

## ABSTRACT

Title of Thesis: CHARACTERIZING rTg4510 MICE MODEL OF ALZHEIMER'S DISEASE ON ADULT NEUROGENESIS AND COGNITIVE FUNCTION

Laurel A. Gordon, Alice Yanhong Lu, Melinda M. Matos, Jennie Se Yeon Seo, Lisa Yi Yeon Seo

Thesis Directed By: Dr. Ricardo C. Araneda, Ph. D  
Associate Professor and Director BISI-PSYS  
Graduate Program  
University of Maryland, Department of Biology

Alzheimer's Disease (AD) is a debilitating illness that affects millions of Americans each year. While there is not one definitive hypothesis that exists regarding how the disease develops, some hypotheses include the cholinergic model, the amyloid beta hypothesis, and the tau pathology model. The rTg4510 (Tg) mouse is a model of AD that over-expresses inducible human mutant tau (P301L), a pathological hallmark of AD. This study characterizes the Tg mouse through analysis of adult neurogenesis in the olfactory system, due to the fact that the olfactory system is one of the first sensory systems of the brain to be affected by AD. For this, we examined the behavioral changes in different age groups, specifically 2 and 7 months. Adult neurogenesis within the granule cell layers and glomerular layers in the main

and accessory olfactory bulb was analyzed by methods of immunohistochemistry using appropriate antibodies and cell counting with confocal microscopy. Several behavioral paradigms, such as habituation/dishabituation, odor detection threshold, and novel object recognition, were executed to assess cognitive function of these mice, especially in relation to markers of olfactory behavior. Our data suggest that there is an age-dependent cognitive impairment but an age-dependent increase in neurogenesis. Further study is required to identify the effects of inducing the mutant tau on neurogenesis in the olfactory bulb (OB) and whether the overexpression of tau directly impacts cognitive decline.

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by

Team Dream

Laurel A. Gordon, Alice Yanhong Lu, Melinda M. Matos, Jennie Se Yeon Seo,  
Lisa Yi Yeon Seo

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Advisory Committee:

Dr. Ricardo C. Araneda, Faculty Mentor

Dr. Kara Duffy

Dr. Quentin Gaudry

Dr. Erica Glasper

Dr. Hadiya Woodham

Dr. David Yager

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Lisa Yi Yeon Seo

Dr. Ricardo C. Araneda

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# Chapter 1: Introduction

## 1.1 Neurodegenerative Diseases

Neurodegenerative diseases are a group of conditions in which there is significant degeneration of the central or peripheral nervous system in individuals (“Neurodegenerative Diseases”). Neurodegenerative diseases affect millions of Americans each year and lead to devastating changes in the individual’s sense of self and ability to perform typical tasks. Neurodegenerative diseases often result in the degeneration or the death of nerve cells, leading to issues with mental functioning, otherwise known as dementia (“What Is Neurodegenerative Disease?”). In this way, dementias form a broad category of neurodegenerative diseases, and can be described as a type of cognitive decline that affects individuals’ daily lives. Dementia is the sixth leading cause of death in America and affects 1 in 9 elderly Americans. Dementia not only affects the individual’s own life but also comes to affect the patient’s family through depression, stress, and financial burdens. Each year, about \$200 billion is spent on healthcare costs related to dementia, half of which. About half of this is accounted to Alzheimer’s disease (AD), the most common form of dementia. Eventually, society as a whole is affected by the neurodegenerative decline that occurs in AD (Alzheimer’s Association, 2017).

## 1.2 Alzheimer’s Disease

### 1.2.1 Definition

Scientifically, AD can be described as “a progressive disease that destroys memory and other important mental functions” (Mayo Clinic Staff Print, 2015). It is estimated that between 11 million and 16 million Americans will have the disease by 2050, and that this will lead to a



proportional increase in the cost of providing care to patients, leading to a huge economic issue in healthcare (Alzheimer's Association, 2017). Evidently, AD is not only an issue in regards to the impacts on the lifestyles of patients and those around them, but also in regards to the economy and healthcare as a whole.

AD is a progressive disorder with no set timeline or rate of cognitive decline. Landmarks may be different for each individual. Phases include the asymptomatic phase, the symptomatic prodementia phase, during which there may be changes in cognitive function, and finally the dementia phase. According to the National Institute on Aging and the Alzheimer's Association, the dementia phase usually marks the diagnosis of AD. It is more difficult to definitively diagnose AD in the earlier phases of the disease (Albert et al., 2011).

During the symptomatic prodementia phase, a decline in cognitive ability may signal AD. Observations include a decline in episodic memory (ability to learn new skills) and functional memory. Cognitive assessment tests like the Rey Auditory Verbal Learning Test and Wechsler Memory Scale Revised are used to diagnose impairment during this phase. There may also be informal methods of testing if formal testing is not feasible. Genetics may also play a role in the diagnosis. If there are mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2), which are autosomal dominant genes linked to AD, the patient is most likely prone to early-onset familial AD. Additionally, medical specialists may look for the presence of an E4 allele in the apolipoprotein E (APOE gene), which helps to identify patients that may be prone to late-onset AD. Other biomarkers involved in AD diagnosis include amyloid beta plaques, tau accumulation in neurofibrillary tangles, neuronal damage, tau in cerebrospinal fluid, oxidative damage, cell death, and hippocampal atrophy. Visualization techniques using fMRI, PET and MRI studies may be involved in identifying biomarkers (Albert et al., 2011).

### **1.2.2 Risk Factors**

According to the Alzheimer's Association (2017), the most important risk factors of AD are age, family history, and heredity. The greatest known risk factor is age, as nearly 1 in 3 people age 85 or older have the disease. However, more research is needed to fully understand the mechanisms responsible for rising AD risks with advancing age. People with family history of the disease are also more likely to develop the disease, which may be due to hereditary genes associated with AD, such as risk genes and deterministic genes. Risk genes are genes that "increase the likelihood of developing a disease," such as APOE-e4 is for AD (Alzheimer's Association, 2017). About 25 percent of AD cases are associated with this gene. More severely, deterministic genes "directly cause a disease"; there are a small number of these genes that cause AD in a few hundred extended families in the world. About 5 percent of people with familial early-onset AD are affected by these deterministic genes (Alzheimer's Association, 2017).

### **1.2.3 Characterization of Alzheimer's**

One way in which AD can be differentiated is by the age of onset, specifically whether a case is early-onset or late-onset. Early-onset AD, in which patients display symptoms before age 65, is the less common type. Early-onset patients will often suffer more severe symptoms and undergo a faster cognitive decline, as early-onset is a more aggressive form of AD. Neurological differences, such as increased gray matter atrophy in the parietal and temporal lobe, are associated with early-onset AD. On the other hand, late-onset AD patients will suffer more damage to the hippocampus, and will undergo slower cognitive decline (Canu et al., 2012).

Another categorization is familial vs. sporadic AD. Most cases of AD are sporadic, showing up in individuals with no specified familial basis. Sporadic AD is also generally

associated with aging, and thus is more likely to occur with late-onset AD. While there has only been one specific gene, APOE, consistently associated with sporadic AD, several susceptibility genes have been linked to the familial form of the disease. Familial AD occurs in individuals with strong familial links to the disease, and is associated with both EO and LO. The genes PSEN1, PSEN2, and APP are correlated with familial AD, and are often used in genetic testing when evaluating individuals' risk for AD (Alzheimer Society of Canada, 2017).

### **1.3 Alzheimer's Disease and Olfaction**

One of the areas affected in AD are the olfactory bulb (OB), which is the neuronal structure primarily responsible for olfaction or sense of smell, and the hippocampus, which is involved in cognitive functions and plays a key role in memory (Zou, Lu, Liu, Zhang, & Zhou, 2016). Therefore, a better understanding of the alteration of neurogenesis and cognitive function within these brain structures in models of AD may be useful in further establishing preventative measures and treatment for AD, alongside other neurodegenerative diseases. Loss of smell is not only evident in Alzheimer's Disease, but also in other neurodegenerative diseases such as Parkinson's Disease (Doty, 2012). The importance of olfaction in various diseases is commonly overlooked and has resulted in several studies that utilize quantitative smell tests.

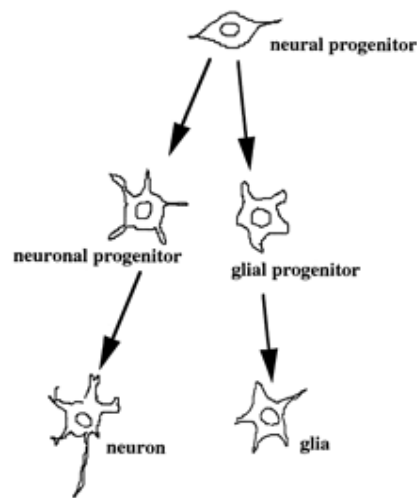
One of the earliest symptoms of AD is the loss of smell. Olfactory dysfunction may be related to the presence of neurofibrillary tangles and amyloid plaques in the OB. Studies have shown that OB tract atrophy increases over the progression of the disease (Thomann et al., 2009). Thus, knowledge regarding the detection of sensory loss in the OB would be helpful in developments towards earlier recognition of AD, and it is relevant to study the OB in an attempt to better understand the pathology behind AD and its development as the disease progresses

(Wesson, Levy, Nixon, & Wilson, 2010).

## 1.4 Adult Neurogenesis

### 1.4.1 Overview of Neurogenesis

The OB is also one of the primary final destinations of neurons that are generated from the process of adult neurogenesis. Neurogenesis is the process of forming new *neurons*. Neural stem cells (NSCs) initially have the ability to differentiate into neurons, astrocytes, and oligodendrocytes. When dividing, a NSC produces one NSC and one neuron. NSC proliferation and differentiation occurs in the adult subventricular zone (SVZ), the rostral migratory streams (RMS), the granular dentate gyrus (DG), and the OB (Seki, 2011). The final destinations of NSCs are the granule cell layer (GCL) and glomerular layer (GL) of the OB. AD ultimately results in a loss of neurons and synaptic contacts due to impaired neurogenesis in the hippocampus and OB. This loss of neurons, or decrease in neurogenesis, is believed to be age-dependent in AD, with older individuals having greater decreases. There are many proposed reasons for the age-dependent decline in neurogenesis (Rodríguez & Verkhratsky, 2011).



**Figure 1.** Neurogenesis (Duman, Malberg & Nakagawa, 2001).

The degeneration of neurons in the areas of brain that are generally regulated by adult neurogenesis contribute to the decline in memory that occurs in AD (Cutuli et al., 2014). In order to better understand these mechanisms behind neurodegeneration in AD and ways to reverse its detrimental effects, researchers have explored a variety of different directions, many of which involve non-human models of AD.

#### **1.4.2 Mouse Models of Neurogenesis**

Many recent studies investigating neurogenesis in both the OB and the hippocampus have used mouse models. Mouse models allow researchers to perform both behavioral studies to assess memory and to quantify cells in neurogenic areas of the brain. Additionally, these models, many of which have mutations in genes related to the pathological hallmarks of AD, allow for studying differential brain functioning for individuals with the disease; such models are useful for understanding adult neurogenesis in humans (Guerin, Sacquet, Mandairon, Jourdan, & Didier, 2009). It is for these reasons that we selected the mouse as our model organism in studying adult neurogenesis in the OB.

