6E-10 antibody detects both compact and diffuse A β plaques. In brief, 25 μm sections were mounted on pre-treated slides and processed through standard heat induced epitope retrieval steps beginning in 25mM citrate buffer (pH 7.3). Sections were incubated overnight at 4°C with the primary antibody; an anti- A β antibody (6E-10 purchased from Signet) diluted 1:2500. A secondary antibody, anti-mouse IgG was used and sections were developed with a NovaRed (Vector) substrate kit. Slides were then brought through a dehydration scheme (beginning with water proceeding through graded series of alcohols and ending in a 5 sec xylene dip). Finally slides were coverslipped with a xylene-based mounting media for microscope analysis.

Thioflavin S staining was used to only detect compact (dense) A β deposits. Sections (25 μm) mounted on gelatin dipped slides were immersed in 1% Thioflavin S in 50% EtOH for 5 minutes. Sections were then immersed in graded alcohols, followed by xylene, and coverslipped for microscope analysis.

Image Analysis. All data collected from the 6E-10 and Thioflavin-S staining was analyzed on a Nikon Eclipse E1000 microscope using either 4x (Thioflavin S) or 10x (6E-10) Plan Flour objective lenses. Images were captured using a Retiga 1300 CCD with a QImaging RGB LCD-slider. For the thioflavin S staining, a Nikon BV-2B fluorescence filter cube was used. Data from both of these stains was obtained from three

equally spaced coronal sections through the dorsal hippocampus including the overlying parietal cortex. Image analysis was performed using customized software written in Visual Basic 6.0 (Microsoft) that used Auto-Pro function calls to segment and quantify images according to the established protocols used by Costa et al (2004). A β deposition was quantified as a percent of area of interest ($=\text{Area stained}_{\text{total}}/\text{Area Measured}_{\text{total}}$).

Neurochemical Analysis

Lipid Peroxidation Measures. Isofurans and 8-isoprostanes are stable by-products of lipid peroxidation and were assayed in post-mortem brain tissue of the Tg- and double transgenic (APP/PS1) mice. The anterior and posterior cortices were sent to Dr. J. Roberts at Vanderbilt University for analysis. Briefly, isofurans and 8-isoprostanes were quantified by stable isotope dilution gas chromatography/negative ion chemical ionization mass spectrometry as described by Fessel et al. (2003).

Protein Markers. Protein level expression was assessed using western blot analysis for several proteins including: APP, APOE, COX-2, and GFAP. Tissue samples from the hippocampus were homogenized in 10mM sodium acetate buffer pH 7.2 containing 0.1% triton X-100 and mammalian protease inhibitor cocktail (Sigma Chemical). Samples were then centrifuged and adjusted to contain identical protein concentration based on the Lowry protein assay. The remaining homogenate was electrophoresed over 4-20% gradient polyacrylamide gels and transferred to polyvinylidene difluoride membranes. Blots were visualized using antibodies specific to each protein, and developed with chemiluminescence detection using horseradish peroxidase-conjugated secondary IgG. Gross differences in expression level were determined by visually comparing blot sizes.

Statistical Analysis

Behavioral Analysis. A total of three sets of behavioral data were collected from the three rounds of RAWM testing. Before statistical analysis of the behavioral data, the data was divided into 3 day blocks to aid in data presentation. Any outliers or non-performers (animals who display consistent behavior inhibiting proper performance) were eliminated from the behavioral statistical analysis. The statistical analysis included only those animals with a full data set (e.g., completed all behavioral testing); as the animals that died during the course of the study were eliminated from all behavioral analyses. The RAWM behavioral measures were analyzed with both one-way ANOVAs and two-way repeated measure ANOVAs. Following ANOVA analysis, *post-hoc* pair-by-pair differences between groups were determined through the Fisher LSD test. Differences between groups were considered significant at $p < 0.05$. Paired T-tests were performed to determine if there were any changes in each group's pre- vs. post-treatment behavior.

Histological/Biochemical. Pathological data analysis from the histological and biochemical portion of the experiment was performed using ANOVA followed by Fisher's LSD post-hoc test. Correlational analyses were conducted using the *Systat* analytical software package. Correlations were done between behavioral data and the histological/biochemical data to determine if any relationships exist.

Results

Behavior

Pre-Treatment Testing. In pre-treatment RAWM testing (Figs. 2 and 3), both Tg+ and Tg- mice performed similarly as evidenced by no overall group effect across all 5 blocks for working memory Trials 4 [$F(1,17)=0.21$, $p=n.s.$] and Trial 5 [$F(1,17)=0.27$, $p=n.s.$]. Animals in both groups collectively showed improved working memory across all 5 blocks of testing, as indicated by highly significant block effects for both Trial 4 [$F(4,68)=3.86$, $p<0.01$] and Trial 5 [$F(4,68)=10.83$, $p<0.00001$]. At individual blocks, the only significant group difference occurred during Block 2, Trial 5 of testing. During the last three blocks of testing, however, Tg- and Tg+ mice were identical in working memory performance (Fig. 2). Across all 15 days of testing, Tg+ and Tg- mice were both able to reduce their “overall” number of errors between the first semi-random trial (Trial 1) and working memory Trials 4 and 5 (Fig. 3). Thus, Tg+ and Tg- mice exhibited similar working memory performance during pre-treatment testing, with both groups able to improve performance across trials and across blocks of testing. When animals in the Tg+ group were assigned to receive hyperoxia or air treatments, the pre-treatment performance of these two sub-groups was identical. Even comparing pre-treatment performance between the 3 APPsw+PS1 and 2 APPsw mice comprising the future hyperoxia group, there were no differences in working memory.

Mid-Treatment Testing. There were no differences in working memory performance between Tg+Con and Tg+O₂ treatment groups overall or during individual blocks of performance for either T4 or T5 (data not shown).

Post-Treatment Testing. Over the 3 blocks of post-treatment testing (Figs. 4 and 5), there was a significant group effect for Trial 5 [$F(2,16)=3.56$; $p\leq 0.05$], with post hoc analysis indicating that Tg+O2 mice (but not Tg+Con mice) had impaired T5 working memory vs. Tg- controls ($p<0.02$). The group effect for T4 approached significance [$F(2,16)=2.45$, $p=0.11$] with post hoc analysis again showing impairment of Tg+O2 mice vs. Tg- controls ($p\leq 0.05$). Closer inspection of individual blocks (Fig. 4) revealed a consistent trend for Tg+O2 mice to have the greatest number of T4 and T5 errors such that, when overall T4 and T5 performance was analyzed (Fig. 5), significantly greater numbers of working memory T4 and T5 errors were evident for the Tg+O2 group vs. Tg- controls. Performance of Tg+Con mice consistently fell between the Tg+O2 and Tg- groups, indicating a non-significant trend for impairment. The impaired working memory of Tg+O2 mice is underscored by comparing overall T1 vs. T5 for each group (Fig. 5). Both the Tg- and Tg+Con groups showed a significant reduction in errors from T1 to T5 ($p<0.00001$ and $p<0.005$, respectively), demonstrating good working memory. By contrast, Tg+O2 mice could not significantly reduce their overall number of errors between T1 and T5 ($p=n.s.$). As was the case for pre-treatment testing, there was no difference in post-infusion performance of the 3 APP+PS1 mice vs. the 2 APP mice that comprised the hyperoxia treatment group (data not shown). Swim speed analysis (the number of seconds per error made) revealed that there were no significant differences between groups in swim speed (data not shown).

Pre – vs. Post-Treatment

To further elucidate any changes in working memory performance resulting from gas treatment, each group's pre-treatment RAWM performance (overall T5 errors) was

compared to their post-treatment performance (Fig. 6). Tg- mice showed a nearly-significant ($p < 0.09$; paired t-test) decrease in post-treatment T5 errors, while Tg+Con mice exhibited stable pre- vs. post-treatment performance. In sharp contrast, Tg+O2 mice made significantly more T5 errors during post-treatment testing ($p < 0.05$). The poorer working memory performance of Tg+O2 mice during post-treatment testing is further exemplified by examining the pre- vs. post-treatment performance of individual animals for all three groups (Fig. 7). The vast majority of animals in the Tg- and Tg+Con groups showed improved or stabilized performance during post-treatment testing, while four of the 5 Tg+O2 mice exhibited poorer post-treatment performance.

