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# A Behavioral Characterization of Transgenic Models of Alzheimer's Disease

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Abstract of the dissertation

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Alzheimer's is a debilitating disease characterized by amyloid plaques and neurofibrillary tangles and neuronal loss, as well as memory loss, disorientation, and other behavioral changes. A common method of modeling aspects of Alzheimer's disease in animals is the transgenic mouse. In these mice, human early onset familial Alzheimer's disease associated genes are inserted into the mouse genome to study the contribution of the human gene mutations to the development of pathology. Many transgenic models of Alzheimer's disease have been created, ranging from single transgenic to quintuple transgenic models. While these transgenic strains are well characterized histologically, little systematic behavioral study has been conducted. One important way that these animals have not been studied is with a parallel, comprehensive behavioral evaluation among separate strains that would allow for direct inter-comparisons between strains. This project applied rigorous behavioral methodology while comparing three strains of transgenic mice; the Tg-2576, one of the earliest and best characterized single transgenic amyloid models; the Tg-SwDI, a recent multiple amyloid transgenic that reproduces well the specific angiopathy in Alzheimer's disease; and the 3x-Tg, a recent and well received triple transgenic animal that allows for the study of the interactions of three important mutations in Alzheimer's disease; tau, presenilin and amyloid. All three strains were assessed on a thorough and interlocking battery of operant tasks, ranging from simple rule learning to delayed recall, as well as a number of operant tests of motor and sensory ability. For comparison, the mice were also assessed on other tests, including the open field, Barnes maze, and Morris water maze, which demanded more spatial orientation and spatial memory abilities than the operant tasks. After behavioral testing, post-mortem studies of the brains of each strain were conducted, to allow correlation between the neuropathology of each strain and its corresponding behavioral deficits. The results of this study allow unique inferences to be made about the contributions of single mutations, as well as combinations of mutations, to the neuropathology and behavioral changes in humans with Alzheimer's disease.

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## Introduction

Alois Alzheimer, a German doctor, published the first scientific account of Alzheimer's disease in 1907. Alzheimer reported on a middle aged female patient who displayed confusion, memory problems and difficulty understanding questions. Upon her death, he performed an autopsy of her brain to see if he could discover the cause of her cognitive difficulties. Upon examination, Alzheimer discovered deposits around and outside the nerve cells, and twisted fibers inside the nerve cells. He published the first paper about what is now called Alzheimer's disease as "*On a peculiar disease of the cerebral cortex*" (translated from the German), which detailed his findings (Alzhiemer, 1907).

One hundred years later, we are still searching for the origin of the pathology that he detailed, now referred to as amyloid plaques (Aβ plaques) and neurofibrillary tangles (NFTs). Much research continues along the same line as Alzheimer's, examining human populations to look for potential genetic and environmental causes. This research attempts to understand posible subtypes of the disorder, to characterize the nature of the syndrome, and to examine the efficacy of various interventions. For approximately the last thirty years, this human work has been augmented by animal studies using primates and rodent models. These models are used to understand the basic issues of etiology and the relationship between neuropathology and behavioral deficits, as well as for screening preclinical drugs and other interventions.

The ability to use in vivo animal models to perform experimental manipulations, both of potential causal factors and potential therapeutics, has yielded advances in basic knowledge. However, to some extent the ability of molecular biologists to create transgenic mouse lines has far outstripped the ability of behavioral neuroscientists to properly evaluate the validity of these models and to make specific intercomparisons between transgenic lines.

## Models

### Early neurochemical models

The use of in vivo animal models for research into Alzheimer's disease emerged slowly in the early 80's, and was generally organized around the prominent cholinergic theory of Alzheimer's disease. This theory suggested that memory loss and other

cognitive disturbances associated with AD were caused by the death of cholinergic neurons in the hippocampus and surrounding nuclei and the attendant loss of acetylcholine inputs to higher cortical structures (Davies & Maloney, 1976). This theory stated that the NFTs and amyloid plaques were associated but not central to disease development until later stages. This theory was based on evidence of early loss of cholinergic markers in hippocampus and parahippocampal cortical areas, and the correlation of the loss of these markers with the degree of memory deficits in Alzheimer's disease (Sherman, Kuster, Dean, Bartus, & Friedman, 1981). The early animal experiments employing lesions of basal forebrain structures and cholinergic receptor antagonist drugs appeared to support this theory.

However, after some initial promise of cholinesterase inhibitors boosting cholinergic neurotransmission and enhancing cognition in Alzheimer's patients, it soon became apparent that while cholinergic treatment did effect some short-term relief of symptoms for some, it did not halt the progress of the disease. The inevitable loss of cognitive function is the same with cholinergic treatment or without (Bartus, 2000). Virtually every therapeutic success of cholinergic targeted interventions in rat and primate models did not transfer to the humans, terminally undermining the cholinergic theory.

**The emergence of transgenic models**

At the same time that these disappointing results from the human cholinergic interventions were emerging, a major change in research emphasis emerged due to advances in molecular biology, genomics, and genetic epidemiology. In 1991, landmark papers began to appear reporting genes related to early-onset familial Alzheimer's disease (EOFAD). Several separate genetic mutations were found (Goate et al., 1991; Chartier-Harlin et al., 1991; Murrell, Farlow, Ghetti & Benson, 1991; Handriks et al., 1992; Mullan et al., 1992). These genetic mutations were often later named after the location of the family in whom the mutation was isolated (Swedish, Dutch, etc.). In a short time, the discovery of these genes led to an attempt at founding specific transgenic mouse lines. Mouse lines were created with human genes for Alzheimer's disease inserted into the mouse's own genome, and promoted with a variety of neural-specific gene promoters.

**Transgenic Models**

Alzheimer's disease is diagnosed and defined by certain characteristics, the most prominent of which is the widespread distribution of amyloid β protein (Aβ) throughout the brain in the form of diffuse plaques and fibrillar plaques in both the vascular and parenchymal tissue. This pathological Aβ is created by serial cleavage of amyloid precursor protein (APP) by β and γ secretases (Selkoe 2001). There are several mutations in the Aβ region and regions flanking the Aβ region of the APP gene that create pathological amounts and formations of Aβ in the brain.

The first group of mutations cause excessive amounts of amyloid β-protein to be produced, which proliferates throughout the brain, causing plaques, tangles, microglial activation, and cell death. A second genetic mutation was later found in presenilin. This mutation causes it to cut differentially more Aβ-42 from the APP protein than Aβ-40 (Sherrington et al., 1995). Because Aβ-42 is the form of Aβ that creates the toxic plaques in the brain, this mutation contributes to a robust proliferation of plaques, and early memory loss. This increase in Aβ-42 contributes to the early onset of pathology in early onset Alzheimer's disease patients. A third relevant mutation related to sporadic AD but not present in EOFAD patients is of Tau, discussed more fully later.

In order to create a transgenic mouse model of AD, promotors must be used to cause the inserted human AD genes to express themselves in brain tissue. Various vectors and promoters are used in the creation of these transgenic strains. A vector is used to insert the human pathological DNA into the mouse's genome, while the promoter regulates the expression of this DNA, both the location and intensity of expression. Without the promoter, the mutant Aβ gene may be incorporated in the genome of the mouse, but without any action of the inserted gene (creation of RNA), or any production of excess Aβ. A promoter is used to increase expression of the gene in a site specific manner, for example, the commonly used Thy-1 promoter causes increased expression of mutant AD genes in the brain of transgenic mice.

One important caveat to the following studies of transgenic models of Alzheimer's disease is that all of these models are based on genes from the Early Onset

Familial form of Alzheimer's disease (all of the models and related genes discussed are summarized in Table 1). The EOFAD form only comprises about 5-10% of all Alzheimer's disease cases worldwide. The remainders of cases are termed "sporadic" and occur without any precise genetic mutation in the patient that has yet been identified. So all of these models, as successful as they may be at reaching their goal of being a good simulation model of EOFAD, are fundamentally limited when seen in the general context of the unknown origin and evolution of plaques and tangles in the other 90-95% of Alzheimer's disease patients.

**Early Transgenic Models: Aβ**

Early transgenic models of Alzheimer's disease in vivo were each breakthroughs, but characterized ultimately with difficulties and missteps. Several early papers (Wirak et al., 1991; Kawabata, Higgins, Grodon, 1991) that seemed to have promising models were retracted because of a later failure to replicate their findings (Jucker et al., 1992; Price et al., 1992). These early attempts at transgenic models largely involved the knock-in of human genes for Aβ or APP. Some of the experimenters in these early attempts at transgenics had not used control groups to detect false-positive results, making their findings difficult to interpret. However, the major difficulty with these experiments was that unlike later models, they did not successfully use appropriate promoters.

The β-APP751 strain, which is created by inserting the human wild type APP gene, was one of the first successful transgenic models of Alzheimer's disease. First published in 1991, Cordell and colleagues began an extensive characterization of the animals. An initial publication (Quon et al., 1991) showed that the 751 amino acid version of the βAPP gene, promoted by NSE (neural-specific encolase) caused frequent compact deposits of Aβ in the cortex and hippocampus, and less frequent deposits in the thalamus and striatum, using the same antibodies used to stain deposits in Alzheimer's disease patient's brains. Also, using the Alz50 antibody, which stains aberrant tau deposits in human Alzheimer's brains, soma and processes of what seemed to be tau could be visualized in β-APP751 brains (Higgins, Catalano, Quon & Cordell 1993). A comparison of young (~3mos) to old (22mos) animals shows that amyloid and (what the researchers refer to as) tau pathology increases in the β-APP751 animals approximately two-fold as they reach old age. These animals were compared to wild type (APP-695)

animals, which develop no amyloid or tau pathology as they age (Higgins, Rodems, Catalano, Quon & Cordell, 1995).

Behavioral tests also compared these transgenic animals at different ages in a variety of tasks. First they passed a full battery of motor and activity tests, with the only difference being that transgenic mice were slightly less active. When it came to memory testing, however, there were differences; in 12 month old animals, the β-APP751 mice had decreased Y-maze alternation. A lack of alternation indicates memory deficits, as mice will tend to explore the unexplored arm. When there is less alternation it is concluded that they do not recall which arm they explored. Similarly, in the Morris Water Maze at six months the transgenics had slightly delayed learning as compared to controls, and twelve-month-old transgenics were more impaired, failing to learn the location of the platform during the course of the experiment (Moran, Higgins, Cordell & Moser, 1995).

These behavioral results seem to show that as β-amyloid accumulation in the brain increases, the animals begin to have greater memory problems. This model of Alzheimer's disease initially seemed to be fairly complete, in that it mimics both the physical and cognitive aspects of Alzheimer's disease, and even more so because it does this with ostensibly the same mechanism by which the disease is caused by in humans. However, after these very promising papers, this model disappeared from the literature, with the main author moving on to presenilin models of Alzheimer's disease.

An important early transgenic model of Alzheimer's disease that is still in use today is the APPSwe model. This model also later evolved into the Tg2576, a very common and highly used transgenic model of Alzheimer's disease. The APPSwe model is based on a familial genetic mutation from a family in Sweden that displayed early onset Alzheimer's disease pathology. In the first model, gene expression was driven in the mouse via a hamster prion promoter (Hsaio et al., 1996).

In histological examination, there was a five-fold increase in Aβ-40 and a fourteen-fold increase in Aβ-42 in older transgenic mice as compared to younger. This increase was concentrated in frontal, temporal and entorhinal cortices, as well as hippocampus, subiculum and cerebellum. The mice at nine months had significant impairments in Morris water maze performance, both in latency to find platform, and in

reduced exploration of the target quadrant when platform was not present. The experimenters verified that this was not a function of decreased swimming ability. At ten months, the mice also showed reduced alternation in the Y maze (Hsiao et al., 1996).

Frautschy et al. (1998) did the first comprehensive examination of glial cells in association with amyloid plaques in this APPSwe model. The experimenters found that there were significantly more microglia in the cortex and hippocampus of transgenic mice, as compared to wild types. They also found that the glia were the most dense in layer 1 of the cortex, and getting less dense in layers 2 to 4, and then less still in 5 and 6. Frautschy and colleagues also found that glia in these areas were clustered around amyloid deposits. This is very similar to the pattern of glial activation in post-mortem studies of patients with Alzheimer's disease. Because microglia take up and degrade Aβ in culture, it is assumed that these microglia are attempting to "clean up" the deposits (Ard, Cole, Wei, Mehrle & Fratkin, 1996). Unfortunately, the extended activities of microglia can be damaging to surrounding tissue.

A positive move in the field that was made first in the APPSwe animals was completing a full battery of simple behavioral tests, prompted by growing discussion about the value of batteries of testing rather than specific bioassay approaches (Crawley and Paylor, 1997; Crawley 2007). King et al. (1991) performed a complete battery, including physical testing (string hang) and memory tests (Y-Maze, Morris water maze) done early in the lives of the mice. The researchers followed up with an additional behavioral battery including circular maze (Barnes maze) and active and passive avoidance testing with shock, at later ages. In contrast to the data found by groups previously, King et al. (1991) found that APPswe mice did not have difficulty alternating in Y maze, or in finding the submerged platform in morris water maze. Morris water maze with a visible platform was not impaired in 3 month old animals, but was progressively more impaired and the 6 and 9 month time points, which indicates non-mnemonic factors.

The outcome of the Swedish mutation in humans is very different from its manifestation in any of the murine models. Physically, the amyloid deposition, and microglial response in the brain seem similar, but the downstream effects are not. This model of amyloidosis does not trigger tau accumulation in mice, so that basic hallmark of

Alzheimer's disease is missing from the model. Also, the behavioral effects of the amyloid load do not seem to mimic the behavioral effects of the full blown disorder in humans. Memory impairments in the mouse model seem to be limited and slight, while in humans the effects of EOFAD are severe and pervasive at a young age. This is perhaps a problem of missing tau expression, so if a later model could include tau in a Swedish model, perhaps its overall promise would be greater as a full model of the human disease.

A separate human genetic disorder that has been used to study Alzheimer's disease pathology is hereditary cerebral hemorrhage with amyloidosis of the Dutch, or Flemish, type. This disorder causes amyloid to deposit in the cerebral vasculature, which often happens in patients with Alzheimer's disease. Howland et al. (1995) created a transgenic mouse model of the Dutch disorder, using a synapsin I promoter. The inserted mRNA expressed itself in hippocampus and cortex, but did not cause amyloid deposition. The Dutch and Flemish strains model a different part of the pathology of Alzhiemer's disease. They do not display amyloid or tau accumulation in brain tissues, or mnemonic deficits in these early models. The validity as a model is based on the observation that mice have the same circulating Aβ symptoms, and circulating Aβ is hypothesized to contribute significantly to mnemonic symptoms in Alzheimer's disease patients. This is not a complete model of Alzheimer's disease, but one part, useful as a contribution to the whole.

Finally, a different (non-APP) genetic mutation that increases the amount of Aβ-42 in the brains of humans is the presenilin mutation. Presenilin is also a gene that is implicated in early onset Alzheimer's disease, and does not affect the APP, but rather the direct manufacture of Aβ. Familial early-onset Alzheimer's disease can often be exacerbated by an additional mutation of the Presenilin 1 or 2 genes.

Mutation of the presenilin genes causes the ratio of different types of Aβ in the brain to change. Aβ-40 has come to be seen as a less harmful version of the peptide, where Aβ-42 seems to be what causes the majority of plaque pathology in the brain. Presenilin causes the ratio of Aβ-42 to Aβ-40 to increase greatly, thus exacerbating the damage (Borchelt 1996; Holcomb et al., 1998; Duff et al., 1996). However, a set of experiments by Janus et al. (2000) showed that though there is an increase in Aβ, and

preferentially Aβ-42, in the presenilin 1 transgenic mouse, there is no impairment in spatial learning in these transgenic animals, with use of open field and Morris water maze.

Currently numerous variations of transgenic AD models exist in the literature, the various models of amyloid deposition discussed above and others. Each model has its own constellation of positive and negative attributes when related to the human disorder, and all have one major negative in common: lack of tau (neurofibrillary tangle) pathology. This brings us to our discussion of tau and modeling of tau disorders in mice.

**Early Models of Tau pathology**

Tau is one of a family of three microtubule associated proteins; tau (aka MAP), MAP1, and MAP2. These three proteins perform similar functions, so much so that if one is knocked out of a transgenic mouse, the other two allow the animal to function well into old age. Only in double KO mice do models begin to show pathology (Iqbal et al., 2005). However, when tau is present in pathological forms, it can create havoc. Pathological hyperphosphorilated tau creates the tangles that are the hallmark of tau pathology in the Alzheimer's brain. The hyperphosphorolization of tau has not yet been explained, but seems to be the result of a faulty interaction with microtubules. When microtubules do not bind to tau, tau is easily phosphorylized (Iqbal et al., 2005). Hyperphosphorilated tau then creates the neurofibrillary tangles that impair cognitive function in Alzehimer's disease.

Tau transgenics alone are not considered to be models of Alzheimer's disease, as the animals develop profound physical impairments from tau gene manipulations, rather than the mainly mnemonic impairments and more mild neurofibrillary tangles of AD. The genes that are currently being used to create transgenic models of tau hyperphosphorilization were actually isolated from families with fronto-temporal dementia and Parkinsonism (Götz et al., 2007). However, the creation of these transgenics that use tau alone are an important stepping stone to the creation of combination amyloid and tau expressing animals, that will create a more complete model of Alzheimer's disease.

One of the initial published reports of a transgenic model of tau pathology is the JNPL3, generated from the familial gene mutation P301L that causes fronto-temporal

dementia and Parkinsonism. This gene expression causes severe NFT's throughout the brain. Homozygotic mice developed motor pathology as young as 4.5 months, with hemizygotic animals developing pathology at 6.5 months. Motor deficits include a lack of righting-reflex, failure at hanging tests, and two weeks after first onset of pathology, an inability to walk (Lewis et al., 2000).

However, though this is a gene that creates tau tangles in the brain, it is not the gene by which tau is created in the Alzheimer's disease brain. So while these experiments can give us an insight into the way that tau functions in the brain, which is important, the information that we gain from this model and others like it do not pertain directly to Alzheimer's disease. This does not seem to be the same function, or same chemical cascade, that forms tau in the Alzheimer's disease brain. What creates the excessive hyperphosphorilization of tau in AD is not conclusively understood.

An alternate method of creating a tau transgenic that seems more applicable to AD transgenics was performed by Andorfer et al. (2003), where, rather than inserting genes for pathological tau hyperphosphorilization into the genome of a mouse, they actually inserted the entire wild type human gene for tau into the mouse. These mice developed NFTs with paired helical filaments in old age, just as a human would. Later investigation (Andorfer et al., 2005) showed that cell death in aged mouse brains is not greater nearer to the NFTs, bringing up questions about cell death in Alzheimer's disease. This also brings up an interesting question about the transgenic tau models. Are we doing ourselves a disservice by studying pathological types of tau? Perhaps a more productive method for studying the tau malfunctions in Alzheimer's disease would be studying wild type human tau.

Aβ, tau, and Presenilin models are each an important part of the larger picture of AD that includes mutations of all three. The combination of the three takes us into the next phase of Alzheimer's transgenic research.

**Recent Transgenic models**

More recently, new techniques have emerged that help take the initial animal models of Alzheimer's disease that modeled a only one part of Alzheimer's disease, and combine them with other transgenic models in the same animal to create a more complete model, as well as interactions between the parts. This allows for the study of interactions

among the genes across development, and perhaps offers greater validity of the model as a comprehensive simulation. Some of these new strains try to mix amyloid models, in an attempt to create a more "perfect" mimic of the amyloid pathology in Alzheimer's disease, while others combine amyloid with tau, or with presenilin, in an attempt to model a more full picture of multiple parts of the disorder in a single animal.

Multiple crosses of several strains of EOFAD genes have been attempted in the years since their isolation. The first were simple breeding crosses of already established lines of APP transgenic mice. One such cross is the Swedish-Indiana mutant TgCRND8 with presenilin 1 (Janus et al., 2000). The mice also had diffuse and severe Aβ, as much as seven times the Aβ as comparable single transgenic strains. This strain of mice has extreme difficulty in learning the Morris water maze as young as at 11 weeks of age (Chishti et al., 2001). The TgCRND8 mice also were impaired in developing a conditioned taste aversion (Janus et al., 2004) and in acquiring radial arm maze (Lovasic, Bauschke & Janus, 2004). As the deposits are of such a swift and severe onset, this model is good for studying brain pathology, but less so behavior.

A comparison between single and multiple transgenic mice was the next logical step for this line of research. APPSw mice, mutant presenilin (Hu PS1 A246E) mice, a combination APPSwe- Hu PS1 A246E mouse, and a combination APPSwe and wild type human presenilin mouse were compared. This comparison allowed researchers to pick apart exactly what each transgene contributed to the overall outcome in the APPSwe-A246E mouse. The outcome had both expected, and some unexpected results. There was a rapid and intense proliferation of amyloid and amyloid plaques in the brains of the APPSwe-Hu PS1 A246E mice as compared to all other mice. The mutant presenilin alone did not cause any amyloid accumulation by 12 months. The APPSwe alone began to accumulate small amounts of Aβ only at 18 months of age. In contrast, the APPSwe-Hu PS1 A246E mouse had small amounts of Aβ accumulation by 9 months, which increased at 10 and 11 months, being very dense by 12 months of age. (Borchelt, 1997)

Behaviorally, APPSwe + PS1 mice exhibit a similar mixed set of results to mice that are just APPSwe. Significant mnemonic results came from the Morris Water Maze, where at 15-17 months, they performed significantly worse. At the same ages, they alternated significantly less in Y-maze than their age-matched wild type counterparts. In

the other behaviors tested, there were no differences found between the APPSwe + PS1 and wild type mice.(Arendash et al., 2001). All in all though, this combination model is an improvement on the APPSwe alone. It develops a more robust Aβ pathology at a younger age, and it includes multiple factors (both the APP and presenilin mutations) that humans with early-onset Alzheimer's disease present with.

Double transgenic models were also created that expressed both tau and Aβ. The first and most prominent tau-Aβ transgenic model came out just a year after the success of the JNPL3 tau model. Lewis et al. (2001) use the same strain of mice, and just cross them with the Tg2576 or Swedish mouse. The resulting mice, dubbed TAPP, have both tau and Aβ pathology. The researchers found that the TAPP mice developed the pathology of both their predecessors, however, NFT's and Aβ did not seem to co-localize. Also, this animal model has severe physical symptoms and mortality at a very young age, similarly to the JNPL3 mice, because it shares the same pathology.

As transgenic technology improved, more genes could be combined such as in an early 3-transgene model of Alzheimer's disease, the Tg-SwDI mouse, which has the Swedish mutation in addition to the Dutch and Iowa. (Van Nostrand et al., 2004). The Tg-SwDI model is unique from its predecessors in its expression of both robust vascular amyloid, and robust parenchemal Aβ. It is likely that the cerebral accumulation in these animals is due to the inability to clear these deposits through the cerebral vasculature. A comparison between the APPSw and Tg-SwDI animals shows that the Tg-SwDI has greater Aβ in the brain, and less circulating in the blood. (Davis et al., 2006) This suggests that lack of efflux of Aβ across the blood brain barrier and into circulation, in familial AD patients may contribute to the pathology.