#### **1.5 Inspiration for Project/ Experimental Approach**

In the brain, the OB is one of the sensory systems affected by changes in neurogenesis. In previous studies, the OB and hippocampus have both served as models for studies in neurogenesis. However, it is believed that the neurodegeneration in AD may occur up to 10 years prior to the onset of loss-of-memory symptoms in patients, and thus it is more promising in identifying AD patients earlier through looking at behaviors that are mediated by neurogenesis, such as olfactory behaviors (Dhilla Albers et al., 2016). Through better understanding the relationship between loss of neurogenesis in the olfactory bulb and olfactory function as

measured through olfactory tests in AD models, better methods of identifying AD patients earlier may be established. Thus, this study will focus on the OB in modeling AD. In order to replicate the effects of one of the most prominent pathologies of AD (i.e., the accumulation of tau), transgenic mice models will be used. These transgenic models have a transgene, which is a gene that is isolated from another organism, in this case humans, and is introduced into another organism (i.e., mice), resulting in their production of the protein coded by the transgene. Through the use of mice models, we will be able to make more realistic observations and conclusions in terms of the effects of the transgene on AD in humans, as opposed to other potential methods such as cell cultures. Thus, we propose this study to characterize the transgenic mice model, rTg4510 (Tg), in terms of the level of cognitive decline expressed as measured through olfactory behavioral tests, and in their levels of neurogenesis within the OB at various ages in the mouse's life. This research will allow us to evaluate the effectiveness in the Tg model in serving as a representative model for AD.

### **1.6 Research Questions and Hypothesis**

This research aims to characterize Tg mice and determine whether it is an appropriate transgenic mice model for further study of AD pathology. Previous research studies, such as Scott et al. (2016), have focused on the hippocampus of Tg mice, but little work has been done regarding effects on the OB in this model, leading us to focus on this sensory system for our research. Specifically, this study will concentrate on quantifying neurogenesis in the OB, measured by using bromodeoxyuridine, or BrdU, a nucleoside that can be incorporated in newly replicated DNA and detected using antibodies. Behavioral tests to assess function of the OB and the ability to discriminate scents will also be carried out in this study. Ultimately, our research

will seek to answer the following questions: (1) Are there olfactory changes that occur in AD? (2) What are these changes (behavior/neurogenesis)? (3) How early do these deficits occur? We speculate that the Tg mouse model will show significantly greater age-dependent decline regarding cognitive function and neurogenesis in the OB compared to the WT mice due to the effect of the transgene that is meant to induce tau pathology that is characteristic of AD.

## Chapter 2: Literature Review

### 2.1 Pathology of Alzheimer's Disease

#### 2.1.1 Tau Pathology

Tau and amyloid beta are the two main proteins involved in AD. Amyloid beta ( $A\beta$ ) is cleaved from APP by  $\alpha$ ,  $\beta$  and  $\gamma$ -secretases and causes the formation of amyloid plaques in the brain. While amyloid beta protein is found extraneuronally, tau is found intraneuronally, and is primarily expressed in the gray matter of the neocortex. In its natural state, tau is a soluble and an unfolded microtubule-associated protein. However, it can become insoluble and build up as hyperphosphorylated filamentous aggregations. Such aggregations have been linked to changes in axonal transport, as hyperphosphorylation renders tau incapable of microtubule interactions. While the mechanism of tau hyperphosphorylation is not entirely understood, some of the kinases believed to be involved are glycogen synthase kinase 3 (GSK3) and casein kinase 2 (CK2) (Demars, Hu, Gadadhar, & Lazarov, 2010). Tau pathology is not restricted to AD, as tau has been implicated in other neurodegenerative diseases.

Six isoforms of tau are found in humans due to differential splicing on the microtubule-associated protein tau (MAPT) gene, and all six have been found in AD brain samples. Tauopathies are not limited only to AD but also found in a variety of neurodegenerative diseases including Down syndrome, encephalopathy, various dementias and brain iron accumulation. Increased levels of tau in cerebrospinal fluid (CSF) has been found up to 15 years before any signs of AD; however, the link has not been clarified (Spillantini & Goedert, 2013). Previous tau-based treatment directions have included inhibiting tau aggregation and phosphorylation, reducing general levels of tau, stabilizing microtubules, and using tau antibodies (Spillantini &



Goedert, 2013).

Tau hyperphosphorylation has previously been linked to many different problems within neurons that contribute to a decrease in neurogenesis. There is evidence suggesting that tau hyperphosphorylation can lead to chromosome aberrations, which include missing, extra, or irregular portions of chromosomes, and that this can prevent mitosis from occurring. In addition to affecting mitosis, hyperphosphorylation of tau may also interfere with the transport of molecules across axons and the maturation of neurons. Specifically, Demars et al. (2010) found that in mice with mutations linked to AD, which showed decreased proliferation of neural progenitor cells and neural progenitor cell differentiation, there were greater levels of hyperphosphorylated tau. Such results suggest that the decreased proliferation of progenitor cells may be due to the intracellular hyperphosphorylation of tau, and that NFTs formed by this hyperphosphorylation are a partial contributor to decreased neurogenesis (Demars et al., 2010).

### **2.1.2 Amyloid Beta Plaques**

As discussed above, the presence of abnormal A $\beta$  peptide plaques is one of several important pathological features of AD. A $\beta$  peptides are naturally produced as metabolic products. They originate from proteolysis of the APP by the beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), a  $\beta$ -secretase, and  $\gamma$ -secretase. Greater rates of production than that of clearance, as well as the aggregation of the peptides, result in the accumulation of A $\beta$  plaques. Previous research indicates A $\beta$  plaques as an implicating factor of AD (Querfurth & LaFeria, 2010).

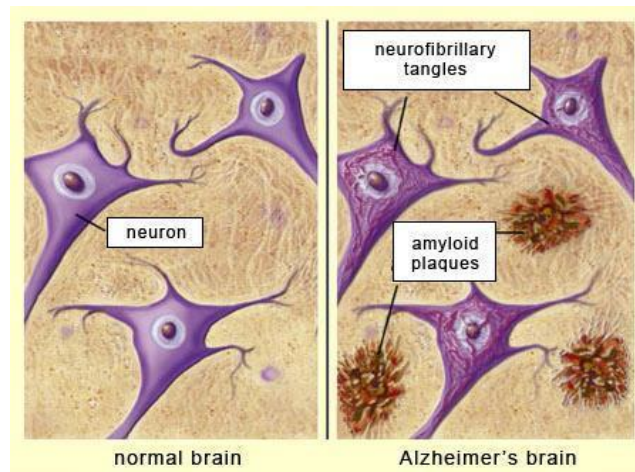
A $\beta$  self-aggregates into different forms, one of which is oligomers that coalesce into intermediate assemblies. It is these oligomers and intermediate assemblies which are known to

be the most neurotoxic forms of A $\beta$  and their numbers are associated with the severity of the cognitive defect in AD. High levels of A $\beta$  at synapses dampen excitatory transmission and prevent neuronal hyperactivity. A reduction in both neprilysin, an endopeptidase that degrades A $\beta$ , and insulin-degrading enzyme, a thiol metalloendopeptidase, causes A $\beta$  accumulation (Querfurth & LaFeria, 2010). Various pathological hallmarks are triggered by Amyloid Beta species such as neuronal death, delayed timing in neuron death, dysfunctional synapses, and instant neuronal death. These pathological hallmarks require the cytoplasmic, tau pathology. The amyloid Beta proteins and tau enhance each other's toxicity overall in the form of a feedback loop.

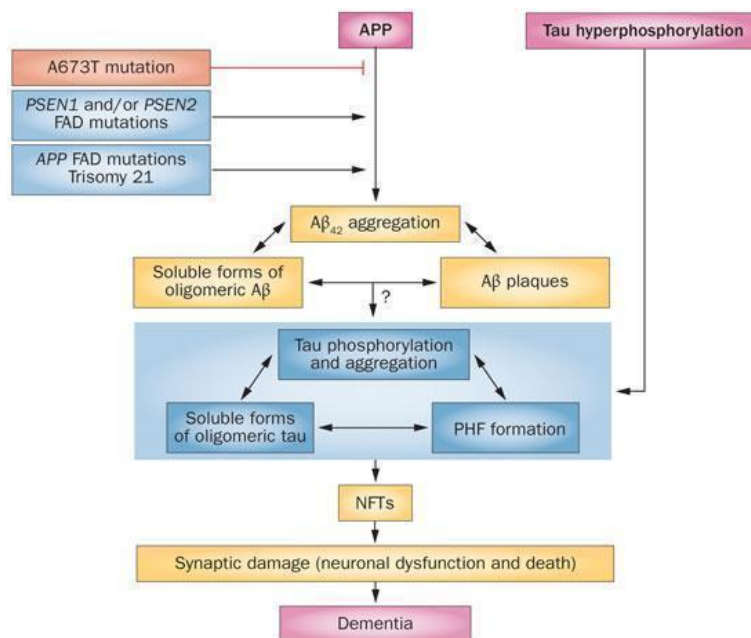
### **2.1.3 Neurofibrillary Tangles**

Mutation of the tau gene can lead to formation of insoluble filaments of hyperphosphorylated tau aggregates called *neurofibrillary tangles* (NFTs), which are considered to be another pathological hallmark of AD. NFTs are not restricted to AD and are also implicated in other neurodegenerative diseases. These mutations cause microtubule destabilization, decrease tau affinity to microtubules, and result in tau aggregation (Rocher et al., 2009). Despite positive correlations between NFTs and cognitive deficits, the direct relationship between them still remains unclear (Santa Cruz et al., 2005). Several previous studies have indicated that NFTs may not be linked to the progression of neurodegeneration in AD and may even play a protective role. One study concluded that structural and functional neuronal changes from expression of mutated tau were independent of NFT formation in mutant tau overexpressing mice models (Rocher et al., 2009). Another study observed NFT formation and extensive cell death in aged mice expressing non-mutant human tau, suggesting that cell death may occur independently of NFT

formation (Andorfer et al., 2005). Although the relationship between NFTs and tau mutations remains elusive, there is wide evidence for NFT formation contributing to the progression of AD.



**Figure 2.** Pathological Hallmarks of AD (Boston University School of Public Health, 2013).



**Figure 3.** Cascade of factors involved in the development of AD (Giacobini & Gold, 2013)

## 2.2 Neurogenesis in the Olfactory Bulb

The OB is multi-layered. The source of newborn neurons is the *subventricular zone* (SVZ) of lateral ventricles and they migrate through the rostral migratory stream (RMS) to the

OB (Lazarini & Lledo, 2011). In the OB, the new neurons will encounter multiple cell layers in between the olfactory tract: GCL (closest to olfactory tract), *internal plexiform layer*, *mitral cell layer*, *external plexiform layer*, *glomerular layer (GL)*, and *olfactory nerve layer* (Doty, 2012). This spatial migration process occurs during the period of six days (Alonso et al., 2006).