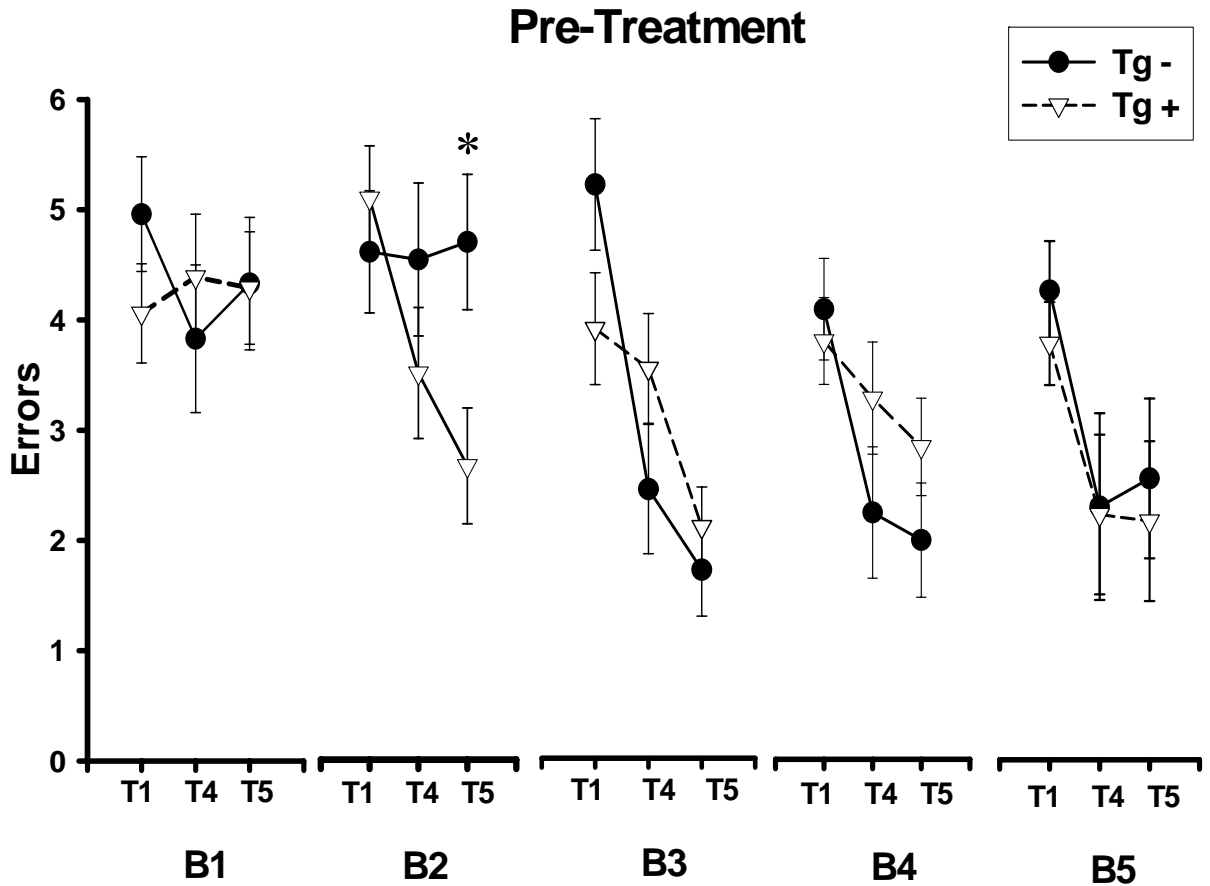


Fig. 2. RAWM pre-treatment acquisition (T1-T4) and memory retention (T5) in Tg+ mice and Tg- mice over five 3-day blocks. * = significant difference between Tg- and Tg+ ($p < .025$) indicating an impairment selectively in Block 2 (B2) in working memory (T4 & T5) for the Tg- group. Otherwise, no other group differences were seen, especially in the last 3 blocks (B4 & B5) indicating similar working memory in the Tg- and Tg+ groups during pre-treatment testing.

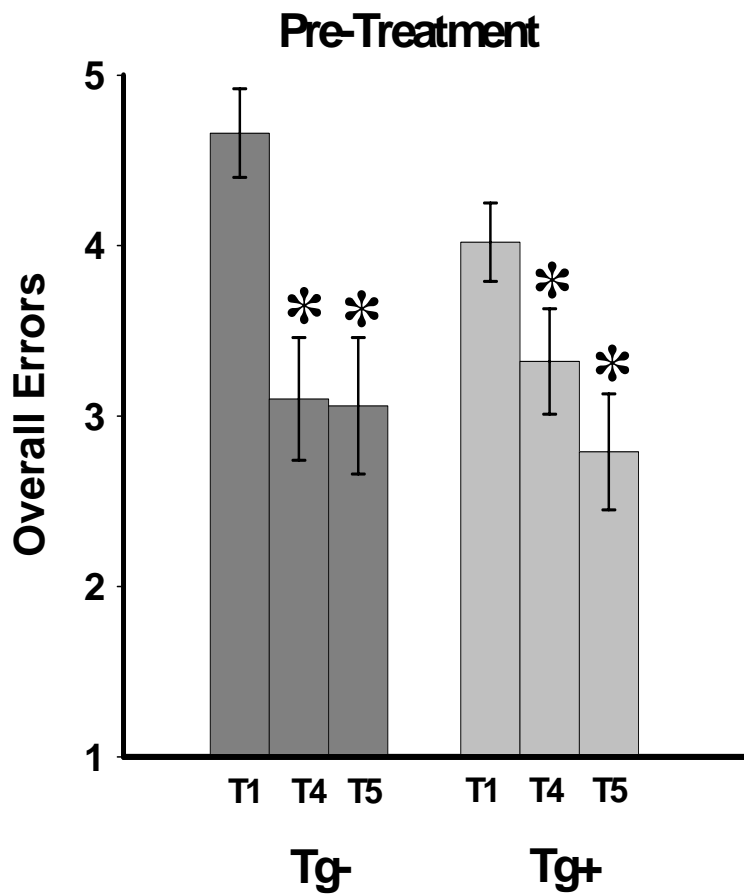


Fig. 3. Pre-Treatment acquisition (T1-T4) and memory retention (T5) overall errors (5 block average) in Tg- and Tg+ mice. Both groups of mice display the ability to significantly reduce their number of errors from T1 to T4 and T5. * $p < .04$ or higher level of significance for both groups vs. T1.

