Beneficial Effects of Quetiapine in the APP/PS1 Transgenic Mice: Implications for Early Intervention for Alzheimer's Disease

by

Shenghua Zhu

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Human Anatomy & Cell Science

University of Manitoba

Winnipeg

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ABSTRACT

Background: Alzheimer's disease (AD) is the leading cause of dementia. Large numbers of amyloid plaques in the brain remain a pathological hallmark of AD. These plaques are primarily composed of amyloid β-protein (Aβ) and are the most important therapeutic target for treatment. It has been postulated that glycogen synthase kinase-3β (GSK3β) activity might exert a central role in the development of AD. GSK3β activity has been implicated in tau phosphorylation, APP processing, Aβ production and neurodegeneration. Quetiapine is frequently used to treat psychoses in AD patients, especially in severe cases of late stage AD and has inhibitory effects on GSK3β activity in mouse brains after acute/subchronic treatment. Therefore, the proposed hypothesis is that chronic quetiapine administration after amyloid plaque onset reduces AD like pathology and alleviates AD like behaviours in APP/PS1 transgenic mice by inhibiting GSK3β activity.

Objective: The purpose of the current study was to determine if quetiapine effectively attenuates the neuropathology and cognitive deficits in an APP/presenilin 1 (PS1) double transgenic mouse model through regulating phospho-Ser^9-GSK3β and inhibiting its activity.

Methods: APP/PS1 transgenic mice were treated with quetiapine (2.5, 5 mg/kg/day) in drinking water starting from 3.5 months of age, for a period of 8 months. One week after behaviour testing, mice were sacrificed at 12 months of age. Half of the hemispheres were rapidly frozen for immunoblot and ELISA analyses and the other half were fixed with 4% paraformaldehyde for histological analyses.

Results: Quetiapine treatment reduced amyloid plaques formation in the cortex and hippocampus of AD mice. It also improved the behavioural deficits in these mice, including attenuating impaired memory and anxiety-like phenotypes. In addition, chronic quetiapine

administration inhibited GSK3β, which resulted in reduced production of Aβ in cortices and hippocampi of transgenic mice. Quetiapine treatment also significantly decreased the activation of astrocytes and attenuated synapse integrity impairment in transgenic mice.

Conclusion: These findings suggest that early application of quetiapine can alleviate memory deficits and pathological changes in the APP/PS1 transgenic mouse model of AD, and further support that modulation of GSK3β activity by quetiapine may be a therapeutic option for AD.

ACKNOWLEDGEMENTS

I would like to take this opportunity to extend my gratitude and appreciation to Dr. Xin-Min Li for his supervision, advice, and guidance. Dr. Li provided me with continuous encouragement and unwavering support.

I gratefully acknowledge Dr. Jiming Kong for his advice and supervision. Dr. Kong's open-door policy, patience, expertise in neuroscience and certainly his keen ear to my ideas were inspiring.

Much gratitude is also due to the other members of my Advisory Committee. I would like to specially thank Dr. Jue He for his exceptional mentorship. Dr. He's expertise on behaviour testing and experimental design was essential for the success of this thesis. I also thank Dr. Maria Vrontakis for her knowledge and critical feedback of this study.

Throughout my training as a Master's student, I have been most fortunate to work with colleagues who not only embraced my ideas and helped with my advancement in neurobiological research in psychiatry, but also shared a memorable friendship. I would like to send special thanks to Dr. Yanbo Zhang. His thoughts were essential to improving the quality of my thesis; and he offered me critical feedback whenever needed.

I thank all of the students and post-doctoral fellows at the Drs. Li and Kong's lab. Dr. Junhui Wang provided me with thought-provoking discussions and invaluable input into the development of my thesis. I thank Dr. Ruiguo Zhang for supporting my endeavours and for his wonderful friendship.

I would like to offer a thank-you to Lynda Kong for her advice and assistance for editing my thesis.

I would also like to thank all staff members in the Central Animal Care Services (CACS) at the University of Manitoba, as well as all research animals of my project for their contribution.

I would lastly like to thank my parents, grandparents and my wife. Their unconditional love and understanding keeps me strong even when I face challenges.

This work was supported by the Canadian Institutes of Health Research (CIHR), the Manitoba Health Research Council (MHRC), and Health Sciences Centre Foundation (HSCF).

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LIST OF ABBREVIATIONS

5-HT	Serotonin
Aβ	Beta Amyloid
AICD	APP Intracellular Domain
AD	Alzheimer's Disease
Akt	Protein Kinase B
APD	Antipsychotic Drugs
APP	Amyloid Precursor Protein
APP-CTFs	Carboxyl-terminal Fragments
APOE	Apolipoprotein E
BACE	β-site APP-cleaving Enzyme
BBB	Blood-Brain Barrier
BDNF	Brain Derived Neurotrophic Factor
CDK5	Cyclin-Dependent Kinase-5
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CTFα	Carboxyl-Terminal Fragment α
CTFβ	Carboxyl-Terminal Fragment β
DA	Dopamine
EPM	Elevated Plus Maze
ETM	Elevated T Maze
FAD	Familial Alzheimer's Disease
FTD	Frontotemporal Dementia
GFAP	Glial Fibrillary Acidic Protein
GSK3	Glycogen Synthase Kinase-3
GSK3α	Glycogen Synthase Kinase-3α
GSK3β	Glycogen Synthase Kinase-3β
MAP	Microtubule Associated Protein
NFTs	Neurofibrillary Tangles

NMDA	N-methyl-D-aspartate
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PBS	Phosphate-buffered Saline
PBS-T	PBS with 0.1% Tween-20
PDH	Pyruvate Dehydrogenase
PKA	Protein Kinase A
PKB	Protein Kinase B
PS1	Presenilin 1
PS2	Presenilin 2
Q2.5	Quetiapine 2.5mg/kg/day
Q5	Quetiapine 5 mg/kg/day
ROS	Reactive Oxygen Species
SAD	Sporadic Alzheimer's Disease
sAPPα	Secretory APPα
sAPPβ	Secretory APPβ
SOD	Superoxide Dismutase
SYP	Synaptophysin
Tg	Transgenic
Non-Tg	Non-Transgenic
VPA	Valproic Acid

CHAPTER 1: INTRODUCTION

1.1. Characteristics of Alzheimer's Disease

Alzheimer's disease (AD) was first described by Alois Alzheimer in the brain of a demented female patient, Auguste D., of about 60 years of age in 1906. It is the most common form of dementia in the elderly, comprising up to 70% - 80% of all cases. AD is a progressive neurodegenerative disorder characterized pathologically by the presence of extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs) and massive neuronal cell and synapse loss at specific sites (Selkoe, 2002; Storey & Cappai, 1999; Suh & Checler, 2002; Van Gassen & Annaert, 2003). The amyloid plaques have been previously shown to be composed of fibrillar β-amyloid (Aβ) peptide and intracellular NFTs contain hyperphosphorylated tau protein.

Clinically, while the symptoms can vary in severity and chronology, they mirror a gradual expansion of degenerative change in the brain, which has been described to occur in six stages (Braak & Braak, 1991). The disease is characterized predominantly by memory loss, followed by a gradual decline in other cognitive functions, namely aphasia (loss of language), apraxia (loss of motor activities), agnosia (loss of object recognition), and executive functioning (Tariot, Profenno, & Ismail, 2004) (DSM-IV-TR, American Psychiatric Association 2000). Early symptoms include experiencing short-term memory loss, confusion, being inattentive, personality changes and language difficulties, often in conjunction with unexplained mood swings. As the disease progresses, there are also a number of psychiatric and behavioural complications (Cantillon et al., 1997; Scharre & Chang, 2002), such as anxiety and depression. More than half of all AD patients show symptoms of apathy, agitation, depression, anxiety and aberrant motor behaviours (Cummings & Zhong, 2006). The symptoms of AD begin insidiously,

typically progress over a period of four to twenty years, and result ultimately in the death of the patients.

The cause of AD is not yet fully understood. A minority of AD patients (under 65 years of age) have a familiar form of AD (FAD, less than 5% of cases) that is largely attributable to autosomal dominant mutations in three genes: *β-amyloid precursor protein* (*APP*), *presenilin* 1 (*PS1*) and *presenilin* 2 (*PS2*). The first genetic mutations causing FAD were discovered in the *APP* gene, whose mutations promoted the generation of Aβ by favouring proteolytic processing of APP (Chartier-Harlin et al., 1991; J. Hardy, 1992; Mullan et al., 1992). FAD-causing mutations later found in *PS1* and *PS2* genes also enhanced the processing of APP to form amyloidogenic Aβ (Scheuner et al., 1996).

The vast majority (>95%) of cases of Alzheimer's disease are sporadic, meaning that the disease does not follow Mendelian inheritance. However, sporadic Alzheimer's disease (SAD) does show significant heritability. The most well known and strongest genetic risk factor is the inheritance of the *ε4* allele of the *apolipoprotein E* (*APOE*) gene (Strittmatter et al., 1993).

Irrespetive of genetics, advancing age is the most significant risk factor for AD. The chance of developing AD doubles every five years after age 65 (Mayeux, 2010). The prevalence increases from 1% among those 60 to 64 years old to up to 40% of those aged 85 years and older (von Strauss, Viitanen, De Ronchi, Winblad, & Fratiglioni, 1999). In Canada, the prevalence of AD is one in 20 in those over 65 years of age ("Canadian study of health and aging: study methods and prevalence of dementia," 1994), making it one of the top leading causes of death for older Canadians (Kung, Hoyert, Xu, & Murphy, 2008). With the increasing longevity of our population, AD is already approaching epidemic proportions with no cure or even preventive

therapy yet available, and as such confers an enormous emotional and economic burden on patients, caregivers, and society.

1.2. The Neurobiological Basis of Alzheimer's Disease

AD appears to develop when the combined effects of various risk factors cross a certain "threshold". At this point they overwhelm the natural brain repair capabilities, thus reducing the brain's ability to maintain healthy neuronal cells. Various hypotheses have been suggested to explain the biological basis of its etiology and pathogenesis of AD.

1.2.1 Amyloid Cascade Hypothesis

The original 'amyloid cascade hypothesis' was first formulated almost over two decades ago. It proposed that accumulation of Aβ peptide, the main component of amyloid plaques in the brain, is the primary influence driving AD pathogenesis (J. A. Hardy & Higgins, 1992). This peptide is derived through the processing of a large transmembrane protein APP that can be processed via two distinct processing pathways (Figure 1): the amyloidogenic pathway that liberates the Aβ peptide and the non-amyloidogenic pathway which precludes the formation of Aβ, and instead generates a secreted form of APP, secretory APPα (sAPPα), and C-terminal fragment C83 (C-terminal fragment α (CTFα)) (Ashe & Zahs, 2010; Ma et al., 2007). C83 can be further cleaved by γ-secretase, producing extracellular fragment p3 and intracellular APP intracellular domain (AICD) (Edbauer et al., 2003; Kim, Ikeuchi, Yu, & Sisodia, 2003). The amyloidogenic process involves two important enzymes: β-secretase (β-site APP-cleaving enzyme, BACE) and a presenilin (PS)-dependent γ-secretase complex (Fukumoto et al., 2004; Lazarov et al., 2005). B-secretase mediates the initial step of Aβ production by β-cleavage of APP, producing secretory APPβ (sAPPβ) and membrane-bound CTFβ (C89/C99) (Fischer, Molinari, Bodendorf, &

Paganetti, 2002). Subsequent cleavage of CTFβ by γ-secretase results in increased production of amyloid peptide Aβ, eventually leads to amyloid deposition.

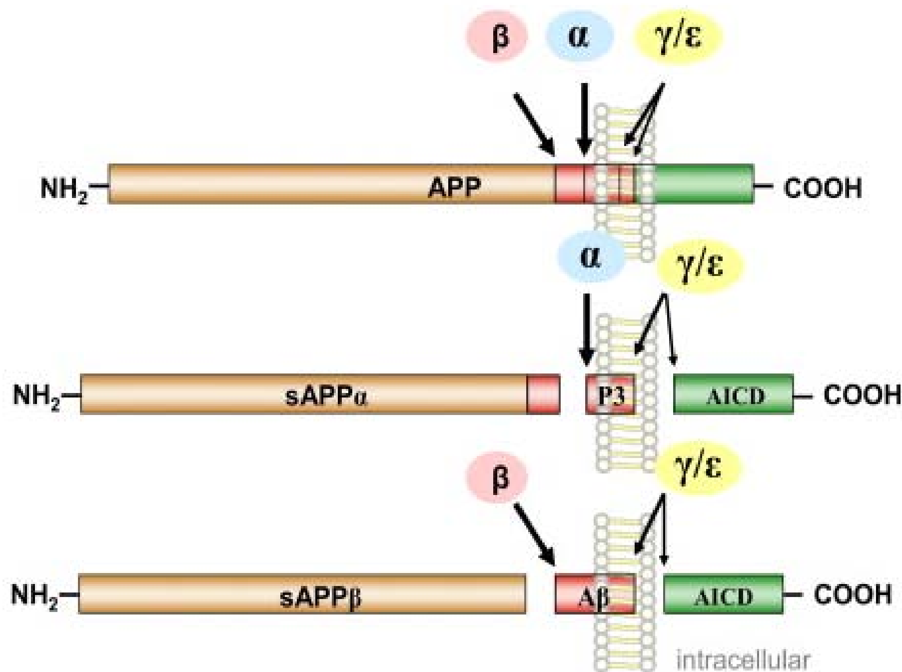


Figure 1. Cleavage of APP
APP can be cleaved in two pathways: the more common, non-pathogenic, non-amyloidogenic pathway, or the pathogenic, amyloidogenic pathway (bottom). In normal conditions, the majority of APP is cleaved within the Aβ domain (small red rectangle) by α-secretase to produce sAPPα and membrane-bound C83. C83 can be further cleaved by γ-secretase, producing extracellular fragment p3 and intracellular AICD. In the pathogenic pathway, APP is first cleaved by β-secretase at the start of the Aβ domain, producing sAPPβ and membrane-bound CTFβ (C89/C99). Cleavage of CTFβ by γ-secretase yields pathogenic Aβ fragments, and intracellular AICD. (Ma, et al., 2007)

A more recent amyloid cascade hypothesis (Figure 2) suggests that soluble Aβ, as determined by an imbalance between its generation versus clearance in the brain, is the primary driver of AD-related pathogenesis, resulting in formation of neurofibrillary tangles containing tau protein, synapse loss, and neuronal cell death (J. Hardy & Selkoe, 2002; Tanzi & Bertram,

2005). Increasingly, in this newer hypothesis, attention is turned to soluble oligomeric Aβ that may be responsible for synaptic dysfunction in the brains of AD patients and in AD animal models (J. Hardy & Selkoe, 2002; Walsh et al., 2002). Whatever the mechanism of neuronal cell loss may be, it is presumably the loss of neuronal function that produces the cognitive and behavioural phenotypes of dementia (Cummings, Vinters, Cole, & Khachaturian, 1998).