A full behavioral characterization of these animals was undertaken by Xu et al. (2007). After a full behavioral battery of testing including Digiscan, open field, novel object, wire hang, inverted wire crawl, light dark box, rotorod and Barnes maze was performed, the only test that showed significant differences between transgenic and wild type mice was the Barnes maze. This impairment in Barnes maze was observed from 3 to 12 months of age, but did not increase. This does not correlate with the increasing levels of Aβ plaques in the brains of the animals during this time period. However, this

could be an experience effect, as it does not seem that a naïve group of mice was used for each round of testing.

A recent and very promising model is the triple-transgenic model of Alzheimer's disease (3xTg-AD) that uses a new technique to create an animal with multiple genetic mutations in the same genetic locus. The APPSwe mutation and P301L tau mutation are microinjected into a presenilin mutant M146V mouse embryo. What is amazing about this mouse is that it contains genetic mutations for all three of the hallmark pathologies in Alzheimer's disease. It has the APP and presenilin mutations that cause a change in Aβ, and a differential accumulation in the pathological plaque form of Aβ, respectively, and a tau mutation, that causes the hallmark NFT's (Oddo et al., 2003).

As would be expected, with this trifecta of pathologies, this model has a variety of impairments. Long term potentiation is reduced in 3xTg's as compared to controls, and as compared to 2xTg mice having both presenilin and tau mutations. Interestingly, when testing was done, mice showed an increase in circulating Aβ, but did not yet display Aβ plaques (Oddo et al., 2003). This lends support to the theory that though the plaques are detrimental, they are not necessary for disruption of neural functioning in Alzheimer's disease patients. Additional research was done on these mice to show that lowering the soluble Aβ levels alone does not attenuate cognitive decline, however decrease of Aβ and tau in conjunction does improve cognitive performance (Oddo et al., 2006).

Another set of experiments by Oddo, Caccamo, Kitazawa, Tseng & LaFerla (2003) showed a variety of important results in these animals related to Morris Water maze training. First, 3xTg mice are impaired on MWM as compared to WT animals beginning at six months of age. This would be expected, because of their pathology. What is interesting is that when trials to acquisition are repeated every 3 months, there is a learning effect on 3-Tg mice, with non-naïve transgenic animals performing better than their naïve age matched counterparts. This learning effect is abolished by 15 months of age, when, presumably, pathology becomes too severe. Most surprisingly, Morris water maze repitition decreased the soluble Aβ and Aβ plaques in 3xTg mice. So overall, a learning task could help preserve cognitive function in mice, and can be correlated with an accompanying decrease in Aβ pathology in the brain (Billings, Green, McGaugh & LaFerla 2007). The utility of this model is just beginning to be tested, but with it the

future for the field of transgenic models of Alzheimer's disease is taking a promising turn.

**Behavioral Testing in Transgenic Models**

A major critique of this field is that the behavioral characterization of the various models, as noted, has been unsystematic and relatively simplistic in its approach. More parallel paths of testing would be useful for being able to compare the various memory and motor deficits in the animals. This would enable researchers to actually tease apart exactly what types of behavioral deficits the animals have, if any, and would allow us to begin to understand what aspects of the disorder are causing the memory deficits. Side by side comparisons of mouse lines in complex tasks have not been undertaken. The debate about whether circulating or plaque amyloid, pre-tangle or tangled tau causes the most dementia could be more effectively evaluated if researchers performed more complete behavioral testing at every phase of the animals lives that was better tailored to the temperaments of the mice, and with a larger N's.

Also, many cognitive tests that are used in mice were not designed for, nor have they had their utility tested on, mice. Most tests that have been employed were rat paradigms that were forced into use in mice by the sudden need in the transgenic mouse field for new behavioral testing paradigms. Swimming, for example, while quite a normal part of the movement repertoire of rats, is much less so for the mouse. Very little effort has been made to develop and validate tests that are less sensitive to the behavioral reactions of the mice to new settings, such as excessive freezing or hyperactivity, and can confound the results.

The movement away from lesion and pharmacological models in favor of transgenic models, while perhaps adding to the face validity in allowing for the assessment of developmental effects, has also fundamentally changed the nature of the experimental designs. Transgenic models are fundamentally longitudinal, correlational designs, in that there are usually multiple brain changes found in the animals that progress over time. However, by and large, analyses of the changes are conducted using the simplest methods. Indeed, histological analyses, if quantified at all, are rarely correlated with measures of behavioral performance, and complex techniques tailored to

these sorts of data sets (e.g. path analysis) are nonexistent in the literature. This results in qualitative, ordinal levels of comparison.

Therefore, the present study set out to fulfill several aims:

Aim 1: To systematically behaviorally characterize several strains of transgenic mice on a complex and interlocking series of operant learning paradigms.

Aim 2: To compare the operant behavioral findings with non-operant tasks that are more commonly used in the field.

Aim 3: To assess how well these operant and non-operant behavioral tests predict pathology in the brains of these animals.

To address these aims, three of the previously discussed strains of transgenic Alzheimer's disease modeling mice were studied. These were the single mutation Tg-2576 mouse, and two multiple mutation models, the Tg-SwDI and 3xTg mice. The hallmark difference between the models is that while the Tg-2576 mouse produces only parenchymal Aβ, the TgSwDI mouse also produces high levels of vascular Aβ and the 3xTg also includes a Tau mutation. Together, the three strains contain both overlapping and non-overlapping genotypes and unique neuropathological endophenotypes. First, all three were tested on a series of operant tasks that assessed learning, memory and other cognitive and non-cognitive constructs. Then, they were tested on non-operant spatial tasks that are established in the literature as reliable tests of memory in these mice. When all behavioral testing was complete, the neuropathology of these strains was compared among the groups.

Table 1: A summary of transgenic animals discussed in this paper

| Model | Genes | Histological Findings | Behavioral Findings | # papers (pubmed) | Comments: |
|---|---|---|---|---|---|
| β-APP751 | Human WT APP | Amyloid increases 2-fold as animals age | ↓ Y-maze alternation<br>↓ MWM performance | 22 | Model no longer used |
| Swedish (Tg-2576) | Swedish | 5-14x increase in Aβ w/age, ↑ glial activation in cortex | ↓ Y-maze, MWM (by some research groups) | 435 | Most highly used APP transgenic strain |
| Dutch or Flemish | Dutch or Flemish | Increased circulating amyloid, but not amyloid plaques | ↑ Aggressiveness, no mnemonic deficits | 202 | Not a model of Alzheimer's Aβ, but a model of cerebral angiopathy |
| Presenilin | Pres-1 (or Pres-2) | ↑ Aβ and preferentially Aβ-42 | No behavioral impairments | 1440 | Changes the ratio of healthy to unhealthy Aβ in the brain |
| Human WT Tau (htau) | Human WT Tau | ↑ NFT's with old age | N/A | 26 | A naturally occurring model of NFT's, rather than pathological |
| Tg SwDI (2004) | Swedish, Dutch, Iowa | ↑ vascular and cerebral amyloid | ↓ Barnes maze learning | 12 | Good model isolating amyloid to vasculature; not comprehensive |
| TgCRND 8 | Swedish, Indiana, Presenilin | Diffuse and severe Aβ accumulation, 7x more than the single trans. | ↓ Conditioned taste aversion, RAM performance | 45 | Early mortality because of fast onset of symptoms |
| APPSwe-Hu PS1 A24E | Swedish, Pres-1 | Cerebral Aβ increased greatly from 9-12 m | ↓ MWM and Y maze performance at 15-17 m | 27 | Combination of APP and presenilin did not cause the expected increase in Aβ-42 |
| TAPP | JNPL3 tau, Tg2576 APP | Increased NFT's in amyloid animals | Severe motor impairments in young | 1 | NFT's and amyloid do not seem to co-localize |
| 3xTg-AD | APPSwe, P301L tau, M146V Pres | Increased tau, amyloid accumulation compared to single transgenics | Severe learning deficits not ameliorated by training at 15 m | 22 | Learning can help ameliorate learning deficits in younger animals |

## Methods

Experiments were performed in three cohorts, each with a separate group of transgenic mice and their wild type controls. All experiments were begun with at least 8 transgenic animals, and 8 wild type controls of the same age and genetic background. There were three groups of transgenic mice; Tg-2576: Swedish mutation (mixed sex), Tg-SwDI; Swedish, Dutch, and Iowa mutations (all male) and 3xTg; Swedish, Presenilin and Tau mutations (all male). Mice of different strains were not studied concurrently because of staggered availability. All efforts were made to make the training and testing identical between all three strains of transgenic mice. Operant training and testing procedures began at six months of age, and concluded at approximately twelve months. Non-operant testing began at approximately twelve months, and concluded at approximately thirteen months. At this point, all mice were euthanized for post-mortem testing.

## Behavioral Characterization

All strains received an identical program of behavioral testing, described as follows:

## Operant behavioral training and testing

The operant chamber was located inside a sound attenuating chamber, with an exhaust fan providing white noise. The chamber was 19x22cm at the floor with two front nose-poke ports that are 4.5cm directly right and left of the water dipper, and one nose-poke port in the rear, directly opposite of the dipper. Nose poke ports were 1.5 cm in diameter, and contained a small light, and a single photo-beam that registered nose-poke responses. The chamber had a metal rung floor, and was lit by a small house light (pic 1 shows the front panel of the chamber).

During the operant testing, all the mice had no access to water for 23 hours, followed by a 30 minute test session in which water was available as reinforcers, and then 30 minutes of free water consumption in their home cage following the test session. Trial initiation and reinforcement procedures were uniform across all behavior tests:

- Trials always began with a nose-poke into an illuminated front nose-poke port.

- Following a correct response, both nose-port well lights were extinguished, and the water reinforcer ladle or "dipper" was made available for 10 seconds. When the dipper was removed, the nose-port lights were illuminated, beginning the next trial.
- Following an incorrect response, both nose-port lights were extinguished for the 10 second intertrial interval, and then re-illuminated to indicate the start of the next trial.

Operant programs progressed in a set order, the same for all mice, as follows;

1. Magazine (dipper) training: In this procedure, both front nose-poke ports were illuminated at the start of all trials. The dipper was activated non-contingently every thirty seconds and remained raised for ten seconds in the testing chamber. A nosepoke response in either port when lights were illuminated produced an additional reinforcer. The mice were exposed to this procedure for two days.
2. Fixed Ratio: In this task, both front nose-poke wells were lit and mice were required to nose-poke in order to get a reward. Mice could poke in either well, at any time when the dipper was not already activated, (and the cue lights off). This program continued until mice made a total of fifteen or more responses in one session, or developed a side bias of at least ten more responses on one side than the other.
3. Alternation: During this task, only one of the front nose poke wells was lit at a time, the lit lamp alternating from right to left. This program forced mice to use both left and right response wells equally. The alternate program continued until animals made thirty or more responses total, fifteen responses to each side during a single session.
4. Light Dark Discrimination: During this task, only one front nose-poke well light was lit at a time, and which well was lit alternated at random. Mice learned to nose-poke correctly by following the light. Program continued until all animals reached 90% correct responses (defined by correct responses/total responses) in a single session.
5. Shuttle: In this task, only one front nose-poke well was lit, and mice learned to poke first the front lit well, then into the rear well that lights after their first

response. The front well that was not lit at the beginning of the trial did light after they poked the rear well, and the last response of the trial was for the mouse to poke their nose into this front well. This program continued for five days.

6. Non-Match to Position: The trial began with one front well lit. Mice learned to poke first into the front lighted well, then into the rear well that lit after their first poke. After the rear poke, both front wells were lit, and the mouse was required to recall which well they poked previously, and poke the opposite well in order to get the reward. This program was identical to shuttle, except that the final response side was not cued. Mice were required to recall which well they were supposed to poke their nose into. This program continued for forty days.
7. Delayed Non-match to Position: This procedure was the same as the Non-match to position procedure, except there was an addition of a delay of 1-19 seconds between the first front nose-poke and the rear nose poke. On day one, there was a three second delay inserted between the two responses. Performance was measured on a daily basis and if error (defined by correct responses/total responses) was less than 25% in a single session, delay was extended for an additional two seconds. If error was greater than 25%, delay was shortened by two seconds. If error was exactly 25%, the current program was continued into the next day. This task continued for twenty-eight days.
8. Reaction Time: At the start of the task, one front nose-poke well was lit, and latency between onset of light stimulus and the nose poke response was measured. Responses were binned into .5 second increments from .5 seconds to 2.5 seconds to see how quickly mice were responding. This program lasted for fifteen days.
9. Signal Detection: At the start of the task, one front nose-poke well was lit for 1 second and the mouse had a 10 second window in which to respond. This sequence repeated throughout the task, with pauses for reward or intertrial interval. If the mouse had less than 25% error (defined as corrects divided by total responses) the stimulus duration was shortened incrementally the following day (to .5s, .3s, .1s). If mice received more than 25% error on a given day, the stimulus duration was lengthened the following day (from 1 to 3 sec). This task

was used to measure attention and visual acuity in the mice. This program continued for ten days.

10. Progressive ratio: At the start of this task, only the left nose-poke well was lit. Mice were required to make an increasing number of left responses to get a single reward. On the first trial, mice were only required to make one response for a reward, and the number of responses required to get a reward increased by one on each trial. The number of trials completed gives us information about the level of the animal's motivation to receive the reward. This program continued for ten days.

After this sequence was finished, mice received at least one full week of rest, with unrestricted food and water access before moving on to non-operant testing.

**Non-Operant Behavioral testing**

After the week of rest, a few non-operant tests were run on each strain to compare performance in tasks in the the animals. The tests are presented here in the order of their completion.

1- Open Field: All animals were placed in a dimly lit room in a large clean tub cage (46cm L x 24cm W x 20cm D), which was placed in a Digiscan machine (Columbus instruments) for 10 minutes of free exploration. The Digiscan quantified movement of the animals by the number of photo beams interrupted during their exploration.

2- Light-Dark box: The light dark box was a large tub cage (see pic 2) with 1/3 of the cage blacked on all sides and covered with a lid, while the other 2/3 of the cage was clear and open (cage size same as above, dark portion is the width of the cage, and 15cm of the 46 cm length). The two parts of the cage were connected by a small floor-level opening (5.5cm x 5.5cm) through which the mice could easily walk. The animals were placed inside the dark area of the cage, and allowed to freely explore both areas for ten minutes, while number of crosses into the light and total amount of time spent in the light was measured. This procedure assessed the sensitivity of the mice to light, as well avoidance of open spaces pitted against approach to novel spaces.

3- Barnes Maze: The Barnes Maze was a large round table (89 cm in diameter) open on all sides, with 8 holes (5cm in diameter) placed equidistantly around the perimeter of the table, 3.5cm from the edge (See pic 3). Mice were placed in the center of the table, and a dark escape box (8cm W x 10cm L. Deep box floor is 9.5cm below the level of the table, shallow is 6cm) is placed under one of the eight holes. The location of the escape box varies between mice, but remains constant across all trials for each mouse. A trial lasts until the mouse finds the escape box, and enters. If the mouse does not enter the box within five minutes, they are gently guided to it, and placed inside. There are two trials per day, fifteen minutes apart, for five or (in the case of slow learning) ten days. The experimenter measures latency to enter the box on each trial. Latency to enter is considered to be an indicator of how well the mouse recalls the location of the box. The Barnes maze tests both short-term spatial memory (on the second trial of the day, just fifteen minutes after the first) and long-term spatial memory (on the first trial of the day, when recall is from the day before).

4- Morris Water Maze: A large shallow pool (1.5 meters in diameter) is filled with water, and a 14 cm in diameter clear platform is placed 1 cm below the level of the water, in the center of one of the four quadrants of the pool (pic 4). The quadrant is different for each successive animal, but remains constant across all trials for a single animal. The mouse is first placed on the platform for thirty seconds, to orient the animal to the maze. Then, after a thirty second interval in a warming cage, the training trials begin. The mouse is placed in the center of the maze, and allowed to swim freely, attempting to find the submerged platform. If the mouse finds the platform, latency is recorded, and it remains on the platform for ten seconds before being moved to a warming cage for thirty seconds. This training trial is repeated four times in succession each day, until criteria for learning has been reached. To reach criteria, the average of the four trials in a single day for a mouse must be less than twenty seconds.

After a mouse reached criteria, it underwent two testing trials; one 1.5 hours after reaching criteria, and one 24 hours after. During a testing trial, the platform is removed from the maze, and the mouse swims freely for one minute

while three measurements are taken; latency to cross platform location, number of platform location crosses, and time in quadrant opposite the target. After the 24 hour testing trial, the mouse resumes training, until all animals have reached criteria. When all animals have reached criteria, all animals are tested again at both 1.5 and 24 hours. This allowed memory to be measured both as soon as the task was learned, and when all animals have received an equal amount of training. This task, similarly to Barnes Maze, measures shorter term (1.5 hour) and longer term (24 hour) spatial memory.

## Methods for Histological Characterization

### Perfusion and tissue treatment:

At the completion of behavioral testing, all mice were deeply sedated with sodium pentobarbital before being transcardially perfused with ice cold saline. Brains were then dissected out, with one hemisphere (cerebellum discarded) being immediately frozen at -80˚C for ELISA. In the case of Tg-SwDI mice, the other hemisphere was submerged in ethanol, and then later fixed in paraffin for sectioning. In the case of the 3xTg and Tg-2576 mice, the brain was submerged in 4% paraformaldehyde for 24 hours, before being submerged in 40% sucrose solution for 48 hours, and finally placed in a mold full of OTC (Optimal Cutting Compound) and frozen at -80˚C, ready for sectioning.

### ELISA (Enzyme-linked Immunosorbent Assay)

Plates were coated with capture antibodies mAb 21F12 for Aβ42 and 2G3 for Aβ-40 and incubated at 4˚C overnight, before being washed and blocked with milk. Samples and standards were then placed in triplicate into wells, and incubated at 4˚C. After washing, samples were incubated in biotinylated reporter antibody 3D6 at room temperature, then amdex-streptavadin-HRP, and washed again. After adding TMB peroxidase, the Spectromax machine and Softmax program were again used to read the plates.

### Tissue Staining

A) **Paraffin sections**:

10µm sections were cut on a sliding microtome, transferred to a warm water bath, and immediately mounted on slides, which were stored at room temperature until

staining began. Paraffin sections were deparaffinized and rehydrated to DI water, then incubated in protease K, and heated in antigen unmasking solution for 30 minutes. After this point, procedures are the same for both types of sections.

**B) Frozen sections:**

14μm sections were cut using a freezing microtome, then floated in PBS, and immediately mounted on slides. Slides were kept frozen at -40˚C until use. After this point, procedures are the same for both types of sections.

**Tau:**

The sections were washed and then incubated in a solution of 1 part 30% hydrogen peroxide, 1 part methanol and 8 parts PBS, followed by Superblock blocking buffer. Sections were then incubated in monoclonal anti-human tau AT8 for 4 hours and anti-mouse IgG for 1 hour. Then, they were incubated in ABC solution (Vector labs), before being developed with DAB, and counterstained with hemotoxylin. Finally, tissue was dehydrated with increasing baths of ethanol and xylene, before being cover-slipped with Permount.

**Fluorescent Aβ and Microglia:**

Sections were washed and then incubated in a solution of 1 part 30% hydrogen peroxide, 1 part methanol and 8 parts PBS, followed by Superblock blocking buffer. Sections were then incubated in anti-Aβ 1-28 polyclonal and Anti-Keratin sulfate 5D4 monoclonal, and then kept covered with aluminum foil when in donkey anti-Rabbit IgG green and donkey anti-mouse IgG red. After secondary antibody, tissue was immediately cover-slipped using crystal mount. Sections were immediately inspected under a fluorescent microscope, and pictures were taken of staining. If sections needed to be stored, they were stored for only a short time, at -80˚C to preserve fluorescence.

**Collagen and Thioflavin:**

The sections were incubated in Protease K 1:5000 for 5 minutes, and then for 15 minutes in Superblock with.3% Triton X 100. They were then incubated overnight in Rabbit Anti-collagen type IV polyclonal at 4˚C, before being washed and incubated for 1 hour in goat anti-rabbit IgG, and washed again. Sections were finally incubated in .0125% Thioflavin S in 40% ethanol and 60% PBS for 5 minutes. Then sections

were washed with 50% ethanol in PBS, and crystal mount was used to coverslip immediately. With these sections, it was important to immediately take pictures of staining under the microscope. If tissue was stored, it was kept in -80 freezer and used within a week.

**Amyloid staining**

Tissue was soaked in .3% Triton X with methanol and hydrogen peroxide for 10 minutes, and then blocked with Superblock for 15 minutes, before being incubated in anti Aβ 1-28 polyclonal for 4 hours. Then tissue was washed, and incubated in Anti-Rabbit IgG for 1 hour, before being incubated in ABC (Vector Labs) for 1 hour. Finally, tissue was developed in DAB for 10 minutes, and counterstained with hematoxylin for 1-2 minutes, before being dehydrated with increasing baths of ethanol and xylene, and coverslipped with Permount.

## Methods for Data Analysis:

Unless otherwise noted, data was analyzed using a within subjects ANOVA using the StatView program by SAS.

## Results

Due to the large amount of data collected in the experiments presented here, a data summary strategy was employed herein. First, non-significant results are presented in the text only. Second, to facilitate intercomparison, some graphs present all transgenic groups in a single figure, though they were studied separately. One way ANOVAs were performed for each pair separately, though presented on the same figure. Finally, where detailed analysis of a simple strain versus its control is desirable, the results from each cohort are presented on a separate panel.

## Operant Results:

### Initial Operant Results:

In the Fixed Ratio task, the mice learned to poke their nose in the well for water reward and successful performance was measured by number of responses per session. The Fixed Ratio's main measurement is of association learning; the mouse needs to associate nose-pokes with the delivery of reward. Three separate one-way ANOVA's were performed, one for each transgenic strain, to compare them to their age-matched wild type. We used data from each day from day one until task completion for each animal, to capture the full impact of the differences between learning in each group.

In this task, the Tg-2576 animals made an average of 3.4 responses per day, which was significantly fewer than their wild-type counterparts who made an average of 12.8 responses per day ($F(1,100)=27.7$, $p<0.0001$). An additional consideration with the Tg-2576 animals is that some mice (two of the eight) were incapable of producing reliable operant responding, making it impossible for them to continue in the study. So, results from subsequent operant tests are from the remaining mice alone, and may be slightly biased in favor of higher performing Tg-2576 animals than if randomly chosen. Tg-SwDI animals also had significantly decreased responses, as compared to their wild types ($F(1,130)=17.2$, $p<0.0001$) but all animals were able to acquire the task. In contrast, the 3xTg animals did not show any differences from their wild types at this task ($F(1,140)=.712$, $p=0.4$). Figure 1 summarizes the results from all three transgenic and wild type groups.