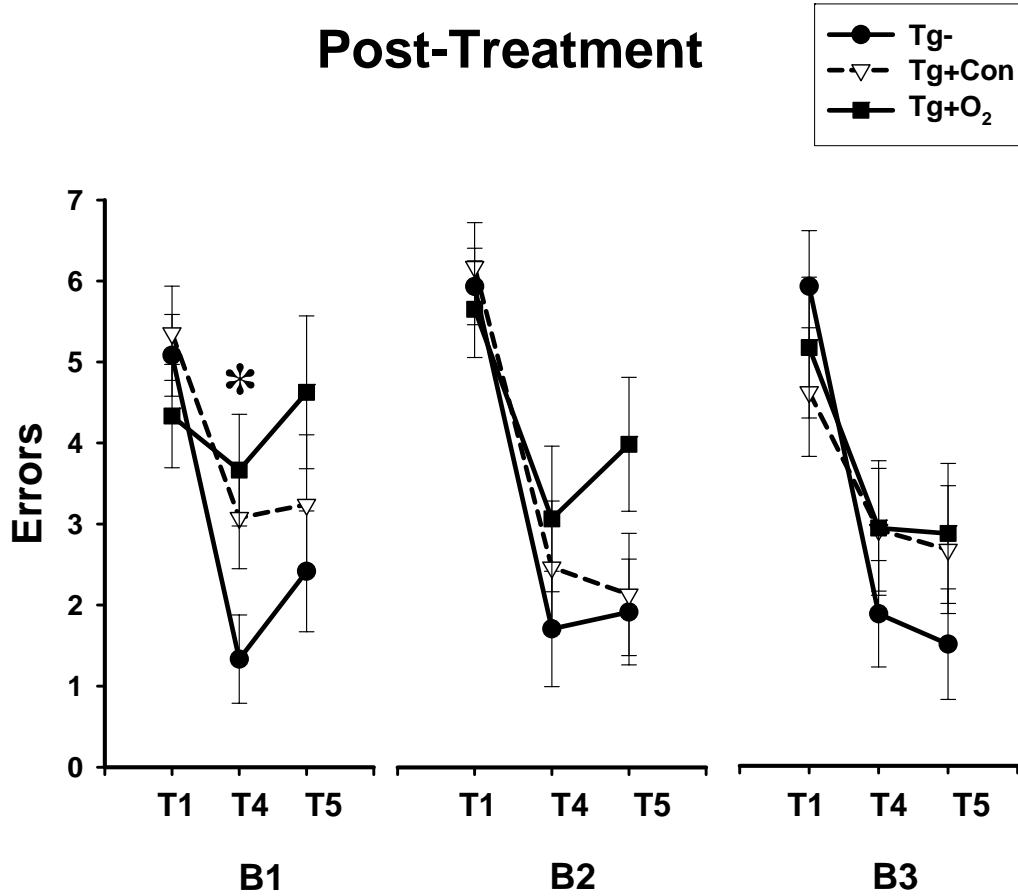


Fig.4. RAWM post-treatment working memory in Tg-, Tg+Con and Tg+O₂ mice over three 3-day blocks. * = significant difference between Tg- and Tg+O₂ ($p < .02$). During working memory trials 4 & 5 a trend for the Tg+O₂ group to make more errors than Tg+Con mice vs Tg- is particularly evident during the first two blocks.

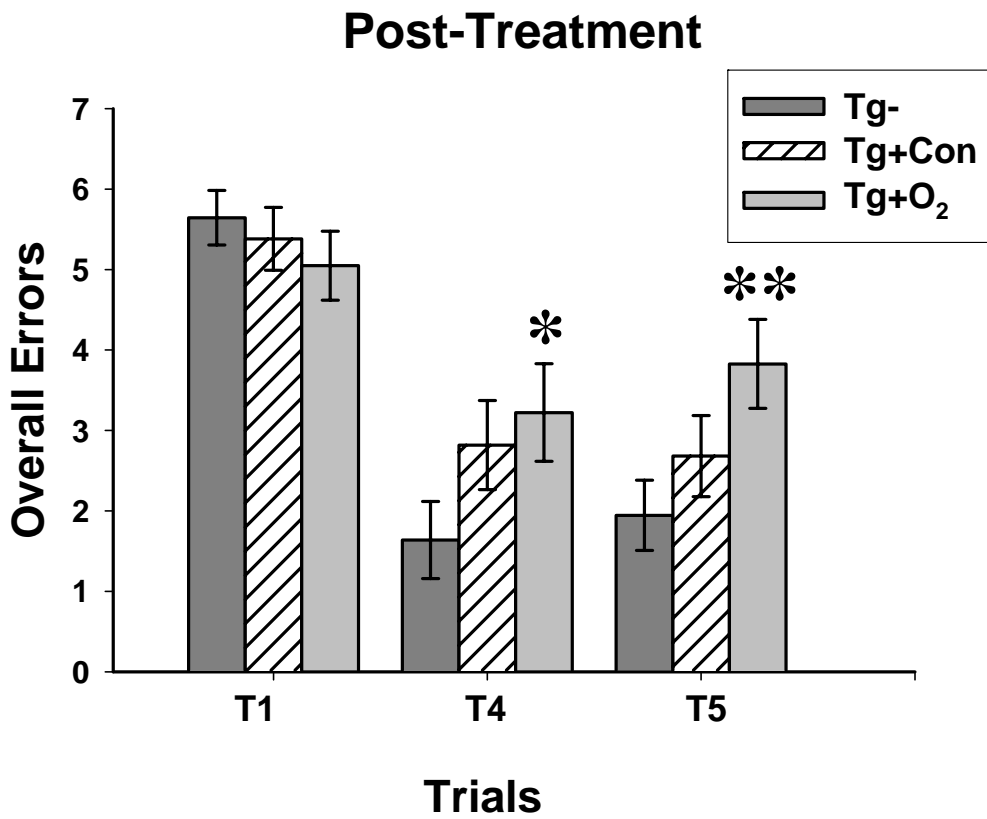


Fig.5. Overall RAWM post-treatment working memory performance across 9 days of testing. Tg+O₂ mice were significantly impaired in comparison to Tg- mice during both T4 overall (* $p \leq .05$) and T5 overall (** $p < .02$). Tg+Con mice remained unimpaired.

Pre- vs. Post-Treatment

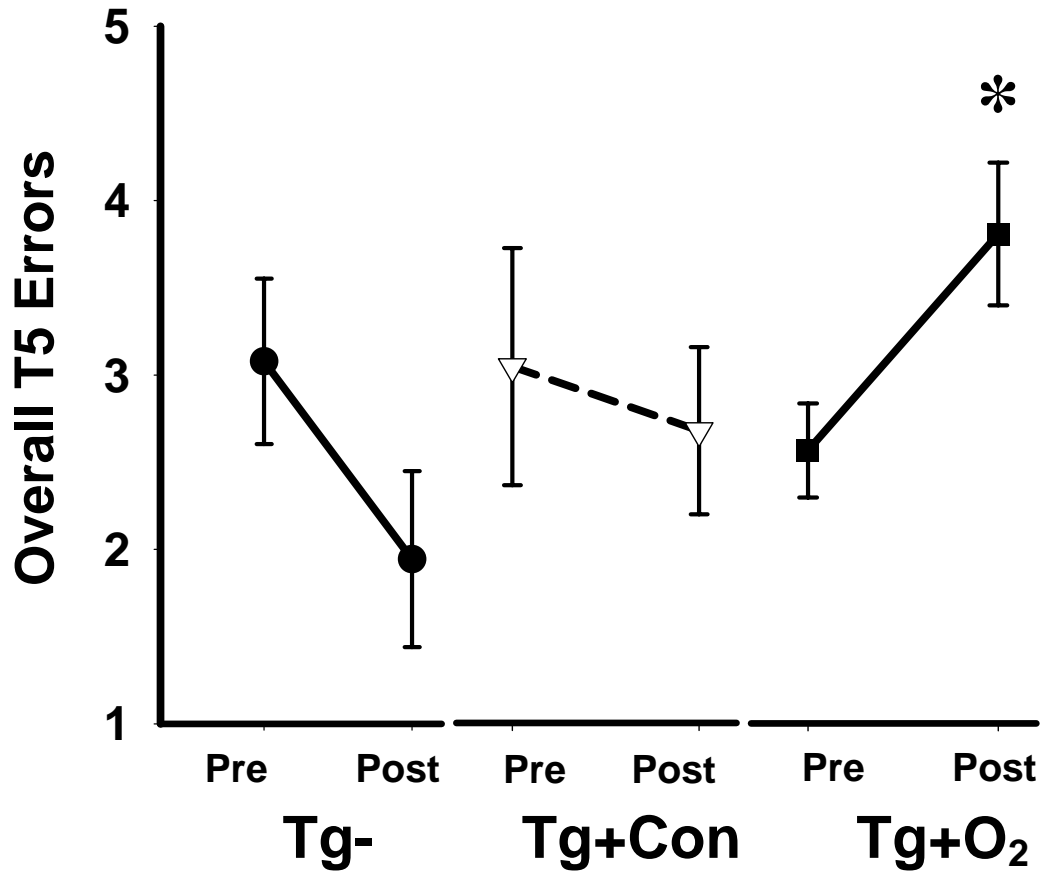


Fig.6. RAWM pre- vs. post-treatment overall T5 Errors for the Tg-, Tg+Con, and Tg+O₂ groups. * $p < .05$ (paired t-test) pre-treatment vs. post-treatment overall T5 errors in the Tg+O₂ group. Both the Tg- and Tg+Con group displayed a trend to reduce their number of errors from the pre- to post-treatment time points.