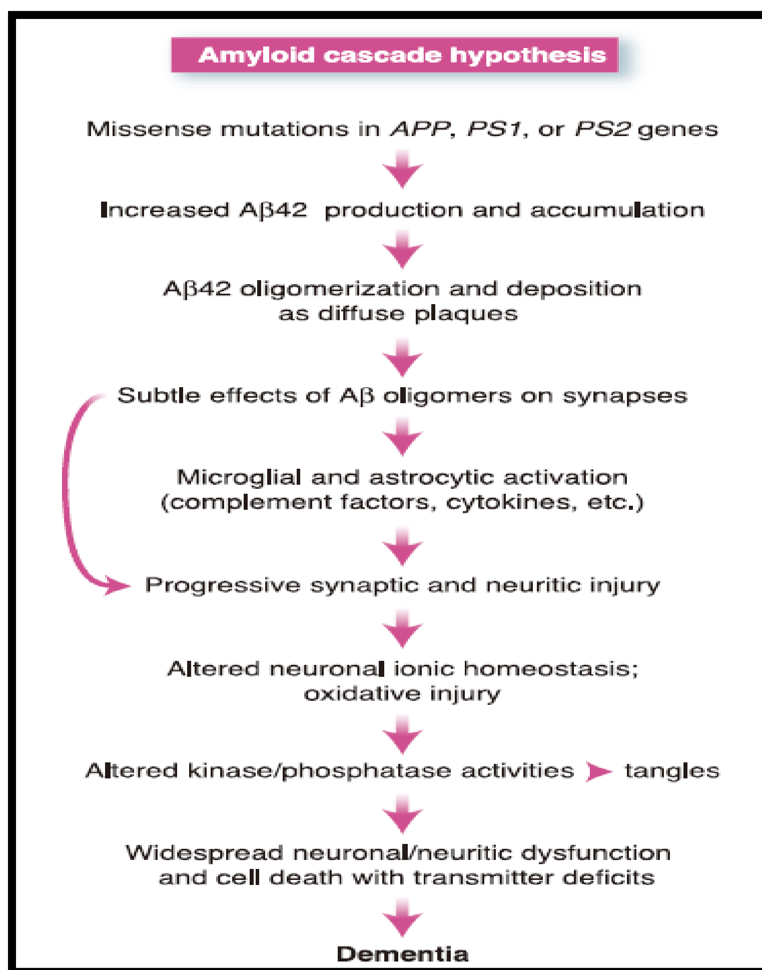


Figure 2. The amyloid cascade hypothesis
The sequence of pathogenic events leads to AD by Aβ. The curved arrow on the perimeter indicates that soluble oligomer Aβ may be responsible for neuritic injury or impaired synaptic integrity. (From J. Hardy & Selkoe, 2002. Reprinted with permission from AAAS.)

There is convincing evidence to support the recent amyloid cascade hypothesis. The most well-documented fact is that APP is believed to be an upstream process, whose mutations will

cause neuropathological changes associated with AD (both plaques and tangles), thus leading to the clinical features of AD in early onset FAD. Also, the apolipoprotein E (APOE) genotype is related to the degree of deposition of Aβ peptide in the cerebral cortex before AD symptoms arise (Polvikoski et al., 1995). Furthermore, the cells and animal models expressing a mutant form of the human APP gene result in increased Aβ generation consistent with that in AD patient (Ding et al., 2008; He et al., 2009; Hsiao et al., 1996; Qing et al., 2008). Therefore, the evidence suggests that APP processing may start at an early stage in the pathogenesis of AD.

Although the amyloid cascade hypothesis offers a broad framework to explain AD pathogenesis, it is currently a simplistic view that carries a number of limitations (J. Hardy & Selkoe, 2002), with certain observations that do not easily fit. The biggest concern is that the number of amyloid plaques in brains does not correlate well with the degree of cognitive function impairment that patients experience clinically (Schmitz et al., 2004). Moreover, the neurotoxic species of Aβ are undefined and the nature of their effects on neuronal function *in vivo* has not been studied (Walsh, et al., 2002). Yet another concern arises from the fact that some transgenic AD mice often do not display clear-cut neuronal loss along with the undergoing progressive Aβ deposition (Games et al., 1995; Hsiao, et al., 1996). These observed discrepancies point to important gaps in our understanding of AD.

1.2.2 Tau Hypothesis

The fact that the amyloid hypothesis has become the focus of much AD research has led some to question whether the other key feature of Alzheimer's disease, NFTs, was perhaps an epiphenomenon. However, a steady influx of research has started to elucidate the significant role of NFTs and their principle protein component, phosphorylated tau, in the brain (Tiraboschi et al., 2004). Along with the inconsistencies present in the amyloid cascade hypothesis, these

observations lay the foundation for the *tau hypothesis,* which states that the key event in the pathophysiology of AD is the hyperphosphorylation of tau and that the tau protein abnormalities initiate the disease cascade (Mudher & Lovestone, 2002).

Tau, normally expressed in axons, is a cytoplasmic protein that binds to tubulin during its polymerization and stabilizes microtubules, the central component of tangles. In AD, altered phosphorylation of tau plays a key role in generation of aggregates of tangles and neuropil threads inside the nerve cell bodies (Gozes, 2010). When this occurs, the disintegrated microtubules break down the neuron's inner transport system, which may result in malfunctions in biochemical communication between neurons, and eventually death of the cells (Chun & Johnson, 2007).

The tau hypothesis is supported by findings from Chun et al., (2007), that tangle accumulation inside the neuron occupies much of the cell, and lead to neuronal death. The number of tangles show strong correlation with the clinical features of AD, such as cognitive state (Nagy et al., 1995). Braak & Braak (1998), discovered that the aggregation of phosphorylated tau occurred in critical regions for memory formation (Braak & Braak, 1998). Further, studies on frontotemporal dementia (FTD) have provided evidence for the abnormal hyperphosphorylation of tau mutations (MAPT) and its subsequent self-aggregation (Gozes, 2010; Heutink, 2000; Rizzu et al., 2000). However, consensus has not been reached on whether tau protein abnormalities initiate the disease cascade.

1.2.3 ‘Amyloid Cascade Hypothesis’ versus ‘Tau Hypothesis’

Whatever the hypotheses of this disease may be, the pathological hallmarks of Aβ and NFTs in disease are significant. The initial step of the disease and these two interactions during the progression of the disease are not completely understood. However, in the last 15 years, the

amyloid cascade hypothesis has been supported by a wealth of studies from worldwide research groups. Alternative existing hypotheses such as tau hypothesis do not have as much experimental support as the amyloid cascade hypothesis. More and more researchers tend to believe that Aβ peptides, especially soluble Aβ peptides, play a central role in AD pathogenesis (Ding, et al., 2008; He, et al., 2009; Qing, et al., 2008).

First, tau mutations caused neurodegenerative disorder (FTD) is characterized by severe deposition of tau in neurofibrillary tangles in the brain, but not deposition of amyloid plaques (Gozes, 2010). The mutations in tau have emphasized that this tau-related pathology can be sufficient to cause dementia; however, this clearly omits the fact that even the most severe tau alternation is not enough to cause the amyloid deposition feature of AD. Thus, the neurofibrillary tangles of wild type tau observed in AD patients' brains are most likely to occur after the generation of Aβ and initial plaque formation, rather than before (J. Hardy & Selkoe, 2002).

Secondly, two double pathology animal models have been created to harmonize the tau and amyloid hypotheses. A double transgenic mouse overexpressing both mutant form of human APP gene and human tau gene has shown increased formation of neurofibrillary tangles and amyloid plaques compared with mice overexpressing tau or APP alone (Lewis et al., 2001). This is not surprising seeing as the mice are derived from parental lines with both pathologies. However, the structure, number and onset of the amyloid plaques is not altered in this double transgenic mouse model whereas the neurofibrillary tangle pathology is substantially enhanced with the presence of amyloid plaques (Lewis, et al., 2001). This finding suggests that abnormal APP processing happens before tau alterations in the pathogenic cascade of AD. In line with this, a recent study showing in mouse hippocampal primary neuronal cultures, Aβ toxicity is tau dependent (Rapoport, Dawson, Binder, Vitek, & Ferreira, 2002).

Thirdly, a lot of evidence indicates that many SAD cases are attributed to Aβ metabolism and clearance due to various genetic risk factors (such as, APOE) (Bertram et al., 2000). Taken together, these above findings are consistent with the amyloid cascade hypothesis that cerebral Aβ accumulation caused by an imbalance between Aβ production and Aβ clearance is the primary influence of AD, resulting in tau tangle formation.

1.3. GSK3β in Alzheimer's Disease

There are two isoforms of Glycogen synthase kinase-3 (GSK3): GSK3α and GSK3β. GSK3β is a multi-functional ubiquitous serine/threonine protein kinase involved in regulating various cellular processes, including cell adhesion, cell division, cell survival, neuronal plasticity and gene expression (Grimes & Jope, 2001). Unlike most other protein kinases, GSK3β is normally constitutively active in all cells, and is primarily regulated via inhibition of its activity (Doble & Woodgett, 2003). GSK3β activity is approximately 200 times higher upon phosphorylation at the tyrosine 216 residue (K. Hughes, Nikolakaki, Plyte, Totty, & Woodgett, 1993), while phosphorylation at the specific serine 9 residue can inhibit the activity of GSK3β (Jope & Johnson, 2004; Lochhead et al., 2006). GSK3β is also regulated by the Wnt signalling pathway and controlling β-catenin levels (Qing, et al., 2008).

Much evidence shows that GSK3β is involved in a wide range of disorders, such as biopolar mood disorders, schizophrenia, stroke, diabetes, and neurodegenerative disorders, including AD (Emamian, Hall, Birnbaum, Karayiorgou, & Gogos, 2004; Grimes & Jope, 2001).

GSK3β has been shown to contribute to the neuropathological mechanisms of AD (Forde & Dale, 2007), including interactions with the Aβ production system associated with AD, participation in phosphorylating the microtubule-binding protein tau that the main component of neurofibrillary tangles in AD, and interactions with presenilin and other AD-associated proteins,

each of which is discussed below. The involvements of GSK3β in a remarkably broad spectrum of AD-associated events are illustrated in Figure 3.

GSK3β levels or activity have been measured in brain tissue in several studies of AD. In human AD patients, increased levels of GSK3β have been found, and GSK3β was discovered to be associated with neurofibrillary tangles in the AD brain (Imahori & Uchida, 1997; Pei et al., 1999; Pei et al., 1997; Yamaguchi et al., 1996). In addition, active GSK3β has been found to accumulate in pretangle neurons (Pei et al., 1999). Taken together, these studies provide strong evidence that alterations in the control of GSK3β may occur or be involved at some level in the AD brain, thus offering a promising therapeutic target for AD (Frame & Cohen, 2001; Martinez, Castro, Dorronsoro, & Alonso, 2002; Medina & Castro, 2008).

1.3.1 Interaction of GSK3β, APP and Aβ

Evidence from several studies has associated GSK3β with the amyloid plaque neuropathology of AD through its interactions with APP, the Aβ peptide product of the proteolysis of APP. The mechanism whereby GSK3β promotes Aβ production probably is associated with the reported phosphorylation of the APP (Aplin, Gibb, Jacobsen, Gallo, & Anderton, 1996). APP is a transmembrane phosphoprotein expressing in a variety of tissues and carrying eight potential phosphorylation sites within its cytoplasmic domain (Lee et al., 2003). GSK3β is thought to mediate the phosphorylation of APP at Thr 668 (numbering for the APP 695 isoform) in neurons (Aplin, et al., 1996; Iijima et al., 2000). This neuron-specific phosphorylation of APP at Thr 668 has been reported to facilitate the BACE cleavage of APP to increase Aβ generation in APP metabolism (Lee, et al., 2003). Therefore, GSK3β promotes Aβ production. The neurotoxic Aβ derived from cleavage of APP activates GSK3β (Takashima et al., 1995). Aβ-induced activation

of GSK3β is reported to lead to phosphorylation of tau (Takashima, Honda, et al., 1998), which may contribute to the accumulation of hyperphosphorylated tau in AD.

Furthermore, *in vivo* studies for the potential interation of GSK3β and APP processing comes from lithium and valproic acid (VPA) treated AD transgenic mice that overexpress the mutant human APP gene. Su et al. found that both lithium as well as VPA, both known to be GSK3β inhibitors, inhibit Aβ production and plaques in the hippocampus region of the brains of the PDAPP (APPV717F) AD transgenic mouse model at clinically relevant plasma concentrations (Su et al., 2004). This inhibitory effect on Aβ production was mediated through the inhibition of GSK3β by lithium. However, in a novel twist, another group has shown that lithium decreases Aβ generation by regulating GSK3α mediating γ-secretase rather than GSK3β (Phiel, Wilson, Lee, & Klein, 2003). Therefore, there are some controversies existing with respect to the effects of lithium in altering the processing of APP.

Aβ activated GSK3β has been found to contribute to neuronal death by inactivating mitochondrial pyruvate dehydrogenase (PDH) (Hoshi et al., 1996) which is involved in the conversion of pyruvate to acetyl-CoA in cholinergic neurons. PDH dysfunction results in reduced synthesis of acetylcholine and mitochondrial dysfunction which contribute to neuronal death (Imahori & Uchida, 1997). These observations suggest that Aβ, through activation of GSK3β, may be responsible for the highly documented finding that there is a severe loss of cholinergic neurons seen in AD (Jope, 1999). Thus, GSK3β contributes to Aβ induced neuronal toxicity and inhibition of GSK3β significantly attenuates the neurotoxiciy induced by Aβ (Alvarez et al., 1999).

1.3.2 Interaction of GSK3β and Tau

Neurofibrillary tangles constitute one of the neuropathological features of AD. These are composed primarily of tau, and tau in these tangles is abnormally hyperphosphorylated. Tau was identified as a substrate of GSK3β, which regulates tau by phosphorylation *in vitro* and in intact cells (Hanger, Hughes, Woodgett, Brion, & Anderton, 1992; Mandelkow et al., 1992). It is of considerable interest that GSK3β can phosphorylate many sites on tau, some of which are the same sites that are abnormally hyperphosphorylated in AD brains (Hanger, Betts, Loviny, Blackstock, & Anderton, 1998). Recent studies have found that GSK3β can phosphorylate tau at Thr 231, a critical site that will impair the ability of tau to bind and stabilize microtubules (Cho & Johnson, 2004). Significant advances on this research come from *in vivo* studies of transgenic mice. Recent studies report the development of mice overexpressing GSK3β, which confirms that tau is an *in vivo* substrate of GSK3β and its phosphorylation increased in this transgenic mouse model (Spittaels et al., 2000). Thus, GSK3β may contribute to the abnormal hyperphosphorylation of tau and promote tau aggregation and its neurotoxicity.