In the second task, Alternation, successful performance was measured by the cumulative number of responses per day, which expressed well how quickly a mouse learned the task. This task measures how well mice are able to generalize the response that they learned in Fixed Ratio to both nose ports. A one-way ANOVA was performed separately for each transgenic group and their wild type group. In this task, there was no difference between the Tg-2576 and their wild type controls (Tg-2576 $M$=16.6, S.E.M.=1.8, WT $M$=15.2 S.E.M.=2.0. $F(1,86)=0.23$, $p=0.6$), the SwDI mice and their wild type controls (Tg-SwDI $M$=19.2, S.E.M.=0.7; WT $M$=19.2 S.E.M.=1.2 $F(1,130)=.001$, $p=.98$), or the 3xTg animals and their wild type controls (3xTg $M$=16.5, S.E.M.=2.3; WT $M$=16.7, S.E.M.=1.7 $F(1,103)=.004$, $p=.95$).

Since successful performance in subsequent tasks was determined by correct responses, the data after this point are expressed as percent accuracy. This measure also corrects for differences in rate of responding, which we found in many tasks. It should be noted that in almost every task, the Tg-2576 mice made fewer responses than their wild types, the Tg-SwDI's fewer than their wild types, and the 3xTg's more than their wild types. There will be further discussion and analysis of this as it pertains to the Non-Match to Position task.

In the Light Dark Discrimination task, there were again differences between groups. The Light Dark Discrimination task measures the ability of mice to learn a contingency to their responses; they may only respond in a lit nose-poke well, and the lit well moves randomly. In this task, analysis was for percent correct, analyzed using a 3 separate ANOVA's across all days that the task was performed. During Light Dark Discrimination is where we begin to see the pattern emerge that develops throughout much of the rest of operant testing. Data from all days until acquisition of task were used, as this seemed to most accurately characterize the results. There were significant differences between the Tg-2576 mice at 71.2% correct and their wild types at 82.8% correct ($F(1,138)=6.7$, $p=0.01$). This trend was less strong in the Tg-SwDI mice, with there being a trend toward these mice making more mistakes than their wild types ($F(1,158)=2.9$, $p=0.09$). Finally, the 3xTg animals, surprisingly, performed *better* than their control animals, with a significantly higher percentage of correct responses at 92.2%, where their wild types only achieved 87.6 ($F(1,93)=5.2$, $p<.05$). Figure 2

compares the percentage correct for all the groups, showing that the Tg-2576 and their corresponding wild types made many errors in this task, the Tg-SwDI and wild types did slightly better, and the 3xTg and wild types made few errors.

The Shuttle task was analyzed separately for each transgenic and wild type group using percent correct and an ANOVA across all five days of the task. In this task, there was the addition of a response requirement in the back of the chamber. The Shuttle task measures complex rule learning, as well as the ability of the mice to follow a sequence of events to completion in order to get a reward. We again see a different relationship between the groups than we see on other tasks (see Figure 3). The Tg-2576 animal, averaged of 88% correct, as compared to their wild types, who made an average of 95% correct responses ($F(1,86)=5.2$, $p<0.03$). Interestingly, in this task, we see a significant difference between the males and females in the Tg-2576 group in the acquisition of the task, with the females acquiring it more slowly (F M=78% SE=5%, M M=98% SE=1%, $F(1,28)=14.5$ $p<.001$). This seems to indicate that the two sexes are starting to diverge in their pathology at this point, with the males accumulating less pathology than the females.

The Tg-SwDI animals actually did better than their wild types at the Shuttle task, making 95.5% correct responses, with their wild types only making 92.5% correct responses ($F(1,72)=5.1$, $p<0.03$). Finally, the 3xTg animals did not perform significantly differently than their wild types, with 97.6% correct, and their wild types 96.2% ($F(1,93)=1.4$, $p=0.23$). It is interesting how the addition of a rear response changes the relative performance of the various groups of mice.

**Non-Match to Position Results:**

The Non-Match to Position task measures spatial working memory; mice have to retain the location of their initial response while making a rear response in the chamber. In this task, when an ANOVA was used for each pair across all 40 days, the Tg-2576 animals had a significantly lower percentage of correct responses than their wild types ($F(1,424)=41.1$, $p<0.001$). Upon further investigation, it is clear that this difference comes from deficient learning of the task over time, and not just from a deficiency in ability at the start. When data is broken down from the 40 days into ten day segments and separate ANOVA's for each 10 days are done to see if there is a difference between

the rate of learning for each group, we can see that; days 1-10 transgenic Tg-2576's receive 40.9% correct, and WT's 50.7 ($F(1,98)=4.7$, $p=0.11$), but there is lengthening of the gap between the groups over time, and by days 31-40 the transgenic receive 60.6% correct, and WT's 77.6% ($F(1,98)=22.6$, $p<0.001$). This shows that the gap between the Tg-2576 and wild type animals only grows as they have more time to learn the task (see Figure 4). There were no sex differences in the Tg-2576 animals at this point.

For the Tg-SwDI animals, there is no significant difference between the two groups in percentage of correct responses when analyzed using an ANOVA across all forty days, with Tg-SwDI mice averaging 62.2% correct, and WT's 64.2% correct ($F(1,627)=0.98$, $p=0.32$). However, when we break the results for the SwDI animals down into ten day increments, we see a very different pattern, again shown by the first and last days of the experiment; days 1-10 there is a large significant difference, with the Tg-SwDI's doing far worse than the WT's ($F(1,155)=11.8$, $p<0.001$). However, during the last 10 days, Tg-SwDI animals actually do better than their WT controls ($F(1,155)=4.3$, $p<0.05$). This shows that though the Tg-SwDI's have trouble acquiring a task at first, they are capable of learning slowly, and eventually doing as well as, or perhaps even better than, the WT's (see Figure 5).

The 3xTg animals showed no significant differences from their wild types when collapsed across all 40 days ($F(1,755)=1.2$, $p=0.26$) However, when broken into increments of 10 days, in the first ten days the transgenics are doing markedly better ($F(1,186)=7.6$, $p<0.01$), but by the last ten days, both groups are performing equally well ($F(1,186)=0.001$, $p=0.98$). This shows that while the transgenics seem to pick up the task more quickly, it doesn't seem that they are actually better at learning and remembering the task over time (see Figure 6).

A graph of the overall percentage of all three pairs of groups together (though they are analyzed in separate ANOVAs) is displayed in Figure 7, which shows all the days percentages averaged to one number. Also, another interesting result was found when analyzing this data; the percentage of correct responses seems to relate to the number of overall responses made by a group. In Figures 8, 9 and 10, the raw responses in the first and last 10 days are displayed for the Tg-2576, ($F(1,115)=26.4$, $p<0.0001$, and $F(1,115)=43.1$, $p<0.0001$), Tg-SwDI ($F(1,150)=26.0$, $p<0.0001$ and $F(1,150)=2.6$,

$p$=0.11) and 3xTg animals ($F$(1,185)=7.5, $p$<0.01 and $F$(1,185)=33.3, $p$<0.0001)respectively, after being analyzed using a within-subjects ANOVA separately by pairs, across the first and last 10 days of the experiment. You can see that the relationship between number of responses per trial, and accuracy per trial is fairly consistent. There will be a discussion of why this relationship might exist in the discussion section.

**Delayed Non-Match to Position Results:**

The Delayed Non-Match to Position task measures short term spatial memory in the animals; they need to remember the location of their first response over a variable delay. The Tg-2576 animals did significantly worse overall than their age-matched wild types at this task, when analyzed with an ANOVA over 40 days for the delay achieved on each day ($F$(1,326)=24.69, $p$<0.001 ). When we break it into week-long segments and do separate within subjects ANOVAs for each week too see if there is a difference in learning for the groups, we see that the source of this significant difference is not in the first week, where Tg-2576 and wild type animals have delays of 1.76 and 1.82 seconds, respectively ($F$(1,75)=0.038, $p$=0.85). The source of the difference is in the later weeks, like week 4, where Tg-2576 animals have a1.67 second delay, and WT's have a much larger 3.39 second delay ($F$(1,75)=8.2, $p$<0.01). See Figure 11 for a summary of these results. Also, at this point we see another significant difference between the sexes in the Tg-2576 group, with the males achieving a significantly longer delay than the females (F M=1.4 sec SE=.13, M M=1.9sec SE=.17, F(1,102)=5.7 p<.05). This further supports the idea that the sexes are developing pathology at different rates.

The transgenic Tg-SwDI animals did comparably to their wild types if one performs an ANOVA for delay time over all 28 days of Delayed Non-Match to position ($F$(1,410)=0.06, $p$=0.8), but if you look at data week by week, it tells a more interesting story. In week one's an ANOVA, the groups perform very similarly, the Tg-SwDI animals with a 2.4 second delay, and the wild types with 2.6 ($F$(1,96)=0.408, $p$=0.52). But a trend seems to emerge as the animals have longer to learn and adjust, and in week four, the Tg-SwDI animals actually have a trend towards doing better than wild types, receiving a 6.4 second delay, as compared to the wild types at 5.1 ($F$(1,96)=2.7, $p$=0.1). This shows that while the SwDI's are initially equal to their wild types, over time it's

possible that they are actually able to retain procedural memories for a longer period of time. (Figure 12)

The 3xTg animals did significantly better overall than their wild types, with the 3xTg's being able to sustain a 4.6 second delay overall, with the WT's only sustaining a 2.9 second delay ($F(1,530)=69.7$, $p<0.001$). The 3xTg animals' relationship with their wild type strain, in contrast to the other two groups, did not change over the course of the 28 days. The week one data show 3xTg animals doing significantly better than their age-matched wild types, sustaining a 2.8 sec delay as compared to the WT's 2.0 ($F(1,131)=13.5$, $p<0.001$). Continuing that trend, the week four data show that while the 3xTg animals sustained a delay of 5.5 sec, the WT animals had only a 3.4 second delay ($F(1,131)=19.4$ $p<0.001$). (Figure 13) For a comparison of all three groups of transgenic and wild type's delays, averaged over all 40 days, please see Figure 14.

**Operant Reaction Time and Attention Results:**

Reaction Time measures how quickly mice can respond to the onset of the stimulus lamp. This reaction time can indicate a few different things; how well mice are attending to the stimuli, as well as their motor ability, and spontaneity. In this task, we quantified raw number of responses, and plotted a reaction time curve, to better illustrate the differences between transgenic groups and their wild types.

For the Tg-2576 animals number of responses per day, and percentage of responses per time bin were analyzed to see the difference in reaction time. There was a significant difference in the number of responses, when analyzed using an ANOVA across the 15 days of the task, with Tg-2576 animals making fewer ($F(1,155)=9.3$, $p<0.01$, Figure 15). However there was only a significant difference in the response curve at 2 seconds, with the wild types making more responses at this point ($F(1,155)=5.3$, $p<0.05$). The curve generally shows that though they make different numbers of responses, these two transgenic strains have comparable reaction times. (Figure 16) There were no detectable sex differences at this task.

For the Tg-SwDI mice, we see a slightly different picture. Though there was no significant difference in the overall number of responses with these mice when analyzed using an ANOVA over all 15 days ($F(1,223)=1.3$, $p=0.25$, Figure 15) there were significant differences in reaction time. Tg-SwDI mice made significantly more

responses at .5 sec (F(1,223)=15.3, p<.001), significantly fewer at 1.5 sec($F$(1,223)=35.1, $p$<0.001), and significantly more again at 2.5 sec ($F$(1,223)=5.3, $p$<0.05). But when we step away from the raw numbers and take a look at the graph, it's clear that what these differences are really pointing to is that the Tg-SwDI animals have a slower reaction time than their WT controls (Figure 17).

For 3xTg mice the reaction time task again shows a different relationship between the strain and its wild type. Predictably, the 3xTg animals made more responses in this task; 63.7 as compared to the wild type's 53.7 ($F$(1,238)=33.3, $p$<0.001). There were also significant differences in the response times in the task with 3xTg animals making more responses in the .5 and 1 second bins ($F$(1,283)=12.5, $p$<0.001and $F$(1,283)=12.6, $p$<0.001), and Wild type animals making more responses in the 1.5 and 2 second bins ($F$(1,283)=22.3, $p$<0.001 and $F$(1,283)=6.1 $p$<0.05). Again, when we look at the reaction time graph, it seems that one group peaks earlier than the other. In this case, it seems that the 3xTg animals have a faster reaction time, though the impression isn't as drastic as it was with the last group. (Figure 18)

In Signal Detection, the task was again (similarly to DNMTP) stepped up and down each day, based on the previous days' performance. The Signal Detection task measures how well mice are attending to the stimuli presented in the front panel. The measurement that is compared between groups for this task is the duration of stimulus, and all pairs were analyzed individually on an ANOVA for the delay achieved on each day.

For the Tg-2576 mice there were significant differences between the two groups in the signal detection task. We're presenting these results, but they need to be taken with caution; two of the three remaining Tg-2576 mice stopped making any correct responses during this task. They still made some intertrial interval responses, so they were not incapable of responding, but they did stop responding to the task. With that in mind, the stimulus needed for response for the Tg-2576 mice was 1.59 seconds, while for the Wild types it was .68 seconds ($F$(1,68)=18.9, $p$<0.001). There were no significant sex differences in this task. For the Tg-SwDI mice, there was no difference in ability to respond to a brief stimulus between the two groups, with Tg-SwDI's needing an average of a .75 second stimulus, while the wild types needed an average of a .77 second stimulus

($F(1,133)=0.21$, $p=0.7$). The 3xTg's also did not show a significant difference between the two groups in this task ($F(1,188)=1.3$, $p=0.25$). This shows that out of the three strains, only one could have had a problem with their detection of stimulus, the Tg-2576 mouse. (see Figure 19)

The Progressive Ratio task measures how many responses a mouse is willing to make for a single reward; this by extension measures the motivation mice have for a single reward. This task was analyzed using separate ANOVA for number of rewards earned by each group pair to get an overall picture of responding. The Tg-2576 animals did not make very many responses, with their average being 8.3 as compared to the wild types 28.6 ($F(1,68)=57.5$, $p<0.0001$), with no sex differences. The Tg-SwDI animals trended towards making more responses then their wild types, averaging 18.3 rewards as compared to their wild type's 17.1 ($F(1, 148)=2.0$, $p=0.16$). The 3xTg animals made a much higher number of responses, and received many more rewards than the other animals, and also many more than their wild type controls, 3xTg's received 32.5 rewards, as compared to their wild types 27.4 ($F(1,188)=12.4$, $p<0.001$). Please see Figure 20 for a graph of these results.

**Non-Operant Results:**

The first measure we take after operant training is one of pure activity level. We use the Digiscan apparatus (Columbus Instruments, Columbus OH) to measure activity level in an objective way-with photo beam breaks. We analyzed the data using 3 separate one-way ANOVAs for number of photo beam breaks recorded as ambulation, comparing each group to their corresponding wild type. We found no difference in ambulation between groups for the Tg-2576 animals and their wild-type controls ($F(1,11)=0.21$, $p=0.66$), while there were no significant sex differences in ambulation. For the Tg-SwDI animals, we found that there was no significant difference in ambulation between the groups, with Tg-SwDI's ambulatory score being 353, and their wild types 358 ($F(1,12)=0.01$, $p=0.91$). The 3xTg animals showed another significant difference from their wild type controls. In this task, they were actually *less* active, getting an ambulatory score of 227 as compared to the wild types' 333 ($F(1,17)=0.02$, $p<0.05$). See figure 21 for a summary of these results.

The next task was Light-Dark box, for which I used two measures to quantify performance. The first measures is the number of times an animal enters into the light side of the box, which is a measure of exploratory behavior or anxiety, and the second measure is the amount of time spent in the light, which is more a measure of anxiety alone. Three separate one way ANOVAs were performed for number of entrances into the light, and for amount of time spent in the light. For the Tg-2576 animals I found a trend toward a difference in number of crosses into the light, with the wild type animals crossing more frequently ($F(1,10)=4.5$, $p=0.06$) but no difference in amount of time spent in the light (Tg-2576 $M=49.0$ S.E.M.=27.1; WT $M=70.8$ S.E.M.=21.7, $F(1,10)=0.40$, $p=0.54$). There were also no significant sex differences for time spent in the light. For the Tg-SwDI animals there was a significant difference between the groups for number of crosses into the light, with Tg-SwDI animals making fewer entrances into the light side of the chamber ($F(1,13)=8.4$, $p<0.05$). However, there was no difference in amount of time spent in the light (Tg-SwDI $M=91.3$, S.E.M.=25.0; WT $M=59.1$, S.E.M.=8.0; $F(1,13)=1.5$, $p=0.23$). For the 3xTg animals there was no significant difference between the two groups in number of times crossed into the light, with 3xTg animals crossing 9.7 times on average, and WT animals crossing 9.4 times ($F(1,17)=0.15$, $p=0.70$). There was also no significant difference between animals on amount of time spend in the light, with 3xTg animals spending an average of 118 seconds, and WT's spending an average of 91 seconds (3xTg $M=106.5$, S.E.M.=17.0; WT $M=91.7$, S.E.M.=19.5; $F(1,17)=0.32$, $p=0.58$). For a graph of the average number of entrances into the light side of the box, please see figure 22.

The next task was Barnes maze, which tests more spatial and long term memory than the previous tasks. Data from this task was analyzed using separate one-way ANOVAs for each transgenic animal as compared to their wild type, on each day. The measure used was latency to enter the escape box. On this task, the 2576 animals failed to learn in five days, so we changed the experiment slightly, and gave them a shallower box (Day 1 $F(1,11)=0.7$, $p=0.4$, Day 2 $F(1,11)=0.3$, $p=0.6$, Day 3 $F(1,11)=.7$, $p=.4$, Day 4 $F(1,11)=.7$, $p=.4$, Day 5 $F(1,11)=.6$, $p=.4$, please see figure 23). When this task was analyzed with a repeated measures ANOVA, the results again showed no significant difference between the groups (Tg-2576 M=260.7 SE=10.9, WT M=231.2 SE=12.9,

F(1,128)=2.9 p=0.8) When this task was run using the same group of mice, but a shallower box, the results are different. With the shallower box, wild type animals performed significantly better at the task (Day 1 F(1,11)=0.4, p=0.4; Day 2 F(1,11)=.1, p=0.8; Day 3 F(1,11)=2.6, p=0.1; Day 4 F(1,11)=7.9, p<0.05; Day 5 F(1,11)=5.9, p<0.05, please see figure 24). A repeated measures ANOVA shows similar results, with an overall significant difference between the two groups on this task (Tg-2576 M=201.3 SE=14.6, WT M=122.3 SE=13.1, F(1,128)=16.3, p<0.0001)This shows that a motor or exploratory deficit in these animals may have contributed to the lack of learning when the task was performed with a deeper box. There was also a significant sex difference between the Tg-2576 animals at this stage, with the females having a longer escape latency than the males (F M=291.2 SE=8.7, M M=111.4 SE=15.3, F(1,58)=103.7).

For the Tg-SwDI animals, there was also a growing, significant difference between groups (day 1 $F(1,13)=4.1$, $p=0.07$, day 2 $F(1,13)=20.3$, $p<0.001$, day three $F(1,13)=8.8$, $p<0.05$, day four $F(1,13)=6.1$, $p<0.05$, and day 5 $F(1,13)=11.3$, $p<0.01$, See figure 25). When a repeated measures ANOVA is completed for this task, the significant difference between the two groups is even more stark (Tg-SwDI M=257.5 SE=10.2 WT M=166.1 SE=11.9 F(1,148)=32.8 p<0.0001) It actually seems to be the second trial of the day that drives the significant differences between the two groups, indicating that while both groups have trouble recalling the trials from the day before, the wild types are more capable of recalling the trial 15 minutes before (**Trial 1**: Day 1 $F(1,13)=2.5$, $p=1.4$, Day 2 $F(1,13)=4.0$, $p=0.07$, Day 3 $F(1,13)=1.2$, $p=0.29$, Day 4 $F(1,13)=1.2$, $p=0.3$, Day5 $F(1,13)= 5.8$, $p<0.05$. **Trial 2**: Day 1 $F(1,13)=3.6$, $p=0.08$, Day 2 $F(1,13)=14.2$, $p<0.01$, Day 3 $F(1,13)=7.1$, $p<0.05$, Day 4 $F(1,13)=9.3$, $p<0.01$, Day 5 $F(1,13)=15.1$, $p<0.01$ figure 26, 27). When the two trials are averaged separately over the 5 days of the experiment, and repeated measures ANOVA is run, it is clear that there is an interaction between the trials and groups (main effect of group: F(1,13)=16.9, p<0.005; group x trial interaction: F(1,13)=8.4, p<0.05, please see figure 28). When a separate ANOVA is run for each group with trials as the independent variable, it is clear that the interaction is caused by the difference in the Wild type animals, and not the SwDI animals (F(1,73)=4.9, p<0.05 and F(1,73)=0.0003, p=0.99, respectively).

The 3xTg mice had surprising results on the Barnes Maze. While the wild type animals improved slowly, but steadily, the 3xTg animals were seemingly incapable of learning the task at all. Because the animals seemed to be taking longer to learn the task, we extended the experiment from 5 days to 10. From day three forward, the WT mice were escaping the maze significantly faster than the 3xTg mice (Using the average of both trials: Day three $F(1,17)=6.3$ $p<0.05$, Day ten $F(1,17)=18.8$ $p<0.001$. See figure 29) The difference between the two groups is extremely significant when analyzed with a repeated measures ANOVA (3xTg M=298.3 SE=0.9, WT M=207.2 SE=8.3, F(1,378)=132.1 P<0.0001) It seemed curious to the experimenters that the 3xTg animals were completely incapable of learning the task, so in addition to measuring the amount of time it took mice to escape, on day 3-5 we measured latency to first find the escape box, and found there was no difference in time to find the correct box. So, we concluded it might be a feature of the box that was preventing the animals from acquiring the task. Based on unpublished observations from Xu et al. we decided to attempt to use a shallower escape box, to see if the 3xTg animals could learn the task using this slight variation. We changed box location, and began the experiment again. This time, we saw a reversal of the progression of the last task. As the experiment progressed, an initially significant difference in latency to escape between the two groups grew smaller and smaller. On day 1 the 3xTg animals had a 300 sec latency, and the WT's 197 ($F(1,15)=9.5$, $p<0.01$). By day 5 it was 179 for 3xTg animals, and 97 for WT's ($F(1,15)=3.0$, $p=0.11$, see figure 30). This shows that perhaps the deficit in learning the Barnes maze was actually an exploratory, anxiety, or motor difference, rather than a deficit in memory alone. However, when analyzed with a repeated measures ANOVA, the difference between the two groups is still significant (3xTG M=247.2 SE=11.2, WT M=152.5 SE=12.8, F(1,168)=30.3 p<0.0001).

To further address the concepts that we looked at using Barnes maze in the 3xTg animals, we used the Morris Water Maze, which brings different motivators to bear on a mnemonically very similar task. For the 3xTg animals, there were no significant differences between groups on any measure. We measured latency to find platform on each testing day, and analyzed the data for each day using a one-way ANOVA and found no significant differences from day 1 ($F(1,15)=0.004$, $p=0.95$) to day 6 ($F(1,15)=0.7$,

*p*=0.42). We also compared the 3 measures (latency to cross, number of crosses, time in opposite quadrant) at 1.5 and 24 hours after individual acquisition, and 1.5 and 24 hours after all mice have acquired the task and found no significant differences. Please see fig 31 for acquisition curve for this task.