Pre- vs. Post-Treatment

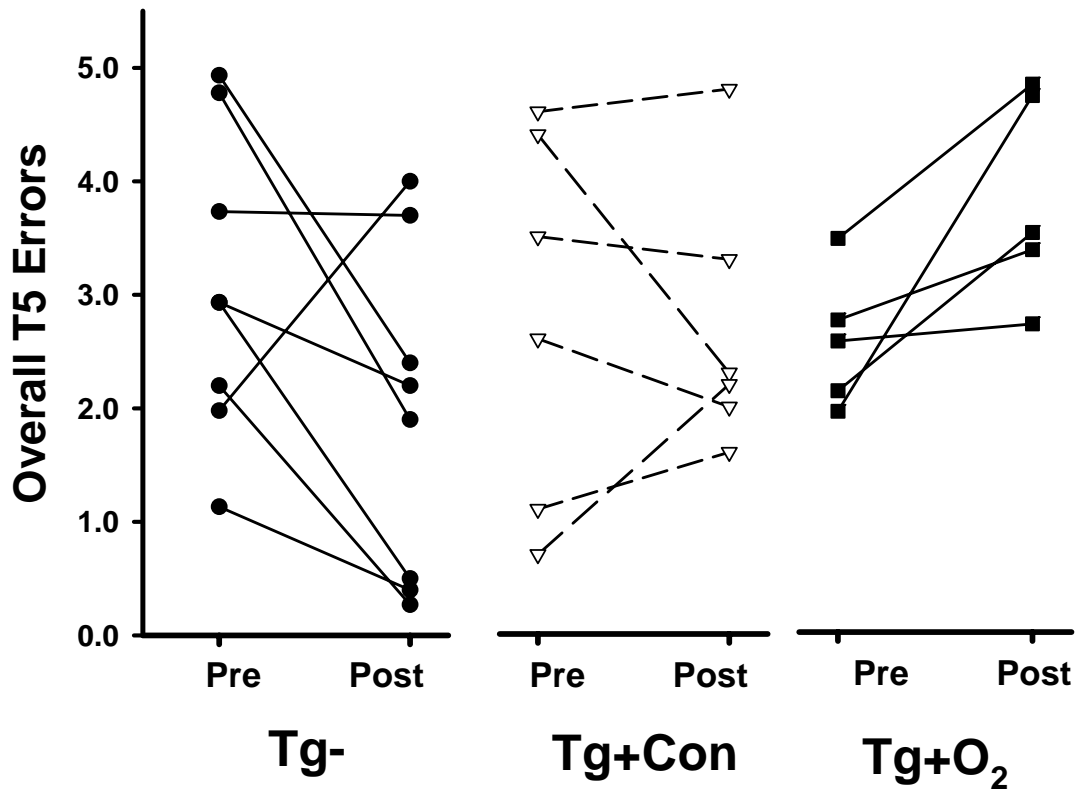


Fig.7. Individual plots of pre- vs. post-treatment overall T5 errors for all animals in each group. All of the animals in the Tg+O₂ group (with the exception of one that stabilized) are shown to increase their number of overall T5 errors.

Neuropathology and Neurochemistry

Degenerative Neuronal Counts. To determine any effects of transgenicity and/or treatment on the number of neurodegenerative neurons in hippocampus, the acid fuchsin/toluine blue method for identifying such neurons was used. As shown in Figure 8, there were no effects of transgenic or hyperoxia treatment on mean numbers of degenerative neurons in CA1&2, CA3 and CA4 hippocampal regions. Utilizing a scale of 0 to 4, semi-quantitative analysis of neurodegenerative neuron numbers in dentate gyrus also yielded no effects of transgenic or hyperoxia treatment (Tg-, 1.6 ± 0.3 ; Tg+Con, 1.1 ± 0.4 ; Tg+O₂, 1.8 ± 0.4). However, correlation analysis revealed a number of significant associations between hippocampal neurodegenerative neurons and post-treatment working memory in Tg+ mice collectively, irrespective of gas treatment. These correlations generally involved overall Trial 5 performance, such as the three positive correlations shown in Fig. 9 between T5 errors and neurodegenerative neuronal counts/rating in dentate gyrus ($p<0.02$), CA3 ($p<0.05$), and CA4 ($p<0.02$). The correlation involving CA3 was even more striking if overall T5 “latency” was used ($p<0.005$, $r=0.861$), rather than T5 errors. Thus, high numbers of neurodegenerative neurons in these hippocampal brain regions were associated with more overall T5 errors.

Neurochemistry. Two novel products of lipid peroxidation, 8-isoprostane and isofurans, were measured in brain tissue from all three groups. As shown in Table 2, analysis of 8-isoprostane revealed no transgenicity or hyperoxia effects in either anterior or posterior cortex. Although no effect of hyperoxia treatment was also seen for isofurans in either cortical area, anterior cortex isofuran levels were significantly reduced in both Tg+ groups (Table 2). Correlation analysis between lipid peroxidation measures and working

memory revealed significant negative correlations between final block T5 performance and iso-furan levels in anterior cortex (Fig. 9). Animals with higher iso-furan levels in anterior cortex exhibited better working memory (less errors). This association was present for Tg⁺ mice alone, as well as for Tg⁺ & Tg⁻ mice combined. Although there were no correlations between lipid peroxidation markers and degenerative neuronal counts in hippocampus for combined Tg⁺ & Tg⁻ mice or Tg⁺ mice alone, several correlations were present for Tg⁺ controls alone (n=6). Higher posterior cortex levels of isofurans were correlated with the number of degenerative neurons in CA1/2 ($r=0.858$, $p<0.05$) and higher posterior cortex levels of 8-isoprostane correlated with the number of degenerative neurons in the CA3 region. Thus, increased lipid peroxidation in posterior cortex was associated with higher numbers of hippocampal degenerative neurons. Preliminary western blots were done investigating the levels of expression of APP, ApoE, COX-2, and GFAP. Visual analysis of the blots for each of these proteins revealed no differences in protein expression levels between groups (data not shown).

Brain A β Deposition. Immunostaining for diffuse A β deposits using the 6E10 antibody indicated that diffuse A β loads in neocortex and hippocampus of Tg⁺ mice were unaffected by hyperoxia treatments (Fig. 10, upper). Similarly, Thioflavin S fluorescent staining for compact A β deposits also revealed no effect of hyperoxia treatments for either brain area in Tg⁺ mice (Fig. 10, lower). Surprisingly few correlations were found between these two A β neuropathology measures vs. working memory, degenerative neuron counts, and lipid peroxidation markers. Indeed, no correlations were evident between either A β neuropathology measure and any working memory measure or degenerative neuronal counts in hippocampus. Although no correlations were also found

between 6E10 staining and lipid peroxidation markers, there was a single significant correlation involving Thioflavin S: Higher cortical Thioflavin S staining correlated with higher anterior cortical isofuran levels ($r=0.627$, $p=0.05$)