1.3.3 Interaction of GSK3β and Presenilin 1

PS1, a transmembrane protein that is necessary for the γ-secretase, is mutated in one form of FAD. Takashima et al. (1998) first reported a direct interaction between PS1 and GSK3β by showing that GSK3β was detected in PS1 immunoprecipitates from human brains (Takashima, Honda, et al., 1998). Further studies show that PS1 mutations that cause FAD increase in its ability to bind GSK3β (Takashima, Murayama, et al., 1998). The same group also found that tau binds the same binding domain of the PS1 protein, implying that PS1 mutations may facilitate GSK3β mediated phosphorylation of tau. Recent studies have suggested that PS1 mutations might compromise neuronal function by increasing GSK3β activity and impairing kinesin-driven

organelle motility (Pigino et al., 2003). In addition to directly binding GSK3β, there is also evidence that PS1 is capable of modulating its activity by regulating Akt (Protein kinase B). Weihl et al. (1999) reported mutated PS1 is associated with a downregulation of the prosurvival Akt activity and subsequent increase in GSK3β activity (Weihl et al., 1999). These imply that mutant PS1 may upregulate GSK3β activity by perturbing its inhibitory control by Akt. Thus, mutanted PS1 may both directly alter GSK3β intracellular localization and indirectly regulate its activity.

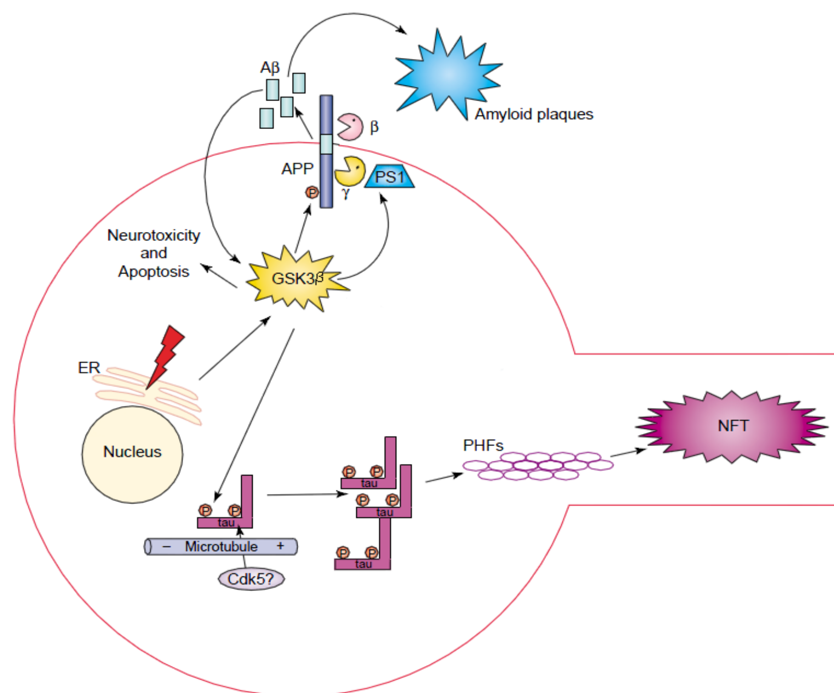


Figure 3. GSK3β is associated with the neuropathology of Alzheimer's disease
GSK3β links many of the major neuropathological mechanisms that are associated with AD. GSK3β phosphorylates APP and neurotoxic Aβ in turn activates GSK3β. Phosphorylation of APP increases Aβ generation in APP metabolism. Moreover, phosphorylation of tau by GSK3β results in decreased microtubule binding that is a possible prelude to neurofibrillary tangle (NFT) formation. Additionally, GSK3β directly binds PS1, which is necessary for the γ-secretase cleavage of APP processing. Taken together, these actions suggest that GSK3β may contribute to the neuropathologies characteristic of AD. (Reprinted from Jope & Johnson, 2004, with permission from Elsevier).

1.4. Potential Factors Involved in AD

1.4.1 Inflammation

Inflammation is well defined as a biological response of the organism to harmful stimuli, such as an invading pathogen, a traumatic event, or in general terms, an injurious agent. This agent can be foreign or self, as inside a cell and the process of inflammation may be classified as either acute or chronic (Wyss-Coray, 2006). Neuroinflammation is often characterized in most neurodegenerative diseases including Alzheimer's disease. Activated microglia and astrocytes are the main cell types that are recruited in this response (Wyss-Coray & Mucke, 2002).

Neuroinflammatory processes have already been considered as another feature of AD and are an important source of oxidative stress in AD patients (Wyss-Coray & Mucke, 2002). A number of inflammatory markers have been shown to be present in the AD brain: elevated inflammatory cytokines and chemokines, and accumulation of activated microglia/astrocytes occurring mainly around amyloid plaques (Matsuoka et al., 2001). Epidemiological studies have indentified long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) as protective against AD by reducing risk for this disorder. These may suggest that there is a close association between AD pathogenesis and neuroinflammation (McGeer & McGeer, 1999). Initially, based upon this evidence, inflammation was proposed as a possible cause of AD. However, NSAIDs failed in clinical trials. Now inflammation is believed as a byproduct of the disease which will not alter the pathogenesis of AD. In other words, it is also possible that little immune response would be beneficial and slow the disease (Wyss-Coray, 2006), which may be the reason a broad inhibition of inflammation drug treating AD failed at phase 3 in clinical trials (Mangialasche, Solomon, Winblad, Mecocci, & Kivipelto, 2010).

A major component of neuroinflammation is the microglial cell, which is a type of immune cell that is well distributed in the CNS and support and protect neuronal functions. They

function as the first and main form of active immune defense. Active microglia will release a variety of pro-inflammatory mediators such as cytokines, chemokines, and reactive oxygen species (ROS) (Tuppo & Arias, 2005). In AD, microglia play a central role to response the AD brain lesions such as amyloid plaques. Aβ can attract and activate microglia, which will lead to form a cluster of microglia mainly around amyloid depositions in the brain. Aβ-activated microglia will release more ROS, which will further damage neurons. Although microglia have some neuroprotective functions (Streit, Walter, & Pennell, 1999), neurotoxic mechanisms involving continuous activation of microglia and toxic factors released by microglia may lead to excessive neuroinflammation. The continuous activation of microglia may result in the inflammation, another feature of AD pathology observed in AD brains.

One of the earliest pathological manifestations in the AD brain is the accumulation of astroglia around amyloid deposits (Wyss-Coray et al., 2003). In the CNS, astrocytes, star-shaped glial cells, are the most abundant cells greatly outnumbering neurons by 10-100 folds in the human brain. They possess a number of important structural and physiological functions, including biochemical support of endothelial cells of the blood-brain barrier (BBB), supplying nutrients to nervous tissue, maintenance of extracellular ion balance, and healing the brain and spinal cord following traumatic injury (Nagele, Wegiel, Venkataraman, Imaki, & Wang, 2004). In addition to accumulating around amyloid deposits, astrocytes have been shown to degrade Aβ *in vitro* and *in situ* (Wyss-Coray, et al., 2003). Current investigations in AD-related pathological potential of astroglia have focused on its role in inflammation. Like microglia, astroglia activated by Aβ release multiple cytokines and chemokines in the CNS, which may lead to neurotoxic damage through generating chronic self-sustaining inflammatory reactions (Paradisi, Sacchetti,

Balduzzi, Gaudi, & Malchiodi-Albedi, 2004). This process may stimulate and even accelerate the progression of AD.

1.4.2 Neurodegeneration

Neuronal degeneration and loss is consistently observed in AD brains (West, Coleman, Flood, & Troncoso, 1994), and in the brains of APP/PS1 transgenic mice (Fonseca, Zhou, Botto, & Tenner, 2004; Rutten et al., 2005), and is presumably the basis of memory loss in AD (Hamos, DeGennaro, & Drachman, 1989). Quantification of neuron loss has shown significant decreases in synaptic density in the cortex and hippocampus of AD brains using electron microscopy or immunohistochemical staining (Davies, Mann, Sumpter, & Yates, 1987), which indicates that synaptic loss in the hippocampus and neocortex is an early phenomenon (Heinonen et al., 1995) and is the major structural correlate of cognitive dysfunction (DeKosky, Scheff, & Styren, 1996).

AD is a slowly progressing mental disorder with a 'silent' clinical period that can be longer than a decade before diagnosis. Likewise, synaptic degeneration might be a slow process and progress during the course of AD. This dynamic process of synaptic degeneration proceeds from the initially reversible downregulation of synaptic function to the irreversiblely marked synapse loss. This raises the possibility that attenuating neurodegeneration may rescue memory and deter cognitive impairment in AD.

1.5. Aβ transgenic Mouse Models of Alzheimer's Disease

Further understanding of the neurobiology of AD comes from transgenic animal models of AD based on the genetic mutations associated with AD patients. Over the last decade, the development of transgenic mouse models of AD has mainly focused on mimicing NFTs and amyloid plaques. Such transgenic models have provided very useful platform for understanding the significances of Aβ deposits and tau phosphorylation in the AD pathogenesis, as well as their

relationship with other pathological features. The following section discusses the utility of Aβ mouse models, and their validity in studying AD.

1.5.1 Relevance of Transgenic Mouse Studies to Clinical Trials

The first transgenic mouse model of AD, which was designed with the PDGF promoter expressing V717F (with valine at residue 717 substituted by phenylalanine) APP, was developed by Games et al. in 1995 and show Aβ amyloid plaques comparable to those in the brains of human AD patients. The PDGF promoter-driven mice (PDAPP) express human $A\beta_{1-40}$ and $A\beta_{1-42}$ that are 5 to 14 times higher than endogenous mouse Aβ (Games, et al., 1995). The PDAPP V717F transgenic mouse progressively develops many of the pathological features of AD, including numerous amyloid plaques, synaptic loss, hippocampus and corpus callosum shrinkage, astrocytosis and microgliosis (Games, et al., 1995; Kobayashi & Chen, 2005). In addition, PDAPP mice demonstrated significant memory impairments on several behavioural tests, such as the Morris water maze, radial arm maze, object recognition and the fear conditioning tasks compared to age matched controls (Kobayashi & Chen, 2005). The cognitive deficits in the older PDAPP mice are well-correlated with increased Aβ levels and reductions in the hippocampus-to-brain ratio. However, similar cognitive declines are also seen in young (3 – 4 months) animals in which there are no apparent Aβ depositions or reductions in hippocampus (Kobayashi & Chen, 2005).

One year after the PDAPP mouse was developed by Games et al., a similar transgenic mouse model, which over-expresses the Swedish double mutant form of APP695, was introduced as the Tg2576 (K670M/M671L) mouse (Hsiao, et al., 1996). Tg2576 mice over-express the 695-amino acid isoform of human Alzheimer APP containing a Lys670 --> Asn, Met671 --> Leu mutation. Similar to the PDAPP transgenic mouse, heterozygous Tg2576 mice

produce a moderate fivefold increase in $A\beta_{1-40}$ and a 14-fold increase in $A\beta_{1-42/43}$ compared with wild type endogenous murine Aβ (Hsiao, et al., 1996). In addition, Tg2576 mice develop amyloid plaques in the cortex, hippocampus and cerebellum. The learning and memory in spatial reference and alternation task impairments do not appear until 9 to 10 months old (Kobayashi & Chen, 2005).

Shortly after the introduction of Tg2576, Sturchler-Pierrat et al. constructed two transgenic mouse lines developing pathological features reminiscent of AD: APP22 and APP23 (Sturchler-Pierrat et al., 1997). APP23 (K670N/M671N) produces higher levels of human APP than does the Tg2576, resulting in an early formation of amyloid plaques in the neocortex and hippocampus. In addition, elevated tau phosphorylation and mild cortical neuronal loss can be demonstrated biochemically in 6 months old animals and increases with age in APP23 transgenic mice. This is the first APP transgenic mouse model to produce the tau pathology (Calhoun et al., 1998; Sturchler-Pierrat, et al., 1997). APP22 mice, however, which express human APP with the combined Swedish and London (V717I) mutations, does not develop plaques until 18 months of age (Sturchler-Pierrat, et al., 1997).

Another mutant mouse line, TgCRND8, exhibits dysfunctions in both β-secretase and γ-secretase activities, and has a very aggressive rate of Aβ deposition because it carries both the Indiana (V717F) and the Swedish (K670N/M671L) mutations (Chishti et al., 2001). Plaque formation begins at around 9 weeks in the hippocampus and cortex.

The first mouse model to examine the role of mutant PS1 (M146L) was produced by Duff et al. in 1996. Presenilin mutations would cause Aβ accumulation as found in FAD patients' brains (Liu, Herukka, Minkeviciene, van Groen, & Tanila, 2004), as it affects the activity of γ-secretase; γ-secretase is an atypical multimeric membrane protein complex

comprised of at least four subunits, including individual enzymes knows as PS1 and PS2 (Qing, et al., 2008). While a single mutation in PS2 does not display Aβ plaques in mouse brains, mutant PS2 transgenic mice have been shown elevations in $A\beta_{1-42/43}$ (Oyama et al., 1998). Similarly, transgenic animals over-expressing human PS1 show high levels of $A\beta_{1-42}$, but amyloid depositions or behavioural alterations do not develop in these animals (Kobayashi & Chen, 2005).

The PS1 knockout mice quickly die after birth suggesting that PS1 plays an important role in development. PS1 deficiency will disrupt early embryogenesis, which results in severe skeletal abnormalities and prenatal death. Besides, severe neuronal loss and haemorrhages are found in the these mouse brains (Shen et al., 1997). Today a few types of PS1 and PS2 transgenic mice could survive after birth and can be used to investigate the biological role of presenilins.

The first double transgenic mouse model of AD was produced to modulate the amyloid accumulation by crossing in human PS1 mutation, also known as the APP/PS1 double transgenic mouse model. Crossing the M146L mutation in PS1 introduced by Duff et al. with the Tg2576 transgenic mouse significantly accelerates the rate of Aβ deposition in the brain, and produces more of the fibrillogenic $A\beta_{1-42}$ species which are 5 times higher by 6 months of age than age matched controls (Holcomb et al., 1998). However, stereological methods demonstrated no significant neuronal loss (He, et al., 2009).

Another multi-gene APP/PS1 mouse model, known as the PSAPP mouse model, was developed by Dineley et al.. The PSAPP is developed by crossing a different mutation in human PS1 (A246E) with the Tg2576 mouse. In these mice, amyloid depositions occur at 7 months old, much earlier than in the Tg2576 mice (Dineley, Xia, Bui, Sweatt, & Zheng, 2002).

There are many other multi-gene transgenic mice models emerging that can offer insight into the mechanism of AD. A mouse model combining Tg2576 with a nitric oxide synthase 2 knock-out model demonstrates Aβ plaques, the hyperphosphorylation of tau, and neuronal loss in mouse brains (Colton et al., 2006). A model of vascular dementia, APPSwDI (K670M/N671L, E693Q, and D694N), shows a rapid progression of Aβ deposition particularly associated with cerebral microvascular accumulation (Davis et al., 2004). It has been suggested that the increased rate of cerebral accumulation is due to deficient clearance of Dutch/Iowa mutant Aβ from brain across the blood-brain barrier (Davis et al., 2006). This is one of the first models to mimic the deficiency of clearance of Aβ, which closely relates to what happens in human AD patients.