**Histological Analysis:**

Only data from the Tg-SwDI and 3xTg animals are presented for post-mortem analysis as data from the Tg-2576 animals are pending.

In the fluorescent double stain for Aβ and activated microglia, it is clear that while SwDI animals have an abundance of intracellular and extracellular Aβ, the 3xTg animals have low to moderate amounts of Aβ, which is mostly intracellular. The SwDI animals have deposits throughout the cortex, hippocampus, thalamus, and subiculum, while the 3xTg animals have deposits concentrated in cortex and subiculum. Also, while there is an abundance of activated microglia in SwDI animals, and indeed microglia associated directly with amyloid deposits, no activated microglia are apparent in the 3xTg tissue. While amyloid and activated microglia were found in all areas mentioned in all Tg-SwDI animals, only cortical Aβ was found in all 3xTg animals, subicular amyloid was found in only 3, and in the form of a single larger aggregation.(please see pics 5-10)

Additional tissue double stained for collagen and fibril amyloid show the unique characteristics of the SwDI strain. There are high concentrations of vessel associated fibril amyloid in the SwDI animals in subiculum and thalamus, and a little vessel associated amyloid in hippocampus and cortex. There was no visible vessel-associated fibril amyloid in the 3xTg animals, and little fibril amyloid overall, only visibly expressed in cortex. (see pics 11-18)

The staining of Aβ alone confirms the findings of the thioflavin and fluorescent stains. Again, SwDI animals have robust intra and extracellular Aβ accumulation in subiculum, hippocampus, thalamus, and cortex, where 3xTg animals have lesser amounts, and what Aβ they have is mostly concentrated in the cortex, and in some animals, the subiculum (please see pictures 19-30).

More quantifiably, ELISA was done for Aβ for both sets of animals, to measure the amount of the two types of Aβ in the mice. The most notable result of this analysis is

the greater amount of Aβ 40 in the SwDI animals as compared to the 3xTg animals (total Aβ 40= soluble+insoluble). For SwDI animals the Aβ 40 concentration was 23,867 pg/mg, where for 3xTg animals it was 427 pg/mg . A one-way ANOVA was performed and we found that this was a significant difference ($F(2,14)=20.4$, $p<0.0001$). This extremely large difference drove the significant differences between the two groups in total soluble and unsoluble Aβ (total Aβ=40+42, $F(2,14)=8.0$, $p<0.01$, see fig 32). This might explain why we found so little Aβ in the staining of the 3xTg animals.

After staining for tau using the AT8 antibody, only a small amount of tau fibrils and cell bodies are apparent in the 3xTg animals, far less than expected. Pictures show examples of the small amount of tau cell bodies, and tau fibrils, which were found in only three of the ten 3xTg animals studied. As expected, there were no visible tau cell bodies or fibrils in the Tg-SwDI animals (pictures 31-34). A positive control for tau is included in the picture section in the form of the cortex of the SwDI-NOS2 KO mouse. The SwDI-NOS2 KO mouse is this the Tg-SwDI mouse, with the addition of a nitrous oxide synthetase knock out, which is known for its robust tau pathology (Colton et al., 2006, Wilcock et al., 2008).

Table 2: Summary of results

| **Task** | **Tg-2576** | **Tg-SwDI** | **3xTg** |
|---|---|---|---|
| Fixed Ratio | Decrease in Responses | Decrease in Responses | No Change |
| Alternation | No Difference | No Sig. Difference | No Change |
| Light Dark Discrim. | Decreased accuracy | Non-Sig Increase | Increased accuracy |
| Shuttle | Decreased accuracy | Increased accuracy | No Change |
| Non-Match to Position | Decreased accuracy | No Overall Difference | No Overall Difference |
| Delayed NMTP | Decrease Delay Ability* | No Overall Difference | Increase Delay Ability* |
| Reaction Time | No Overall Difference | Slowing of Response | Speeding of Response |
| Signal Detection | Longer Stim. Needed+ | No Sig. Difference | Shorter Stim needed+ |
| Progressive Ratio | Fewer responses | No Sig. Difference | Increased Responses |
| Digiscan | No Difference | No Sig. Difference | Decreased Exploration |
| Light/Dark Box | Increased Crosses # | Decreased Crosses # | No Difference |
| Barnes Maze (deep) | No overall difference | Longer Escape Latency | Longer Escape Latency |
| Barnes Maze (shallow) | Longer Escape Latency | | Decreasing Latency |
| Morris Water Maze | | | No Sig. Difference |
| Aβ Concentration | | High | Low |

* Delay Ability= the amount of time delay there can be between subsequent nose-pokes and still allow mouse to maintain 75% correct responses

+ Stimulation needed=the length of light stimulus required for a mouse to maintain 75% correct

# Crosses= the number of times a mouse entered the light side of the light dark box during exploration

## Discussion

The goals of this research program were twofold. The first goal was to perform a novel comprehensive behavioral comparison of three important strains of transgenic mice thought to model aspects of Alzheimer's neuropathology. The three strains we chose to work with were the commonly used Tg-2576, the Tg-SwDI; a model of cerebral amyloid angiopathy, and the new and much hyped 3xTg mice. Each of these strains has unique characteristics and model different aspects of Alzheimer's disease. More comprehensively, the second goal was to explore the utility of this program of operant and non-operant behavioral testing for dissociating strain differences.

Previous methods for behavioral characterization in Alzheimer's transgenic animals have tended to rely on a limited number of behavioral tests, involved stressful test conditions for mice, and also tended to test only spatial long term memory (e.g. Morris Water Maze). These many previous studies could be characterized as employing a "behavioral bioassay" approach, where a behavioral test is employed simply as an external marker for the status of a brain system (Wilner, 1984). In contrast, the present study sought to employ a true neuropsychological battery approach , by using a complex set of interlocking cognitive operant tasks to test a variety of aspects of cognitive, sensory, and motor faculties in these Alzheimer's transgenic model animals. By implementing and analyzing these tests, experimenters acquired a more complete picture of what mnemonic, and non-mnemonic changes are manifested in these transgenic animals with a marked improvement in our ability to elucidate the exact cognitive impairment that a transgenic mouse expresses.

Several working hypotheses guided the design of this work. First, there was the concern that all three strains of mice would be so highly impaired that they would not be able to learn the tasks, or that they would perform extremely poorly on all tasks. This would have made it difficult or impossible to distinguish the exact mnemonic and behavioral differences between the strains using our strategy. This hypothesis was caused by the existing behavioral literature showing, for example, comparable and pronounced deficits in the learning of the Morris water maze in the Tg2576 and 3xTg strains (e.g. Hsaio et al., 1996; Oddo et al., 2003). Alternately, it was hypothesized that the severity of observed deficits might depend on the number of different transgenes, and

therefore the number of different types pathology a strain of mouse had. That is, the Tg-2576 mice, with only the Swedish mutation and parenchymal Aβ buildup, would perform fairly well in perhaps all except the most challenging tasks, while the Tg-SwDI, who have the Swedish, Dutch and Iowa mutations, and have vascular amyloid as well as parenchymal amyloid, would perform less well. Following this logic, the 3xTg animals, with the Swedish, Presenilin, and Tau mutations, and parenchymal amyloid as well as tau, would perform worst of all. Finally, it was hypothesized that the impairment in the tasks might be directly related to the amount and severity of pathology in the brains of the animals (the endophenotype), rather than a function of the number of mutations carried. This final hypothesis is the one that was best supported, as discussed in the following section.

## Behavioral Results:

### Behavioral Results:Tg-2576

Behaviorally, the Tg-2576 animals showed the highest degree of impairment on all tasks, as well as striking variability in their responses. The animals first showed impairment in simple response-reward contingency learning, the Fixed Ratio task. Wild type animals did much better than Tg-2576 animals, who were slower to respond, and therefore to learn the task. There were also animals that *could not learn* this task, and the Tg-2576 animals were the only transgenics who had group members who were unable to learn this, the simplest rule. We can conclude that the mice have a basic deficit in their ability to make associations between stimuli-response-reward, which is the basis of learning.

The second task that Tg-2576 mice had difficulty learning was Light Dark Discrimination. This is a measurement of response flexibility (i.e a simple executive function), and a deficit in this task shows that Tg-2576 animals were more likely to perseverate on a single side, and less likely to have a flexible response set, thought to be indicative of intact frontal cortical function (Seamans et al., 1995).

The Shuttle task added an additional layer of complexity by requiring the completion of three distinct responses (front port nose-poke, rear port nose-poke then front port alternation response nose-poke) for a single reward in a response chain. This

task also required mice to generalize to a response in the rear of the chamber, which they had not done previously. Tg-2576 animals again did less well than their wild types at this task, showing that they might have some spatial memory deficits (as they could not learn to move front, then rear, then front again) or perhaps the mice were unable to coordinate a sequence of events from front, to rear, and back again. Tg-2576 mice performed less well than wild types throughout the five days of this task. This deficit would be similar to procedural memory deficits found in Alzheimer's disease patients.

The Tg-2576 animals also had difficulty with Non-Match to Position. This task is identical to Shuttle, except that it requires remembering of the sample response to guide a subsequent later choice response. This is because the second front response is not signaled, so the animal needed to recall their initial "sample" response so as to alternate ("non-match"). It is a more complex conditional discrimination problem, so it was not surprising that the Tg-2576 animals did much worse than controls at this task. That the two groups begin at a similar starting point, but progressed by the end of the 40 days to be significantly different, shows that while both groups struggled with the task at first, the Tg-2576 animals continued to struggle when their wild types were able to perform the task fairly well.

In the Delayed Non-Match to Position task, where the retention interval was varied to a different value for each animal for each session to maintain a comparable 75% reinforcement rate, a significant difference between groups was again evident. The pattern showed that in the first week, groups perform comparably, but by the last week, Tg-2576 mice were performing less well than their age-matched wild types. The wild types were generally able to progress to longer delays, indicating specific short-term memory impairments in the Tg-2576's unrelated to changes in motivation or other secondary processes.

After DNMTP, operant tasks designed to assess the general ability of animals to respond were employed. The simple Reaction Time task revealed only slight differences between the two groups in their reaction time distributions. This task rules out response differences as explanations for the deficits in earlier tasks. However, evidence that they may have difficulty in detecting stimuli or sustaining attention to stimuli was shown by the Signal Detection task. Tg-2576 animals generally required longer stimulus duration

in order to react accurately. It should also be noted that there was considerable variability of response in the remaining Tg-2576 animals here, a general characteristic of this strain.

Finally, in the Progressive Ratio task, Tg-2576 animals made far fewer responses than did the wild types, indicating a deficit in motivation. This finding isolates this consistent overall reduced rate of responding from some of the transgenic mice across the operant tasks, and is an observation indicated for the first time in this well studied strain due to the nature of the current approach (Hsaio et al., 1996, Adriani et al., 2006, Barnes and Good, 2005).

Also, in Non-Operant tests, Tg-2576 mice showed no difference in their exploratory behavior in the open field, and only a trend toward less activity in the light/dark box. Neither of these results can explain the many large significant differences between the Tg-2576 mice and their wild type animals. In the Barnes maze, with the regular depth box it was a surprise to see that there was no difference between the groups. However, on examination of data, this lack of difference between the groups appeared to be due to a lack of learning of the task by either group. When a shallower box was substituted, the wild type animals performed significantly better than Tg-2576 animals, showing a deficit in spatial memory.

It also seemed that across the later memory-dependent tasks, females began to have more difficulty in the tasks, showing decreased performance at the Shuttle, Delayed Non-Match to Position, and Barnes Maze tasks. This seems to indicate that as the Tg-2576 animals aged, the female animals may have had increased pathology as compared to their male counterparts.

In summary, 2576 animals seemed to have comprehensive and multi-faceted deficits in many cognitive constructs, such as learning rules, switching rules, navigating spatially during a task in the operant chamber or in a maze, and retaining procedural information throughout task or over a delay. A consistent motivational deficit in the absence of motor problems and gross physical difficulties was also observed. There seemed to be some variation between the animals of opposite sex. These deficits were expressed in extremely different levels in age matched animals, consistent with the range of ages that neuropathogical signs are evident in these transgenic animals reported in the literature (eg. von Kleinlin et al., 2004).

**Behavioral Results:Tg-SwDI**

In contrast to the Tg-2576 mice, the Tg-SwDI animals showed a varied set of strengths and weaknesses on the behavioral tasks. On the first task, Fixed Ratio, Tg-SwDI mice did significantly worse than their age-matched wild types, showing that these transgenic mice are slower to learn a stimulus-response relationship. There was also a trend towards Tg-SwDI mice having trouble task-switching to Light Dark discrimination, which would show that they were perseverative and had trouble learning a new rule, but this difference was not significant.

Interestingly, on the Shuttle task, Tg-SwDI animals actually performed significantly *better* than their wild type controls. This result shows that the transgenic animals were perfectly capable of retaining a sequence of events, and navigating spatially in the chamber. When looking at the more difficult Non-Match to Position task, which adds a memory component, again strong differences between the groups were not evident. The same outcome was observed in the Delayed Non-Match to Position task, with both groups performing similarly.

While significant group differences were evident in Shuttle, these results considered with those from the other complex operant tasks seem to indicate at best a mild behavioral difference in the Tg-SwDI animals. While the mice certainly had a transient difficulty in complex rule-learning to begin with, it seems that once they learned a rule, they performed comparably to wild types.

When Tg-SwDI animals were assessed for non-mnemonic changes, some additional subtle differences were evident. In particular, Tg-SwDI animals had slower reaction times in the Reaction Time task. Again emphasizing the selectivity of deficits in this strain, no differences in the motivation in Progressive ratio or Signal Detection, and no difference in activity level in the Digiscan were evident. In the context of these findings, the reduced entries of the Tg-SwDI mice to the lit area of the Light-dark box may indicate hesitation to enter lit areas or even different sensitivity to light levels.

This finding is most relevant to understanding the pronounced and reliable deficits that the Tg-SwDI animals showed in the Barnes maze. (Xu et al., 2007) The increased latency to find and enter the escape box during Barnes Maze, which is usually

thought to indicate impaired short (within day) or long term (between day) spatial memory, but that may be more reflective of a different manner in which the Tg-SwDI explore, and therefore, learn about a novel, spatial environment. However, the nature of the deficits indicate a slower rate of performance improvement in these mice, and strong learning in the WT animals, rather than the lack of improvement evident in the Tg-2576 animals. In summary, while Tg-SwDI animals showed some difficulty in learning tasks initially, the global impairments evident in the Tg-2576 strain were not evident.

**Behavioral Results: 3xTg**

Perhaps the most surprising findings of the study were in the 3xTg animals. One could say that they showed the opposite of the pattern of responses that might be expected, given that they have the highest number of different mutations to their genome. In short, after beginning with no difficulty in learning the early tasks as compared to their wild type controls, the 3xTg animals went on to do as well as, or better than their wild type controls in many of the operant tasks. In particular, the 3xTg animals made fewer errors during Light Dark Discrimination, which shows increased flexibility with responses and rule learning. They also showed increased ability to make correct responses after the delay in Delayed Non-Match to Position. One possible explanation is that they show increased reactivity and startle to stimuli, an explanation supported by the observations that the 3xTg animals have faster responses in Reaction Time than their wild types, need a shorter stimulus during Signal Detection, and make more responses during Progressive Ratio.

Interestingly, when not responding to a learned stimulus but just left to explore novel environments in the Digiscan and Light-Dark box, the 3xTg animals are not more reactive and actually somewhat less active. A previous study by Giminez-Llort et al. (2007) showed that the 3xTg mice (and Alzheimer's transgenic mice in general) have decreased ability to deal with mild stressors, or novelty. They also find that reduced exploratory behavior is common, and associated with slower habituation and neophobia. Giminez-Llort et al. also assert that the difference in exploratory behavior is due to this neophobia. This theorized trait may have carried over also into Barnes Maze. While the 3xTg animals did the same or better than controls in the operant tasks, they performed strikingly *worse* than wild types in the Barnes maze. When taking into consideration the

potential of neophobia, we decided to explore this result further, and used a shallower box that was easier to explore from the edge of the opening prior to entry. When the difference between the two groups, significant at day 1, decreased in significance over the course of 5 days, we concluded that the depth of the box was a factor that was contributing to the differences between the two groups. Finally, the lack of an impairment in the Morris Water maze further supports the idea that the performance differences in the Barnes maze is not due to memory impairment. This Morris Water Maze result was especially noteworthy given the histological findings described below, and reports in the literature detailed deficits in MWM in these animals (Billings et al., 2007).

In conclusion, the 3xTg animals performed better on both mnemonic and non-mnemonic aspects of operant training than their age-matched wild types. They also had decreased activity in the Digiscan open field. Finally, though they had severe deficits on the deep box Barnes Maze, this deficit diminished when using a shallower box, which supports a difference in manner exploration, rather than a mnemonic deficit as the cause. This hypothesis is further supported by the lack of difference between the 3xTg animals and their wild types on the Morris Water Maze.

## Histological Results

### Histological Results: Tg-2576

Literature shows that the Tg-2576 mice have Aβ pathology in many areas of the brain, including the frontal, temporal and entorhinal cortices, as well as the hippocampus, subiculum, and cerebellum. Aβ is most dense in the Tg-2576 mouse in the cortex, subiculum, and pre-subiculum (Hsaio et al., 1996). Associated with these Aβ deposits are activated microglia in the cortex and hippocampus, which are not present in non-plaque containing brain areas (Frautschy et al., 1998). There is no tau present in Tg-2576 animals.

The robust accumulation of Aβ in these animals in hippocampal and cortical areas, along with the extensive activation of microglia in these areas seems to be sufficient to cause severe memory deficits in the Tg-2576 animals. It seems possible that the extensive cortical accumulation in this strain, more than in the other strains, is what contributes to the Tg-2576 mouse's more severe behavioral symptoms. Perhaps while

the severe hippocampal densities of Aβ and microglia contribute to the deficits in spatial memory, it is the addition of robust plaques in cortex that contribute to the severe procedural memory deficits.

**Histological Results: Tg-SwDI**

Tissue analysis of the current group of SwDI mice agrees with published accounts of their pathology (Miao et al., 2005, Davis et al., 2004). There was intracellular and extracellular Aβ found in the cortex, hippocampus, subiculum, and thalamus. In the cortex, deposits tended to be diffuse and non-vessel associated, while in the subiculum and hippocampus they were dense and vessel associated. In the thalamus, there were moderate amounts of plaque, with some fibril and vessel associated, and some parenchymal. Also associated with these plaques are activated microglia. Microglia are robust in the subiculum and hippocampus, and present but not as robust in the thalamus and cortex.

The selective nature of the neuropathology and behavioral deficits in this mouse provide an interesting opportunity to isolate the possible contribution of vascular amyloid and the downstream effects to limited behavioral domains. This is clearly not the case for the Tg-2576.

**Histological Results:3xTg**

Tissue analysis of 3xTg animals yielded surprising results. Though previously published reports showed diffuse Aβ deposits and tau fibrillization and cell bodies in many parts of the brain (see Oddo et al., 2003a, 2003b), our analysis did not corroborate these reports. Contrary to these reports, little amyloid was observed in the current 3xTg animals, and it was mostly concentrated in the subiculum, and the cortex. Also, little tau was found, with only 3 of the 10 animals showing any tau staining at all, and those showing only one or two cell bodies, and a few patches of fibrillization. We also could see little to no activated microglia in these animals.

The histological results in these animals, while surprising, do seem to explain the lack of operant behavioral deficits in the 3xTg animals, as well as the lack of deficit in maze learning that could not be explained by exploratory deficits. However, that some behavioral deficits were observed suggest that other aberrant pathological processes may account for these deficits.

**Recommendations for the field: Mice**

It seems, after our full analysis, that the most useful role of the Tg-2576 mice is as a baseline for comparison to other strains. They have severe behavioral impairments that any strain compared to them can be shown to have subtle impairments, rather than the floor effect that we see with these animals, which is kind of an "end state" Alzheimer's disease model, rather than a model of progressing AD. In the comprehensive set of tasks these animals show an effect on almost every measure, which does not allow us to show what sparing there is in the strain. Also, there are some things that make these animals less than ideal for behavioral testing. The strain has a high mortality rate (25% during the course of the present experiment) which makes them less ideal for longitudinal experiments, unless the increased mortality is planned for. Also, there is the troubling matter of some animals not being able to learn basic tasks, preventing more subtle, selective analyses of cognitive processes. It seems that the animals that dropped out of the experiment were probably the animals that were the most impaired, and the loss of their measurements to some extant may have biased the group averages in the direction of more sparing of function.

Tg-SwDI mice had somewhat surprising results overall. Based on previous reports showing reliable impairments in the Barnes maze with these animals, one might have expected more substantial impairments in the operant tests. Instead, the operant tests provided convincing evidence against characterization of these animals as severely memory impaired. In some ways, the moderate impairment we see in the Tg-SwDI animals is a great demonstration of the utility with our technical approach employed presently. It highlights that one should not make assumptions that impairment in a task (e.g. the Barnes maze) thought to measure a construct (e.g. spatial working memory) would necessarily manifest in all other tasks thought to measure that construct. Instead, we are reminded that behavioral tasks are unique procedures, and measure a variety of constructs, and that dissociations can often be very revealing of this.

Perhaps the most striking lesson comes from the 3xTg animals. It seems that the inheritance of the transgenes is unstable in these animals, and the effects are not expressing in subsequent generations. Initial reports on this line, showed tremendous

promise in these animals, though the utility of this strain has to be thoroughly re-evaluated.

**Operant Paradigm Neuropsychological Assessment**

Throughout the three sets of experiments in the operant chamber, and finally on non-operant tasks, many benefits have been found to working with the operant paradigm in conjunction with later spatial testing. One of the major insights in these experiments is how much *sparing* of function can be revealed by this approach. In a "behavioral bioassay" approach, one seeks to find an assay that is consistently, strongly impaired by manipulations of genotype. While there are certainly merits to this approach, such as high-throughput and standardization properties, behavioral bioassys are not suited for neuropsychological characterization, and as discussed above, can at times be misleading in that regard. The present approach, in contrast, encourages the development of a more nuanced characterization, dissociating what is impaired in these animals, and what is left unaffected. This encourages researchers to look at the transgenic mice in ways more consistent with the complex and fluid nature of the deficits that emerge along the progression of Alzheimer's disease.

Another strength of this approach is that smaller changes may be detected in these mice, due to sampling in each task over multiple days. Other tests used in the Alzheimer's modeling field have a single trial per day, which gives only a few measurements, and less to analyze. This paradigm gives us multiple trials per day and multiple trials per animal over the course of several days, which allows us to apply more complex statistical analyses when needed to see smaller differences between the groups. Furthermore, the animals in the operant task are behaving under familiar, repetitious conditions, in an enclosed environment. All of these factors presumably serve to minimize stress and distraction due to novel and threatening test conditions. Also this paradigm allows us to separate learning problems from memory problems using a task such as the DNMTP, where the retention interval component can be separated from the learning of the task procedures.

Even the ability of the mice to learn such complex tasks is part of what makes this paradigm unique; more can be done with applications of this approach. In particular,

training of the animals in tasks earlier, before pathology manifests itself, would allow the animals to serve as their own controls as well. Also, mice could be re-tested at later ages, as pathology progressed.