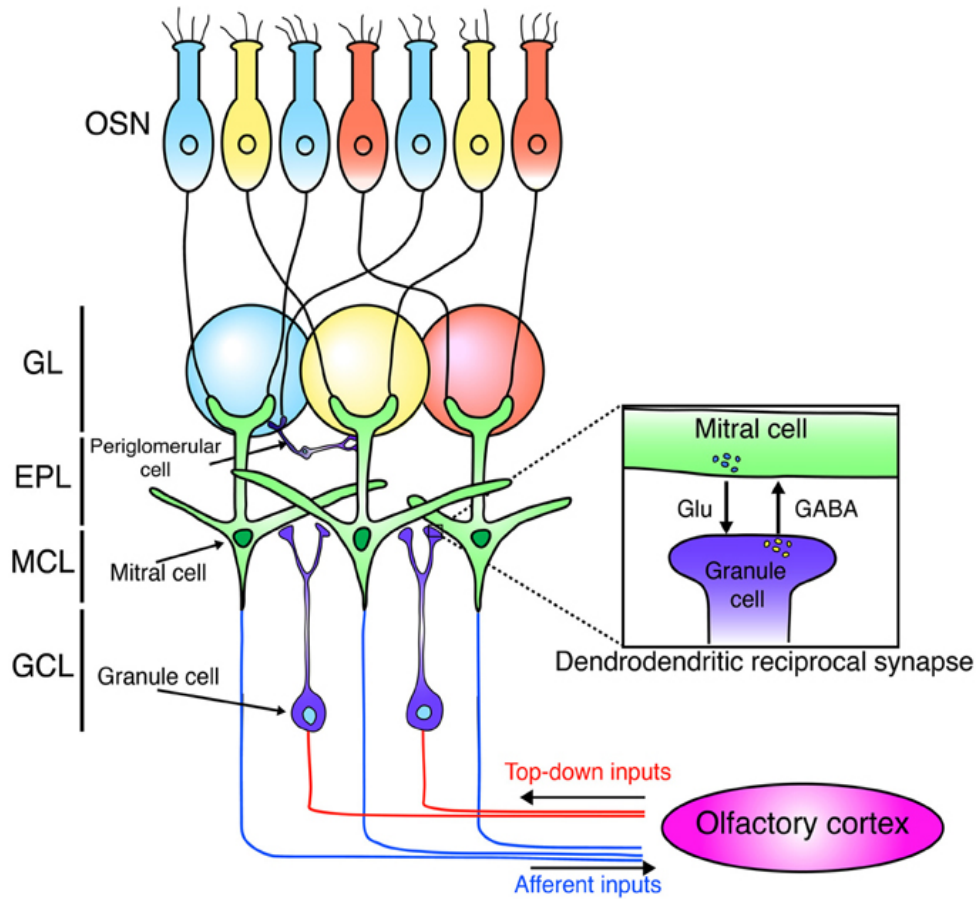
As the young neurons migrate from the SVZ to the OB, they differentiate into granule cells (GCs) and periglomerular cells (PCs). A majority of the GCs in the OB will be generated postnatally and continue generation into adulthood. Due to apoptosis occurring in the layers where young neurons are placed, these newly generated GCs replace older cells. The GCs begin to form into sheets for the GCL. Short neurites extend into deeper sections of the GCL and a large dendrite extends in the direction of the external plexiform layer (EPL). The role of GCs is to monitor the mitral and tufted cells to optimize olfactory function. They do so by decreasing possible overlap for odor representations executed by mitral and tufted cells. The tufted cells receive input from receptor cells in the olfactory epithelium (found in the nose) to aid in the sense of smell. Mitral cells' response to odor is shaped by GCs in the form of activity-dependent mechanisms which leads to an increase in olfactory discrimination (Petreanu & Alvarez-Buylla, 2002).

There are five stages in the development of newborn neurons. The classifications are: 1) tangentially migrating neuroblasts (days 2-7), 2) radially migrating young neurons (days 5-7), 3) GCs with a simple unbranched dendrite (days 9-13), 4) GCs with a non-spiny branched dendrite in the EPL (days 11-22), and 5) mature GCs (days 15-30), and periglomerular interneurons. After 15 to 22 days after birth, newly-generated GCs will display a mature morphology (Petreanu & Alvarez-Buylla, 2002).

Stimulation of adult neurogenesis has been seen to improve long-term memory and

olfactory learning. For example, Lepousez et al. (2014) found that increase in olfactory learning leads to higher spine density of adult-born GCs. Olfactory learning also stimulates restoration of excitatory and inhibitory inputs in the dendritic domain of adult-born neurons. Both *associative* (classical and *operant conditioning*) and *nonassociative* (*habituation* and *sensitization*) forms of olfactory learning require adult neurogenesis. In turn, adult-generated neurons are needed to shape olfactory memory circuits. Odor deprivation generally decreases the survival and excitability of adult-generated OB neurons as well as olfactory memory performance (Alonso et al., 2006; Singer et al., 2009).

The olfactory epithelium, specialized sensory tissue inside the nose, provides chemical information to the OB circuit. Sensory neurons transform this chemical signaling into electrical patterns (Lazarini & Lledo, 2011). Centrifugal fibers found in the OB contribute to context-dependent modulation of circuit activity, such as attention, reward, learning, and memory (Lazarini & Lledo, 2011). Before a certain critical period, odor exposure does not affect the survival of newborn neurons, suggesting that the lack of activity does not have an effect on generation, migration, and early differentiation of GCs. However, when the GCs mature and presumably become synaptically connected, their survival depends on the level of activity. The dependence of mature GCs on odor exposure may allow construction of specific OB circuits and replacements (Petreanu & Alvarez-Buylla, 2002).



**Figure 4.** The neuronal circuit of the OB (Aimone et al., 2014).

### 2.3 Therapeutic Approaches for Neurodegenerative Diseases

During the early stages of neurodegenerative diseases, changes in neurogenesis are observed. This suggests that interventions preserving neurogenesis could be helpful for presenting therapeutic strategies to slow down cognitive decline with the diseases (Rodríguez & Verkhratsky, 2011).

The main focus of brain repair is to increase the amount of new neurons into the brain to fix any possible brain lesion damages. In addition to understanding neurogenesis and brain repair, research concluded that progenitors of new neurons are present in the subventricular and subgranular zones of the hippocampal DG. The generation of neuroblasts in the SVZ travel to

reach the adult OB and become neurons. Through this, certain types of grafted neurons are able to travel through the brain (Lois & Kelsch, 2014).

## **2.4 Recent Research on Neurodegenerative Diseases Using Mice Models**

Many studies target the A $\beta$  hypothesis of AD, either by attempting to decrease initial production or increase eventual clearance. A 2014 study used the Tg2576 familial AD mouse model. After undergoing immunotherapy by intraperitoneal antibody injection to target specific oligomers in A $\beta$  plaques, plaques were not reduced although synapse loss was alleviated. The study hypothesized that cognitive functions, as a result, may increase (Dorostkar et al., 2014).

Another theory of treatment focuses on the behavioral aspect of AD, focusing on the idea that increased cognitive stimulation might delay or alleviate symptoms of AD. A 2015 study of 3xTg-AD mice with both APP and tau301L transgenes indicated that, if training began at p3mo, postnatal three months, mice were able to perform better on hippocampus-dependent spatial tests. This training was episodic and continued throughout the aging process. These tests included the Morris Water Maze and the novel object recognition memory task (Martinez-Coria et al., 2015).

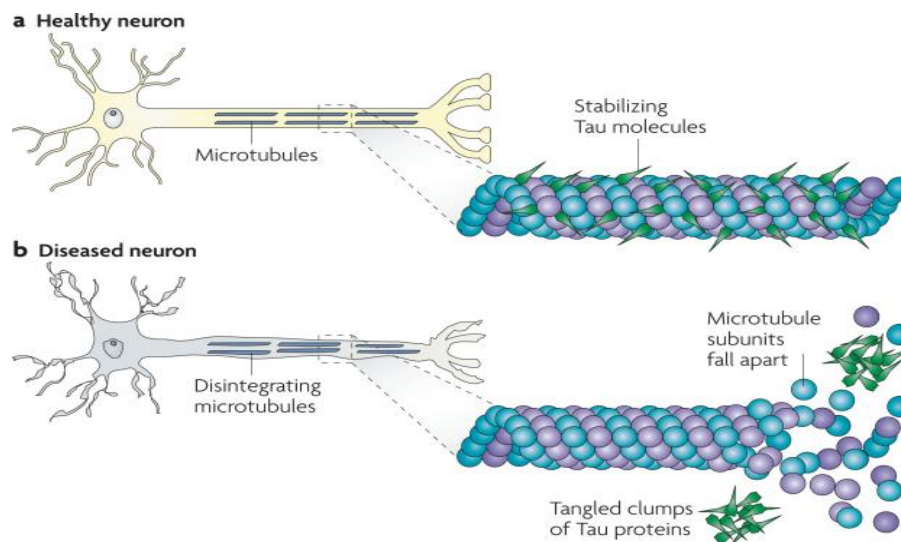
In addition, a 2014 study investigated the relationship between memory deficits upon tau expression in the hippocampus using the rTg4510 model expressing the tau301L transgene. Behavioral tests such as hippocampal-dependent incremental repeated acquisition (IRA) and MWM were conducted to identify memory deficits. Tetracycline transactivator (TTA) allowed a conditional model of tau P301L expression; tau is expressed when tetracycline is absent. They found the presence of tau pathology after 3 months of tau expression in the hippocampus. The behavioral test results aligned with these findings as the Tg mice showed greater memory deficits

with tau expression (Hunsberger et al., 2014).

## 2.5 Selection of a Mouse Model

We took many considerations when deciding which mouse model would be used for our study. Primarily, it was important to have a model that was in fact representative of AD through its pathology.

There has been a great deal of evidence that there is a loss of synaptic connections in AD, which partially contribute to the cognitive decline. While it was previously thought that the main protein responsible for cognitive decline in AD was strictly  $A\beta$ , there is more support due to recent studies for an equally important role of tau (Pooler, Noble, & Hanger, 2014). Specifically, in AD, tau becomes hyperphosphorylated, and it is no longer able to interact correctly with microtubules. Additionally, the soluble form of tau is thought to be neurotoxic and lead to synaptic generation, though the mechanism is not yet fully understood (Pooler, Noble, & Hanger, 2014).