Ultimately, these transgenic mouse models of AD provide researchers with opportunities to investigate the underlying mechanisms of AD, and develop therapeutic agents which effectively target these processes.

1.5.2 Behavioural Deficits of the APP/PS1 Transgenic Mouse Model of AD

Within the abundance of AD mouse models, the APP/PS1 double transgenic model is the most widely used in studying drug effects and gene function in AD, and is the mouse model chosen for this study. A review of the literature shows that APP/PS1 mice exhibit a number of cognitive deficits specially when tested in the Y-maze, with fewer alternations. While younger animals tend to perform as well as wild type controls in the Morris water maze, the animals start to show spatial deficits by 6 months of age (He, et al., 2009). In addition, young and old APP/PS1 mice have been tested in the elevated plus maze (EPM), elevated T maze (ETM), light/dark box test, and open field test, and are found to display significant changes in anxiety levels (He et al., 2006). This increased level of anxiety is associated with the onset of the neuropathology, Aβ

plaque deposition, as well as learning and working memory impairment (He, et al., 2009). Meanwhile, the mice show increased motor activity in an open field test and locomotion test.

1.6. Quetiapine's Effects in Alzheimer's Disease

1.6.1 Atypical Antipsychotics

Antipsychotic drugs (APD) have been the first pharmacotherapeutic line for the treatment of schizophrenia for approximately half of a century; this began with the discovery and introduction of chlorpromazine (Lehmann & Ban, 1997). APDs are generally divided into three groups: first generation APDs, known as conventional or typical APDs, and second generation APDs, known as atypical APDs, and third generation APDs. Typical antipsychotics include chlorpromazine and haloperidol. Atypical antipsychotics are represented by clozapine, olanzapine, quetiapine, and risperidone. While both typical and atypical APDs can alleviate positive symptoms, clinical studies have found atypical APDs are more effective for the treatment of negative and cognitive symptoms (Cuesta, Peralta, & Zarzuela, 2001; Jann, 2004; Purdon, Malla, Labelle, & Lit, 2001; Velligan et al., 2002).

Considering atypical antipsychotics' efficacy and its excellent tolerability in clinical uses, they are commonly used for a wide range of neuropsychiatric conditions other than schizophrenia, including those associated with psychotic depression and mood disorder, obsessive-compulsive disorder, body dysmorphic disorder, bipolar disorder (Jeste & Dolder, 2004), posttraumatic stress disorder (Pae et al., 2008) and psychosis in Alzheimer's disease (Madhusoodanan, Shah, Brenner, & Gupta, 2007). Olanzapine, quetiapine, and risperidone all have better treatment effects on neurocognitive function in patients with early-psychosis (Keefe et al., 2007). Quetiapine is also used to treat psychotic symptoms and improve cognition in Parkinson's disease without worsening patients' motor function (Juncos et al., 2004).

Neurotransmitter receptors are the primary molecular targets of atypical and typical antipsychotics, and the blockade of dopamine D_2 receptor is important for normalizing neurotransmitter imbalances that may be responsible for the reversal of positive symptoms and acute manifestation of schizophrenia (Wong & Van Tol, 2003). The difference is that atypical antipsychotics can also bind to serotonin (5-HT) receptors. These different binding affinities for dopamine D_2 and 5-HT_2A receptors may give us some insight to understand some of the different therapeutic effects of atypical antipsychotics (Kapur, Zipursky, & Remington, 1999; Seeman & Tallerico, 1999). Although a potential relationship between receptors binding and therapeutic properties of antipsychotics can be established, the picture is complicated by the fact that the drug-receptor binding profiles are very complex. The mechanisms underlying their therapeutic effects may extend beyond the dopamine and serotonin receptor blockade effects. Therefore, it is reasonable to hypothesize that the functional outcome of these complex mechanisms is not only due to direct drug-receptor blockade effects, but is rather the results of downstream interactions with other signaling pathways and transcriptional factors.

The "anatomy" of schizophrenia has been extensively investigated. Recently, it has been suggested that progressive neuropathological changes, such as neuronal atrophy and/or cell death, ventricle enlargement and shrinkage of specific brain regions (for instance hippocampus and prefrontal cortex), occur over the course of the disease (Arnold & Trojanowski, 1996; DeLisi et al., 1997; Waddington et al., 1991; Woods et al., 1990). Cognitive defects usually occur early in the course of schizophrenia, and the severity of deficiency is predictive of the long-term treatment outcome for patients (Green, 1996). Neural injury or neurodegeneration may be causative of cognitive deficits in schizophrenia (Harvey, 1998). Therefore, the beneficial effects

of atypical antipsychotics on behaviour may also come from their possible downstream neuroprotective effects.

1.6.2 Neuroprotective Effects of Quetiapine

Quetiapine *(Seroquel®)*, is a novel atypical antipsychotic drug that was approved by the U.S. Food and Drug Administration (FDA) in 1997 for the treatment of patients with schizophrenia. It effectively alleviates positive and negative symptoms, cognitive dysfunction in patients with schizophrenia (Purdon, et al., 2001; Velligan, et al., 2002). It is a dibenzothiazepine, and competitively antagonizes both dopamine-2 (D_2) and serotonin-2 ($5\text{-}HT_2$) receptors, but with a much higher affinity for $5\text{-}HT_2$ receptor than D_2 receptor (Gefvert et al., 2001). In addition to the high binding ratios of $5\text{-}HT_2$:D_2 receptor, quetiapine also has affinity for $5\text{-}HT_{1A}$, $5\text{-}HT_6$, histamine-1 (H_1), α1- and α2-adrenergic receptors. It also has some moderate inhibitive ability on serotonin reuptake (Hirsch, Link, Goldstein, & Arvanitis, 1996).

In vitro studies have shown that quetiapine protects PC12 cells from cell death induced by serum withdrawal or by the addition of hydrogen peroxide, β-amyloid peptide, or N-methyl-4-phenylpyridinium ion (MPP^+) (Bai et al., 2002; Qing, Xu, Wei, Gibson, & Li, 2003; Wei, Bai, Richardson, Mousseau, & Li, 2003; Wei, Mousseau, Richardson, Dyck, & Li, 2003). In addition, quetiapine shows some antioxidant properties. Quetiapine is able to increase the superoxide dismutase (SOD1) gene expression in PC12 cells and reduce cell death induced by serum withdrawal (Bai, et al., 2002). This antioxidant effect of quetiapine may be the reason why it can prevent PC12 cell death induced by various insults. Furthermore, research has demonstrated that quetiapine and olanzapine are able to prevent PC12 cells from Aβ-induced apoptosis and the overproduction of intracellular reactive oxygen species, attenuate Aβ-induced activity changes of the antioxidant enzymes (SOD1, catalase, and glutathione peroxidase), and block Aβ-induced

decrease in mitochondrial membrane potential in PC12 cells (Wang, Xu, Dyck, & Li, 2005). Taken together, all these *in vitro* studies suggest the neuroprotection of quetiapine and other atypical antipsychotics may be partially due to their antioxidant properties.

In *in vivo* studies, quetiapine has been shown to attenuate methamphetamine-induced memory impairment and neurotoxicity (He, Xu, Yang, Zhang, & Li, 2005; He, Yang, Yu, Li, & Li, 2006), and alleviate anxiety-like behavioural changes induced by a neurotoxic regimen of dl-amphetamine in rats (He, et al., 2005). Quetiapine has some beneficial effects on phencyclidine-induced reference memory impairment and decrease the Bcl-X_L/Bax ratio in the posterior cingulate cortex of rats (He, Xu, et al., 2006). These findings confirm previous findings that quetiapine alleviates psychotic symptoms and improves cognitive function without worsening motor function in patients with Parkinson's disease (Juncos, et al., 2004). Although quetiapine is used to effectively alleviate psychoses in patients with AD, its possible beneficial effects on cognition in AD remain unclear (Caballero, Hitchcock, Scharre, Beversdorf, & Nahata, 2006; Scharre & Chang, 2002). Furthermore, there is no data published with respect to its possible beneficial effects on the pathological changes associated with AD.

Quetiapine has also been shown to up-regulate the level of brain derived neurotrophic factor (BDNF) that is an important neurotrophin in brain neurons. Reduced expression of neurotrophins may cause abnormalities during development. After a certain period of stress exposure in rats, quetiapine is able to reverse stress induced reductions of BDNF expression level in the hippocampus (Xu et al., 2002). The above studies demonstrate that quetiapine possesses extensive neuroprotective properties, which are partly exerted through antioxidant activity and the modulation of growth factors.

1.6.3 Effects of Quetiapine on the Akt/GSK3β Signaling Pathway

Akt is a serine/threonine kinase, and the activation of Akt involves phosphorylation of its regulatory threonine residue (Thr 308) and serine residue (Ser 473) by different kinases (Andjelkovic et al., 1997; Jacinto et al., 2006; Scheid & Woodgett, 2001). Akt has been shown to inhibit GSK3β in response to some growth factors, including BDNF, IGF, and insulin (Cross, Alessi, Cohen, Andjelkovich, & Hemmings, 1995; Frame & Cohen, 2001).

Over the past few years, a serial study has provided evidence for involvement of the Akt/GSK3 signalling pathway in the mechanism of certain antipsychotic drugs, including atypical antipsychotics beyond their dopamine and serotonin receptor blockade effects (Beaulieu et al., 2005; Beaulieu et al., 2004; Emamian, et al., 2004).

In an *in vitro* study, the atypical antipsychotic clozapine has been shown to be able to enhance the Akt/GSK3 signaling pathway in SH-SY5Y human neuroblastoma cells (Kang et al., 2004). *In vivo*, acute or subchronic administration of several atypical antipsychotics in mice, including olanzapine, risperidone, quetiapine, clozapine, and ziprasidone, mediated phosphorylation of GSK3β through phosphorylation at Ser 9 that reproduces the similar action of Akt in different brain regions (Alimohamad, Rajakumar, Seah, & Rushlow, 2005; Li et al., 2004; Roh et al., 2007).

The mechanism by which quetiapine inhibit GSK3β is still unknown. Because both dopamine and 5-HT can affect the activity of GSK3β (Beaulieu, Gainetdinov, & Caron, 2009) and quetiapine antagonizes both receptors, it remains uncertain whether any or both of these neurotransmitters are responsible for the regulation of GSK3β by quetiapine. It seems that quetiapine may regulate this Akt/GSK3β signaling pathway that is a common downstream target of quetiapine via both dopamine and serotonin. Meanwhile, GSK3β has been found to play a

very important role in the pathological changes in AD, it may be very promising to investigate the modulation of Akt/GSK3β by quetiapine as a therapeutic target in the treatment of AD.

1.7. Overall Goals of This Research

The overall goals of this thesis are to investigate the effects of chronic quetiapine treatment after pathology onset on the AD pathogenesis in transgenic mice and to expand our understanding of GSK3β's role in the effects of quetiapine in AD treatment. Thus, the general hypothesis is that chronic quetiapine adminstration after pathology onset slows the progression of Alzheimer's disease by regulating GSK3β.

We anticipate that quetiapine will attenuate memory impairment and cerebral pathology in the APP/PS1 transgenic mouse model of AD. We also anticipate that quetiapine will decrease cerebral GSK3β level and activity in AD mice. The expected findings are important because they may highlight the potential treatments for its symptoms and pathologies involved in AD. This study will not only contribute to our understanding of the pathophysiology of AD, it also has implications for early intervention in AD patients, particularly because the animals in the current study began to receive treatment after the onset of amyloid plaques. Lastly, the present study contributes to the field of AD research by addressing the possible underlying mechanism of quetiapine in AD.

CHAPTER 2: MATERIALS AND METHODS

2.1. Animals and Genotyping

The single APP and PS1 transgenic mice used in this study were originally obtained from AstraZeneca R&D. These mice express a chimeric mouse/human APP containing the K670N/M671N Swedish mutations and a mutant human PS1 carrying M146L under the control of mouse prion promoter elements, directing transgene expression predominantly to CNS neurons. $APP_{K670N/M671N}/PS1_{M146L}$ double transgenic mice were generated by crossing single transgenic mice expressing human mutant $APP_{K670N/M671N}$ (Hsiao, et al., 1996) and mutant $PS1_{M146L}$ (Duff et al., 1996). The mouse colonies were located in the Central Animal Care Services (CACS) at the University of Manitoba. At weaning (21 days old), animals were genotyped by PCR using genomic DNA isolated from tail biopsies. This study used a total of 48 mice, 26 females and 22 males (Table 1), which were maintained until 12 months of age.

All animals were housed at 12h day/12h night cycle with free access to water and food. All procedures used in this study were in accordance with guidelines established by the Canadian Council on Animal Care according to standard animal care protocols and maintained in a pathogen-free environment at the University of Manitoba. The animals were randomly assigned for treatment and coded, and the experimenters and data analyzer remained blinded to which treatment they received, until the code was broken.

After weaning at 3 weeks of age, mice were anesthetized with isoflurane and ear marked. A tissue sample was taken from the tip of the tail. Tissue was digested in 300 µl of lysis buffer (50 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 20 mM NaCl, 1% SDS) with 7 µl of 20 mg/mL Proteinase K (Roche) at 55°C overnight. The next day, samples were centrifuged at 14,500 rpm for 10 min and DNA was precipitated by transferring the supernatant to a clear tube with equal

volumes of cold 95% or 100% ethanol. DNA was pelleted by centrifugation at 14,500×g for 10 min, the supernatant was discarded and pellets washed twice in 70% ethanol, dried, and finally spun down at 14,500 rpm for 5 min. DNA pellet was resuspended in 100 μl distilled water. Genotyping was performed by PCR with the following primers: human APP forward primer hAPP1 5'-GCC GTT GAC AAG TAT CTC GAG ACA CCT GG-3'; human APP reverse primer hAPP2 5'-GTG TCT CCA CCA GCT GCT GTC TCT CGT TGG C-3'; human PS1 sense primer 5'-GAC AAC CAC CTG AGC AAT AC-3'; and human PS1 anti-sense primer 5'-CAT CTT GCT CCA CCA CCT GCC-3'. APP and PS1 positive mice displayed two bands, with the positive bands being 250 bp and 145 bp in size, while non-transgenic mice displayed no bands (Figure 4).