This current work also revealed that there are some downsides to this approach. As we mentioned earlier, mortality in mouse strains, which happens often with transgenics, is a problem because of the longitudinal nature of the task. Also, because of the longitudinal nature, there are carry-over effects between tasks. This problem is especially difficult when one group learns a task less well then another and both groups subsequently move to a new task that is based on the previous task. One could, during the course of the experiment, train each individual mouse up to a certain criteria, such as a number or percentage of correct responses, and then move them to the next task at that point, rather than having criteria of a certain number of days. The problem with this strategy is that some mice would finish within a very short time span, while it would take other animals closer to six months to complete the sequence. This would create both handling and age effects in the mice. Another possible strategy is to "vacation" the mice when they reach a certain criteria, and then add them back into the experiment when all mice have reached that criteria. Of course, this strategy also has its problems; there would be a difference in handling and exposure to the test chamber, as well as the possibility of the mice that were given breaks forgetting rules learned earlier.

Another problem with this procedure is the difference in rate of responding that we have observed between the groups. We have observed on almost all tasks that whichever group has a higher percentage correct, also has more responses. Because we can't decrease the number of responses and see if this is causing mice to learn the task better, we can only theorize as to why this occurs. Differences in responding during these tasks could be due to activity level; which we assessed by performing Digiscan, motivation for reward; which we assessed by using progressive ratio, or sensory deficits; which we assessed by using light/dark box and signal detection. It seems in the end, that the difference in responding may just be related to the reinforcement; an animal that makes more correct responses gets more rewards, and is therefore more likely to continue to make more responses.

In conclusion, this set of experiments sets a precedent in the field for a different type of behavioral testing. This testing strategy encourages researchers to focus on both impairments and sparing to paint a fuller picture of the behavioral symptoms in their animals. The strategy is very adaptable for use in different situations, and to find out different information about mice. The application of this approach to the study of other transgenic lines and for treatment studies will broaden the depth of analyses available for this field.

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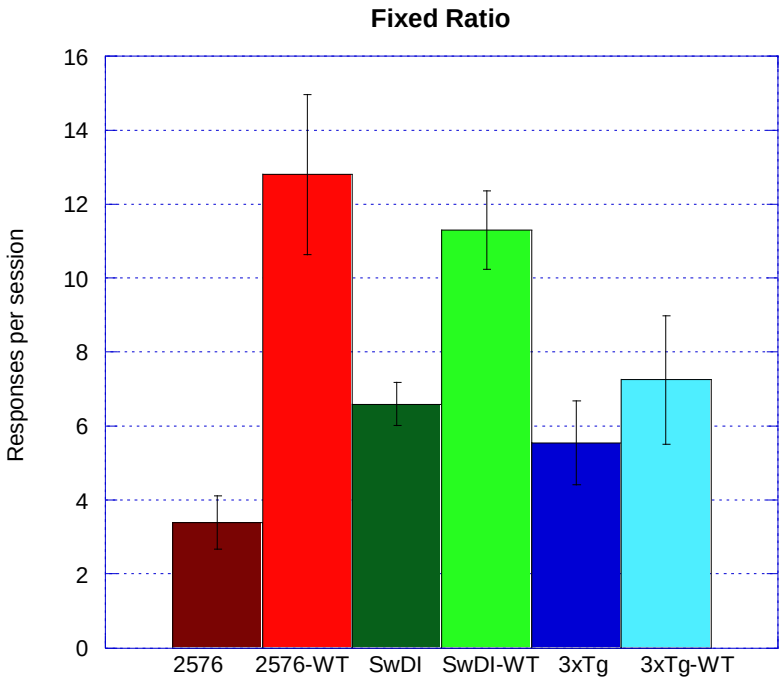


Figure 1: Average number of responses per session for each transgenic mouse, and their corresponding wild type. Tg-2576 and Tg-SwDI mice made significantly fewer responses than their wild types.

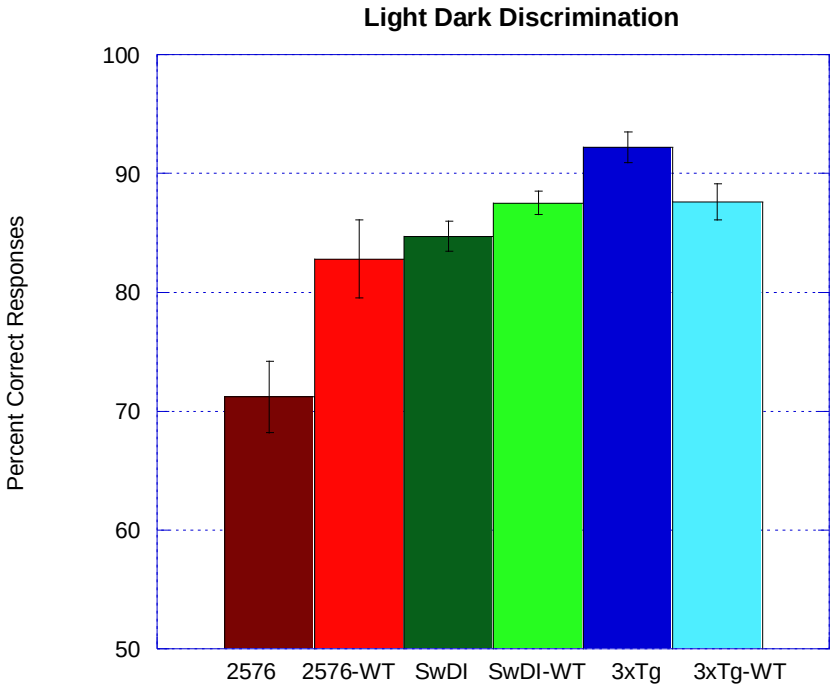


Figure 2: Percentage of correct responses, averaged over all days of Light Dark Discrimination for each transgenic and their wild type. The Tg-2576 mice made significantly fewer responses than their wild types, while 3xTg mice made significantly more responses than theirs.

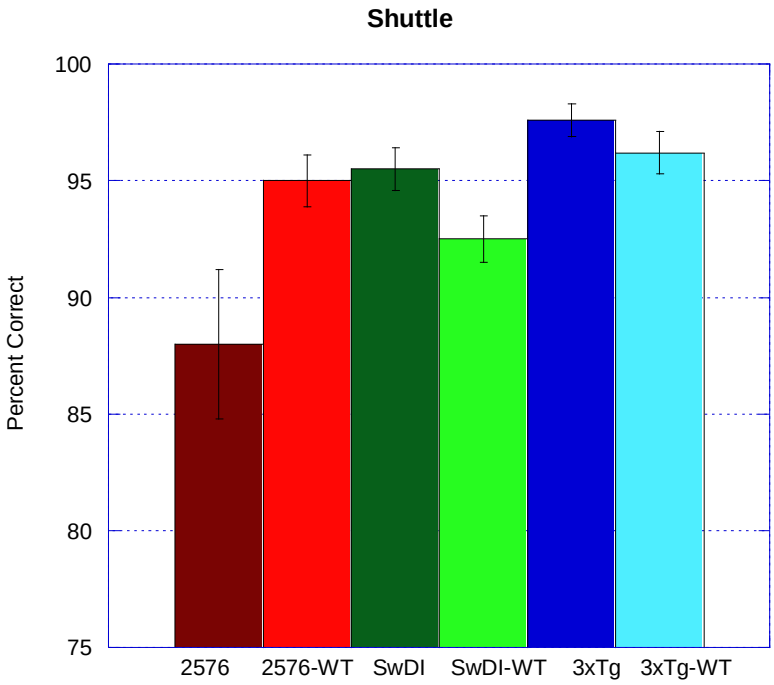


Figure 3: Percentage of correct responses averaged over all 10 days of the Shuttle task. Tg-2576 mice were significantly less accurate than wild types, while Tg-SwDI mice were significantly more accurate.

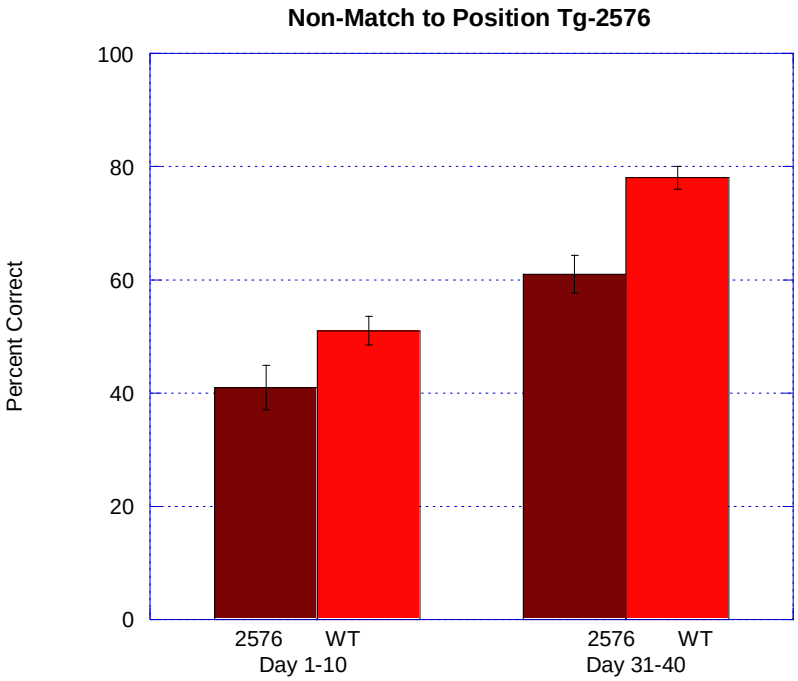


Figure 4: Percentage of correct responses for Tg-2576 on the first 10, and last 10 days of the Non-Match to Position task. The difference is non-significant on days 1-10, and significant on days 31-40.

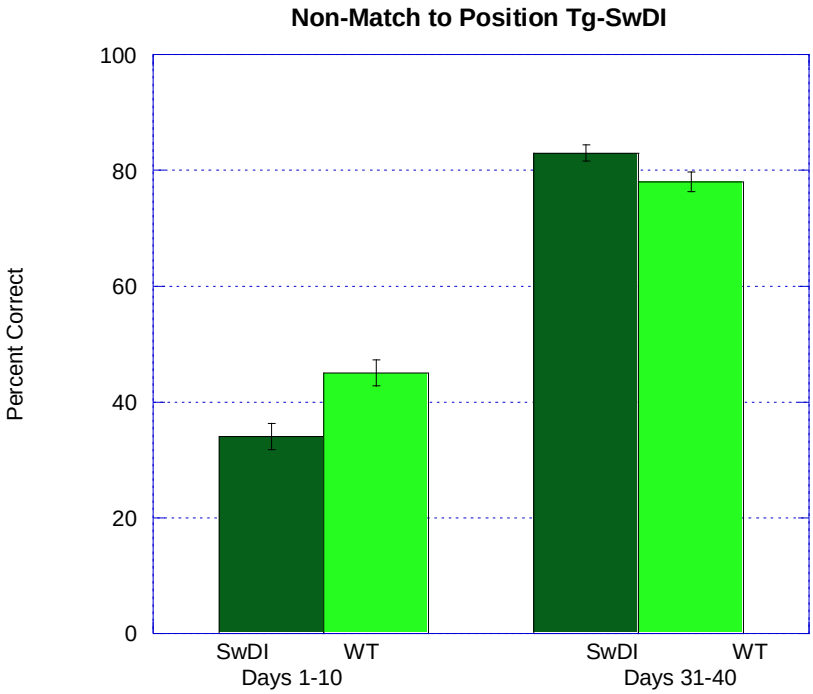


Figure 5: Percentage of correct responses for Tg-SwDI mice on the first 10, and last 10 days of the Non-Match to Position task. For the first 10 days, WT's are significantly better, for the last 10 there's no significant difference.

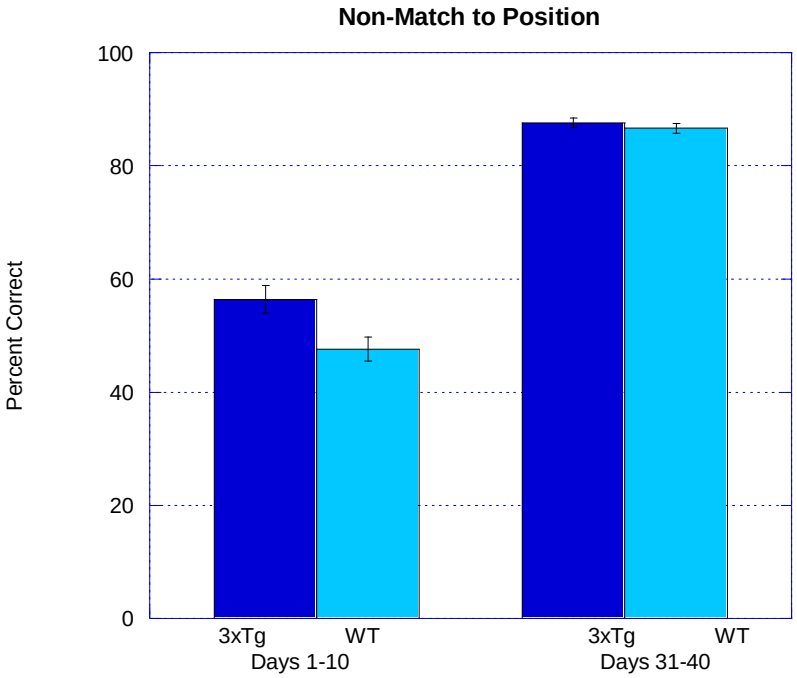


Figure 6: Percentage correct for 3xTg mice and their wild types on the Non-Match to position task on the first 10, and last 10 days. 3xTg mice do significantly better the first 10 days, but not the last 10 days.

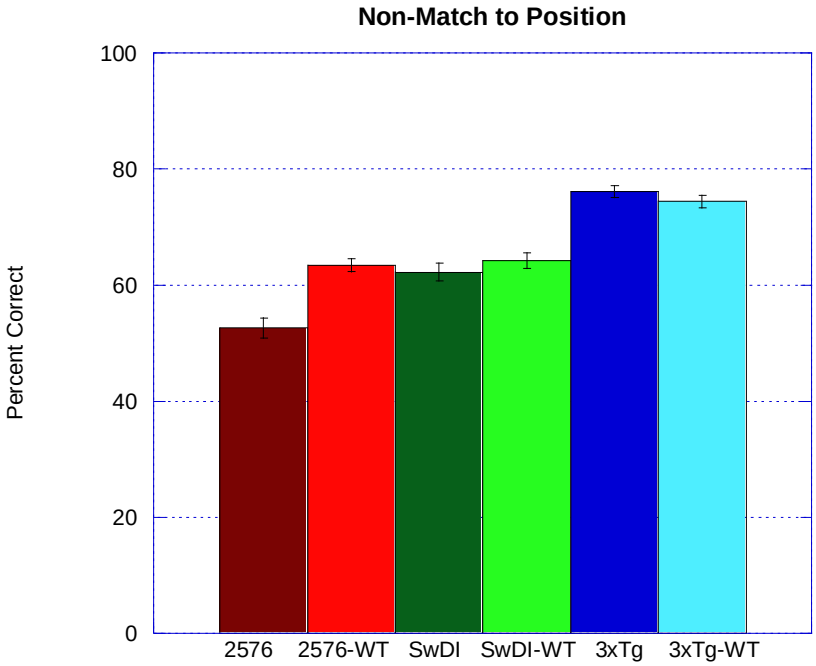


Figure 7: Overall percent correct for each transgenic and their corresponding wild type, averaged over all 40 days of Non-Match to Position. Tg-2576 mice did significantly worse than their wild types.

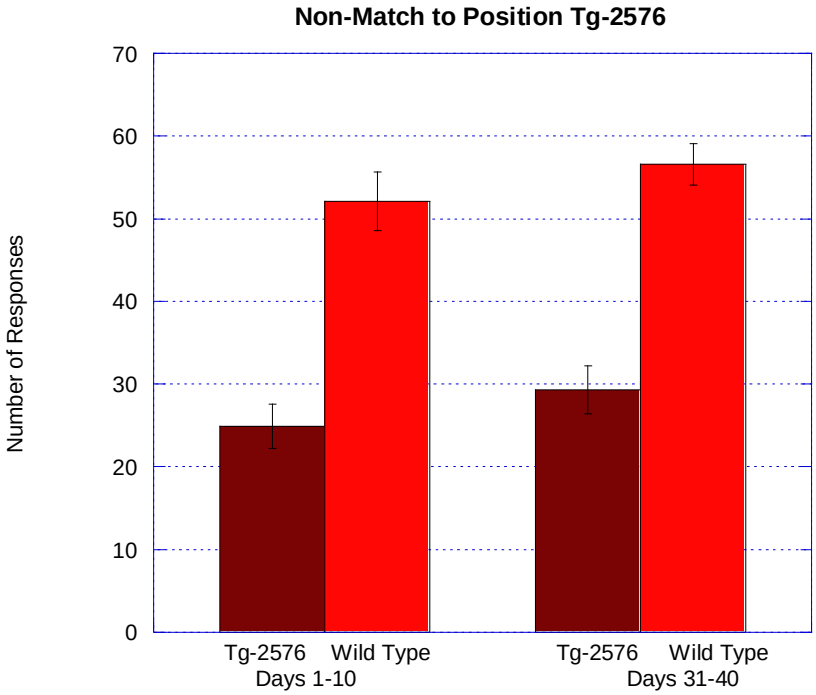


Figure 8: Average number of responses made by Tg-2576 mice in a single session, averaged over the first and last 10 days of NMTP. Wild type animals made significantly more responses than Tg-2576 mice.

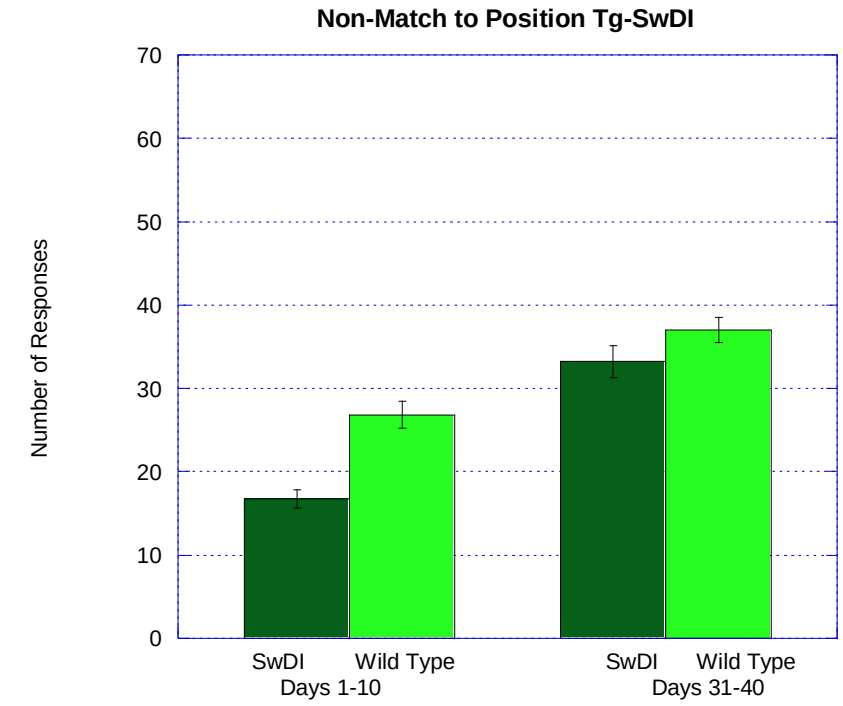


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Figure 9: Average number of responses per session for Tg-SwDI animals and wild types, averaged over the first 10 (significant difference), and last 10 (non-significant) days of Non-Match to Position.

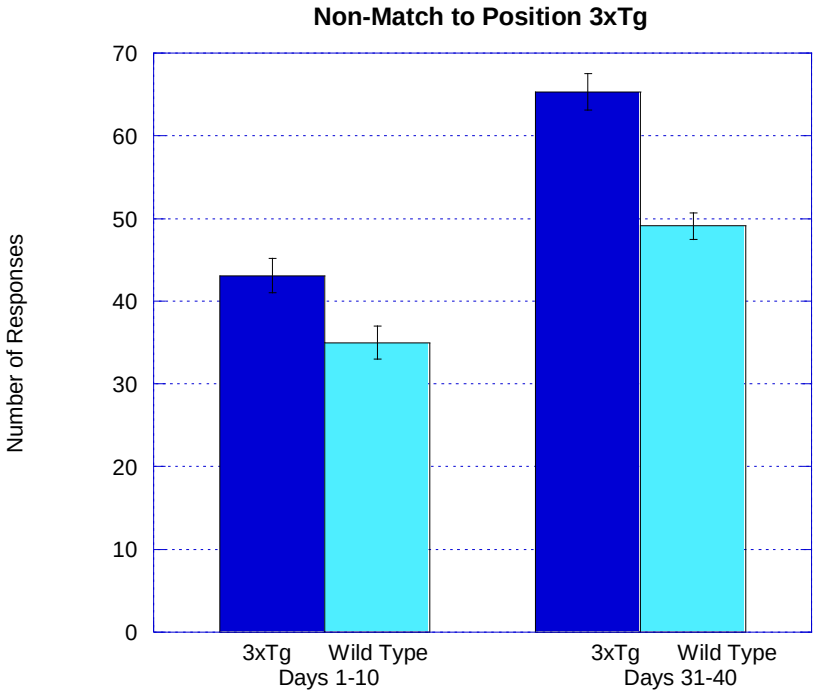



Figure 10: Average number of responses in a single session of NMTP averaged for the first 10 and last 10 days, for the 3xTg animals. Results were significant for the first and last 10 days.

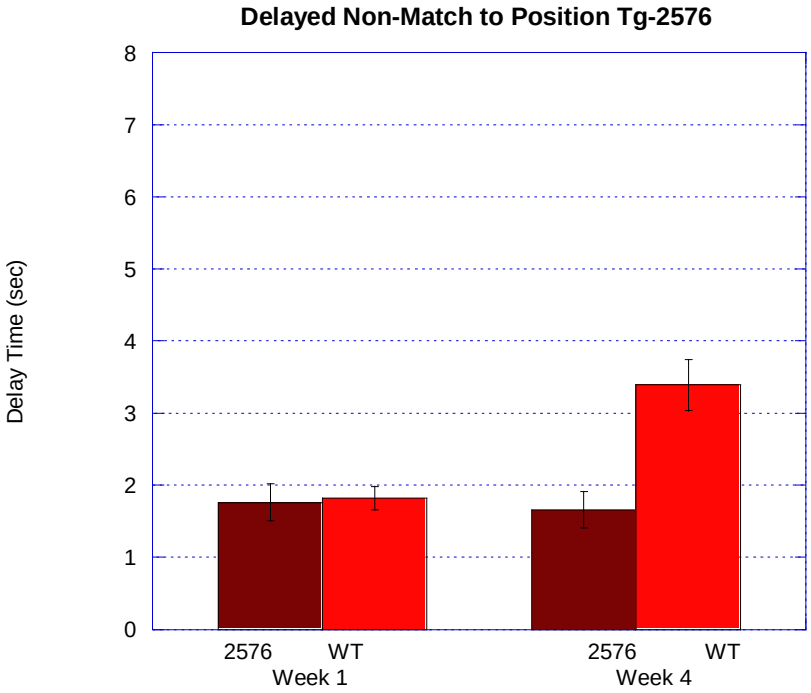



Figure 11: Average delay achieved by Tg-2576 and WT in weeks one and four of Delayed Non-Match to Position. Delay difference was non-significant in week 1, with WT's sustaining a significantly longer delay in week 4.