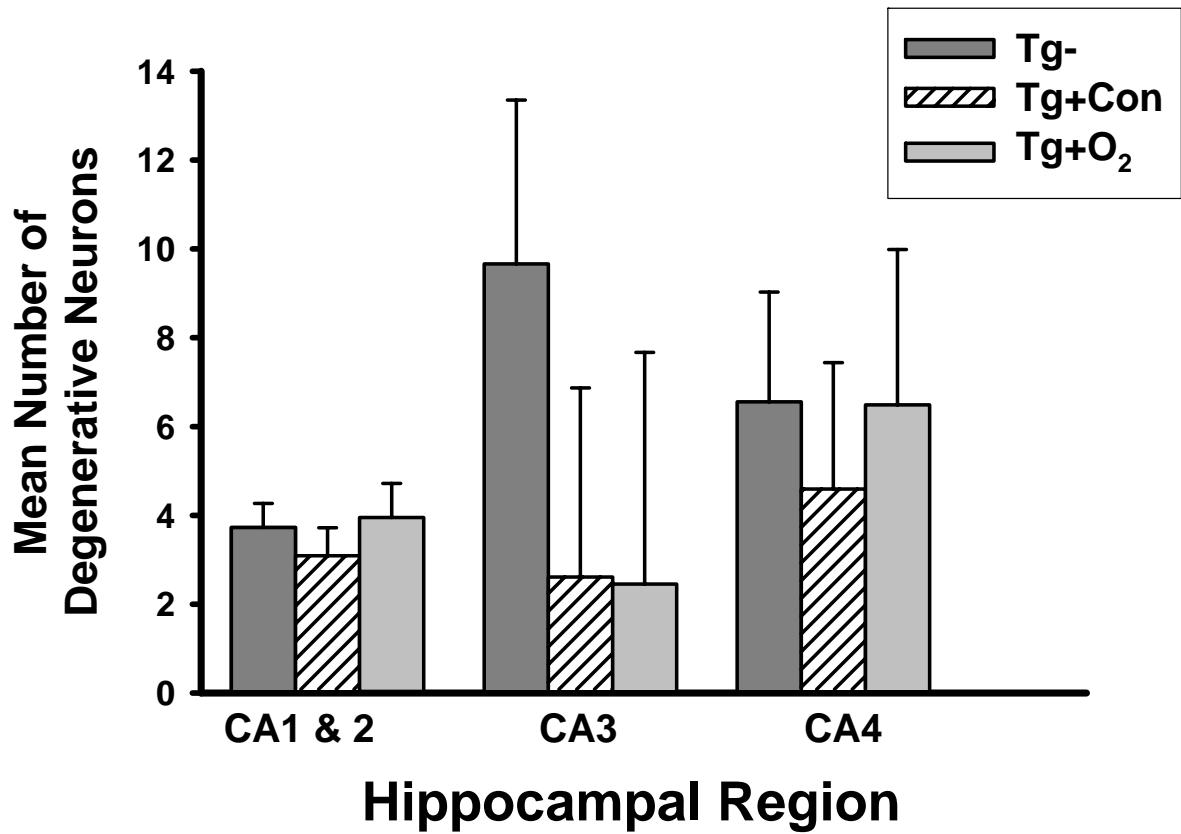


Fig.8. The mean number of degenerative neurons in various hippocampal regions using the acid fuchsin and touilidine blue method. No significant differences between groups were found.

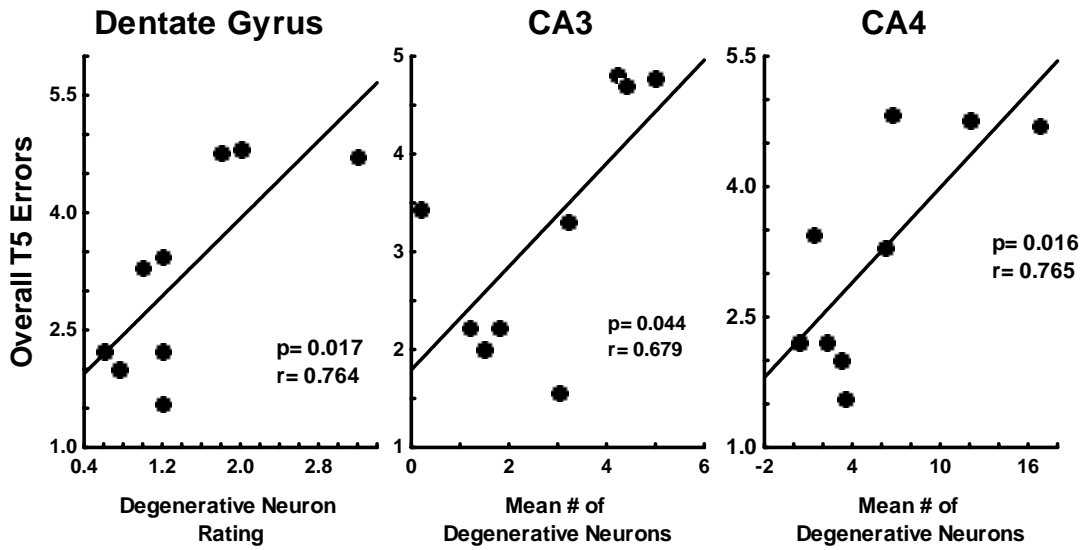


Fig.9. A series of correlation scatter plots illustrating that the degree of neuronal degeneration in the dentate gyrus and the number of degenerative neurons in, CA3, and CA4 region of the hippocampus were positively correlated with working memory impairment in Tg+ mice, irrespective of gas treatment. More degenerative neurons were seen in Tg+ mice making high numbers of working memory (Trial 5) errors overall.

Lipid Peroxidation Markers

	<u>Tg-</u>	<u>Tg+Con</u>	<u>Tg+O₂</u>
8-IsoProstane(ng/g)			
Anterior Cortex	3.4 ± 0.3	3.1 ± 0.3	3.2 ± 0.4
Posterior Cortex	3.9 ± 0.9	3.4 ± 1.0	5.0 ± 1.3
Iso-Furans(ng/g)			
Anterior Cortex	6.5 ± 1.0	3.2 ± 1.1*	2.5 ± 1.4*
Posterior Cortex	8.7 ± 3.1	4.5 ± 3.6	9.4 ± 4.4

Table 2. The mean amount (results presented as ng/g wet weight of tissue) of 8-IsoProstane and Iso-Furan, both markers of lipid peroxidation, in anterior cortex and posterior cortex from each group of animals. Lipid peroxidation in neocortex was unaffected by hyperoxia treatments in Tg+ mice, although anterior cortex isofuran levels were significantly reduced in both groups of Tg+ mice. * = p<0.05 vs. Tg- for iso-furan measurement in the anterior cortex.