**Figure 5.** Tau Function in Healthy vs. Diseased Neurons (Brunden, Trojanowski, & Lee, 2009)

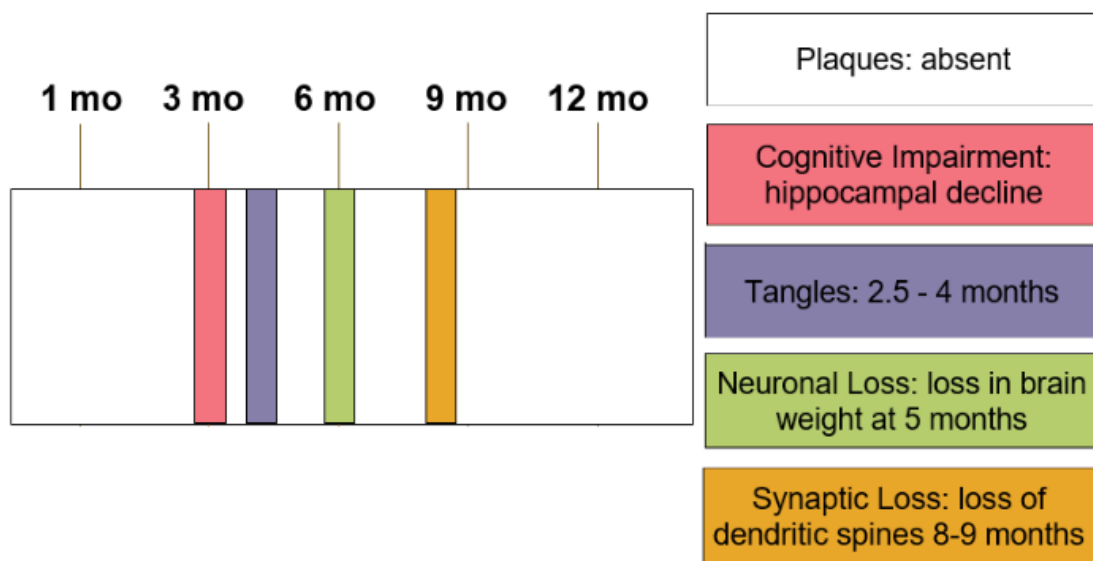


Although A $\beta$  is usually considered the main pathological hallmark of AD, tau is equally important due to its role in the formation of neurofibrillary tangles. While there are several mouse models that are created to represent the A $\beta$  hypothesis, specifically those that have mutations in APP or the presenilin genes, these models do not allow for the study of the effects of NFTs as produced by hyperphosphorylated tau (Chin, 2011). Thus it is important to choose a model that allows for the isolation of the effect of hyperphosphorylated tau pathology without the presence of A $\beta$  aggregates.

It is currently not known why mice that are Tg in the APP gene (responsible for the formation of amyloid-beta aggregates) do not always develop NFTs, nor are the interactions between A $\beta$  and tau pathologies understood very well (Chin, 2011). If models can be used that contain only transgenes involved in the formation of hyperphosphorylated tau and tau aggregates, the role of tau in cognitive impairment and neurodegeneration can be better understood.

For our experiments, we selected the Tg4510 mouse model, which express a repressible form of the MAPT P301L mutation in the genome, responsible for the dementia pathology (SantaCruz et al., 2005; Hunsberger et al., 2014). The expression of this transgene leads to impairments in a wide range of areas, including spatial reference memory, forebrain atrophy, and pathologies of neurofibrillary tangles (Ramsden et al., 2005). The transgene was confirmed to be strongly expressed in structures present in the forebrain, specifically the neocortex and the hippocampus, and thus it is a model relevant to studying diseases that involve dysfunctional tau protein, such as AD and frontotemporal dementia with parkinsonism (Ramsden et al., 2005). Importantly, the expression of this transgene is suppressible, meaning that it can be turned off with the administration of doxycycline (Ramsden et al., 2005).

Following the generation of this mouse model, several experiments have been performed to study the effects of the expression of the P301L transgene on the Tg mouse model. One particular study looked at the neuronal network activity in the hippocampus of Tg mice at various ages. The study found that there was an age-dependent formation of the NFTs and loss of neurons in the hippocampus, as well as an impaired memory function, which was tested with the MWM, beginning as early as 2.5 months of age (Scott, Kiss, Kawabe, & Hajos, 2016). A similar study found that compared to WT littermates, Tg mice had significant cognitive impairments at the age of 4 months, in addition to a loss of neurons in the hippocampus (Ramsden et al., 2005). In this mouse model, pre-tangles were also found in the cortex and the basal ganglia by the age of 6.5 months (Lewis et al., 2000).



**Figure 6.** Timeline of the Neurodegeneration of the Tg mouse model (Crimins, Rocher, & Leubke, 2012; Menkes-Caspi et al., 2015; Santa Cruz et al., 2005; Spires et al., 2006).

These studies collectively suggest that this mouse model is a useful model for comparison with human tauopathy and that it may be established as a standard method of studying the buildup of tau for application to human neurodegenerative diseases including AD.

## Chapter 3: Methodology

### 3.1 Research Design

This study aims to characterize the olfactory decline of Tg tau pathology mouse model through behavioral tests and neurogenesis analysis.

#### 3.1.1 Animals

All of the animal procedures and experiments for this study were performed in accordance to the guidelines of the Institutional Animal Care and Use Committee of the University of Maryland. All experiments including the behavioral, perfusion, and immunohistochemistry were performed rTg4510 female and male mice which were littermates (1-7 months old). This strain of mice was obtained from the Jackson Laboratory (stock #015815). Since cortical cell loss occurs around 8 months in this strain, we performed experiments within the strain from the time of 60 days postnatal (PD60) through the age of 7 months (Spires et al., 2006). Because the Tg strain have a transgene inserted that causes the development of AD related pathology, we referred to these mice as the “AD group,” or our experimental group, and the same observations and injections were conducted in parallel for the non-positive littermates. Both female and male mice were used in experimental groups, with roughly equal numbers of each sex per trial group.

#### 3.1.2 Environmental Conditions

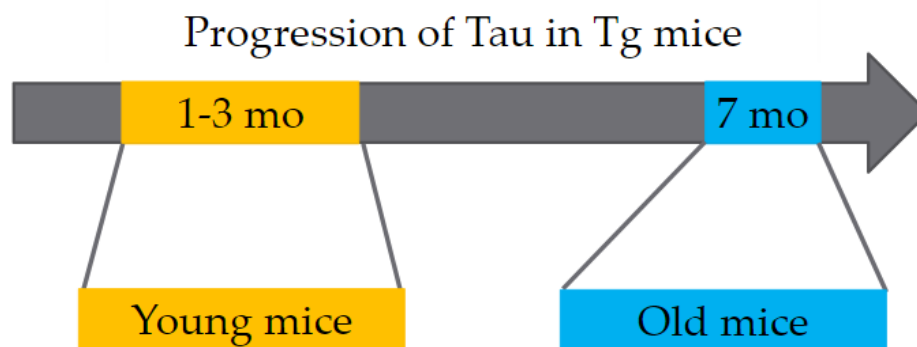
It is important that mice have the same environmental conditions to prevent confounding factors. Animals were kept on a cycle with 12 light/12 dark cycle. All behavioral experiments were performed during the dark cycle of the day for the mice, during which time the mice are

known to be more active. Three to five mice were housed per cage. The mice were housed at 72 degrees Fahrenheit and the humidity was controlled to 60 plus or minus 5%, in accordance with standards of mice studies. The mice were monitored daily to ensure healthy mass and food intake.

### 3.1.3 Treatment Groups

All experiments were performed on four treatment groups, each consisting of three to seven mice. The groups are composed of WT or Tg strain at 2 and 7 months. These mice are referred to as “Young” and “Old,” respectively. Some behavioral testing was performed on various younger mice groups (1 and 3 months) based on availability at the time of the experiments.

	WT	Tg
Young	p60 WT	p60 Tg
Old	p7mo WT	p7mo Tg

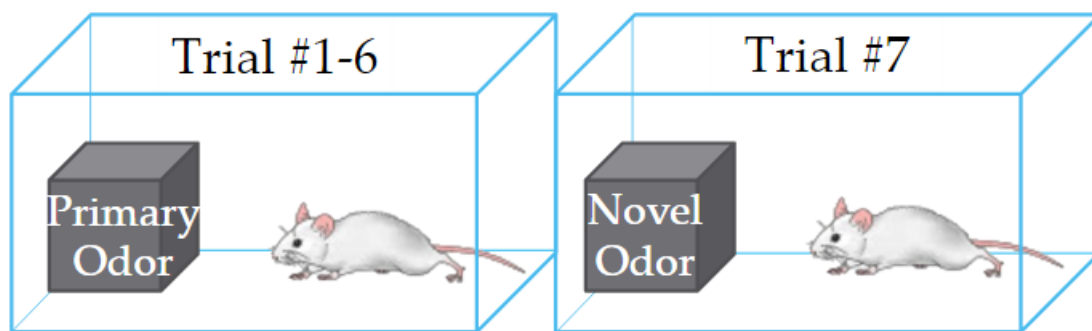


**Figure 7.** Treatment groups of WT and Tg mouse models.

### 3.1.4 Behavioral Tests

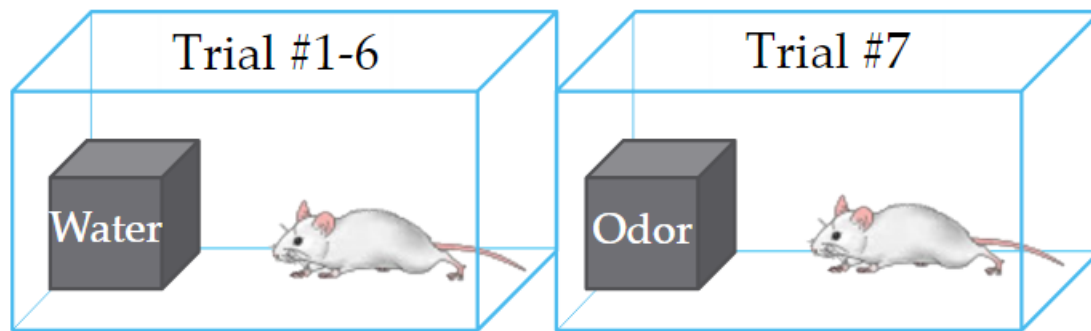
Several behavioral tests were performed in order to determine cognitive learning in the mice.

**Habituation/dishabituation.** The habituation dishabituation protocol is used to analyze the ability to discriminate between odors. A 1:1000 dilution of the primary odor is presented on a small wooden cube and placed within the mouse's cage after a thirty minute period of habituation to the cage with a plain cube. The mouse is presented with a repeated odor for several trials. The amount of time in two minutes that the mice spend investigating the face of the cube with the odor is recorded. The inter-trial interval approximately 30 seconds. On the seventh trial, this odor is replaced by a novel odor. If the mouse is able to discriminate between odors, it will demonstrate dishabituation. Ideally, the mice should have decreased investigation time as trial six approaches, and an increased investigation time in the seventh trial. That would indicate the mouse's ability to distinguish between the two isomers. The habituation/dishabituation test serves as a general control, ensuring that the mice used in later behavioral experiments have normally functioning olfactory systems. It observes olfactory memory and learning by using two isomers, L-carvone and D-carvone. A two-tailed t-test was used for data analysis.