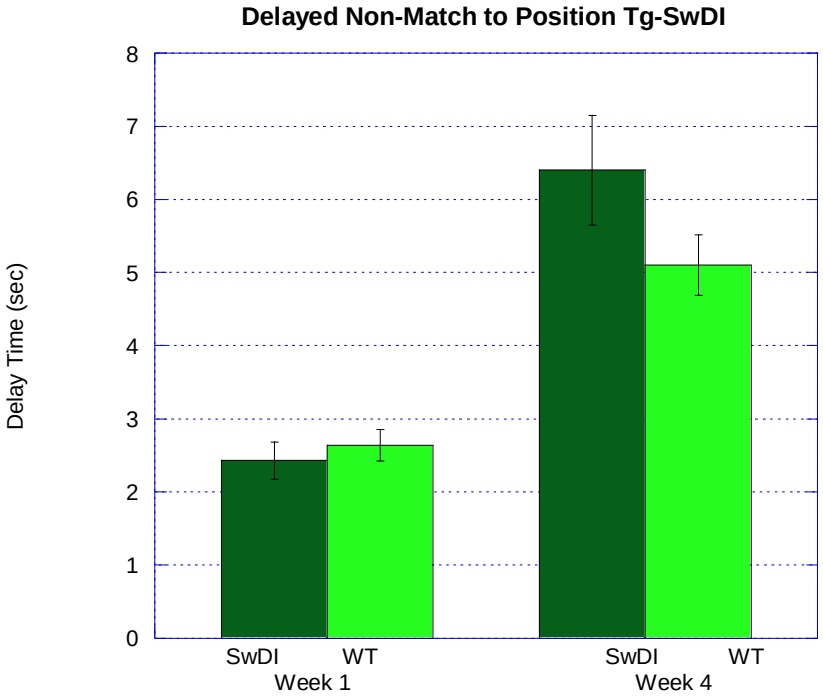



Figure 12: Average Delay achieved by Tg-SwDI mice and their wild types in weeks one and four of Delayed Non-Match to Position. Results are significant week 4, but not week 1.

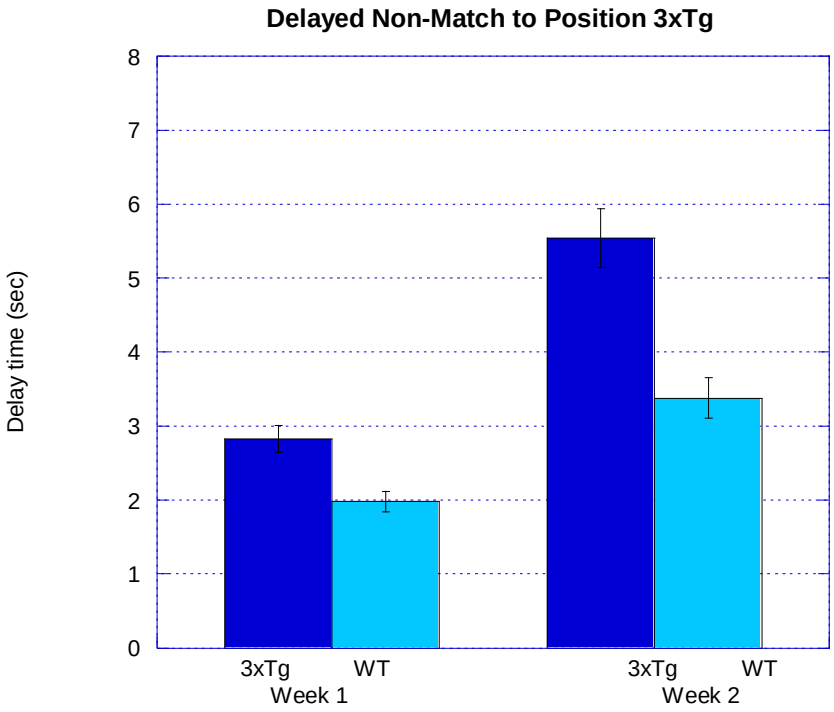



Figure 13: Average delay achieved by 3xTg and wild type mice in weeks one and four of Delayed Non-Match to position. Results are significant at both weeks 1 and 4.

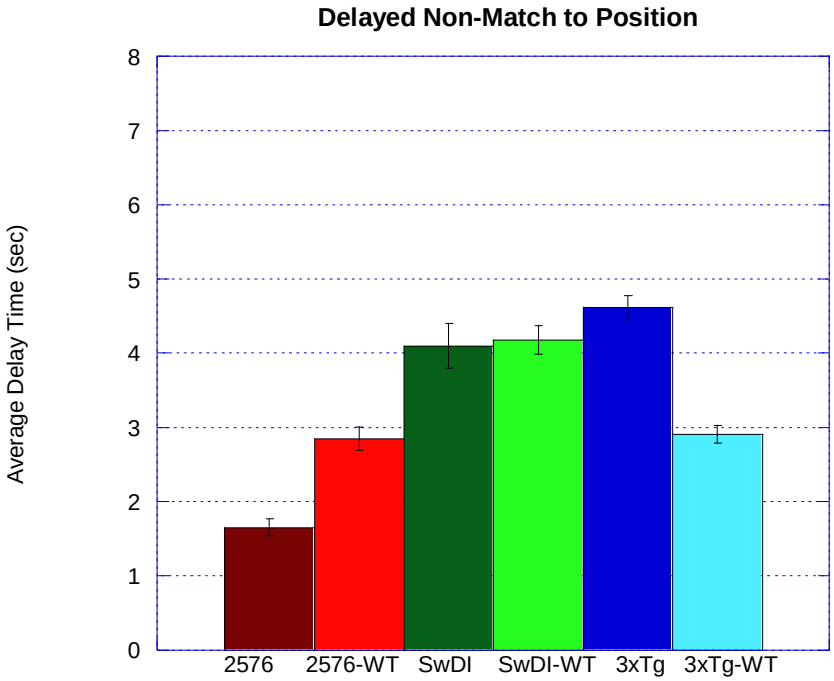



Figure 14: Average Delay time achieved by all six groups, averaged over all 40 days of Delayed Non-Match to Position. Tg-2576 mice did significantly worse than their WT's, and 3xTg's significantly better than their WT's.

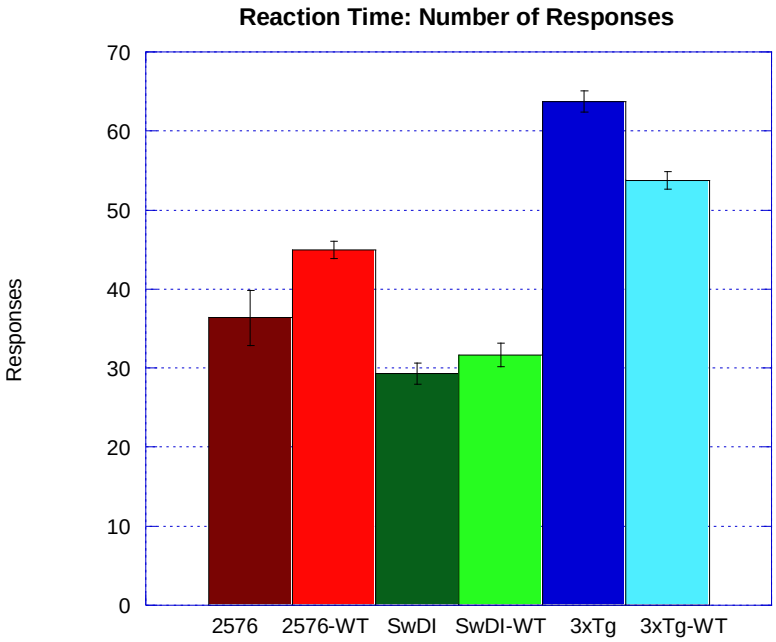



Figure 15: Average number of responses made per session for each of the three transgenics, and WT's. Tg-2576 mice made significantly fewer responses than their WT, and 3xTg's significantly more.

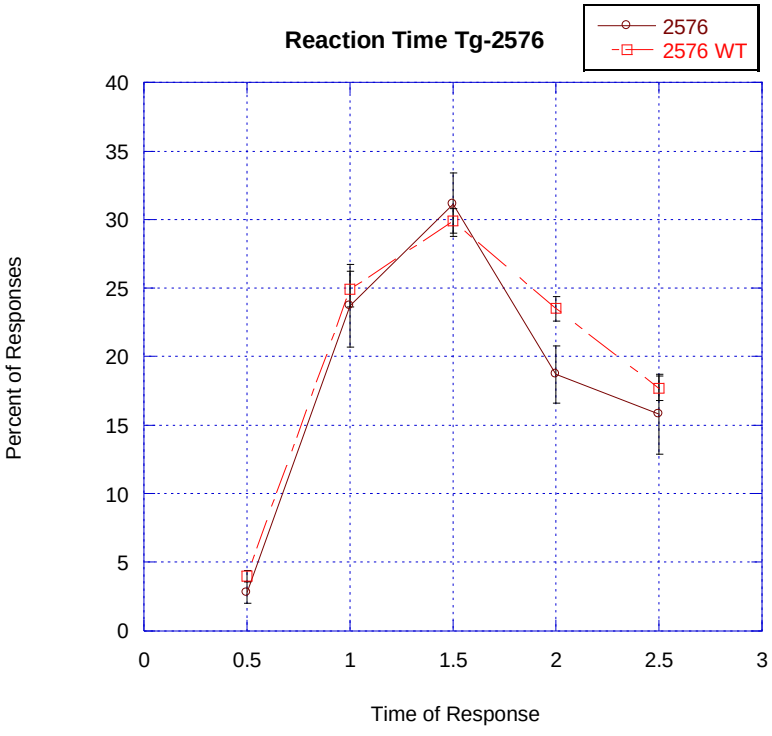



Figure 16: Response times for Tg-2576 and WT are displayed as percentage of responses at each time point. There is a significant difference between groups at 2 seconds with WT's making more responses.

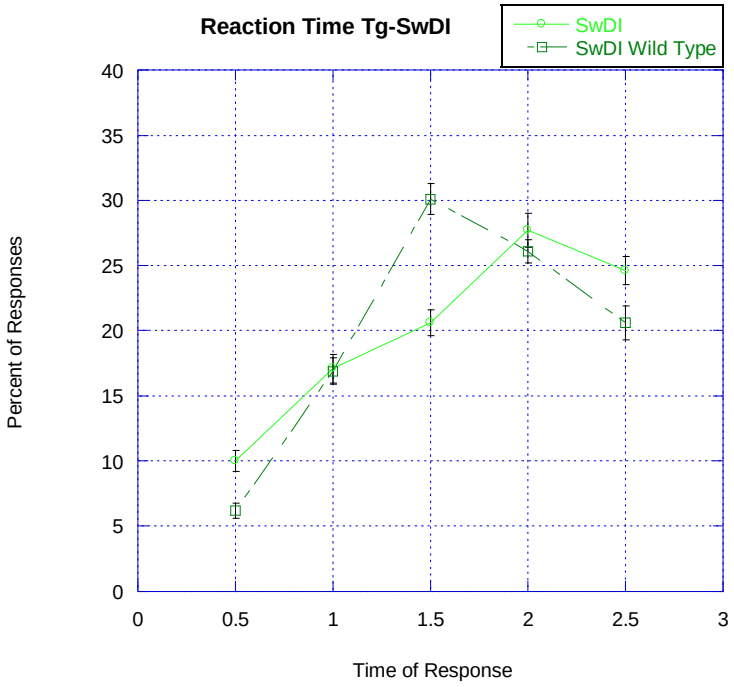



Figure 17: Response times for Tg-2576 and WT; Tg-SwDI peaked slightly later, significant differences at .5, 1.5, and 2.5 seconds.

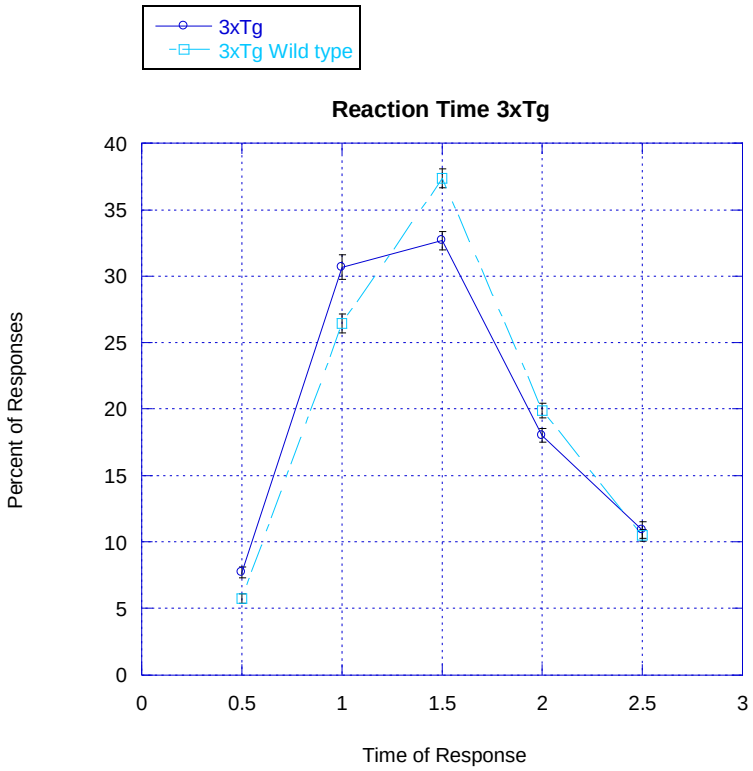



Figure 18: Response times for 3xTg and WT, WT's peaking slightly later. Significant differences at 1.5 and 2 sec. with WT's making for responses.

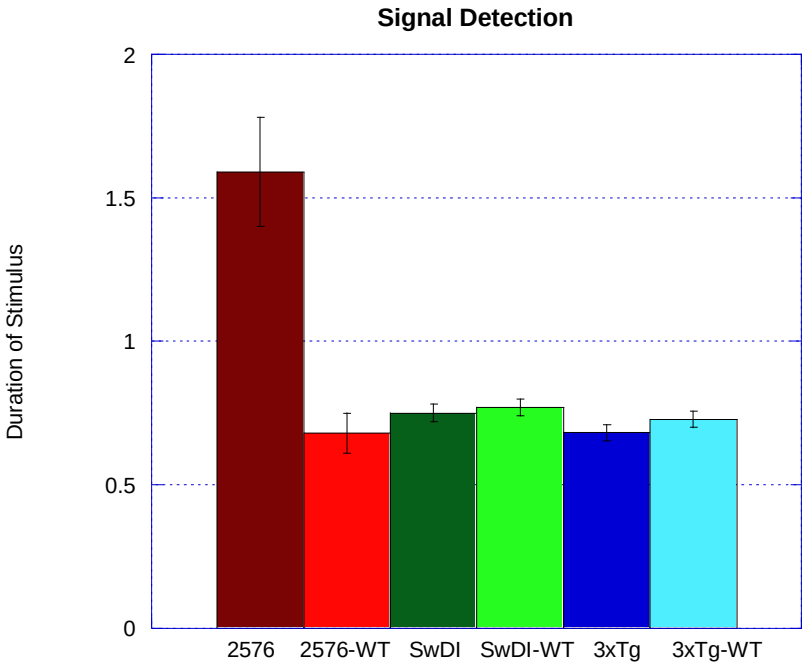



Figure 19: Average stimulus needed for a response, averaged over 10 days. Tg-2576 mice needed a significantly longer stimulus.

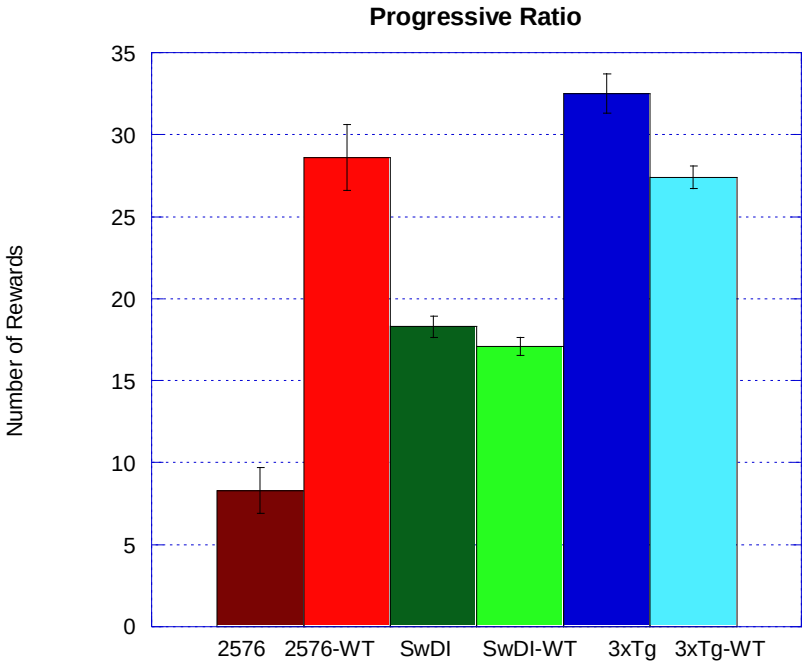



Figure 20: Average number of rewards received for all groups over 10 days of Progressive Ratio. Tg-2576 mice received significantly fewer rewards than WT, 3xTg significantly more.

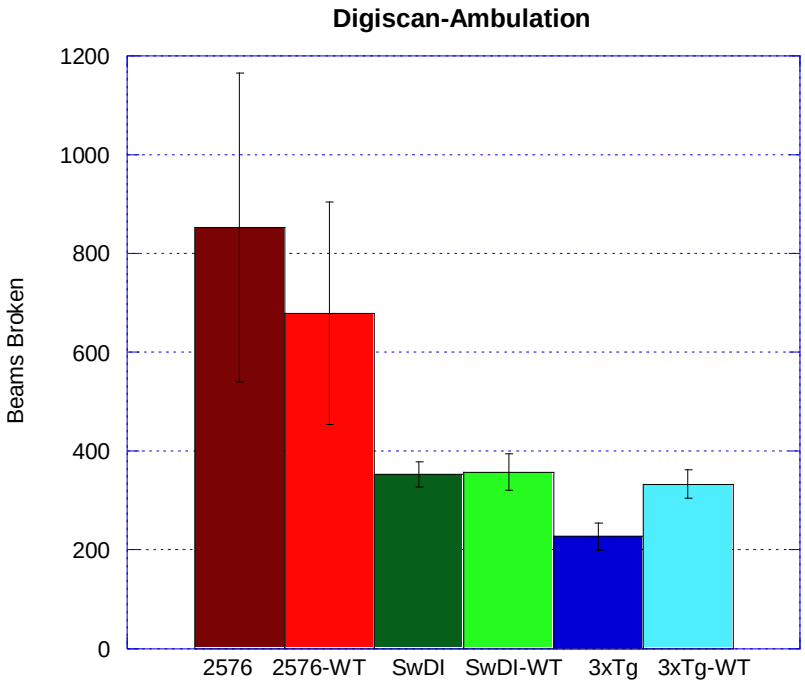



Figure 21: Average number of photo beams broken during ambulation, 3xTg animals broke significantly fewer photo beams than their wild types.

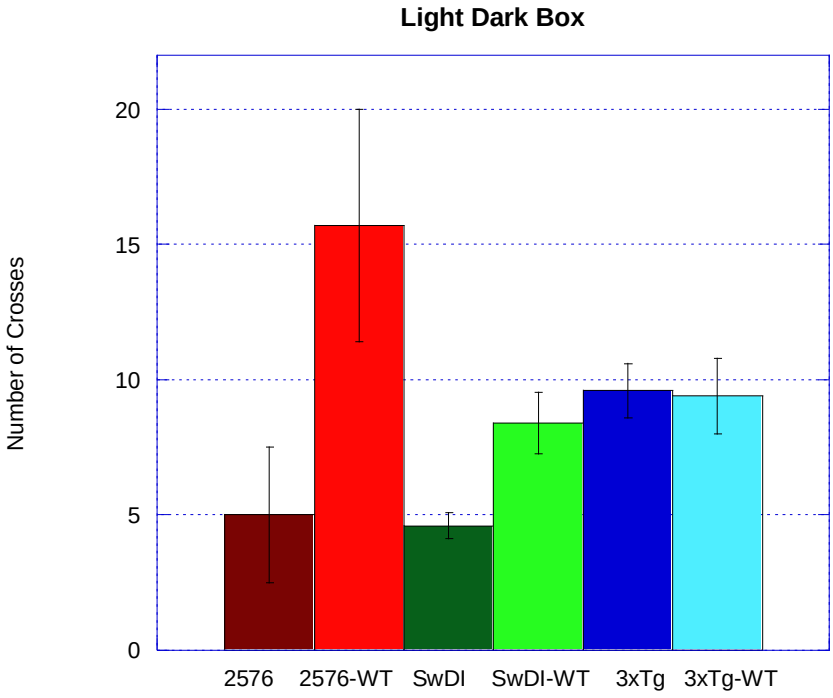



Figure 22: Average number of crosses into the light side of the box during light/dark box per group. Both Tg-2576 and Tg-SwDI mice crossed into light fewer times than their wild types.

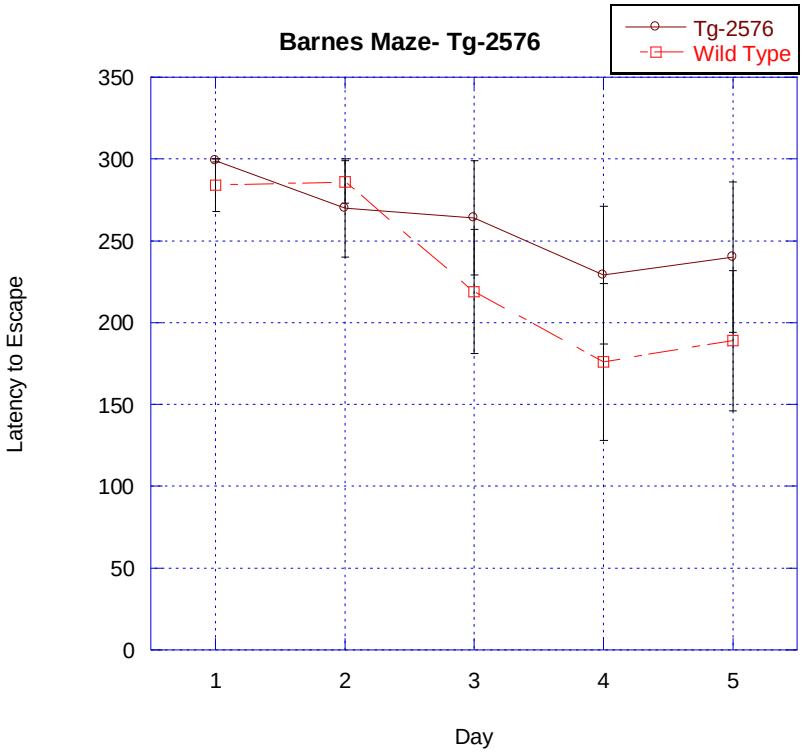



Figure 23: Daily average escape latency for Tg-2576 mice. No significant differences.