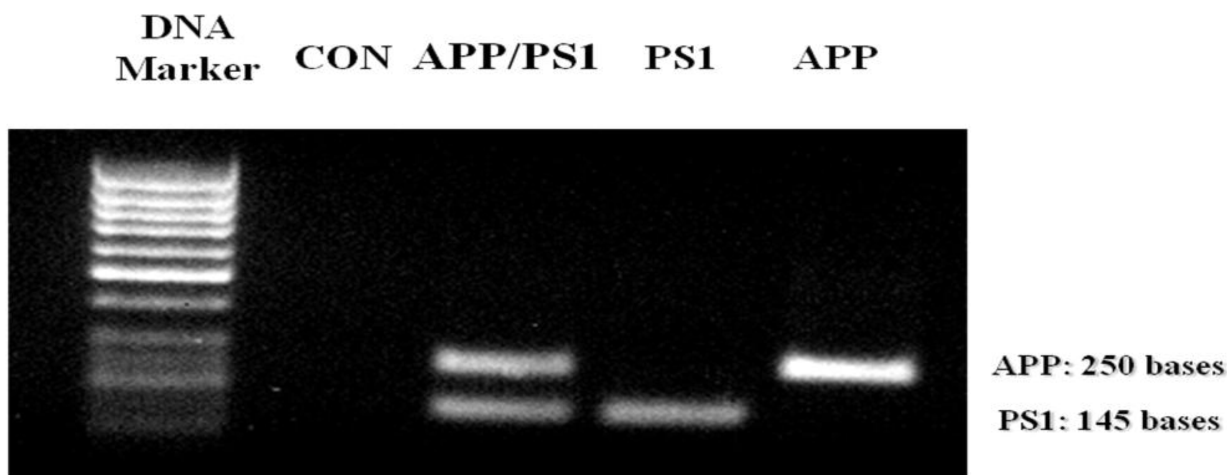


Figure 4. PCR of complementary DNA obtained from double APP/PS1 mouse carrier and single transgenic APP or PS1 mice carrriers
Con: Control or non-transgenic mice; APP/PS1: APP/PS1 double transgenic mice; APP: APP single transgenic mice; PS1: PS1 single transgenic mice.

2.2. Drug Treatment

Three to four months old APP/PS1 transgenic mice and wild-type littermates were randomly assigned into 6 groups: non-transgenic+water (Con), non-transgenic+quetiapine 2.5 mg/kg/day (Q2.5), non-transgenic+quetiapine 5 mg/kg/day (Q5), transgenic+water (Tg),

transgenic+quetiapine 2.5 mg/kg/day (Tg+Q2.5) and transgenic+quetiapine 5 mg/kg/day (Tg+Q5). Quetiapine (provided by AstraZeneca Pharmaceuticals, Macclesfield, UK) was dissolved in double distilled water (2 mg/100 ml water, 1 mg/100 ml water) and delivered in bottles, available *ad libitum* as drinking water. This was freshly prepared every other day. Treatment started when the mice were 3 - 4 months old and was continued until they were 12 months old. The doses and duration of quetiapine, whose pharmacokinetics had been extensively studied in rodents, were chosen based on pilot studies. Our pilot studies found that chronic quetiapine treatment for 6 - 7 months at this dosage markedly mitigated the body weight loss of transgenic mice (data not shown) and there was no associated morbidity/mortality with the treatment. Meanwhile, there was no significant difference between the groups regarding the amount of water that the mice drank. On average, a 30-g mouse drank 7.5 ml a day. The use of 3 - 4 months old APP/PS1 mice receiving the quetiapine treatment was based on previous reports demonstrating that these mice began to have Aβ plaques as early as 2.5 months of age and had a high Aβ load in hippocampal and cortical subareas by 6 months of age (Ding, et al., 2008; He, et al., 2009).

	Con	Q2.5	Q5	Tg	Tg+Q2.5	Tg+Q5
12 month						
Male	4	4	5	3	3	3
Female	6	4	4	4	4	4
Total	10	8	9	7	7	7

Table 1. Number of mice in each group
Number of female and male control and APP/PS1 transgenic mice used to generate behaviour testing data.

2.3. General Guidelines for Behaviour Testing

All animals received standard husbandry care during testing, including cage enrichment (PVC pipe, half a Nestlet square and/or shredded paper, and paper hut) and *ad libitum* access to food and water. All testing occurred during the light cycle. The testing included tests of motor function, anxiety, and learning and memory, which occurred on a 10-day schedule in the following order: Y-maze, open field, light-dark box, EPM, and water maze (6 days) (Figure 5). While a standard test battery has one week inter-test intervals, the rapid test battery used facilitates the rate of studying, reduces operating costs and does not significantly affect behavioural performance (Paylor, Spencer, Yuva-Paylor, & Pieke-Dahl, 2006).

All animals were tested at 12 months of age, providing a longitudinal study of behaviour. For all behaviour tests, mice were transferred from their holding room in the CACS to the behaviour testing procedure room via covered push cart, and allowed to rest for a minimum of 30 min before testing. All apparatuses were cleaned with 75% ethanol wipes before each behavioural assessment for each mouse, mainly for hygiene purposes and to remove odors that may have created biases. We used an overhead CCD camera and computerized tracking system, ANY-Maze™ Video Tracking Software (Stoelting Co., IL, USA), to record the behaviours for the open field test, Y-maze, and water maze tests. This tracking device and overhead camera provides a systematic, standardized and objective measure of behavior, it also minimizes bias from environmental stressors, especially by the experimenter's presence.

Test Day									
1	2	3	4	5	6	7	8	9	10
Y-maze	Open Field	L/D Box	EPM	Water Maze					

Figure 5. Behaviour testing schedule
Testing occurred on a 10-day schedule, in the following order: Y-maze, open field, light-dark box, EPM and then water maze tests (6 days).

2.3.1 Open Field Test

The open field test measures behavioural response including spontaneous locomotor activity and exploratory behaviours. The first open field apparatus was described to measure spontaneous locomotion of rats in a given time period (Broadhurst, 1961). This technique was later successfully adapted to study locomotor activity in the mouse (DeFries, Hegmann, & Weir, 1966). While commonly used to measure locomotor activity, the open field test is also used as a measure of anxiety-like behaviour in rodents. The brightly lit, exposed Inner zone of the open field arena is considered an aversive environment that acts as an anxiogenic stimulus. As such, mice with higher anxiety levels tend to avoid the highly illuminated, novel Inner zone (Holmes, 2001).

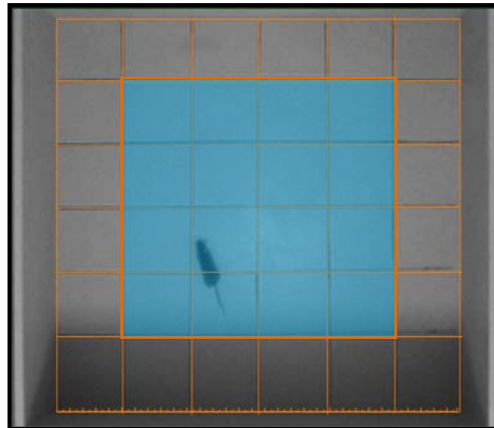


Figure 6. Open feld apparatus
Calibration image as viewed in ANY-Maze™ Video Tracking Software (version 4.63). The open feld apparatus consists of a bare square box (36" by 36") with 35 cm high black walls. The open field box is partitioned into 36 equal sized squares, and divided into 20 outer border squares and 16 inner zone squares. A tracking camera views the box from above. Orange lines are zone boundaries. The blue area is defined as the Inner zone, while the perimeter is defined as the Outer zone.

The open field test was performed in a bare square box (36" by 36") made of compressed wood and painted in grey. The open field box was partitioned into 36 equal sized squares, and divided into 20 outer border squares and 16 inner zone squares (Figure 6). The test procedure

was the same as that previously described by He et al. (He, et al., 2005). Mice were placed in a particular corner of the arena and were tracked using ANY-Maze^{TM} Video Tracking Software (version 4.63) with a digital camera. Mice were allowed to explore the maze for 5 min, after which they were returned to their home cage. The maze was cleaned with 75% ethanol wipes before commencing testing with the next mouse. Total distance and average speed were analyzed as measures of motor ability. The number of rears, latency to first rear, latency to first enter the Inner zone, time in the Inner zone, and number of entries into the Inner zone were taken as measures of anxiety.

2.3.2 Y-Maze Test

The free running Y-maze spontaneous alternation task has been used to study mouse behaviour for over 30 years (Kokkinidis & Anisman, 1976). Spatial working memory was assessed by the Y-maze test as previously described in our laboratory (He, et al., 2009; Xiao et al., 2008). It is based on the principle that a mouse tends to explore the maze systematically, entering each arm in turn. The ability to alternate requires that a mouse remembers which arm it has recently occupied (R. N. Hughes, 2004). For instance, if the three arms of the Y-maze are labelled A, B, and C, and the mouse starts in arm A and proceeds to arm B, in order to successfully explore the last arm C the mouse must remember which two arms it had already entered. This measure is called spontaneous alternation, and is defined as entries into three different arms on consecutive choices in overlapping triplet sets (R. N. Hughes, 2004). Percentage of alternation was defined as the number of sequential triplets containing successive entries into all three arms (ABC, ACB, constituted a sequential triplet, while B-C-B or A-C-A did not) during the session as a proportion of the maximum possible alternation (equivalent to the total number of arm entries minus - 2) × 100 (Holcomb, et al., 1998). For example, the 9-step sequence CABCABABA consists of 4 sets:

(1) CAB, (2) ABC, (3) BCA, and (4) CAB, out of a possible 7 sets (total number of entries minus 2), for an alternation rate of 57%. Previous studies have shown that Y-maze is a highly hippocampal function dependent memory performance task (Dillon, Qu, Marcus, & Dodart, 2008; Reisel et al., 2002). Different spatial working memory of all groups can be elucidated by comparing alternation rates of different groups.

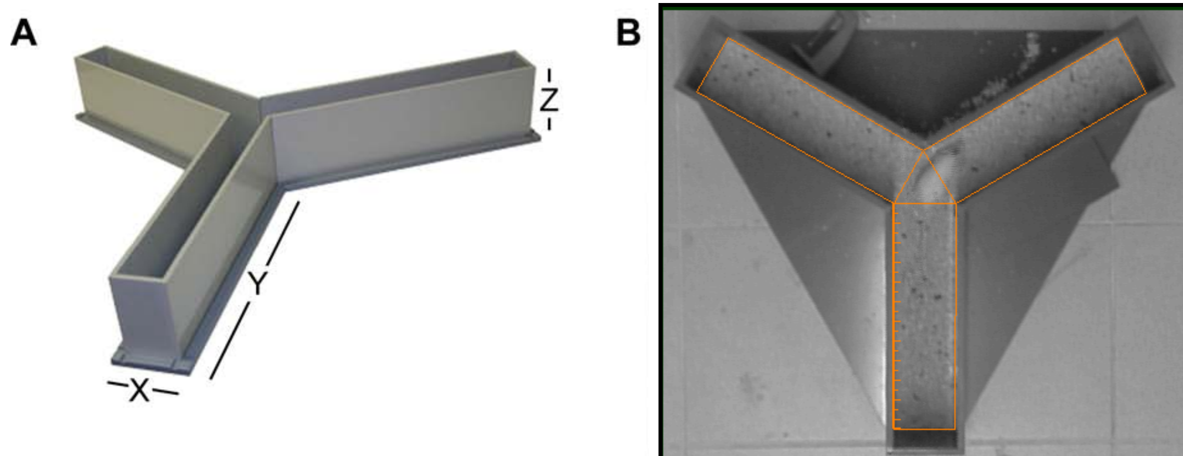


Figure 7. Y-maze apparatus
(**A**) Image of the Y-maze apparatus with specifications. x = 5 cm; y = 35 cm; z = 10 cm (image © Stoelting Co., used with permission). (**B**) Calibration image as viewed in ANY-Maze™ Video Tracking Software (version 4.63). Orange lines represent zone boundaries between 3 arms. The center zone is undefined.

The experiment used a Y-maze with three arms diverging 120° from the central point, each 5 cm wide and 35 cm in length, and shielded with 10 cm high walls (Figure 7). The testing was performed in a dimly illuminated behaviour room, and the apparatus was placed 40 cm above the floor. Mice were individually placed at the end of the start arm and tracking of the mice commenced automatically using ANY-Maze™ Video Tracking Software (version 4.63). Mice were allowed to explore the maze freely for 8 min, after which they were returned to their home cage (Yamada et al., 1996). The maze was cleaned before commencing testing on the next mouse. A mouse was considered to have entered an arm when all 4 paws were positioned in the

arm runway. Distance/average speed and total arm entries were analyzed as measures of motor ability, while spontaneous alternation was analyzed as a measure of spatial working memory. Percent spontaneous alternation was calculated as the percentage of the number of alternations performed out of the total number of alternations possible, based on the number of arm entries per mouse. Mice whose total entrance number was less than 15 times during the test were not taken into the final data.

2.3.3 Morris Water Maze

The Morris water maze is the most frequently used behavioural test of learning and memory in rodents. While it was originally introduced to investigate spatial learning in the rat (R. Morris, 1984), studies have shown that the test is highly hippocampal dependent in both rats (R. G. Morris, Garrud, Rawlins, & O'Keefe, 1982) and mice (Logue, Paylor, & Wehner, 1997). However, it is generally believed that mice have impaired water maze performance compared to rats due to the better swimming ability of rats. The test has since been widely used in studies of transgenic and knockout mice, especially in studies of AD transgenic mice.

Water maze testing occurred in a circular and galvanized pool (120 cm diameter × 50 cm height, Figure 8) with a white interior, with a water temperature adjusted to 24 ± 1ºC. The surface area of the water pool was divided into four equal quadrants. Mice received hidden platform trials from Day 1 to Day 5 for 5 days, and a probe trial on Day 6. Tracking of animals was achieved using ANY-Maze™ Video Tracking Software (version 4.63). Mice that floated for greater than 20 sec during any trial or spined in any trial were eliminated from the analysis.

In the hidden platform (acquisition) trials, the clear Plexiglas® platform (10 cm in diameter) was placed in one of the four maze quadrants and submerged 1 cm below the water surface in a fixed position (30 cm away from the side wall) in the northwestern/third quadrant.

Its location was kept constant at the same (target) quadrant throughout entire acquisition trials process. Mice were required to find the hidden platform using spatial strategies based upon the distal cues posed in the behaviour testing room. Conditions and room layout were kept constant throughout the whole experiment. Mice were gently released into the water and always faced to the inside wall. In each trial, mice were started in different positions. A mouse was allowed to swim until it climbed onto the platform or for a maximum of 60 s. Once the mice reached the platform, they were allowed to stay on it for 10 - 20 s. Then, they were taken out, dried, and placed into a separated cage for approximate 60 min before the next trial (Ding, et al., 2008; Qing, et al., 2008). If a mouse was not able to locate the hidden platform within 60 s, it was gently guided by the experimenter to the platform and allowed to stay there for 10 - 20 s. Between each animal trial, the water tank was cleaned by a strainer and stirred as well to erase the olfactory traces of the previous animal. Mice were trained for 3 trials per day with an inter-trial interval of 1 h for 5 consecutive days. All the animal spatial memory scores were assessed by assessing the average latency time (in seconds) and path length of a mouse reaching the hidden platform.