**Figure 8.** Habituation Dishabituation Protocol. Primary odor, L-carvone; Novel odor, D-carvone

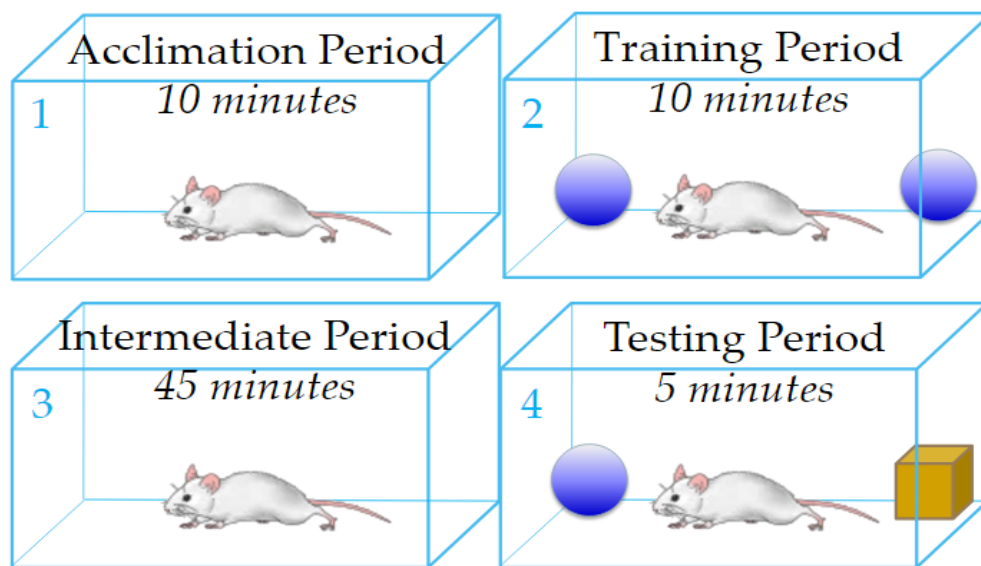
**Odor detection threshold.** The Odor Detection Threshold (ODT) was determined by testing the investigation time of four groups of mice at different concentrations of L-carvone. The purpose of the test was to identify the lowest concentration at which the mice could detect different odors. The groups consisted of 1 month WT, 1 month Tg, 7 months WT, and 7 months Tg. Each mouse was placed into a clean cage with a small, wooden cube placed into the center for 30 minutes to allow it to become habituated to the cube. For the first trial, a new cube with 100 microliters of water placed on one surface of it was inserted into the cage. The investigation time of the mice was recorded with a timer for 1 minute and this is influenced by the amount of time the mice spent investigating the face of the cube with the water present. This procedure with the water is repeated until the seventh trial. On the seventh trial, 100 microliters of serial dilutions of either 1:100,000, 1:150,000, 1:200,000, 1:200,000, 1:250,000, or 1:300,000 of two isomers, L-carvone or D-carvone.



**Figure 9.** Odor Detection Threshold Protocol. Odor, either L-carvone or D-carvone.

**Novel object recognition.** The Novel Object Recognition (NOR) task was adopted from the protocol used by Bevins and Besheer (Bevins & Besheer, 2006). The paradigm was carried out for four different groups of mice, including 2 month WT and Tg, as well as 7 month WT and Tg. The day of the testing, we placed the mice in an unfamiliar cage (the cage in which the

experiment would take place) for an acclimation period of 10 minutes. After 10 minutes, the mouse was removed and temporarily placed in a cup while we placed the training objects, which were two blue marbles, on opposite sides at the back of the cage. These marbles were taped to the bottom of the cage so that they could not be moved by the mice. The mice had a training period of 10 minutes during which time they were placed in the cage with the marbles, and the amount of time spent investigating each marble was recorded with the use of a stopwatch. The marbles were then removed and the mouse remained in the empty cage for a 45 minute period. We then placed the trained object, the blue marble, in the back corner of one side of the cage, and introduced the novel object, a yellow wooden cube of similar size, in the opposite corner. The side of the cage in which the novel object was placed was randomized. The mouse then underwent a 5 minute testing period with the trained object and the normal object, and the time spent investigating each object was again recorded. During the testing interval, both objects were taped down in the cage to prevent them from being moved. The NOR serves as a control for general neurogenesis and cognitive ability in the mice.



**Figure 10.** Depiction of phases for novel object recognition test.

### **3.1.5 BrdU Injections**

Cell tracer 5'-bromo-2'-deoxyuridine (BrdU) is a thymidine analog that becomes integrated into the cell during cell division and is subsequently passed down to daughter cells (Wojtowicz & Kee, 2006). BrdU marks cells undergoing cell division and newly formed neurons at stages of proliferation, differentiation and maturation, and can thus be used to trace neurogenesis from proliferation to maturation. BrdU prepared in phosphate-buffered saline (PBS, 10 mg/mL) was injected intraperitoneally at 100m/kg per injection for a total of three times, every two hours to 1 month old mice (Nunez-Parra, Pugh & Araneda 2010). BrdU was used to visualize newly born adult neurons.

### **3.1.6 Perfusion**

As previous studies have indicated that newly born neurons have differentiated into mature GCs and periglomerular cells (PG) approximately 30 days after their formation (Nunez-Parra, Pugh & Araneda 2010). Mice were anesthetized with isoflurane and the brains were fixed by transcardial perfusion with about 40mL of cold PBS until the fluid was clear of blood when leaving the body of the mouse. Cold 4% paraformaldehyde solution (PFA, Electron Microscopy Science) was prepared in PBS in 1:4 ratio. After perfusion, mice brains were carefully dissected to preserve whole, intact OB, and post fixed in 4% PFA overnight at 4°C in order to minimize damage to the OB (Nunez-Parra, Pugh & Araneda 2010).

### **3.1.7 Tissue Preservation**

After perfusion fixation, the mice brains were removed and cryoprotected overnight with a solution of 30% sucrose in PBS until they were in equilibrium with the solution and sunk to the bottom. These brains were then sectioned into right and left OBs and hippocampi. All four



sections were embedded in Tissue-Tek O.C.T. (Electron Microscopy Science) and *cryopreserved* in -80°C. The hippocampal sections were preserved for further reference due to time constraints of the experiment (Nunez-Parra, Pugh & Araneda 2010).

### **3.1.8 Cryostat and Slicing**

Randomly chosen OB sections of each preserved brains were sliced in 20µm thick sagittal sections using a Leica CM1850 cryostat. Four sections were mounted on each of six frozen tissue slides (Fisher) and stored at -20 °C until use (Nunez-Parra, Pugh & Araneda 2010).

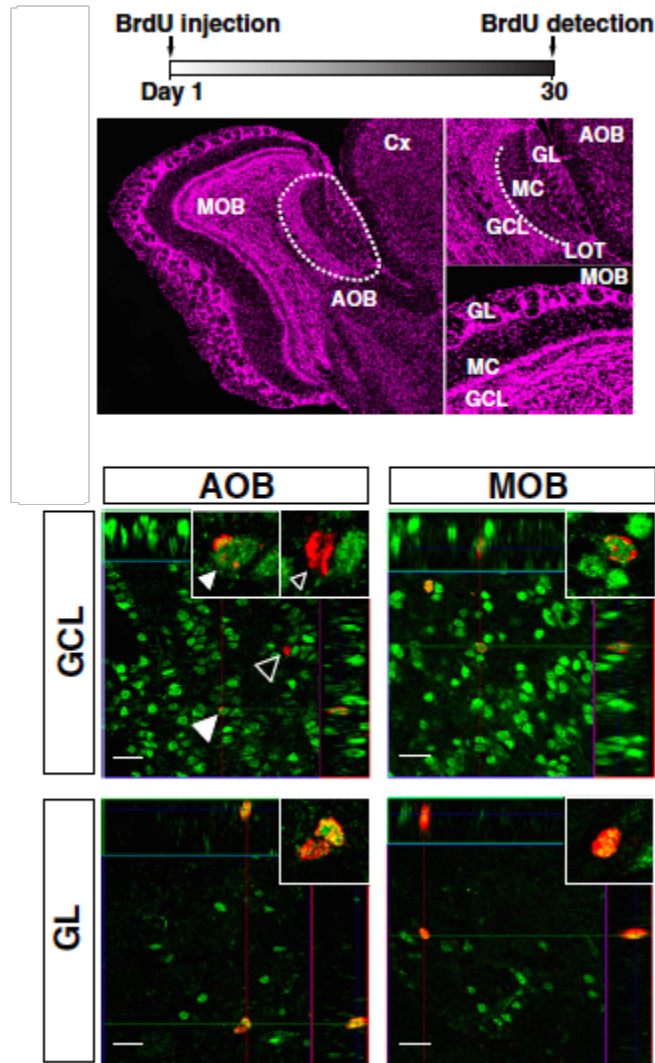
### **3.1.9 Immunohistochemistry**

The brain slices containing the accessory and main OBs (AOB and MOB, respectively) were prepared for histology under the confocal microscope using immunohistochemical staining methods. These slices were rinsed with PBS twice and double distilled water once after being warmed to room temperature. Then, they were placed in hydrochloric acid solution at 37°C for one hour to denature the DNA and facilitate staining by antibody binding. After rinsing three times with sodium tetraborate buffer for neutralization, the slices were washed twice with PBS and once with PBS-T (PBS solution with Triton-x-100). 10% donkey serum in PBS-T was used to block nonspecific sites of the brain slices for one hour. Next, they were incubated with rat anti-BrdU to label newly born cells and mouse anti-NeuN to label all neurons diluted in PBS-T and donkey serum overnight at room temperature. The slices were rinsed four times with PBS-T the next day and incubated with secondary antibodies donkey anti-Rat Alexa-594 and donkey anti-Mouse Alexa-488 diluted in donkey serum and PBS-T for two hours at room temperature in the dark. Finally, they were rinsed with PBS-T once, PBS three times, mounted with Vectashield, sealed coverslips with clear nail polish, and stored in a dark place (Nunez-Parra,

Pugh & Aranedo 2010).

### **3.1.10 Confocal Microscopy**

Three out of the four sagittal sections per animal containing the AOB and MOB GL and GCL layers were used for quantification. We used the Leica SP5X confocal microscope provided by Imaging Core, with white light laser (470-670 nm in 1 nm increments) at 70%, xyz scanning, and z-Galvo configurations. We used 63x oil immersion objective at 3 $\mu$ m optical steps. In order to focus and navigate, we also visualized the specimen through the eyepiece under filter sets GFP and RFP. For each sample, we only imaged if one or more neurons could be visualized in filter set GFP and RFP. We used double labeling with BrdU, indicating newly generated neurons in red, and NeuN, indicating all neurons in green. Cells which expressed both indicated the presence of neurogenesis (Wojtowicz & Kee, 2006).



**Figure 11.** Confocal images of sagittal sections contained AOB and MOB GL and GCL layers. RFP is used to visualize BrdU (newborn neurons), and GFP is used to visualize NeuN (all neurons). When there is colocalization between red and green, cell is positive for both BrdU and NeuN (Nunez-Parra, Pugh & Araneda, 2010).

### 3.1.11 Statistical Analysis

A total of twenty-four sagittal brain slices were produced of either right or left brain hemispheres for each mouse. The twenty-four serial sagittal sections were divided into six slides, four sections per slide, and one out of the six slides were used for quantification. Only three

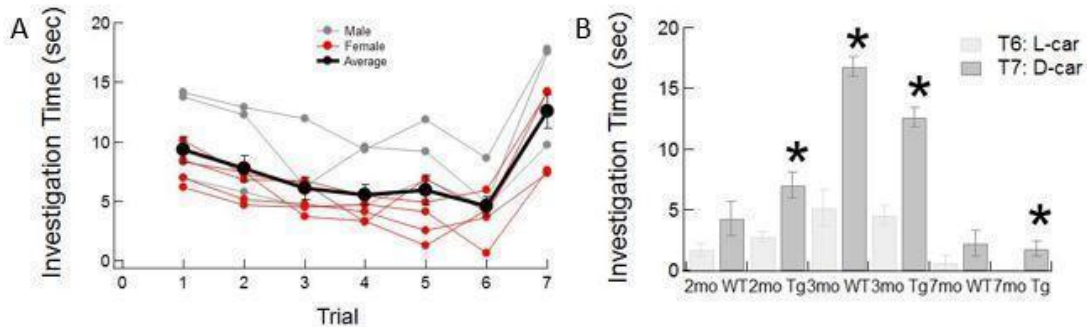
sections out of the four on the randomly chose slide were used for confocal analysis. For each section, BrdU positive cells were quantified in a total of fourteen regions, including the GCL and GL of the AOB and MOB, 2 to 4 Z-stack images, respectively. Data was expressed as the mean number of BrdU positive cells normalized to the area of the Z-stack in  $\text{mm}^3$  (BrdU positive cells/ $\text{mm}^3$ )  $\pm$  standard error for each region (Nunez-Parra, Pugh & Araneda 2010). Statistical significance was determined using two-tailed t-tests on excel. Graphs were produced using the Igor software.