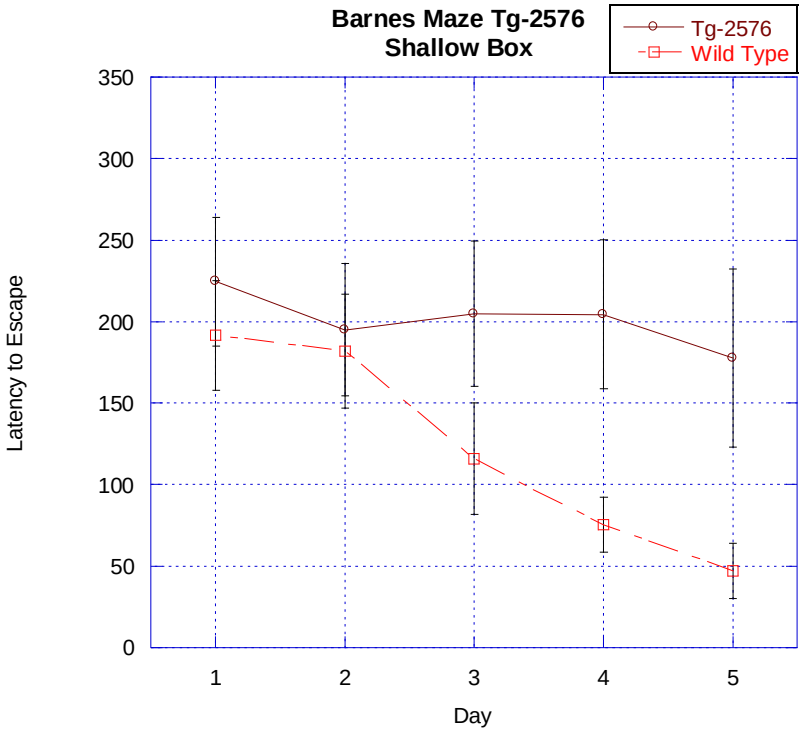



Figure 24: Daily average escape latency for Tg-2576 mice in the shallow box. Note the growing significant difference between groups, with wild types performing better.

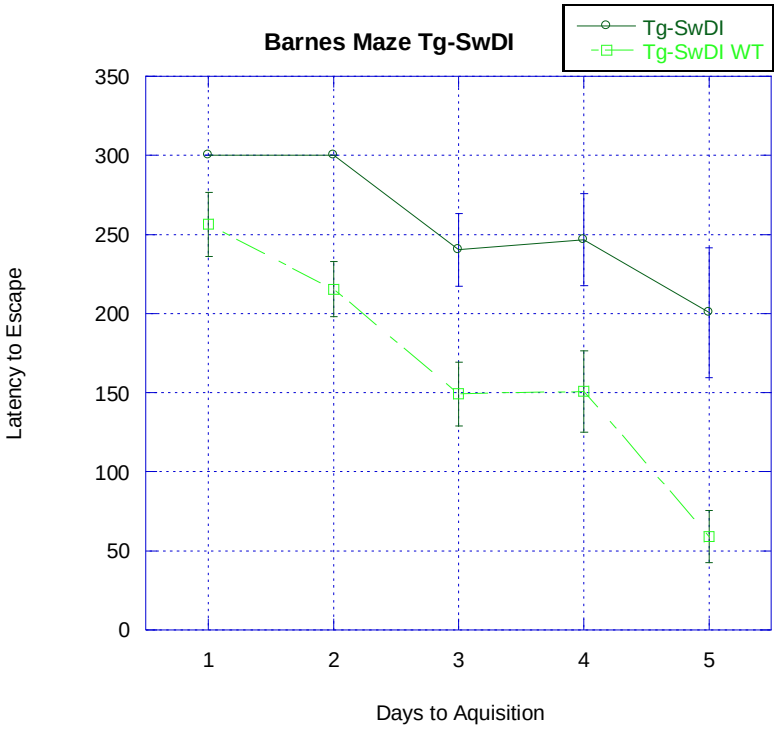



Figure 25: Daily averages for latency to escape for Tg-SwDI mice and wild-types, WT's escaped significantly more quickly.

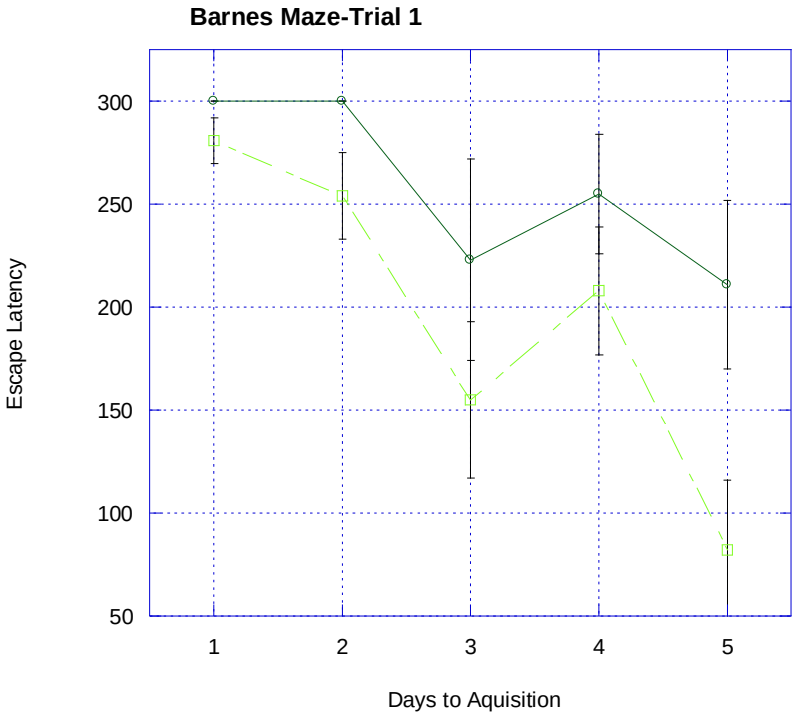



Figure 26: This is the average latency for trial one for Tg-SwDI's, mice are recalling the location of the escape box from the previous day. There were significant differences on days 1, 2, and 5.

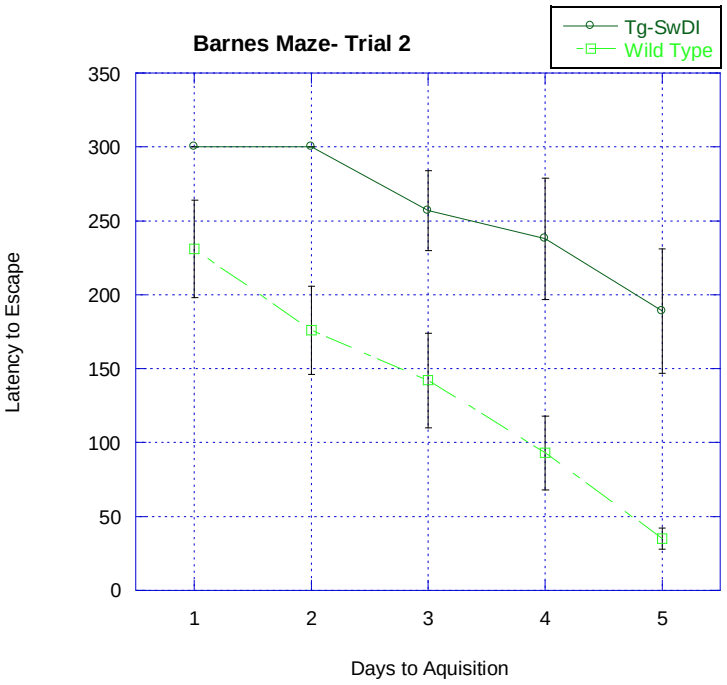



Figure 27: This is the average latency for trial 2, where Tg-SwDI's are recalling the location of the platform from 15 minutes prior. There were significant differences on all days.

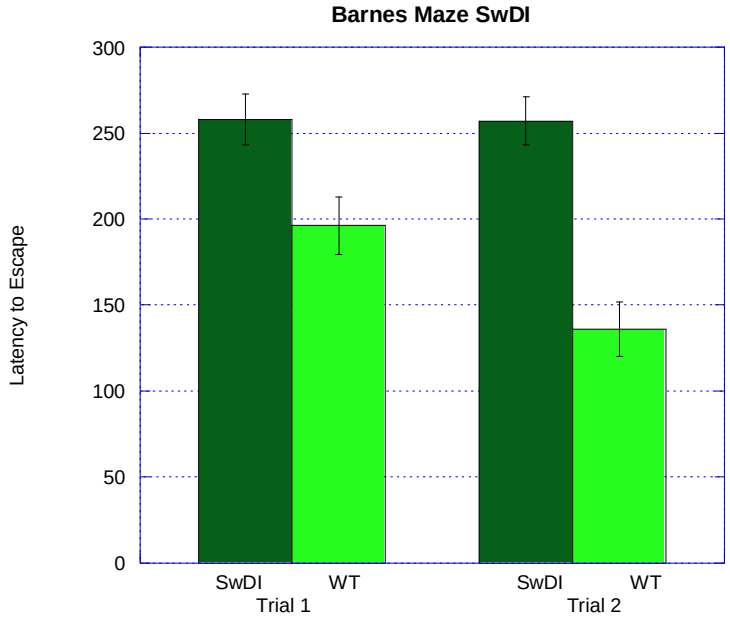



Figure 28: Average Escape latencies for the second and third trials per day. There is a significant difference between Tg-SwDI and WT's on each trial, as well as a significant difference between WT on trial one and two, but not between Tg-SwDI on trial one and trial two.

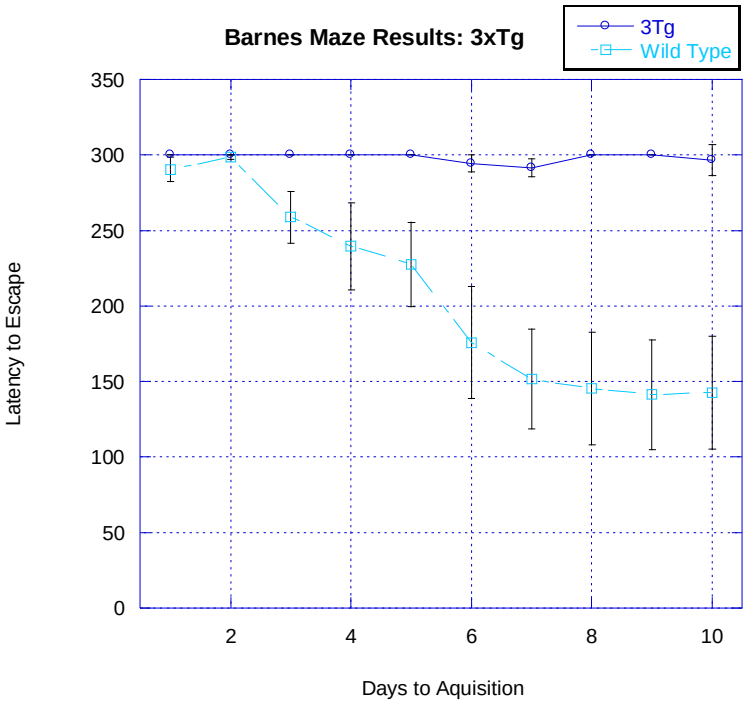



Figure 29: The average latency to escape per day for 3xTg mice and their wild types over 10 days. There were significant differences from day 3 to day 10.

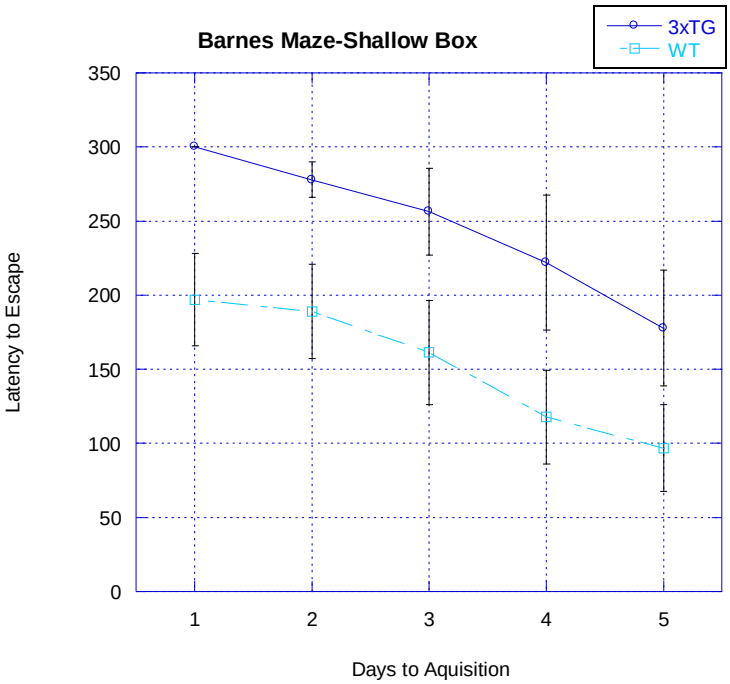



Figure 30: Average daily shallow box acquisition for 3xTg and wild types. Note that curves grow closer. Differences are significant days 1-3, but not days 4 and 5.

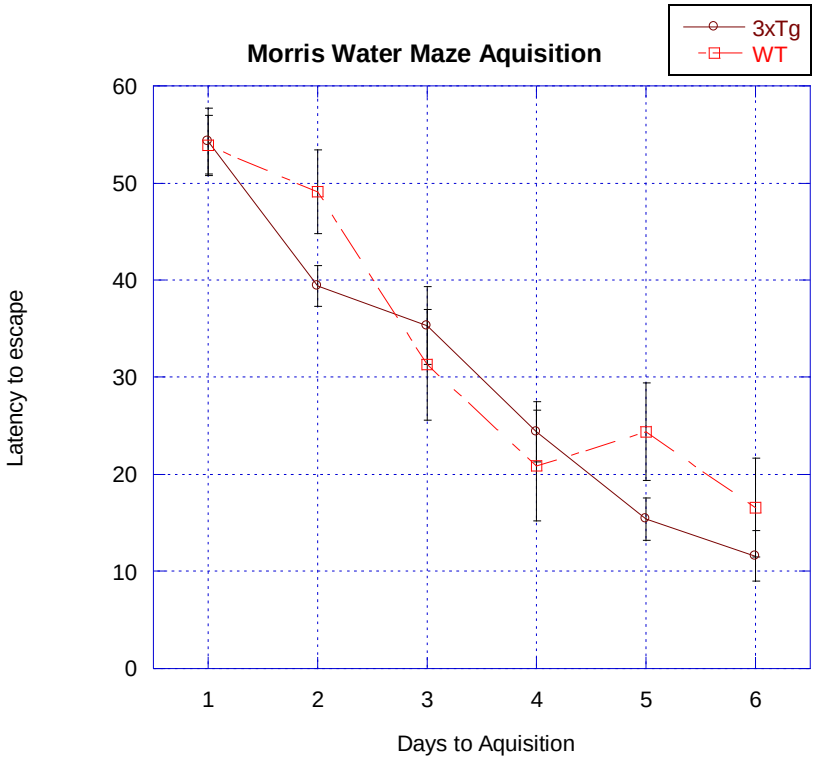



Figure 31: Average daily time to escape water maze per group. Results are all non-significant.

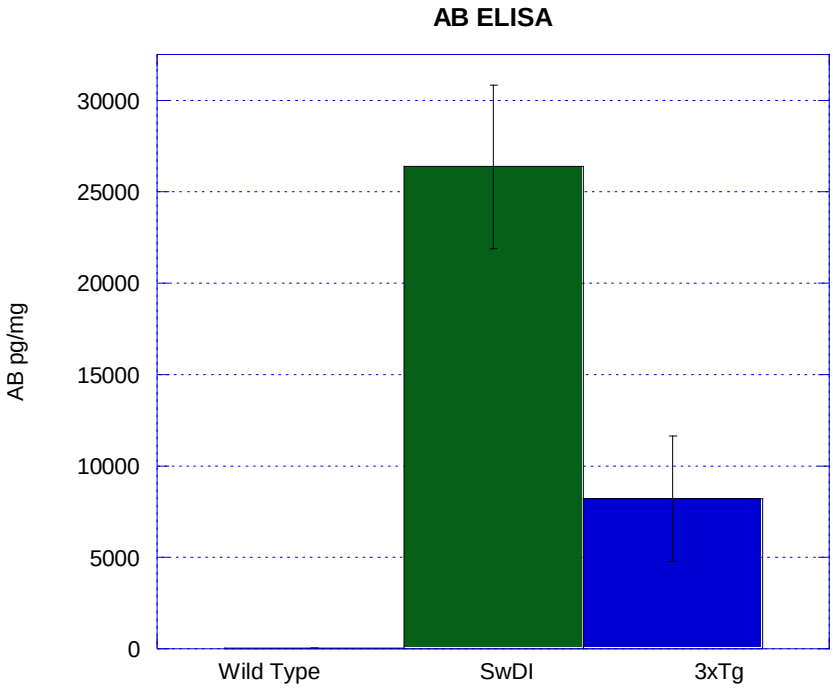


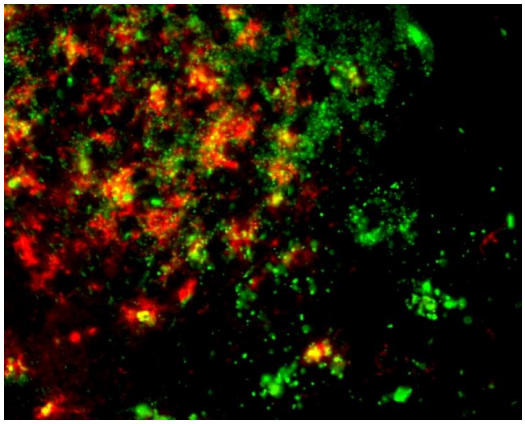

Figure 32: Average amount of total Aβ in a Wild type (close to 0) SwDI, and 3xTg animal. There are significant differences between all groups.

Picture 1 shows the front panel of the operant chamber. To the right and left are the 2 nose-poke ports, and in the center is the reward well.

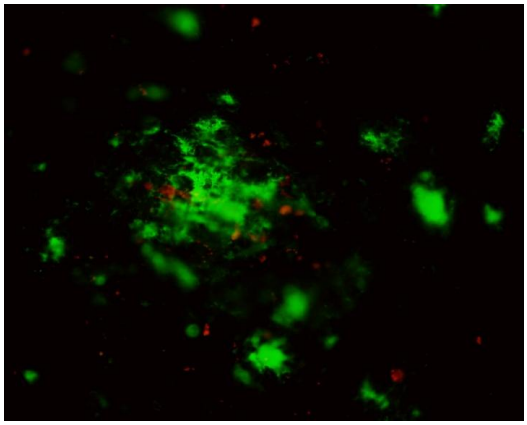
Picture 2 shows the light dark box. During testing, the dark side of the box is covered by a lid.

Picture 3 shows the Barnes Maze. During testing, there is an escape box under one of the eight holes.

Picture 4 shows the Morris Water Maze, the submerged platform is not visible in this view.

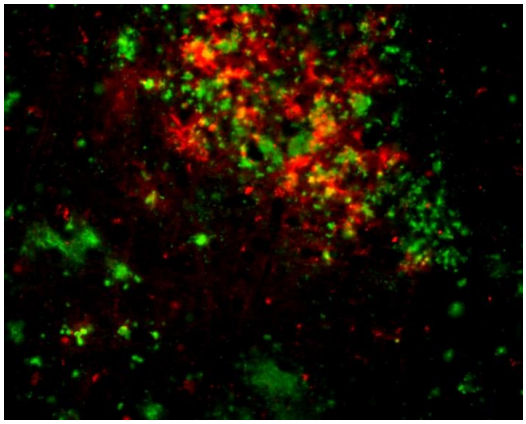


5

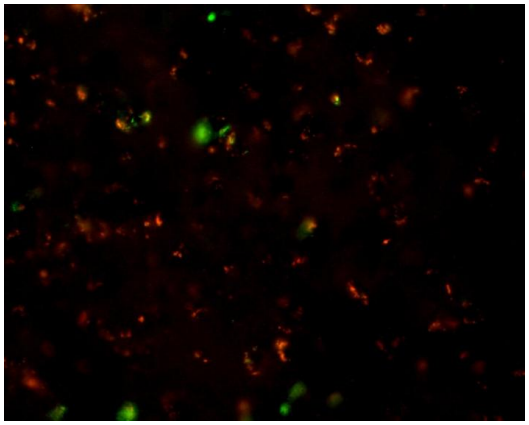


6

Pictures 5 and 6 show subicular Aβ and Microglia at 40x magnification in Tg-SwDI and 3xTG animals, respectively. Note the greater Aβ and activated microglia in the Tg-SwDI section, and more intracellular Aβ in the 3xTg section.

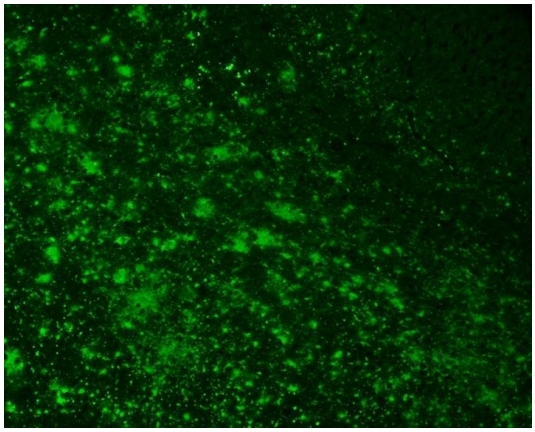


7

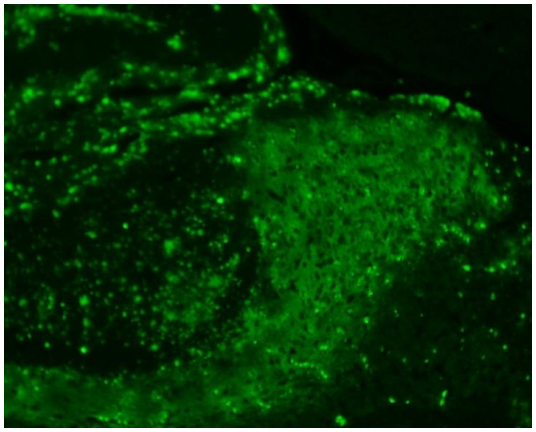


8

Pictures 7 and 8 show 40x magnification of cortical Aβand Microglia in Tg-SwDI and 3xTg animals, respectively. Note the greater Aβ in the Tg-SwDI animals, as well as the extensive activated microglia in the Tg-SwDI, which is completely missing in the 3xTg animal.