In the probe trial, the platform was removed from the water pool and mice were allowed to swim for 60 s in the pool to assess memory consolidation 24 h after the last hidden platform trial. In this trial, mice were released at the same position. The time spent in the target quadrant and the number of target area (the previous platform location) crossings was analyzed as measures of degree of spatial memory. Tracking of animal movement were achieved with a camcorder mounted above the tank and the data was analyzed by an observer blinded to which experimental group each animal belonged.

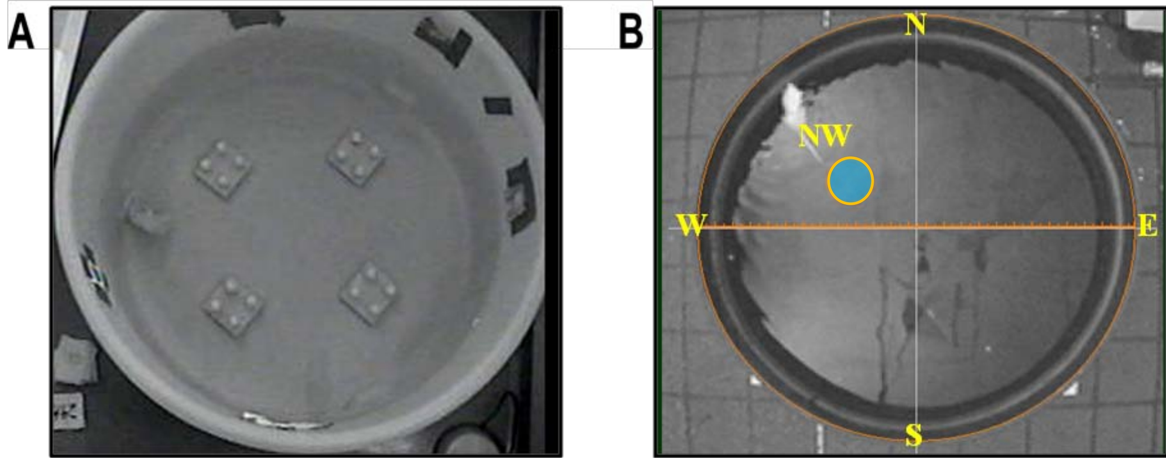


Figure 8. Water maze apparatus
(**A**) Image of the water maze pool as used for hidden platform trials. The 10 cm platform was submerged 1 cm below the water surface. The starting direction of the mouse varied across trials (NE, SW, SE), while the platform was kept constant at the same location. As shown, the platform is in the NW quadrant. (**B**) Calibration image as viewed in ANY-Maze^{TM} Video Tracking Software (version 4.63). Orange lines represent zone boundaries. The platform zone was defined as one location: NW. Tracking was achieved through a camera above the pool. During the probe trial, the platform was removed. As shown, the platform (the blue area) is in the NW quadrant.

2.4. Collection of Cerebrospinal Fluid (CSF)

On the day following behaviour testing, CSF was obtained from each mouse, using the method adapted from that of Liu et al. with minor modifications (Liu & Duff, 2008; Liu, et al., 2004). Following anesthesia by isofluorane, an incision was immediately made from the top of the skull to the dorsal thorax. The subcutaneous tissue and muscles in the surrounding area were separated by blunt dissection with forceps to clearly expose the meninges overlying the cisterna magna. 4 - 8 µl of CSF flew into the tube under the positive pressure as a result of blood pressure after a capillary tube was used to penetrate the translucent meninges. The CSF samples were injected into sterile PCR tubes, frozen immediately on dry ice and then transferred into a -80°C freezer until ELISA assays were performed.

2.5. Tissue Processing and Histology

After collecting CSF, animals were perfused through the ascending aorta with cold 0.1M phosphate-buffered saline (PBS, pH 7.4). Brains (excluding cerebellum, pons, and medulla oblongata) were divided sagitally. The right hemisphere was separated into the following segments: frontal, middle, and posterior cortex, hippocampus, striatum and then snap frozen and stored at -80°C for protein and other biochemistry analyses. The left hemisphere was postfixed with freshly depolymerized 4% paraformaldehyde (PFA) in PBS for an additional 48 h at 4°C, and then was equilibrated overnight in 30% sucrose (He, et al., 2009; Qing, et al., 2008). The serial coronal sections were cut into 30 μm on a freezing sliding microtome (Leica Microsystems, Wetzlar, Germany) for immunohistochemistry and histology analysis.

2.5.1 Amyloid Plaque Quantification

The presence of amyloid in tissue sections was investigated after being stained with a commercially available amyloid stain, the Congo Red kit (Sigma–Aldrich, St. Louis, MI), according to the manufacturer's instruction. Quantifications were performed blindly in four coronal sections per animal, spaced 180 μm apart. The total number of amyloid plaques in the cortex and the hippocampus was counted manually under a Zeiss Imager-A1 microscope at 100× magnification. The size of the total counted area was collected using a Zeiss Imager-A1 microscope and analyzed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The plaque number was reported as the total number of plaques counted in the cortex and hippocampus (He, et al., 2009). The size of plaques was presented as the average area fraction positive to Congo red staining of the cortex and hippocampus (Toledo & Inestrosa, 2010).

2.5.2 GFAP Immunohistochemistry

For immunohistochemistry (GFAP), three 30 μm coronal brain sections per mouse (spaced 180 μm apart) were stained with the ABC Peroxidase Staining Kit (Pierce) in accordance with the manufacturer's instructions. Briefly, endogenous peroxidase activity was quenched with 3% hydrogen peroxide in PBS for 20 min. The sections were then incubated for 30 min in blocking buffer [5% goat normal serum (Invitrogen) and 0.3% Triton X-100 (Sigma) in PBS] at room temperature and subsequently incubated overnight at 4°C in an appropriately diluted primary antibody: a rabbit polyclonal GFAP antibody (DakoCytomation, 1:4000). After the sections were rinsed, secondary biotinylated antibody against the corresponding specie was used at a dilution of 1:1000 (Vector Laboratories, CA). After being washed 3 times using PBS with 0.1% Tween-20 (PBS-T), the slides were incubated with ABC reagents for 30 min. The chromagen was diaminobenzidine (Thermo Scientific). Slides were then air-dried in the dark, mounted and viewed with an Axio-Imager A1, camera Axiocam, and the software Axio-Vision version 4 for image acquisition (all from Carl Zeiss, Jena, Germany). The immunohistochemical controls were performed as above, but with the omission of the primary antibodies. No positive immunostaining was found in any of the controls.

2.6. Quantification of $A\beta_{1\text{-}40/42}$ Levels by ELISA

Brain tissues (frontal cortex and hippocampus) were homogenized in 7 - 8 volumes of modified RIPA lysis buffer including 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MI), 1% phosphatase inhibitor cocktail 2 (Sigma-Aldrich, St. Louis, MI) and 1% phosphatase inhibitor cocktail 3 (Sigma-Aldrich, St. Louis, MI) and then centrifuged at 100,000 × g at 4ºC for 1 hour. Soluble fractions of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were measured in the supernatants. For quantification of Aβ in the insoluble fraction, the pellets containin insoluble Aβ were extracted with 8 volumes of

5.0 M guanidine-HCl in 50 mM Tris-HCl, pH 8.0 by 50-100 µl aliquots and ground thoroughly with a hand-held motor (Fisher, K749540-0000) after each addition. The mixed homogenates were left at room temperature for 3 - 4 hours on a shaker at 90 rpm and then diluted upon appropriate factors with ice cold Dulbecco's phosphor-buffered saline (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8.0 g/L NaCl, 1.150 g/L Na_2HPO_4, pH 7.4) containing 0.5% bovine serum albumin, 0.05% Tween 20 and protease inhibitor (BSAT-DPBS) and centrifuged at 16,000 × g for 20 min at 4ºC. This guanidine-HCl-extractable fraction is hereafter referred to as insoluble Aβ. Each sample was assayed in duplicate at appropriate dilutions so that relative luminescent units fell within the linear range of standard curves (Hellstrom-Lindahl, Viitanen, & Marutle, 2009).

The levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were quantified using the Signal Select Human Aβ ELISA kits (Invitrogen, Carlsbad, CA, 92008, USA) according to the manufacturer's protocol. The plate was read at 450 nm on a BioTek PowerWave XS Microplate Spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). All measurements were done in duplicate. The $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were calculated by comparison with a standard curve of synthetic human $A\beta_{1-40}$ and $A\beta_{1-42}$. For each Aβ species quantified, all samples from the all groups were run in the same ELISA. The levels of Aβ were corrected for total protein of brain tissue and dilution factor, and expressed as µg (Aβ) /g (total protein) in the transgenic mice. Finally, the levels of Aβ in each group were standardized to that in the Tg group (as 100%).

CSF samples were directly diluted upon appropriate factors with ice cold BSAT-DPBS buffer. Then the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ were quantified using the Signal Select Human Aβ ELISA kits (Invitrogen, Carlsbad, CA, 92008, USA) according to the manufacturer's protocol. The level of Aβ was expressed as µg (Aβ) /µl (total volume) in the transgenic mice. Finally, the level of Aβ in each group was standardized to that in the Tg group (as 100%).

2.7. Immunoblotting

Brain tissues (frontal cortex and hippocampus) were homogenized in ice-cold lysis buffer containing 20 mM Tris-HCl pH 7.6, 150 mM NaCl, 2 mM EDTA, 1 mM EGTA, 0.1% v/v Triton X-100, 20 mM NaF, 0.02% NaN_3, 1 mM PMSF (all obtained from Sigma), 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MI), 1% phosphatase inhibitor cocktail 2 (Sigma-Aldrich, St. Louis, MI) and 1% phosphatase inhibitor cocktail 3 (Sigma-Aldrich, St. Louis, MI). Homogenates were sonicated briefly and centrifuged at 15,000 × g for 30 min. The protein concentration of the samples in the supernatants was determined using a Bicinchoninic acid (BCA) Protein Assay Reagent kit (Pierce). Equivalent amounts of total proteins of brain tissues were boiled and resolved on 12% SDS-PAGE under reduced conditions. Proteins were transferred to a polyvinylidene difluoride (PVDF, GE Healthcare) membrane (200 mA for 2 h). The membrane was blocked with 5% skim milk in 0.1% Tween 20/TBS for 1 h and then incubated with specific antibodies such as a polyclonal rabbit C-terminal APP (1:3000) antibody (Sigma), a polyclonal rabbit PS1 (1:1000) antibody (Cell Signaling Technology), a monoclonal mouse tau-1 (1:2000) antibody (Santa Cruz Biotechnology), a monoclonal rabbit GSK3β (1:2000) antibody (Cell Signaling Technology) and a monoclonal rabbit phosphorylated (p)-S^9-GSK3β (1:1000) antibody (Cell Signaling Technology) overnight at 4°C. To measure the levels of GFAP and synaptophysin, membranes were incubated with a monoclonal mouse GFAP (1:6000) antibody (Millipore) and a monoclonal mouse synaptophysin (1:15,000) antibody (Santa Cruz Biotechnology) for 1 hour at room temperature. After being washed, immunoreactive proteins were detected using the corresponding HRP-conjugated secondary antibodies (1:10000 for rabbit, 1:5000 for mouse; Invitrogen, Carlsbad, CA, 92008, USA) for an hour at room temperature, using an ECL kit (PerkinElmer, MA, USA) according to the manufacturer's instructions. Blots subsequently labeled with a mouse monoclonal β actin

antibody (1:4,000; AC-15, Sigma) as an internal control for the concentration of protein loaded were made followed the same procedures as above. Bands were analyzed using Quantity One software (Bio-Rad). Quantitative results were expressed as a ratio of specific protein to its internal control.

2.8. Statistics

All results are expressed as means ± SEM. One-way ANOVA was performed followed by Tukey's Multiple comparisons or Dunnett's Multiple comparisons *post hoc* analysis to identify significant effects of quetiapine on the percentage of alternation in the Y-maze test, retention memory in water maze probe trial, and anxiety level in the open field test. Differences were considered significant at $p < 0.05$. Specific details on the statistical analyses used for each test can be found in the figure captions.

Two-way Repeated Measures ANOVAs with a *post hoc* analysis were performed to examine significant effects of quetiapine on learning memory in the water maze hidden platform trials. Statistical significance was set at $p < 0.05$.

The significant differences of the effects of quetiapine on Aβ levels in the frontal cortex, hippocampus and CSF, on the plaque load in the hippocampus and cortex, and on specific protein levels in the frontal cortex and hippocampus, were determined by one-way ANOVA using PRISM 4.0 software (GraphPad Software, Inc, CA, USA). Tukey's or Dunnett's *post hoc* test for multiple comparisons was conducted. Specific details on the statistical analyses used for each analysis are described in the figure captions. A p value of less than 0.05 was regarded as statistically significant. A two-tailed t-test for independent samples was used for two-group comparisons.

CHAPTER 3: RESULTS

3.1. Transgenic mice appear grossly healthy

Similar to previous descriptions of quetiapine on mice by He et al. (He, et al., 2009), after 8 months, chronically quetiapine treated transgenic and non-transgenic mice in this study appeared grossly normal and healthy. Overall, most mice in all groups stayed healthy for the 12 months of study. There was no associated morbidity/mortality with the treatment and no significant difference with respect to animals suffering from dermatitis or serious illnesses that resulted in euthanization or death (1 transgenic and 1 non-transgenic). Transgenic and non-transgenic mice with quetiapine treatment grew as well as non-treated control mice during the 12 months of life, and there was no significant difference within the transgenic groups and non-transgenic groups (Figure 9).

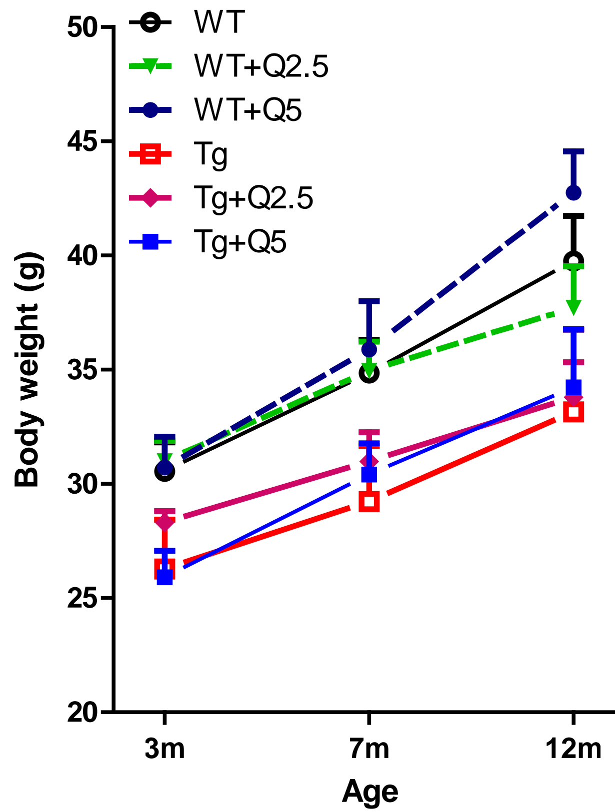


Figure 9. Transgenic and non-transgenic mice weight during the 12 months of life
Mice were weighed at 3 months (3m), 7 months (7m), and 12 months (12m) of age. Two-way Repeated Measures ANOVAs followed by Tukey's Multiple comparisons found no significant differences at 12m of age. (data shown as means ± SEM).