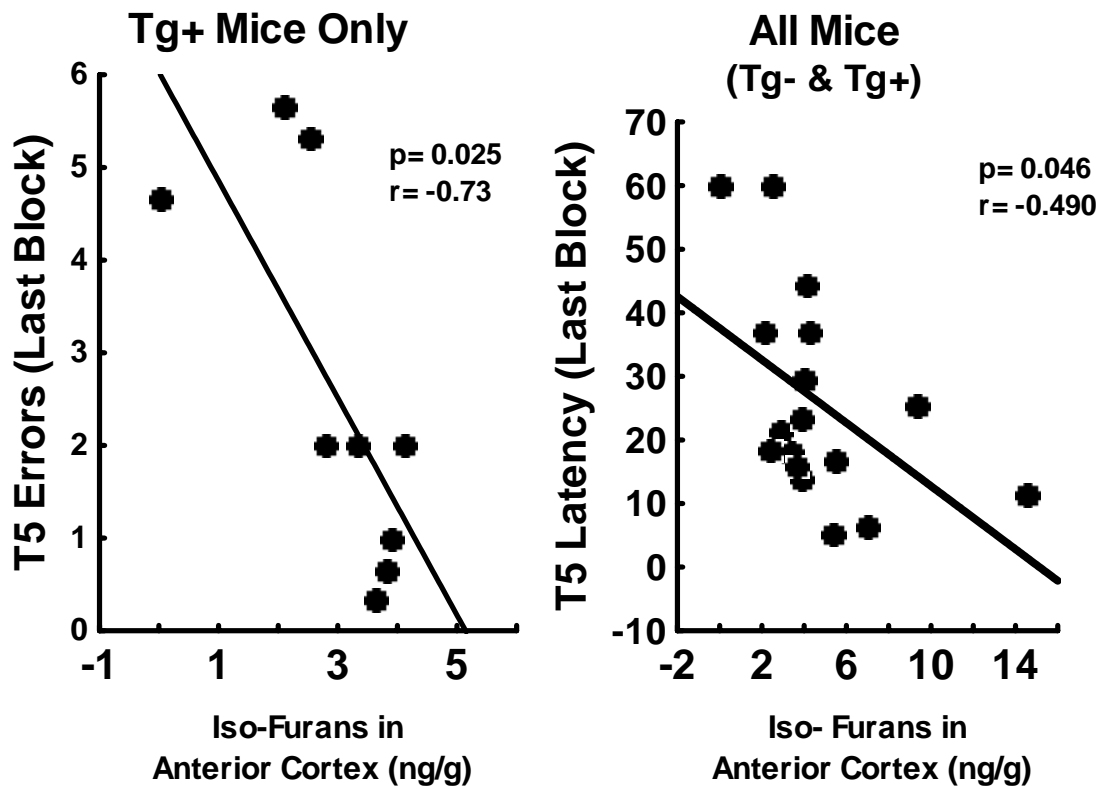


Fig.10. A pair of correlation scatterplots illustrating that an inverse correlation was present between iso-furans in anterior cortex and working memory for Tg+ mice (left), as well as for combined Tg- and Tg+ mice (right). Mice with higher iso-furan levels actually had better working memory (made less errors) than those with lower iso-furan levels.

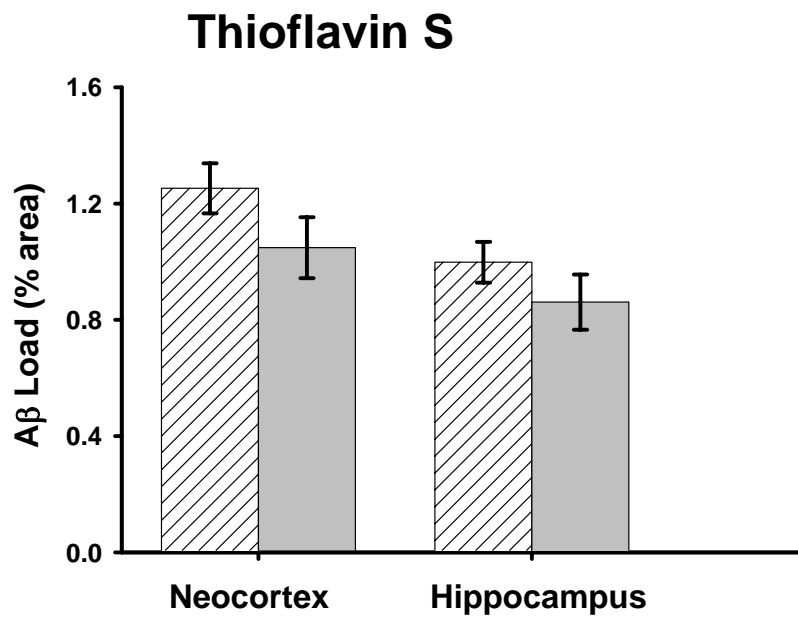
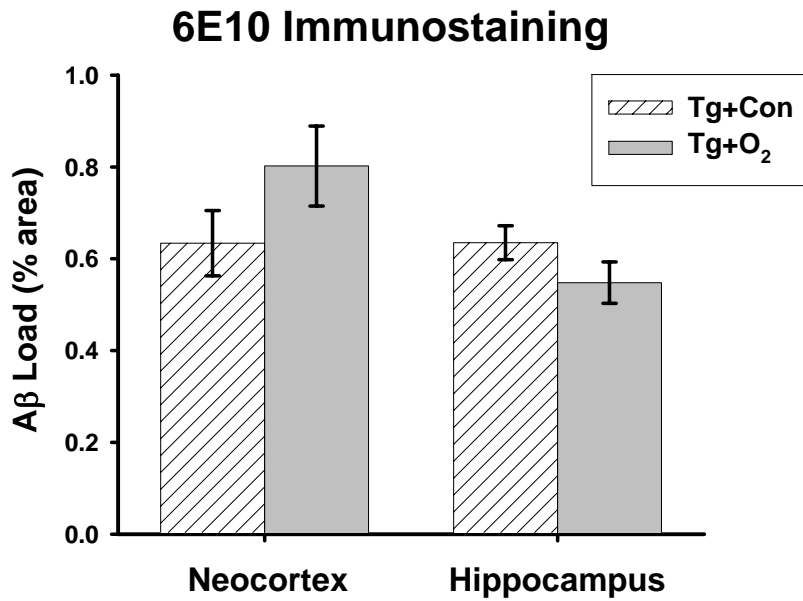


Fig.11. A β loads in neocortex and hippocampus of Tg+ mice were not affected by hyperoxia treatments. Hyperoxia had no significant effect on A β deposition in either brain region, as determined by both 6E10 immunostaining and Thioflavin-S staining. Brains from Tg- mice exhibited no staining with either method.

Discussion

General Summary. In the present study, we evaluated the effects of hyperoxia on AD transgenic mice in the RAWM. We determined that exposures to 100% oxygen (hyperoxia) trigger working memory impairment in Tg⁺ mice that otherwise would have been unimpaired. Hyperoxia induced memory impairment in Tg⁺ mice did not involve changes in brain A β deposition, degenerative cell numbers in hippocampus, neocortical lipid peroxidation, or hippocampal levels of APP, ApoE, COX-2, or GFAP. The combination of excess A β and hyperoxia could have induced greater cerebral vasoconstriction than either one alone, resulting in a pathologic cerebral hypoperfusion that triggered subsequent cognitive impairment. These results suggest that humans genetically pre-disposed to AD and those with increased brain A β levels have increased risk of developing cognitive impairment following hyperoxia treatment. This is a novel finding that calls into question the wide use of 100% oxygen treatments in aged individuals at high risk for developing AD following major surgery.

Behavior. In pre-treatment RAWM testing at 3 months of age, Tg⁻ and Tg⁺ mice were identical in working memory performance. Both groups were able to reduce errors from T1 to T4 and T5. Therefore, the Tg⁺ animals were not impaired in working memory at 3 months of age (no overt plaque deposition). Post-treatment RAWM testing at 7.5 months of age revealed a cognitive impairment selectively in the Tg⁺O₂ group vs. the Tg⁻ group in overall errors for both T4 and T5. The Tg⁺Con group trended towards impairment, however, remained non-significant. The data shows this consistent trend through all 3 blocks of post-treatment testing, where the Tg⁺Con group consistently made more errors than the Tg⁻ and less errors than the Tg⁺O₂ group. The three

hyperoxia treatments given to the Tg+ mice induced an earlier cognitive impairment in animals that would have otherwise remained unimpaired. This is evidenced by the fact that the Tg+Con group of animals (littermates of the Tg+O₂ group) were not impaired (as compared with the Tg- group) at the post-treatment test point.

In comparing pre- vs. post-treatment behavior in the three groups of animals the effect of hyperoxia becomes even more apparent. While both the Tg- and Tg+Con groups either stabilize or improve their performance in overall T5 errors from pre- to post-treatment testing the Tg+O₂ group significantly increased their number of errors from pre- to post-treatment testing. The behavioral finding, that hyperoxia induces impairment in rodents has been reported on previously in a publication by Fukui, et al (1999). In that study the authors exposed a rat model for oxidative stress (rats were fed a vitamin E deficient diet) to 48 hours of hyperoxia and tested cognition and memory in the Morris water-maze. They reported that the animals exposed to hyperoxia were not capable of “relearning”, and although the testing methods were somewhat questionable, their findings that hyperoxia had a negative effect on behavior corroborates the findings of the present study. As far as the knowledge of this laboratory permits this is the only study investigating the effects of hyperoxia in a rodent model for behavior.