## Chapter 4: Results

### 4.1 Behavioral Data

#### 4.1.1 Habituation/Dishabituation

The habituation/dishabituation paradigm was conducted for six different groups of mice, including 2 month WT and Tg, 3 month WT and Tg, and 7 month WT and Tg. 3 month Tg mice were compared by gender and the female Tg mice generally showed lower investigation times in all seven trials than male Tg mice did (Fig. 12A). Behavioral data by gender can be further tested in future studies to determine significance of gender in habituation and dishabituation. Overall, the investigation time of the novel odor used in the seventh trial increased for all six groups as compared to that of the odor used in the sixth trial (Fig. 12B). Of the six groups, 2 month Tg, 3 month WT, 3 month Tg, and 7 month Tg mice showed statistically significant increase between the two trials and 2 month WT and 7 month WT did not show any significance (Fig. 12B).



**Figure 12.** Habituation/Dishabituation results. (A) Investigation time in seconds across 7 trials of 3 month Tg mice for gender comparison. Female Tg mice have lower investigation time in general when compared to male Tg mice. (B) Investigation time in seconds across three age groups (2 months, 3 months, 7 months), WT vs. Tg. Light gray indicates investigation time for trial 6, L-carvone, the same odor used for trials 1-5. Dark gray indicates the novel odor, D-carvone. There should be a significant increase in investigation time. Significance is indicated with an asterisk (t-test; \*,  $p < 0.05$ ).

Trial 6 (sec)	Trial 7 (sec)	Number of mice	p-value
4.6 ± 0.8	12.6 ± 1.4	8	<0.05*

**Table 1:** Figure 12A data

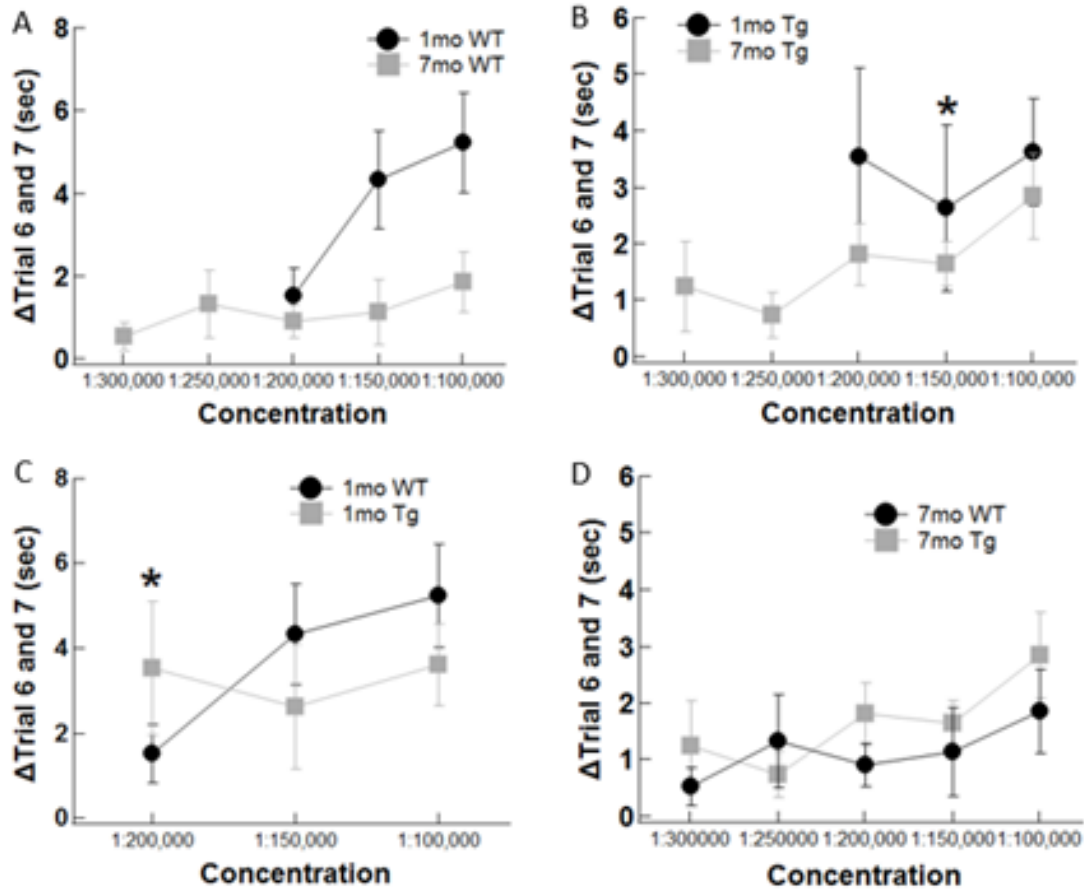
Age/Genotype	Trial 6 (sec)	Trial 7 (sec)	Number of mice	p-value
2mo WT	1.7 ± 0.5	4.3 ± 1.5	4	0.19
2mo Tg	2.8 ± 0.4	7.0 ± 1.1	12	<0.05*
3mo WT	5.1 ± 1.5	16.8 ± 0.8	4	<0.05*
3mo Tg	4.6 ± 0.8	12.6 ± 0.8	8	<0.05*
7mo WT	0.6 ± 0.6	2.2 ± 1.1	2	0.24
7mo Tg	0.0 ± 0.0	1.8 ± 0.6	4	<0.05*

**Table 2:** Figure 12B data

#### 4.1.2 Odor Detection Threshold

Previous studies show a correlation between olfactory dysfunction and neurodegenerative diseases (Albers, Tabert & Devanand, 2006; Enwere, 2004). The groups were compared in four different combinations and the data showed a general increase in the investigation time difference between the sixth and seventh trials as the concentration of L-carvone increased (Fig. 13). Significance was shown in Fig. 13B comparing 1 month and 7 month Tg mice at 1:150,000 concentration and in Fig. 13C comparing 1 month WT and Tg mice at 1:200,000 concentration. No significance was seen in Fig. 13A comparing 1 month and 7 month WT mice at all

concentrations , Fig. 13B at 1:100,000 and 1:200,000 concentrations , Fig. 13C at 1:100,000 and 1:150,000 concentrations , and Fig. 13D comparing 7 month WT and Tg mice at all concentrations .



**Figure 13.** Odor Detection Threshold of different age and genotypic groups. (A) Investigation time in seconds of mice at trial 6 and 7, where the odor is changed, recorded of five different concentration of L-carvone comparing 1 month and 7 month WT mice (B). Comparing 1 month and 7 month Tg mice at five different concentrations. (C). Comparing 1 month WT and 1 month Tg mice at three different concentrations. (D). Comparing 7 month WT and 7 month Tg mice at three different concentrations (t-test; \*,  $p < 0.05$ ). Time difference in trial 6 and 7 increases as concentration increases in all mice but 1 month WT mice generally have higher time differences than older Tg mice.

Concentration	1mo WT Δ Trial 6 & 7 (sec)	Number of mice	7mo WT Δ Trial 6 & 7 (sec)	Number of mice	p-value
1:200,000	1.5 ± 0.7	2	0.9 ± 0.4	4	0.48
1:150,000	4.3 ± 1.2	2	1.1 ± 0.8	4	0.18
1:100,000	5.2 ± 1.2	2	1.9 ± 0.7	4	0.21

**Table 3:** Figure 13A data

Concentration	1mo Tg Δ Trial 6 & 7 (sec)	Number of mice	7mo Tg Δ Trial 6 & 7 (sec)	Number of mice	p-value
1:200,000	1.5 ± 0.7	2	1.8 ± 0.5	3	0.08
1:150,000	2.6 ± 1.5	8	1.6 ± 0.4	4	0.02*
1:100,000	3.6 ± 1.0	6	2.8 ± 0.8	4	0.31

**Table 4:** Figure 13B data

Concentration	1mo WT Δ Trial 6 & 7 (sec)	Number of mice	1mo Tg Δ Trial 6 & 7 (sec)	Number of mice	p-value
1:200,000	1.5 ± 0.7	2	3.5 ± 1.6	8	0.03*
1:150,000	4.3 ± 1.2	2	2.6 ± 1.5	8	0.25
1:100,000	5.2 ± 1.2	2	3.6 ± 1.0	6	0.41

**Table 5:** Figure 13C data



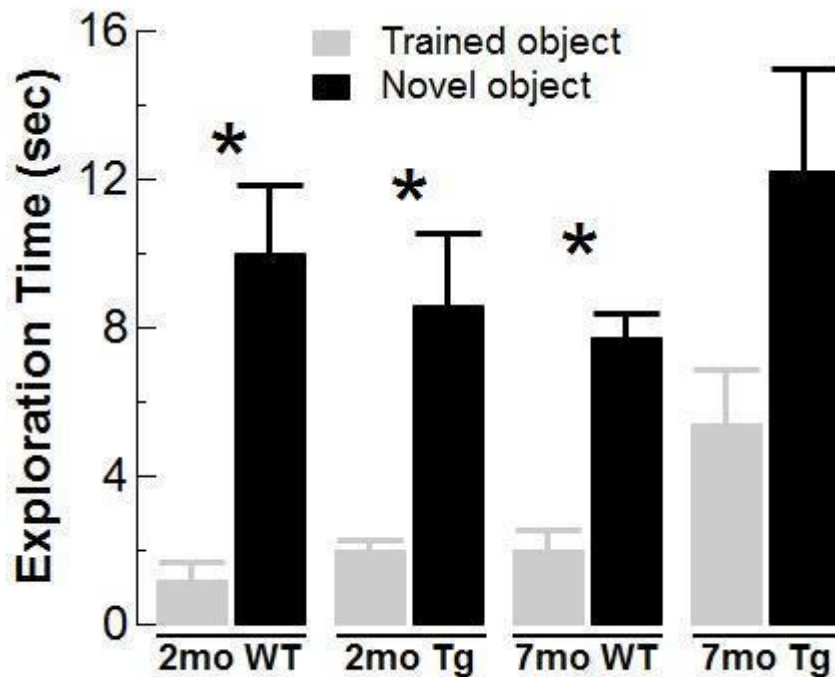
Concentration	7mo WT Δ Trial 6 & 7 (sec)	Number of mice	7mo Tg Δ Trial 6 & 7 (sec)	Number of mice	p-value
1:300,000	0.5 ± 0.3	4	1.2 ± 0.8	3	0.14
1:250,000	1.3 ± 0.8	4	0.7 ± 0.4	3	0.37
1:200,000	0.9 ± 0.4	4	1.8 ± 0.5	3	0.12
1:150,000	1.1 ± 0.8	4	1.6 ± 0.4	4	0.19
1:100,000	1.9 ± 0.7	4	2.8 ± 0.8	4	0.06

**Table 6:** Figure 13D data

#### 4.1.3 Novel Object Recognition

The results of the novel object recognition test showed that there were significant differences in investigation times between the trained and novel objects for the 2 month WT, 2 month Tg, and 7 month WT mice (Fig. 14). No statistical significance was shown in 7 month Tg mice (Fig. 14). Despite the statistical significance, all mice groups had higher exploration time of the novel object as compared to that of the trained object. Discrimination index was calculated using a formula taken from a paper by Antunes and Biala in order to confirm that the mice could successfully discriminate the trained and novel objects. The formula used was  $DI = (\text{Novel Object Exploration Time} - \text{Trained Object Exploration Time}) / \text{Total Exploration Time}$  (Antunes & Biala, 2012). A positive score indicated more time spent with the novel object whereas a negative score indicated more time spent with the familiar object. A zero score indicated a null preference (Antunes & Biala, 2012). Calculations were all positive with the highest DI for 2

month WT and lowest DI for 7 months Tg mice (2 month WT, 0.786; 2 month Tg, 0.623; 7 month WT, 0.576; 7 month Tg, 0.382; not shown in figure).