3.2. Quetiapine alleviates AD-like behaviours in APP/PS1 transgenic mice

The APP/PS1 AD mouse model is a mouse model in which mice develop age-onset memory deficiencies associated with Aβ production (Arendash et al., 2001; Chen et al., 2000; Holcomb, et al., 1998; King & Arendash, 2002). Since quetiapine alleviates psychotic symptoms and improves cognition in Parkinson's disease (Juncos, et al., 2004), we evaluated whether quetiapine treatment protects mice from Aβ-associated behavioural impairments using several behavioural tests.

3.2.1 Quetiapine reduces heightened anxiety shown in transgenic mice in the open field test

The open field test was used to measure anxiety in APP/PS1 transgenic mice. The anxiety level of mice in an unfamiliar environment of an open field was associated with the ratio of the ambulation inside the inner squares over the total ambulation, with lower values indicating higher levels of anxiety. Lower values of time spent inside the box indicated higher levels of anxiety. Decreased interaction with the Inner zone was taken as a measure of heightened anxiety, as described in the methods (Section 2.3.1). To evaluate whether quetiapine treatment significantly influenced results, data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age were subjected to one-way ANOVA with Dunnett's *post hoc* test.

Transgenic mice showed heightened anxiety compared to non-transgenic mice (WT), as measured by spending less time in the Inner zone, which demonstrated an anxiety-like phenotype that developed in AD mice at 12 months of age (15.23 ± 3.200 vs. 26.88 ± 3.697, $p < 0.05$,

Figure 10A). Due to high variability, quetiapine did not significantly affect the time spent in the Inner zone by transgenic mice (26.58 ± 4.206 vs. 15.23 ± 3.200, $p < 0.0753$, Figure 10A).

General locomotor activity was examined by looking at total distance travelled in the open field test. To evaluate whether quetiapine or genotype significantly influenced results, data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age were subjected to one-way ANOVA. There was no difference in the total ambulation among all the groups [$F(3,26) = 0.3766$, $p = 0.7706$, Figure 10B].

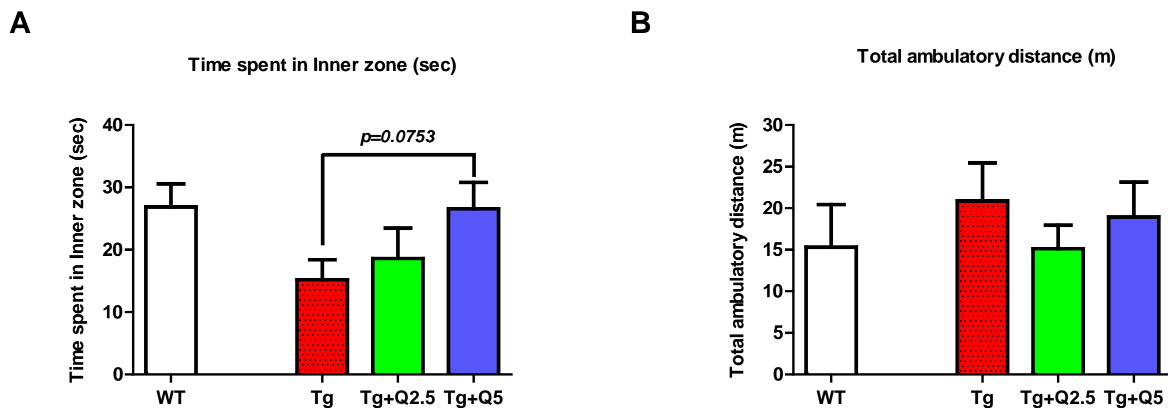


Figure 10. Quetiapine reduces heightened anxiety shown in transgenic mice in the open field test
(**A**) Time spent in Inner zone. To evaluate whether quetiapine treatment significantly influenced this results, data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age was subjected to one-way ANOVA with Dunnett's *post hoc* test. Transgenic mice showed heightened anxiety compared to WT, as measured by less time spent in the Inner zone. However, quetiapine did no significant affect the time spent in the Inner zone by transgenic mice. (**B**) Total distance travelled. To evaluate whether quetiapine or genotype significantly influenced results, data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age was analyzed with one-way ANOVA. There was no difference in the total ambulation among the all the groups. Results are expressed as means ± SEM.

3.2.2 Quetiapine prevents impaired spontaneous alternations performance shown in transgenic mice in the Y-Maze test

The Y-maze was used to examine spatial working memory. As described in the methods (Section 2.3.2), total arm entries were analyzed as a measure of general locomotor activity to differ across groups. Mice whose total entrance number was less than 15 times during the test were not taken into the final data. When prior spontaneous alternations performance was taken into account, no differences in total arm entries were found [$F(3,21) = 0.1175$, $p = 0.9488$, Figure 11A]. Thus this factor was unlikely to confound the Y-maze results.

The percentage of spontaneous alternations, as calculated by the number of alternations performed out of the total number of all alternations possible based upon the number of arm entries per mouse, was the unit of measure. Data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age were subjected to one-way ANOVA [$F(3,23) = 3.770$, $p = 0.0246$, Figure 11B]. Since differences were found, a Tukey's multiple comparisons *post hoc* analysis was conducted. The alternation behaviour in the transgenic mice was significantly less than that in the non-transgenic wild type mice (49.98 ± 6.932 vs. 70.08 ± 3.136, $p < 0.05$, Tg vs. WT, Figure 11B). Quetiapine (5 mg/kg/day) significantly prevented the decrease of the alternation performance in the transgenic mice (71.54 ± 5.842 vs. 49.98 ± 6.932, $p < 0.05$, Tg+Q5 vs. Tg, Figure 11B). However, the lower dosage of quetiapine (2.5 mg/kg/day) did not have a significant increase when compared with transgenic mice (60.92 ± 4.741 vs. 49.98 ± 6.932, $p > 0.1$, Tg+Q2.5 vs. Tg, Figure 11B).

Y Maze

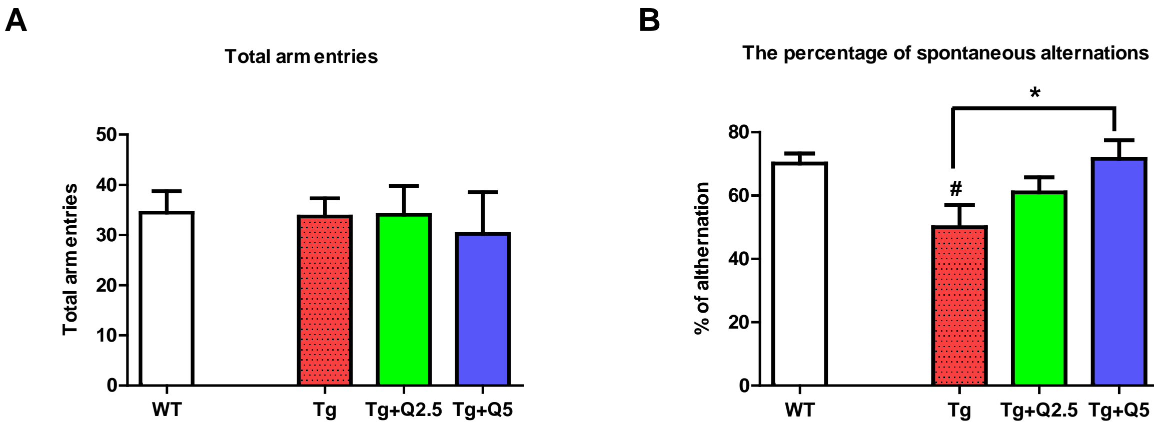


Figure 11. Quetiapine prevents impaired spontaneous alternations performance shown in transgenic mice in the Y-Maze test
(**A**) All mice have the same level of general locomotor activity in the Y-maze. Data shown as means ± SEM. Data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups were subjected to one-way ANOVA. No difference in total arm entries were found in all groups at 12m of age ($p > 0.05$). Thus the locomotion was unlikely to confound the Y-maze results. (**B**) The percentage of spontaneous alternations. Data shown as means ± SEM. All data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups were subjected to one-way ANOVA followed by a Turkey's multiple comparisons *post hoc* analysis. The alternation behaviour in the transgenic mice was less than that in the non-transgenic wild type mice ($^{\#}p < 0.05$, Tg vs. WT). Quetiapine (5 mg/kg/day) significantly prevented the decrease of the alternation performance in the transgenic mice ($^{*}p < 0.05$, Tg+Q5 vs. Tg). However, the lower dosage of quetiapine (2.5 mg/kg/day) did not have a significant increase when compared with transgenic mice.

3.2.3 Quetiapine alleviates deficiencies in spatial memory shown in transgenic mice in the Morris water maze test

The Morris water maze was used to examine potential differences in spatial learning and memory in APP/PS1 transgenic mice. It is the most widely accepted behavioural test of hippocampus-dependent spatial learning and memory (R. Morris, 1984). The hidden platform tests on Days 1 through 5 examined task acquisition, while the probe trial on Day 6 examined recall.

In the hidden platform trials, spatial memory acquisition in the mice was assessed by the escape latency in the hidden platform test of five days of training trials. A two-way Repeated Measures ANOVAs showed that quetiapine and training [$F(4,80) = 20.14$, $p < 0.0001$] have effects on the escape latency. A *post hoc* analysis indicated that the escape latency on Day 5 for the transgenic mice was higher than that for the non-transgenic wild-type mice (30.333 ± 3.377 vs. 18.616 ± 2.291, Tg vs. WT, $p<0.05$, Figure 12), and that quetiapine (5 mg/kg/day) significantly prevented the increase of escape latency in the transgenic mice (17.279 ± 2.470 vs. 30.333 ± 3.377, $p < 0.05$, Tg+Q5 vs. Tg, Figure 12). However, no significant differences were found between quetiapine (2.5 mg/kg/day) treated transgenic mice and non-treated transgenic mice (22.658 ± 3.629 vs. 30.333 ± 3.377, $p < 0.05$, Tg+Q5 vs. Tg, Figure 12).

The transgenic mice showed impaired acquisition of spatial learning. These learning memory impaired transgenic mice failed to use available distal cues to locate the submerged escape platform, indicated as a slower improvement in the escape latency across consecutive trials (red line, Figure 12) when compared with the performance of non-transgenic wild type mice. In contrast, quetiapine (5 mg/kg/day) treated transgenic mice were able to locate the hidden platform, as demonstrated by significantly reduced escape latency across trials (blue line, Figure 12). Meanwhile quetiapine (2.5 mg/kg/day) treated transgenic mice did not show a significantly decreased escape latency trend (green line, Figure 12).

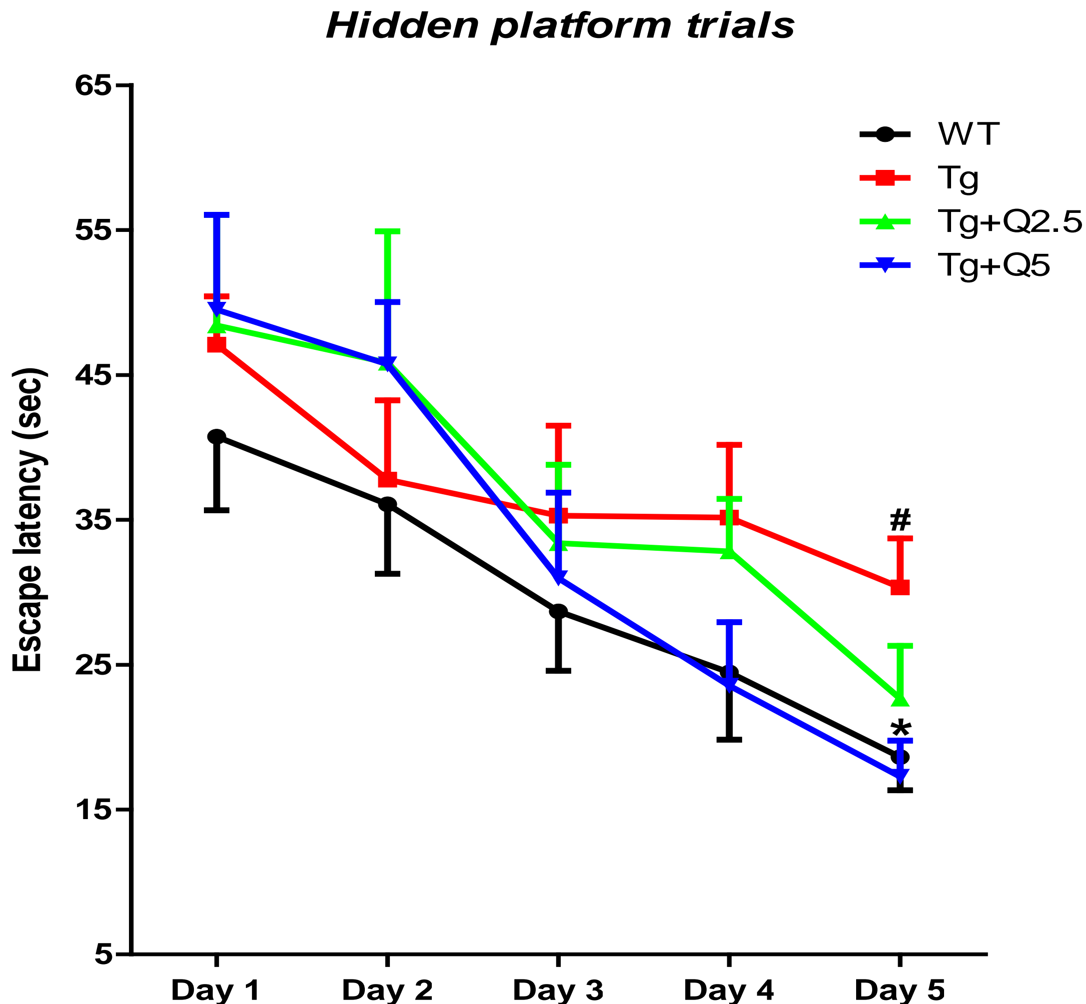


Figure 12. Quetiapine treated transgenic mice shows reduced escape latencies in water maze hidden platform trials
Data shown as means ± SEM. Data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups were subjected to two-way Repeated Measures ANOVAs followed by a *post hoc* analysis. On Day 5 transgenic mice showed higher escape latency than the non-transgenic wild type mice; and quetiapine (5 mg/kg /day) significantly prevented the increase of escape latency in the transgenic mice. However, no significant differences were found between quetiapine (2.5 mg/kg/day) treated transgenic mice and non-treated transgenic mice. n = 5–10 in each group; $^{\#}p < 0.05$ Tg vs. WT; $*p < 0.05$ Tg+Q5 vs. Tg.