Studies done with humans and hyperoxia have reported that hyperoxia has a beneficial effect on short-term memory in normal adults. In 1998 a study done by Moss et al., (1998) reported significant improvements in immediate and delayed word recall in individuals exposed to hyperoxia for 1 or 3 minutes prior to the start of testing. A study done by Scholey et al., (1999) reported that individuals exposed to hyperoxia for 1 minute prior to testing recalled more words and had faster reaction times. These studies,

alongside other similar studies (Prior & Chander 1982 & Sholey et al., 1998) suggest that hyperoxia may be beneficial to short-term memory when administered directly prior to testing. It is important to note that these studies are only tangentially related to the current study. The long term effect of hyperoxia was not investigated in any of these studies, and more importantly the studies utilized young healthy adults as the test subjects. Only one study done by Prior & Chander (1982) investigated the effects of hyperoxia in elderly patients. In that study the researchers exposed elderly patients to 12 hours of post-surgery hyperoxia and then performed cognitive testing immediately following the treatment. This study reported that the hyperoxia exposure did not have a deleterious effect on cognition. It is important to note that only a single test was given directly following treatment, with no follow up studies investigating behavioral changes once the patient returned home. Thus, while previous literature has suggested that hyperoxia may be either benign or even beneficial under specified narrow conditions, the long-term effects of hyperoxia given to patients at high risk for developing AD has not yet been established.

Pathology. Insoluble A β was quantified using two staining techniques; antibody staining for compact and diffuse A β deposits was done using the 6E-10 antibody from Signet, and Thioflavin-S staining was done for compact A β deposits. Both staining techniques yielded no differences between the amount of insoluble A β in the Tg+Con group and the Tg+O₂ group. Therefore, hyperoxia does not increase A β deposition in the mouse model utilized in this study. Prior studies using APP models have reported finding a correlation between A β deposition and RAWM performance (Arendash et al., 2001; Leighty et al., 2004). However, this study did not yield any correlations between

A β deposition and RAWM performance. While the data from the two staining techniques mentioned above did not show any differences it would be interesting in a follow up study to quantify soluble A β in an ELISA assay. Some evidence has linked hyperoxia treatment to potentially increased A β production in rodents. A study done by Wen, et al (2004) found that cerebral ischemia in rats caused a 30% increase in β -secretase activity. The cerebral vaso-constriction caused by hyperoxia would hypothetically induce mild ischemia, or hypoperfusion in the brain. β -secretase, as mentioned prior, is the enzyme responsible for the pathological cleavage of APP that results in the toxic A β fragment. Therefore, an increase in production of β -secretase caused by severe restriction of blood flow to the brain could lead to an increased amount of deposited A β . The expression level of β -secretase would also be interesting to look at in any possible follow up studies.

The APP/PS1 mouse model utilized in this study has been shown to exhibit the AD hallmarks of elevated soluble A β levels alongside A β deposition into compact plaques by 6 months of age (Takeuchi et al, 2000). A β deposition is seen as early as 12 weeks in the cortex and hippocampus of this animal model (Holcomb et al, 1998). The hippocampus plays a strong role in spatial and memory related tasks, such as the RAWM. The sensitivity of the task to the hippocampus and A β deposition makes the task extremely fitting for the present study. The present study aimed to use APP transgenic mice at an age when they were on the verge of displaying cognitive impairments, yet remained unimpaired. The hyperoxia treatments were designed to push such animals into cognitive impairment at an earlier age than when the animals naturally would have developed an impairment. An animal model that is in a state just prior to the onset of

cognitive impairment models an elderly patient that is in the latent or possibly the prodromal stage of AD. As mentioned previously, A β accumulation and deposition is thought to occur many years prior to disease diagnosis. Thus, aged individuals at a high risk for developing AD, have increased amounts of A β in their brain. Therefore, the animal model used exhibits both an imminent decline in cognition alongside the presence of an abundance of the toxic A β protein. The marked decline in working memory in the Tg+O₂ group may therefore, be predictive of what an elderly patient may experience after being treated with hyperoxia.

Prior literature has suggested a specific vulnerability of the neurons of the hippocampus to ischemia (Pulsinelli et al, 1985; Kirino et al, 1985). Both Pulsinelli and Kirino exposed a rat model to 10-30 min. of ischemia (through a 4 vessel occlusion technique) and found that the neurons in the CA1 region of the hippocampus were selectively vulnerable to the treatment. Kirino et al, 1985 related two theories explaining this selective vulnerability. The first theory has been coined the “vascular theory” and stated that the design of the local vasculature and the location of the hippocampus in the watershed area between the carotid and vertebrobasilar territories lends the hippocampus to selective vulnerability due to ischemia. The second theory hypothesized that the neurons of the hippocampus were selectively vulnerable to ischemia due to differences in their physical and chemical characteristics. Prior literature has suggested that the hippocampus is also selectively vulnerable to injury induced by hypoxia (Gorgias et al, 1996). In a study by Gorgias et al. (1996), rats exposed to extreme hypoxia (3%O₂) for 6 minutes displayed selective injury to the neurons of the CA1 region of the hippocampus.

This study suggested that the neurons of the hippocampus, specifically in the CA1 region, are particularly vulnerable to changes in oxygen availability.

The treatment given in the present study, hyperoxia, is a strong generator of free radicals as well as a potent vasoconstrictor of cerebral vessels that may result in mild ischemia. It was therefore, hypothesized that in the present study there would be evidence of increased neuronal degeneration in the hippocampus in the Tg+O₂ group due to oxidative damage and decreased cerebral blood flow (at least during hyperoxia treatments). However, acid fuchsin and touilidine blue staining for degenerative neurons did not reveal any group differences at euthanasia (10 days following final hyperoxia treatment). Cell counts performed in the CA1/2, CA3 and CA4 regions of the hippocampus, as well as in the dentate gyrus, did not reveal any differences between the three groups. Therefore, there was no effect of transgenicity or hyperoxia on hippocampal neuronal degeneration. Current literature reports no evidence of significant cell loss in the APP/PS1 model in cortical and hippocampal areas at 3 to 12 months of age (Takeuchi et al, 2000). Therefore, it was not expected that there should be a transgenic effect for this histological measure at the 8 month sacrifice timepoint. Nonetheless, a recent study by Sadowski et al. (2004) reported that in 22 month old APP/PS1 mice there was a 35.8% dropout of neurons on the CA1 region of the hippocampus that was detected using stereological techniques. Also, a study by Schmitz et al. (2004) reported significant hippocampal neuronal loss in 17 month old APP/PS1 transgenic mice bearing both the Swedish and London APP mutations. Perhaps, the absence of significant hyperoxia effects on neuronal degeneration was due to three 3-hour hyperoxia treatments being insufficient to induce degeneration detectable by the methods

used. Or there may have been an increase in degenerative neurons immediately following hyperoxia that was missed due to the 10 day delay in sacrifice. In future studies, a more lengthy treatment time and immediate sacrifice should be considered. Clearly, the hyperoxia-induced cognitive impairment found in this study was not attributable to hippocampal neuronal degeneration that was detected by acid fuchsin and touilidine blue staining. However, a more thorough investigation of the cellular integrity in the hippocampus, or stereological analysis should be performed before this can be ruled out as a probable cause of the impairment.

It is possible that, with more sensitive techniques a treatment or transgenic effect on hippocampal neurons may be detectable. For example, it is possible that by using scanning electron microscopy, changes in cellular morphology (including organelle integrity) could be identified. A study done by Urano, et al (1997) using a rat model investigated the morphological changes through electron microscopy in the brain associated with exposure to 100% oxygen for 48 hours followed by immediate sacrifice. They found swollen astrocytes around vessels, deformed nerve cell nuclei, swollen mitochondria, and an abnormal accumulation of synaptic vesicles in swollen nerve terminals. These cellular changes may be the underlying causes of the cognitive impairment reported on here.

Correlational analyses of cell count data with RAWM data revealed an interesting correlation for all Tg+ mice combined. The number of overall T5 working memory errors was highly correlated with the degenerative cell scoring in the dentate gyrus, and the mean number of degenerative neurons found in the CA3 and CA4 region of Tg+ animals. Tg+ animals that had a high number of degenerative neurons were found to

make more overall T5 errors. This correlational analysis provides limited evidence that the amount of neuronal degeneration in the hippocampus is linked to working memory in Tg+ animals.