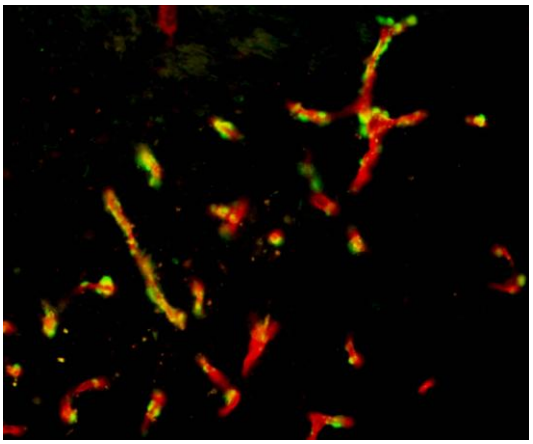


9

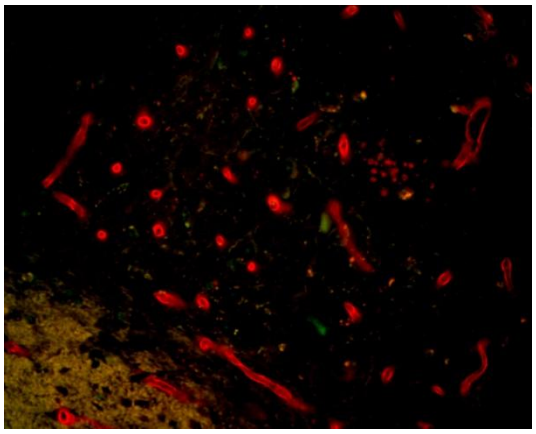


10

Pictures 9 and 10 show overviews of cortex and subiculum, respectively, in a Tg-SwDI animal. Note the extensive intracellular and extracellular staining of Aβ in these animals. These views could not be taken of 3xTg animals, as there was not enough staining to be visible at this magnification.

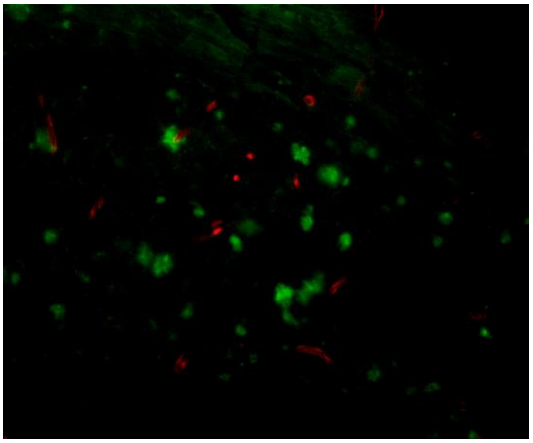


11

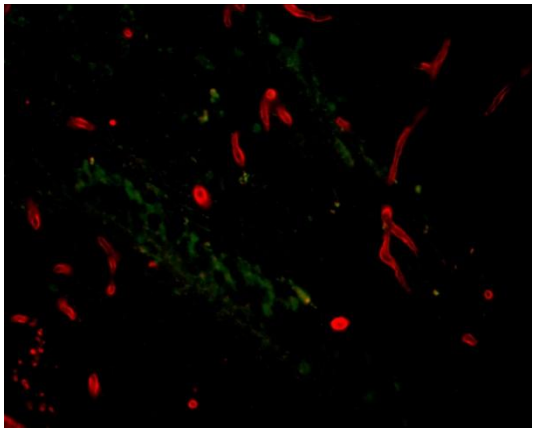


12

Pictures 11 and 12 show subiculum at 40x magnification in Tg-SwDI and 3xTG animals, respectively. These sections were stained for collagen (vessels, in red) and fibrillar amyloid. Note the extensive vessel associated amyloid in the Tg-SwDI animals, and lack of vessel associated amyloid in the 3xTg animals.

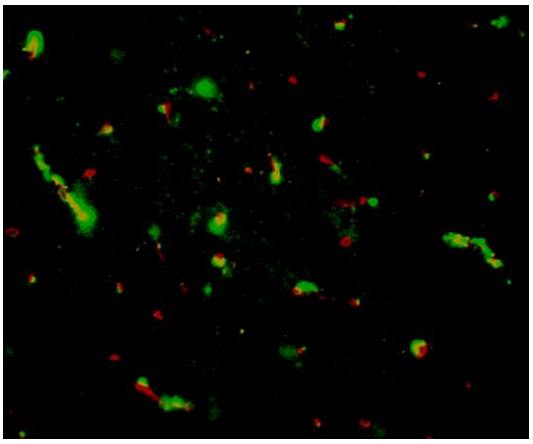


13

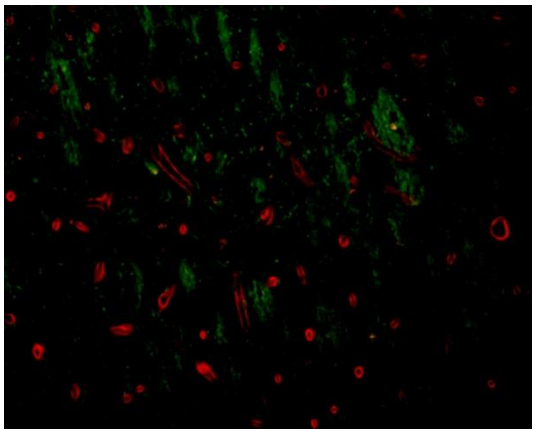


14

Pictures 13 and 14 are of hippocampus in Tg-SwDI and 3xTg tissue, respectively, at 40x magnification stained for collagen and fibrillar amyloid. Note the amyloid in Tg-SwDI animal, though non-vessel associated, and the lack of amyloid in the 3xTg animal.

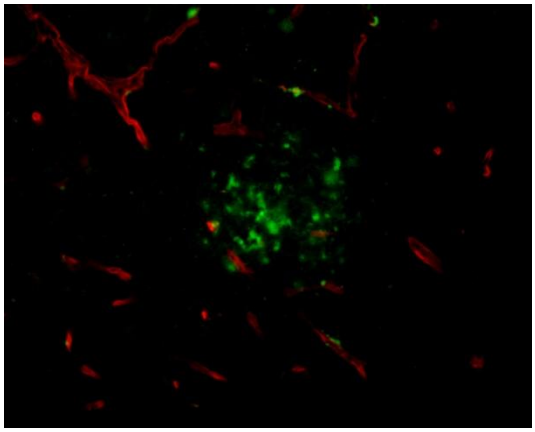


15

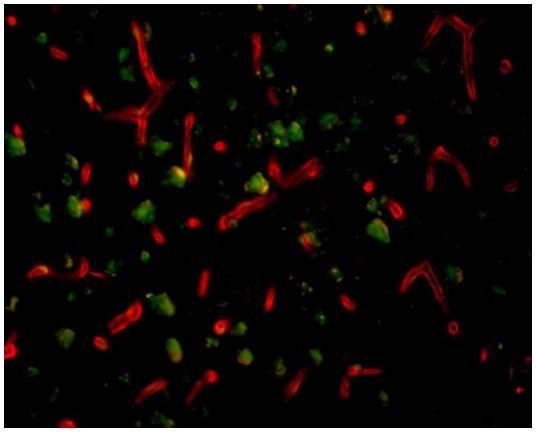


16

Pictures 15 and 16 show Thalamus in Tg-SwDI and 3xTg animals, respectively, at 40x magnification. Note the vessel-associated fibrillar amyloid in the Tg-SwDI animal, and lack of fibrillar amyloid in the 3xTg animal.

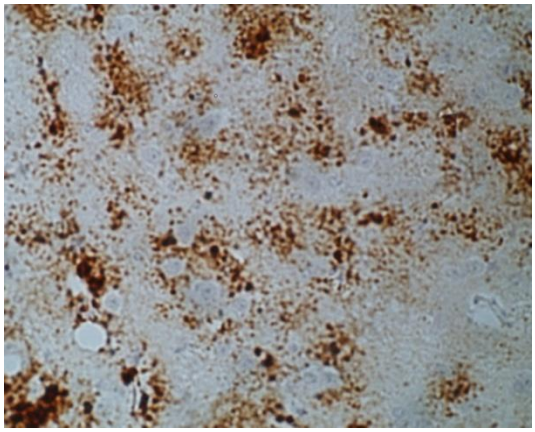


17

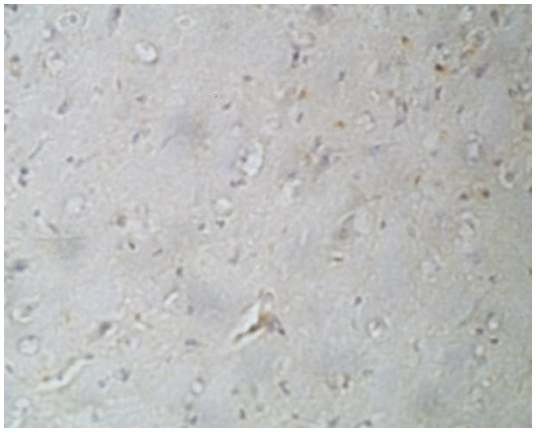


18

Pictures 17 and 18 are 40x magnifications of the cortex of Tg-SwDI and 3xTg animals, respectively. Note that there is fibrillar amyloid in both animals, but it is not vessel associated in either.

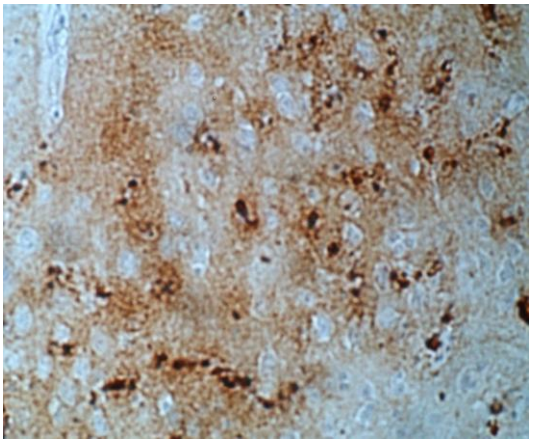


19

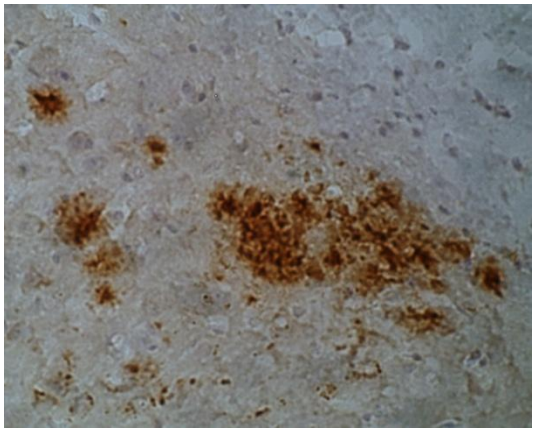


20

Pictures 19 and 20 show the average amount of Aβ alone, in the thalamus of Tg-SwDI and 3xTg animals, respectively, at 40x magnification.

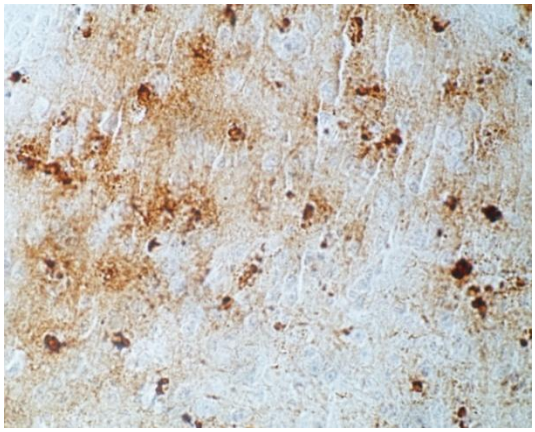


21

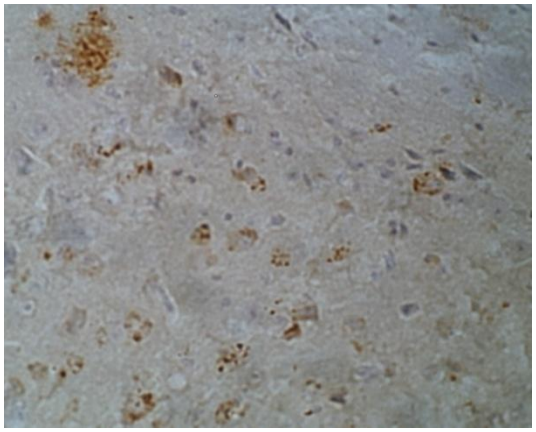


22

Pictures 21 and 22 show subiculum at 40x magnification in Tg-SwDI and 3xTg animals, respectively. These figures represent tissue from each group that had a higher level of staining than most. Note the diffuse Aβ in the Tg-SwDI animal, and the small deposit of dense amyloid in the 3xTg animal.

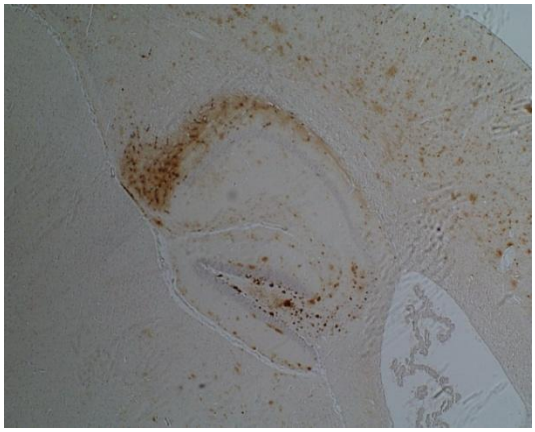


23

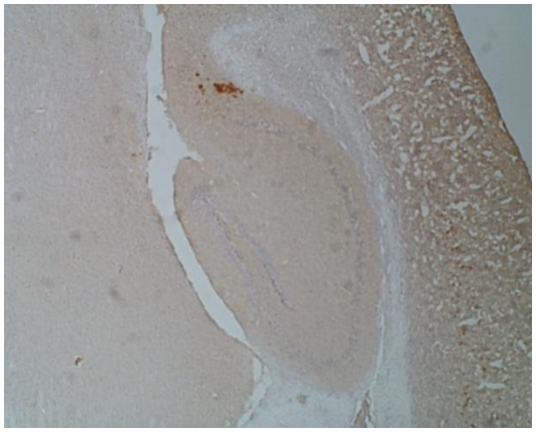


24

Pictures 23 and 24 represent the average accumulation of Aβ in the subiculum of Tg-SwDI and 3xTg animals, respectively, at 40x magnification.

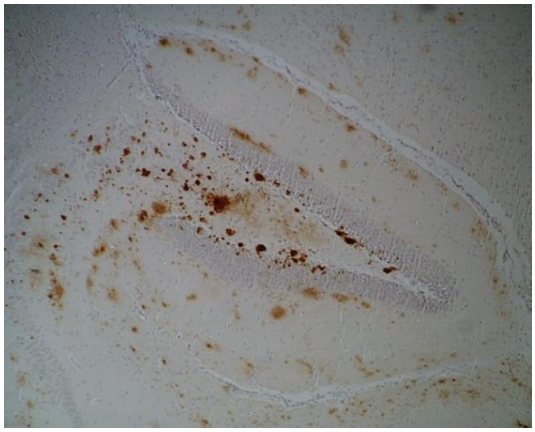


25

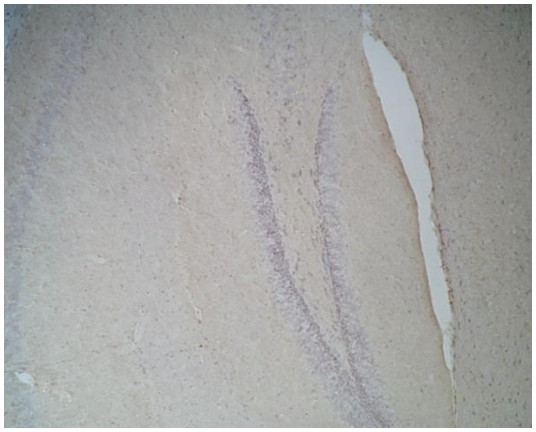


26

Pictures 25 and 26 show the hippocampus of a Tg-SwDI and 3xTg animal, respectively, at 4x magnification. Note the dense Aβ staining throughout the hippocampus, and subiculum of the Tg-SwDI animal, and lack of staining except for a deposit in the subiculum of the 3xTg animal.

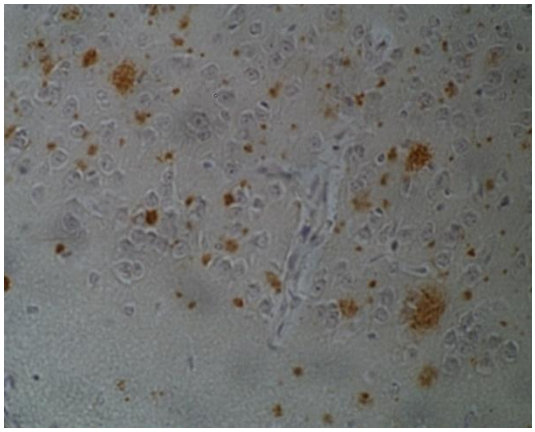


27

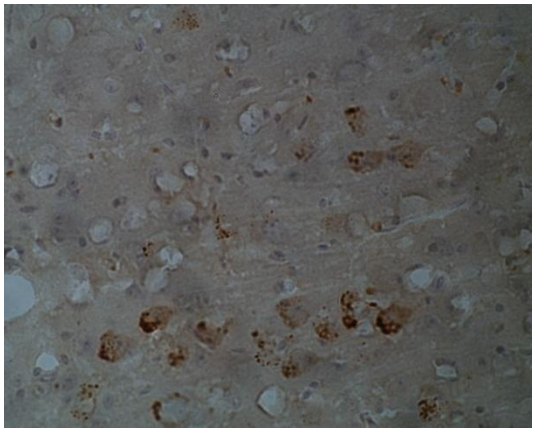


28

Pictures 27 and 28 show the hippocampus of a Tg-SwDI and 3xTg animal, respectively, at 10x magnification. Note the Aβ in the Tg-SwDI animal, and lack thereof in the 3xTg animal.

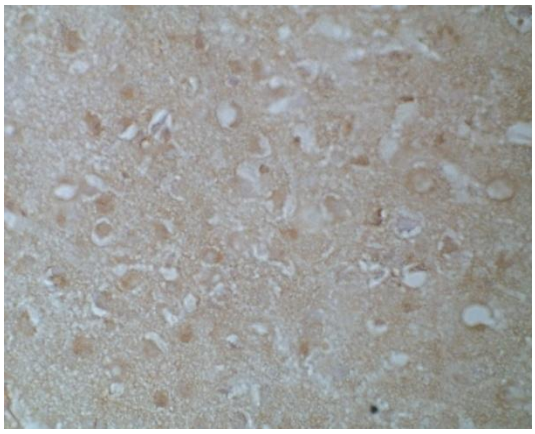


29

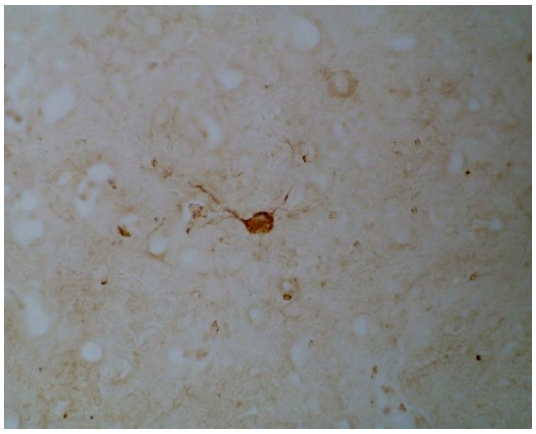


30

Pictures 29 and 30 show the cortex of Tg-SwDI and 3xTg animals, respectively, at 40x magnification, stained for Aβ. Note the difference in Aβ staining.



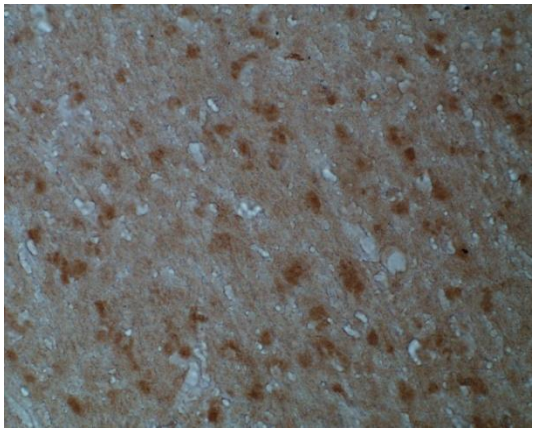
31



32

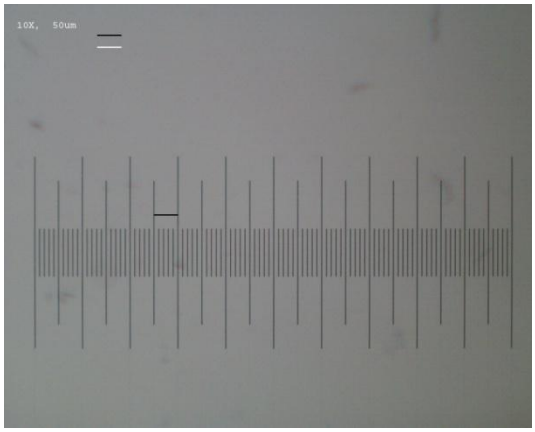
Pictures 31 and 32 show Tau cell body staining at 40x magnification in Tg-SwDI and 3xTg animals, respectively. There are no tau cell bodies in Tg-SwDI animals, while there are few in 3xTg animals, and only in some animals.

33

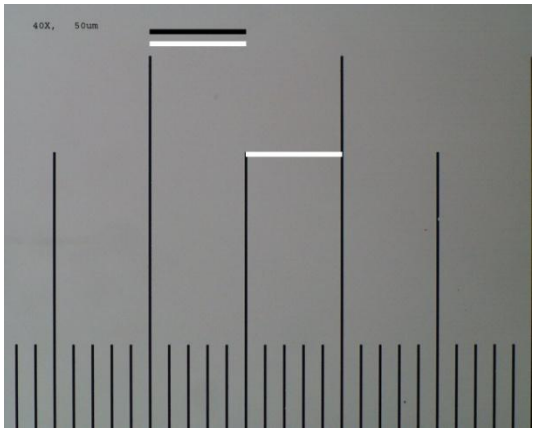


34

Picture 33 Shows tau fibrils at 40x magnification in a 3xTg animal. Fibrils were only found in 3 3xTg animals.
Picture 34 shows Tau cell bodies in a NOS3 KO mouse at 40x magnification. This animal was used as positive control to show tau staining. This animal did not show tau fibrils.




35




36

Pictures 35 and 36 show scale Bars for 10x (left) and 40x (right) magnifications. Black and white bars indicate 50 µm.