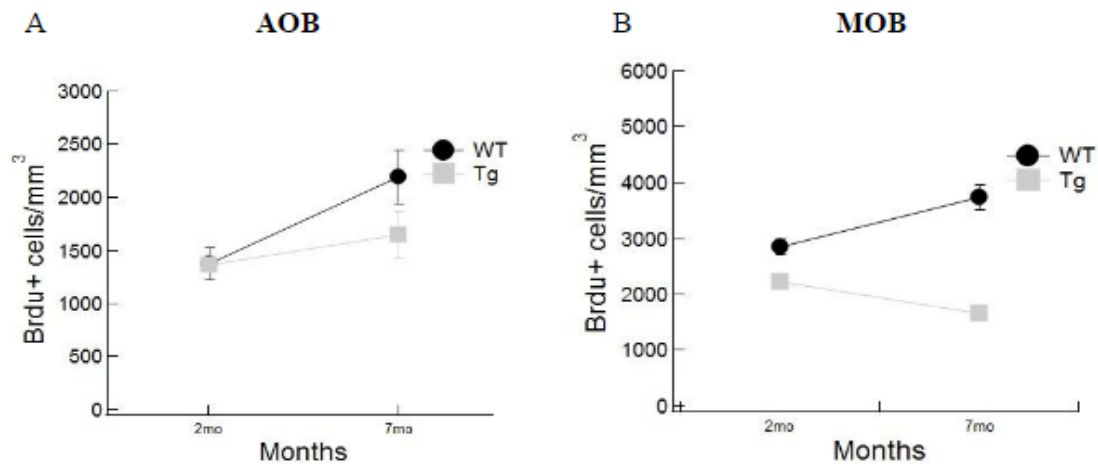
**Figure 14.** Novel Object Recognition Test of 2 and 7 month old WT and Tg mice (t-test;\*, p<0.05, 2mo WT, n = 6, 2mo Tg, n = 4, 7mo WT, n = 6, 7mo Tg, n = 3). Longer exploration time in seconds of novel object in all mice groups.

Age/Genotype	Exp time of TO (sec)	Exp time of NO (sec)	Number of mice	p-value
2mo WT	1.2 ± 0.4	10.0 ± 1.8	6	0.003*
2mo Tg	2.0 ± 0.2	8.6 ± 1.9	4	0.038*
7mo WT	2.1 ± 0.5	7.8 ± 0.6	6	0.00003*
7mo Tg	5.5 ± 1.4	12.3 ± 2.7	3	0.108

**Table 7:** Figure 14 data

## 4.2 Confocal Data

The confocal imaging data consisted of sections of the AOB and MOB to examine the correlation between age, genotype, and number of BrdU+ cells. No statistical significance was observed in all four groups in the AOB and the MOB (Fig. 15).



**Figure 15.** Confocal data visualized under Leica SP5X (confocal microscope). BrdU+ cells/mm<sup>3</sup> represents the number of cells that stained positive for BrdU and NeuN, or the number of newly differentiated and colocalized neurons. (A) The AOB exhibited a surprising increase in BrdU+ cells from 2 to 7 months of age, although WT mice had greater neurogenesis than Tg mice did at 7 months of age. (B) The main OB neurogenesis count increased for WT mice, while it decreased for Tg mice, over age. No statistical significance was observed (*t*-test, \*, *p*<0.05).

Age/Genotype	BrdU+ cells/mm <sup>3</sup>	Number of mice	p-value
2mo WT	1369 ± 157	3	0.996
2mo Tg	1365 ± 6	3	
7mo WT	2195 ± 248	3	0.692
7mo Tg	1647 ± 222	3	

**Table 8:** Figure 15A data

Age/Genotype	BrdU+ cells/mm <sup>3</sup>	Number of mice	p-value
2mo WT	2855 ± 148	3	0.583
2mo Tg	2213 ± 17	3	
7mo WT	3733 ± 228	3	0.167
7mo Tg	1650 ± 104	3	

**Table 9:** Figure 15B data

## Chapter 5: Discussion

### 5.1 Behavioral Data

Multiple behavioral tests were conducted to analyze the cognitive deficits in the Tg mice when compared to the control.

#### 5.1.1 Habituation/Dishabituation

The habituation/dishabituation protocol was chosen as a standard baseline test that controls for the mouse's ability to discriminate between odors. If the mouse has this ability, it demonstrates dishabituation upon the seventh trial's introduction of the novel odor. Neurogenesis continues in the OB, where neurons mature and integrate. This indicates that the mice have functional olfactory processing systems, as well as olfactory learning and memory, and can be accurately used for behavioral paradigms such as ODT and NOR.

The habituation/dishabituation protocol analyzed the ability to distinguish odors at 2, 3, and 7 months of age. 2 month Tg mice actually had higher investigation times than 7 month WT mice. At 2 months, it is likely that the effects of neurodegeneration have not yet materialized in the mouse model. Mice are not finished with the developmental phase at this age. In many other mouse models for AD, the effects of neurodegenerative disease do not become apparent in behavioral testing until around 3 or 4 months of age. At 3 months, there is a markedly significant difference. WT mice investigated the novel odor for a longer time than did the Tg mice group. Overall, the 3 month group investigated the novel odor for a longer time than the 2 month age group did; this may be attributed to brain and OB maturation. At 7 months, both WT and Tg mice groups show a marked decline in NOR. The WT group has a slightly higher investigation time. The results of the habituation/dishabituation paradigm seem to support our hypothesis that

the age-dependent cognitive decline and behavioral test performance due to development of tau introduced by the transgene.

### **5.1.2 Odor Detection Threshold**

Odor detection protocol observed the ability of the mice to detect minute difference in odor and to determine the ODT. The 1 month WT mice group showed significant increased levels of investigation time with the different concentration of L-carvone compared to the 7 month WT group who showed only a small increase in investigation time for the different concentration of L-carvone. The 1 month Tg mice showed a higher increase in investigation time as concentrations increased compared to the 7 month Tg mice which may be due to the fact that the 7 month mice are older and may show a decline in odor recognition. The 1 month WT group of mice showed a higher investigation time as the concentrations of the odor solution increased compared to the decreased and increased investigation time by the 1 month Tg group as the concentration of the L-carvone odor solution increased. There was only a slight difference between the investigation time as the concentrations increased and the 7 month Tg group actually showed a slightly higher investigation time compared to the WT group.

These results may indicate that aging in both mice types are correlated to decreased investigation time in detecting the change in odor concentration of the L-carvone solution. This is in relation to the hypothesis of observable behavioral changes between 1 month and 7 month old mice. WT mice have also shown a significantly higher amount of investigation time to the increased concentration of the odor solution which may support our hypothesis discussing the differences between WT and Tg mice at each age related to the age-dependent cognitive decline and decrease in neurogenesis due to the introduction of the tau gene. A decrease in GCs due to

decreased neurogenesis from AD can be contributing to these results. A decrease in GCs can lead to a decrease in odor discrimination abilities in mice due to their role in coding odorant information (Gheusi et al., 2000).

### **5.1.3 Novel Object Recognition**

NOR protocol observed the ability of the mice to detect differences of objects and therefore indicates their ability to explore the environment and detect changes (Bevins & Besheer, 2006). This task is meant to be reflective of neural processes that are involved in memory storage, and thus serves as a reflection of the activity of the hippocampus, the main brain structure associated with object recognition.

The NOR task was selected for our research due to its ability to promote the natural tendency of mice to explore their environment and new objects, and because it is in no way aversive to the animals. In this way, we are more confidently able to attribute the results directly to the cognitive function of the mice, rather than to any effects of fear or stress in the mice. Because the mice of the 2 month WT, 2 month Tg, and 7 month WT were able to significantly distinguish the novel object in their environment, this suggests that the mice do not have impaired functioning of the hippocampus, or at least not to an extent that results in an inability to distinguish between objects. Also, 2 month WT mice showed the highest positive DI, meaning this mice group spent more time investigating the novel object compared to the trained object, hence showing discrimination. Although our sample size was small, if we had a large sample size and less deviation, the fact that the 7 month Tg mice were unable to distinguish the two objects while the 2 month Tg did would suggest that there is cognitive impairment in the hippocampus and memory storage functioning that develops within the Tg mice sometime

between these two age groups, while no such deficits develop for WT mice. The 7 month Tg mice also had the lowest positive DI indicating that it spent the least amount of time investigating the novel object as opposed to the trained object compared to other mice groups, hence they were worse at discriminating the objects. Such findings support the results of similar studies that found Tg mice to have reduced dentate gyrus spike amplitude and worse performance on memory tasks such as the MWM (Scott, Kiss, Kawabe, & Hajos, 2016; Roof et al., 2010).

As the NOR serves to supplement our characterization of the Tg mouse model in addition to the behavioral tests and cell quantification directly related to the functioning and neurogenesis of the OB, these results suggest that if we were to perform neurogenesis quantifications within the hippocampus of these mice, we would perhaps find significant differences in neurogenesis between 2 and 7 month old Tg mice. Such an experiment could be carried out within the dentate gyrus, the location of neurogenesis within the hippocampus, in order to determine whether this decline in hippocampal function is due to the lack of new neuron formation within the region or whether there are other neuronal processes with which tau is interfering. Ultimately, our NOR results support our hypothesis that there are significant differences in cognitive function between 2 month and 7 month Tg mice, and that the effect of tau is cumulative.

## **5.2 Confocal Data**

Confocal data sought to analyze the effects of tau development in Tg mice on neurogenesis, as indicated by the density of BrdU+ cells/mm<sup>3</sup> in sagittal sections of the OB. The AOB showed a lower-than-expected value of neurogenesis with 1000-1500 BrdU+ cells/mm<sup>3</sup> at 2 months for both WT and Tg mouse models. In conclusion, there was a small increase in



neurogenesis from 2 to 7 months in the AOB, although the WT mice had the characteristically higher neurogenesis rate at 7 month. The MOB displayed a 2000-3000 BrdU+ cells/mm<sup>3</sup> count for 2 months. This value increased to between 3000-4000 BrdU+ cells/mm<sup>3</sup> count for WT mice, and decreased to about 1500 BrdU+ cells/mm<sup>3</sup> count for Tg. Our results were not significant and not consistent with results from previous studies (Nunez-Parra, Pugh & Araneda, 2010).