While there is no single measure used to assess the overall level of oxidative stress in an organism, an assay of iso-furans and 8-isoprostanes (stable by-products of lipid peroxidation uniquely regulated by oxygen tension) is used to assess the amount of lipid peroxidation tissue has undergone. Iso-furans, in particular, are increased in conditions of elevated oxygen concentrations such as that induced by hyperoxia (Roberts L & Fessel J, 2004). The current study investigated levels of iso-furans and 8-isoprostanes in both the anterior and posterior cortices of all animals. There was no effect of hyperoxia treatment for either marker. As well, there were largely no effects of transgenicity on these lipid peroxidation markers with one exception. There was significantly less iso-furan in the anterior cortices of Tg+ animals. This reduction was somewhat surprising as previous literature has reported increased lipid peroxidation in AD transgenic animals (Schuessel et al, 2005; Pratico et al, 2001). The study done by Schuessel et al, (2005) found that in APP transgenic mice, the lipid peroxidation marker 4-hydroxynenal was significantly increased by 3 months of age. The study done by Pratico et al, 2001 examined isoprostanes (specifically, 8,12-*iso*-iPF_{2α}-VI) in urine, plasma, and brain tissue in the APPsw mouse model. The authors reported increases in isoprostanes universally by 8 months of age, months before overt Aβ deposition in such mice. Increases in lipid peroxidation in AD transgenic mice may be due in part to the presence of Aβ. A study by Matsuoka et al, 2001 found that the lipid peroxidation marker 4-hydroxy-2-noneal (HNE) increases in APP/PS1 mice in relation to an age-

associated increase in amyloid load between 7 and 30 months of age. This study suggests a significant role for A β in modulating oxidative stress.

A β has also been found to generate free radicals (Hensley, 1994). Increases in free radicals lead to increased oxidative stress with subsequent oxidative damage such as lipid peroxidation. This fundamental idea coupled alongside the previous literature provided the foundation for the hypothesis that the Tg⁺ animals would have increased levels of the lipid peroxidation markers examined. Our hypothesis that the treatment group (Tg+O₂) would have the highest amount of lipid peroxidation incorporates the increases in oxidative stress previously reported in AD Tg⁺ animals with the addition of hyperoxia. Hyperoxia is also a known producer of free radicals, “about 5% of inspired oxygen is converted to the dangerous superoxide radical” (Fridovich, 1983). Superoxide as mentioned above was shown in a study by Johnson (2001) to react with NO to form the toxic peroxynitrite (ONOO⁻) radical. Peroxynitrite is a potent oxidant that attacks DNA, proteins, and lipids leading to cellular damage. Thus, the Tg+O₂ animals, being exposed to two sources of increased oxidative stress, were hypothesized to exhibit a heightened amount of lipid peroxidation markers. However, as mentioned previously, the data presented here does not support this hypothesis. Our finding that Tg⁺ animals had significantly decreased amounts of iso-furan in the anterior cortex may be due to decreases in brain metabolism. A study done by Sadowski et al, 2004 reported finding significant decreases in hippocampal glucose metabolism in 22 month old APP/PS1 mice. Given the fact that glucose metabolism can generate free radicals, a significant decrease in metabolism could easily explain a decrease in oxidative stress markers. Another possible explanation for the decrease in lipid peroxidation in Tg⁺ animals might involve

the role of A β itself. As mentioned previously, there is growing evidence that points towards the antioxidant capabilities of A β , as demonstrated by its ability to prevent lipoprotein oxidation in the CSF (Kontush, 2001). These findings are in accord with earlier work that has found A β plaque load in humans to be inversely correlated with oxidative stress, where oxidative damage is quantitatively greatest in the early stages of the disease and reduces with disease progression (Nunomura, 2001). Thus, decreased lipid peroxidation in AD brains was associated with increased A β deposition. The Tg⁺ animals may have had decreased lipid peroxidation as compared with non-transgenic littermates due to the presence of A β in their brains. The majority of literature does not support this idea. However, as the exact role of A β has yet to be fully elucidated, it is relevant to mention this hypothesis that A β is acting as an antioxidant as it has not yet been disproven. In any event, a possible explanation for the lack of a treatment effect may be simply due to the fact that the animals were sacrificed 10 days post-treatment. The effects of hyperoxia on lipid peroxidation may therefore be transient. A follow-up study in which animals are sacrificed immediately following hyperoxia treatment is necessary to answer this question.

The lipid peroxidation data yielded several interesting correlations when analyzed alongside the behavioral and cell count data. In Tg⁺ mice alone, as well as for combined Tg⁺ and Tg⁻ mice, higher iso-furan levels correlated with lower T5 errors. Thus, an animal that performed well in the last block of RAWM testing (exhibited by a low number of errors) had a higher amount of iso-furan in their anterior cortex. Perhaps, more cognitively active animals utilize more oxygen resulting in increased iso-furan. This data suggests that animals with increased iso-furan have better cognition than

animals with lower levels. In Tg⁺ control animals alone, a correlation was found between higher posterior cortex levels of iso-furans and the number of degenerative neurons in CA1/2; as well, higher posterior cortex levels of 8-isoprostane correlated with degenerative neurons in CA3. Thus, increased lipid peroxidation in posterior cortex was associated with higher numbers of hippocampal degenerative neurons in Tg⁺ untreated animals. In a yet broader picture, increased degenerative cell numbers in the hippocampus correlated with both poorer cognitive performance and increased lipid peroxidation. The exact relationship between these markers is still unclear. More research needs to be done on these novel markers to discover their underlying function with regards to memory and cognitive functions.

Vasoactive role of A β . Another hypothesis presented in this study focuses on the vasoactive role of A β . Numerous studies (Thomas et al, 1997; Crawford et al, 1998; Arendash et al, 1999) have reported that freshly solubilized and vascularly injected A β enhances cerebral vasoconstriction in rat models. This enhanced cerebral vasoconstriction results in decreased cerebral blood flow (Suo et al., 1998). The study by Suo et al., (1998) utilized fluorescent microspheres to monitor both cerebral blood flow and cerebrovascular resistance in a rat. This technique could be utilized in a future study to monitor the effects of hyperoxia on cerebral blood flow. In conjunction with these murine studies, a human study done by Mentis et al., (1996) reported that PET scanning techniques show decreases in resting cerebral blood flow in patients with AD. Thus, patients with an increased amount of A β in their brains may have chronic decreased cerebral blood flow resulting in a hypoperfusion of the brain. It has also been suggested by Aliyev et al., (2005) that chronic cerebral hypoperfusion may be an initiator of AD.

Hyperoxia is also a potent cerebral vasoconstrictor (mentioned above). In the present study, hyperoxia and A β may have worked in concert with one another to promote a greater hypoperfusion than either one alone, resulting in subsequent cognitive impairment. It would be interesting in a follow-up study to collect data regarding this hypothesis. During hyperoxia treatment, cerebral blood flow can be monitored using laser-Doppler flowmetry; this data would be able to provide evidence that the hyperoxia treatment induces vasoconstriction in AD transgenic mice. In post-mortem tissue, white matter lesions and the extent of activated microglia and astroglia could be analyzed as markers of hypoperfusion (Shibata et al., 2004).

Clinical Implications of the Hyperoxia Findings. Currently, two main risk factors that are widely accepted for developing AD are age and the inheritance of the ApoE4 allele. Of course, many other risk factors have been put forth involving an individuals' health, health history, and lifestyle (discussed above). It has been previously suggested by Ahua-Haim et al., (1998) that a link between surgery in the elderly and precipitation of AD exists. Surgery incorporates many potentially harmful components including administration of various drugs, anesthesia, changes in blood pressure, and possible perioperative hyperoxia treatments. Results from the present study suggest that perioperative hyperoxia treatment and a pre-disposition to AD are additional and synergistic risk factors for AD-related postoperative cognitive impairment. These findings will hopefully trigger subsequent studies that further elucidate the role of hyperoxia in patients at risk for developing AD, as well as call into question the wide use of this potentially toxic treatment.

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