The results of quantification of neurogenesis was not exactly as expected, as it was hypothesized that the Tg mouse model would show a significant decline in neurogenesis for both WT and Tg groups (Nunez-Parra, Pugh & Araneda, 2010). This marked decline in neurogenesis occurs only for the Tg MOB from 2 to 7 months. To conclude, there was an increase in neurogenesis in the WT from 2 to 7 months in both the AOB and MOB. Although exact explanations remain unclear, this could be due to a defect in the WT mice group at 2 months since the WT are littermates of the Tg mice. The littermates may have expressed transgenes that resulted in the low amount of new neurons in the OB.

An explanation for the increase in neurogenesis of Tg in the AOB could be due to the onset of tau expression. Based on the 2014 study, behavioral deficits were observed before neuronal loss was readily detectable in the hippocampus of adult-onset P301L tau expression (Hunsberger et al., 2014). Although it is unclear how adult-onset tau expression affects neurogenesis in the OB in the Tg model, we can relate the findings of the 2014 study to speculate that early-onset of tau expressed our Tg mice could have resulted in observation of behavioral deficits before neuronal loss was detected.

## Chapter 6: Limitations

### 6.1 Behavioral Limitations

There were several limitations to this study that may potentially explain the concluding results.

#### 6.1.1 Cube Recognition

In additional experiments performed in Dr. Araneda's lab to study any interesting behaviors of the Tg mice, mice were presented with several trials of a cube with water, similar to what is done in the odor discrimination test. However, in these experiments in which no diluted odors were actually presented (as only water was spread on the cube each trial), it was found that the mice displayed an increased investigation time towards the cube each time a new cube was placed in the cage, rather than the same cube that had been previously presented in a former trial. Thus, it is possible that in our odor detection threshold and habituation/dishabituation experiments, Tg mice had an increased investigation time due to both the presence of the new cube and the presence of a new odor, not strictly the presence of a new odor itself. As a result, it is not definitively conclusive to say that an increased investigation time in response to a cube with an odor, whether it be a new or diluted odor, is strictly due to the presence of that odor and thus that the mice were able to differentiate these odors.

#### 6.1.2 Defining Wildtype Mice

Although exact explanations remain unclear, one plausible explanation for the increase in neurogenesis from 2 to 7 months in both the MOB and AOB of the WT mice was that our WT mice were littermates of the Tg mice which were not positive for the transgene. Since the Tg

mice were heterozygous and the WT mice were homozygous for the transgene, the genotype of the WT mice may have been affected. Further experiments should include the use of a more well-established WT mice such as the C57 mouse model that includes mice that are not littermates of the positive Tg mouse in order to confirm the validity of our results.

### **6.1.3 Sample Size Limitations**

In order to gather data that adequately represents the characterization of the Tg mice, we should increase the sample size of each treatment groups, and observe whether there are any deviations in the effects we observed in a smaller sample. A 2011 study that quantified the olfactory neurogenesis in the C57/BL6 mouse models used around 3 to 6 mice per treatment group. Since our experiments were also analyzing olfactory neurogenesis, an appropriate sample size would be in this range (Nunez-Parra, Pugh & Araneda, 2011).

### **6.1.4 Use of the rTg4510 Mice Model**

The Tg mice was chosen in order to observe the effects of isolated tauopathies and NFTs in mice with the aim of better understanding the interactions of tau in the OB on a cellular level as well as understand its cognitive repercussions. It is important to characterize different strains of AD mice since each has differences regarding baseline learning ability and activity; thus, other models of tauopathy in AD that exist, such as the JNPL3, Htau, and TAPP models, may be important to use in order to verify our results and observe the cognitive and neurogenesis effects of tau in mice of different learning capabilities.

## Chapter 7: Conclusion

Characterization of Tg mice models to be used specifically for AD research in the OB is still in its infancy. Experimental results from this study are inconclusive as to whether Tg mice models are ideal for OB research and can serve as a representative model of the olfactory changes that occur in AD. However, behavioral results are promising and therefore more histological research should be performed in order to fully understand the mice models' characterization and potential. Future research in addition to this study may be beneficial and necessary to ascertain the current results.

## Chapter 8: Future Directions

This study aimed to characterize a relatively new AD Tg mice at 2 and 7 months for behavioral and neurogenesis qualities. Since this study used both genders of the mice and differences in sex have not been addressed particularly, more research should be conducted that compares the behavioral and neurogenesis differences between the sexes of the mice. Since our results showed that neurogenesis increased in older mice in general, which is not explained by any other studies done on AD Tg mice models to our knowledge, more trials of the experiments should be conducted with a larger sample size. In addition, in order to gain more insight into whether the WT littermates were affected by the transgene, we can conduct more experiments using a more well-established WT model such as C57/BL6 as the control. Furthermore, more experiments using mice at different ages can be conducted to characterize the onset of cognitive deficits and neuronal loss in the olfactory bulb. Lastly, Tg mice have been previously tested using doxycycline to suppress the Tg tau and observed to see whether there were behavioral,

biochemical, and histological changes in these mice (SantaCruz, 2005). Doxycycline treatments can be used to test for changes in neurogenesis in different age groups in the OB as well as the hippocampus, including treatments based on the onset of tau expression.

## Glossary

**AMPA receptor:** a transmembrane receptor for glutamate that is involved in fast synaptic transmission in the nervous system.

**Amyloid- $\beta$ :** peptides of 36-43 amino that serve as the main component of amyloid plaques found in Alzheimer's patients' brains.

**Apoptosis:** the process of programmed cell death observed in multicellular organisms.

**APP (Amyloid Precursor Protein):** gene involved in familial AD

**Assay:** an investigative procedure used to determine the amount of a certain activity in a target area.

**Associative learning:** any learning process in which a new response becomes associated with a particular stimulus.

**Autoradiograph:** recorded on a photographic film, an image produced by emitted radiation from a specimen, such as a section of tissue, treated with a radioactively labeled isotope.

**BACE-1:** B-site amyloid precursor protein (APP) cleaving enzyme; results in amyloid plaques

**Bromodeoxyuridine (BrdU):** a reagent often used for cell proliferation assays incorporated into DNA during the S-phase of mitosis.

**Cognition:** the process of gaining knowledge through thought and experience.

**Confocal microscopy:** an optical imaging technique for increasing optical resolution and contrast of a micrograph.

**Cryopreserve:** to maintain viability of cells, tissue, organs, etc. by storing them at very low temperature.

**Dentate gyrus (DG):** a structure in the brain that is part of the hippocampus and is known to have high rates of neurogenesis.

**Deterministic genes:** genes that directly cause the disease; inheritance of the genes guarantees one will develop the disease

**Dopamine:** a natural hormone and neurotransmitter responsible for cognitive alertness.

**Early onset (EO):** display of symptoms of AD before age 65

**External plexiform layer:** the layer of cells in the olfactory bulb between the glomerular layer and mitral cell layer. Contains mostly dendrites of mitral cells and GABAergic GCs.

**GABA:** gamma-aminobutyric acid. The main inhibitory neurotransmitter in the mammalian nervous system.

**Glomerular layer:** the region of the olfactory bulb where the synapses form between the terminals of the olfactory nerves and dendrites of mitral, periglomerular and tufted cells.

**Granular layer:** thin layer of cells in the epidermis.

**Habituation:** the diminishing of a physiological or emotional response to a frequently repeated stimulus.

**Hippocampus:** section of brain known as the center of emotion, memory, and autonomic nervous system.

**Immunohistochemistry:** the process of detecting antigens in cells of a tissue by utilizing antibodies that bind specifically to said antigens.

**Internal plexiform layer:** layer of cells in the olfactory bulb between the mitral cell layer and the GCL. Consists mostly of GC dendrites, but also includes some axons and dendritic process of short-axon cells.

**Late onset (LO):** display of AD symptoms after age 65

**MAPT (microtubule-associated protein tau):** gene involved in development of tau isoforms

**Mitral cell layer:** a narrow band of cells in the olfactory bulb that sends its primary dendrites to

the glomerular layer where it forms a tuft within the layer and synapses with primary olfactory nerve axons.

**Neurodegenerative disease:** a condition primarily associated with death of neurons in the brain.

**Neurons:** a cell in the brain that can process and transmit information both chemically and electrically.

**Neuroplasticity:** changes in neural pathways and synapses due to factors like the environment, bodily injury, or behavior.

**Neurotrophins:** proteins that influence the survival, development, and function of neurons.

**Non-associative learning:** progressive diminution of behavioral response probability with repetition stimulus.

**Noradrenaline:** a hormone produced naturally by the body that also serves as a neurotransmitter and is associated with strong concentration.

**Nucleoside:** contains the nitrogenous base and ribose/deoxyribose but not the phosphate group that a nucleotide would have.

**Olfactory bulb:** a section of the brain located on the inferior area and is primarily responsible for the sense of smell.

**Olfactory epithelium (OE):** specialized epithelial tissue inside the nasal cavity that is involved in smell.

**Olfactory nerve layer:** the layer of neuron cells in the olfactory epithelium that receive direct input and relay it into the olfactory bulb.

**Operant conditioning:** a type of learning in which an individual's behavior is modified by its antecedents and consequences.

**Peritoneum:** a thin membrane lining the inside of the abdominal cavity



**PSEN1 (presenilin-1):** gene on chromosome 14 that enables the cleaving action of gamma secretase; implicated in familial AD

**PSEN2 (presenilin-2):** gene on chromosome 14 that enables the cleaving action of gamma secretase; implicated in familial AD

**Psychotropic:** affecting the mental state.

**REM Behavior Disorder (RBD):** a disorder characterized by loss of body paralysis during REM sleep.

**Risk genes:** genes that increase the likelihood of developing the disease; does not guarantee one will develop the disease

**Rostral migratory stream:** extends from the subventricular/subependymal zone of the anterior horn of the lateral ventricle along the ventricular extension into the olfactory bulb.

**Rostro-caudal:** between head and tail.

**Sagittal:** relating to suture on top of the skull that runs between the parietal bones in a front to back direction.

**Saturation point:** the point at which there is no longer any room to hold any more of a certain liquid, object, or physical material

**Sensitization:** repeated administrations of a stimulus results in the progressive amplification of a response.

**Serotonin:** a neurotransmitter found in the central nervous system of humans commonly associated with maintaining mood balance.

**Sliding microtome:** a microtome in which the object to be cut is fixed and the knife is carried obliquely across it

**Subventricular zone (SVZ):** a layered brain structure that is known to have high rates of adult

neurogenesis and contains a large amount of proliferating cells.

**Synucleinopathies:** a group of neurodegenerative diseases characterized by neuronal or glial inclusions of alpha-synuclein.

**Tauopathies:** neuronal inclusions or neurofibrillary tangles in AD made of microtubule associated protein tau.

**Tg2576 mice (APP Tg mice):** Tg mice with a mutation that causes overproduction of amyloid in the brain which is also associated with impaired learning, loss of working memory, and changes in synaptic plasticity.

**Tryptophan hydroxylase (TPH):** a rate-limiting enzyme that affects the rate of serotonin synthesis in the brain.

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