In the probe test, spatial memory retention was assessed in two units of measure, both the time spent in the target quadrant (the platform had been located during the training trials) (He, et

al., 2009) and the number of target area (the previous hidden platform location) crossings (Qing, et al., 2008) taken as measures of degree of spatial memory. When time spent in the target quadrant was analyzed, data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age were subjected to one-way ANOVA [$F(3,16) = 4.667$, $p = 0.0158$, Figure 13A]. Since differences were found, a Dunnett's multiple comparisons *post hoc* analysis was conducted. The transgenic mice spent less time searching for the target quadrant compared to the non-transgenic wild type mice (9.500 ± 3.380 vs. 19.94 ± 1.578, Tg vs. WT, $p < 0.05$, Figure 13A). Quetiapine (5 mg/kg/day) significantly prevented the decrease of time spent in the target quadrant in the transgenic mice (20.05 ± 2.60 vs. 9.50 ± 3.38, $p < 0.05$, Tg+Q5 vs. Tg, Figure 13A). However, the lower dosage of quetiapine (2.5 mg/kg/day) did not have a significant increase in time spent searching for the target quadrant as compared to transgenic mice (11.43 ± 3.507 vs. 9.500 ± 3.380, $p > 0.1$, Tg+Q2.5 vs. Tg, Figure 13A). While the number of target area crossings was analyzed, all data were subjected to one-way ANOVA [$F(3,17) = 1.313$, $p = 0.3027$, Figure 13B]. All mice had a similar number of target area crossing in the probe trial. Quetiapine had no effects on the number of times the mice traveled.

Probe trial

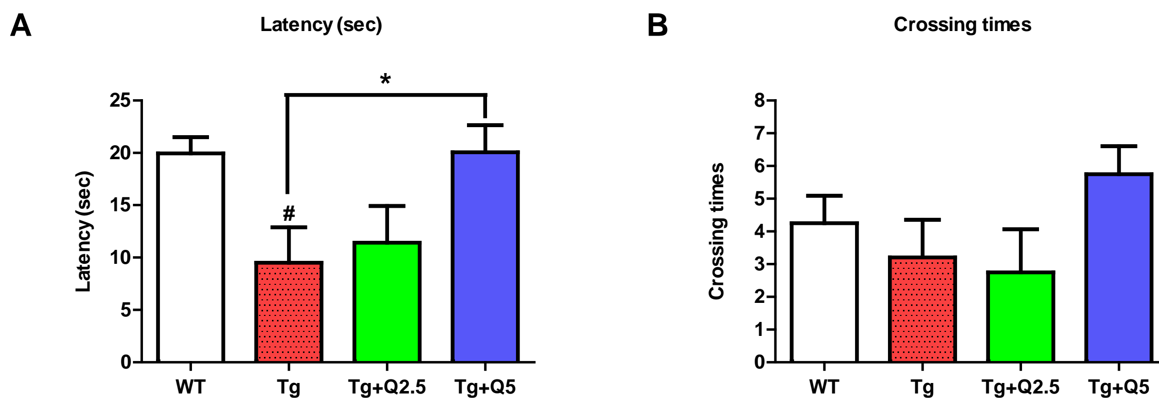


Figure 13. Quetiapine prevents the decrease of time spent in the platform quadrant shown in transgenic mice in the probe trial

(**A**) Time spent in the target quadrant. Data shown as means ± SEM. Data from Con, Tg, Tg+Q2.5 and Tg+Q5 groups at 12 months of age were subjected to one-way ANOVA followed by a Dunnett's multiple comparisons *post hoc* analysis. Transgenic mice spent less time searching for the target quadrant compared to the non-transgenic wild type mice ($^{\#}p < 0.05$, Tg vs. WT). Quetiapine (5 mg/kg/day) significantly prevented the decrease of time spent in the target quadrant in the transgenic mice ($^{*}p < 0.05$, Tg+Q5 vs. Tg). However, lower dosage of quetiapine (2.5 mg/kg/day) did not result in these mice significantly increasing time spent searching for the target quadrant as compared to transgenic mice. (**B**) The number of target area crossings. Data shown as means ± SEM. Data were subjected to one-way ANOVA. No significant differences were seen, in either the higher dosage (blue bar) or lower dosage (green bar) quetiapine ($p > 0.05$).

Overall, quetiapine treatment not only significantly promoted learning during the hidden-platform trials but also significantly promoted improved memory retention during the probe trials 24 hours after the last trial (Figure 12, 13).

In the Morris water maze test, all the observed deficits in the acquisition trials and in the probe test were not attributable to non-cognitive factors, because there was no difference in term of the swimming speed and escape latencies pattern (Figure 14) amongst all the groups in the acquisition trials and probe test. Moreover, quetiapine treatment was stopped 24 hours prior to all the behavioural tests and there was no treatment during the behavioural tests. Therefore, the effect of quetiapine on the behavioural performances in the mice was not just acute, but also long lasting.

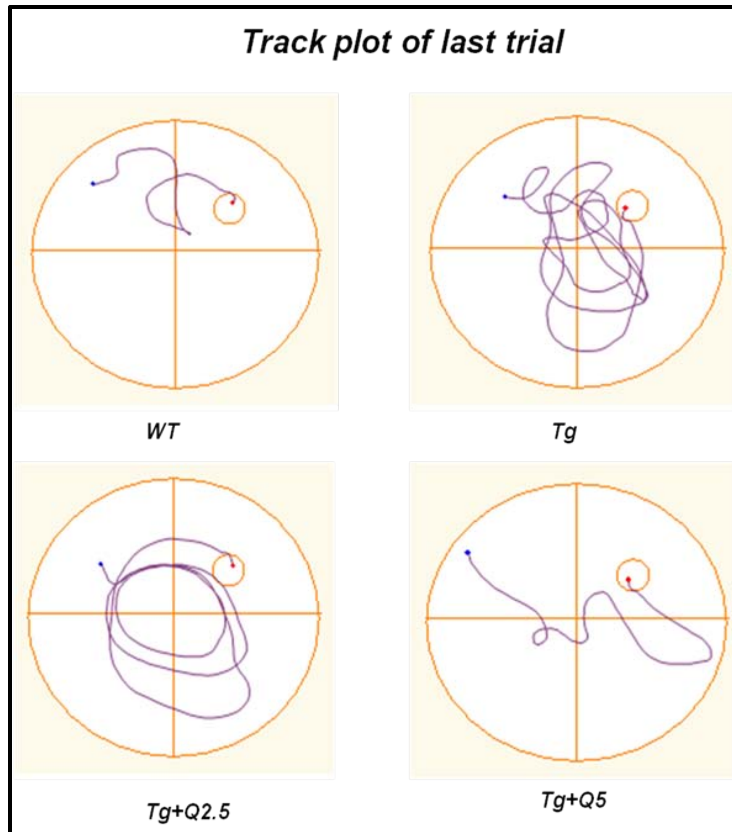


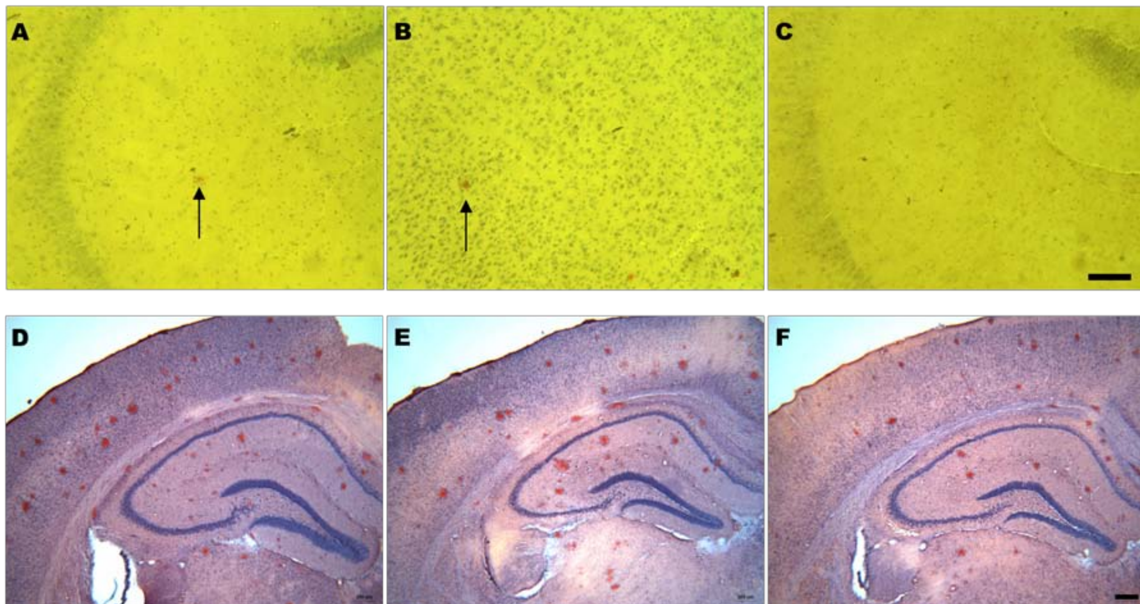
Figure 14. Mice perform similarly in the water maze the hidden platform trials
There was no significant difference in term of the swimming speed and escape latencies pattern among all the groups in the acquisition trials and probe test. Representative tracking plot of last hidden platform trial.

3.3. Quetiapine decreases the number of Aβ plaques in the brain of transgenic mice

A pathologic hallmark of AD is the formation of Aβ plaques. Aβ plaque deposition in the cortex and hippocampus of the APP/PS1 transgenic mice start to be detectable as early as 2.5 months of age (Blanchard et al., 2003; Ding, et al., 2008; He, et al., 2009). At 3 months of age a moderate level of pre-existing Aβ deposition in the cortex and hippocampus was demonstrated in our previous study (Figure 15 A: Hippocampus and B: Cortex). There were no detectable plaques in the non-transgenic mice up to 12 months of age (Figure 15C). Quetiapine has been shown to

inhibit fibrillar aggregation of Aβ *in vitro* (Xu et al., 2008). However, its therapeutic effect on Aβ deposition in the transgenic mouse model has not been fully documented.

Coronal brain sections were stained by a Congo red kit following the standard protocol accompanying the product. The number of total Congo Red-positive plaques in the transgenic mice was quantified. One-way ANOVA showed a significantly change on the number of total plaques in the cortex of 12 months old transgenic female and male mice, $F(2, 12) = 4.228$, $p = 0.0408$ and hippocampus, $F(2, 12) = 4.422$, $p = 0.0364$. A *post hoc* analysis indicated that quetiapine (5 mg/kg/day) decreased the number of plaques in the cortex and hippocampus of transgenic mice. Figure 15 (D-F) illustrates representative photomicrographs of Congo Red staining showing Aβ plaques in the brain of 12 months old APP/PS1 transgenic mice.



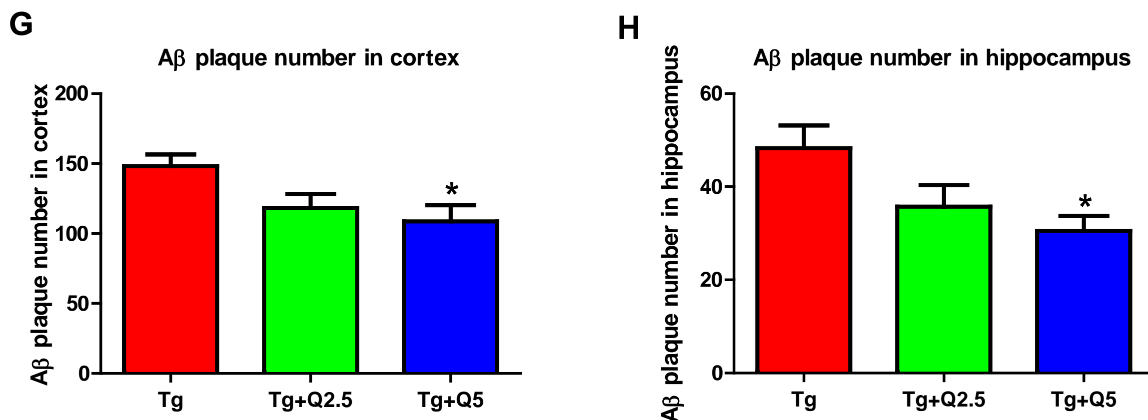


Figure 15. Effects of chronic quetiapine administration on the number of Aβ plaques in the brains of transgenic mice
(**A-C**) Stereological assessment of Aβ plaques burden in 3 month old transgenic mice (**A** hippocampus and **B** cortex of transgenic mice) compared with wild type mice (**C** cortex of wild type counterparts) using Congo red stainning. (**D-F**) Representative photomicrographs of Congo Red staining showing Aβ plaques in the 12 months old (**D**) transgenic, (**E**) transgenic + quetiapine 2.5 mg/kg/day (**F**) and transgenic + quetiapine 5 mg/kg/day mice. Stereological quantification of Aβ plaque number in cortex (**G**) and hippocampus (**H**). The scale bars represent 90μm (**A-C**) and 200μm (**D-F**). Results are expressed as means ± SEM; n = 4-6 in each group; $*p < 0.05$ vs. Tg.

3.4. Quetiapine decreases $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in transgenic mice

Since the principal components of Aβ plaques are Aβ peptides, total $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the transgenic mice were measured.

In the frontal cortex, one-way ANOVA showed that quetiapine produced a significant change in the level of total $A\beta_{1-40}$ [$F(2,6) = 5.120$, $p = 0.0504$, Figure 16A], and a Dunnett's multiple comparisons *post hoc* analysis indicated that quetiapine (5 mg/kg/day) significantly decreased the total $A\beta_{1-40}$ level (56.68% ± 4.537% relatively to Tg 100%, $p < 0.05$, Figure 16A) in the 12 months old transgenic mice. Meanwhile, quetiapine showed a tendency to decrease the total $A\beta_{1-42}$ level in the 12 months old transgenic mice, although the decrease was not significant [$F(2,6) = 3.948$, $p = 0.0711$, Figure 16B].

In the hippocampus, one-way ANOVA showed that quetiapine produced a significant change in the level of total Aβ$_{1-40}$ [$F(2,8) = 5.429$, $p = 0.0324$, Figure 16C]. Since differences were found, a Tukey's multiple comparisons *post hoc* analysis was conducted. Quetiapine (5 mg/kg/day) significantly decreased the total Aβ$_{1-40}$ level (69.59% ± 5.732% relatively to Tg 100%, $p < 0.05$, Figure 16C) in the 12 months old transgenic mice. Meanwhile, quetiapine showed a tendency to decrease the total Aβ$_{1-42}$ level in the 12 months old transgenic mice, although the decrease was not significant [$F(2,9) = 2.247$, $p = 0.1617$, Figure 16D].

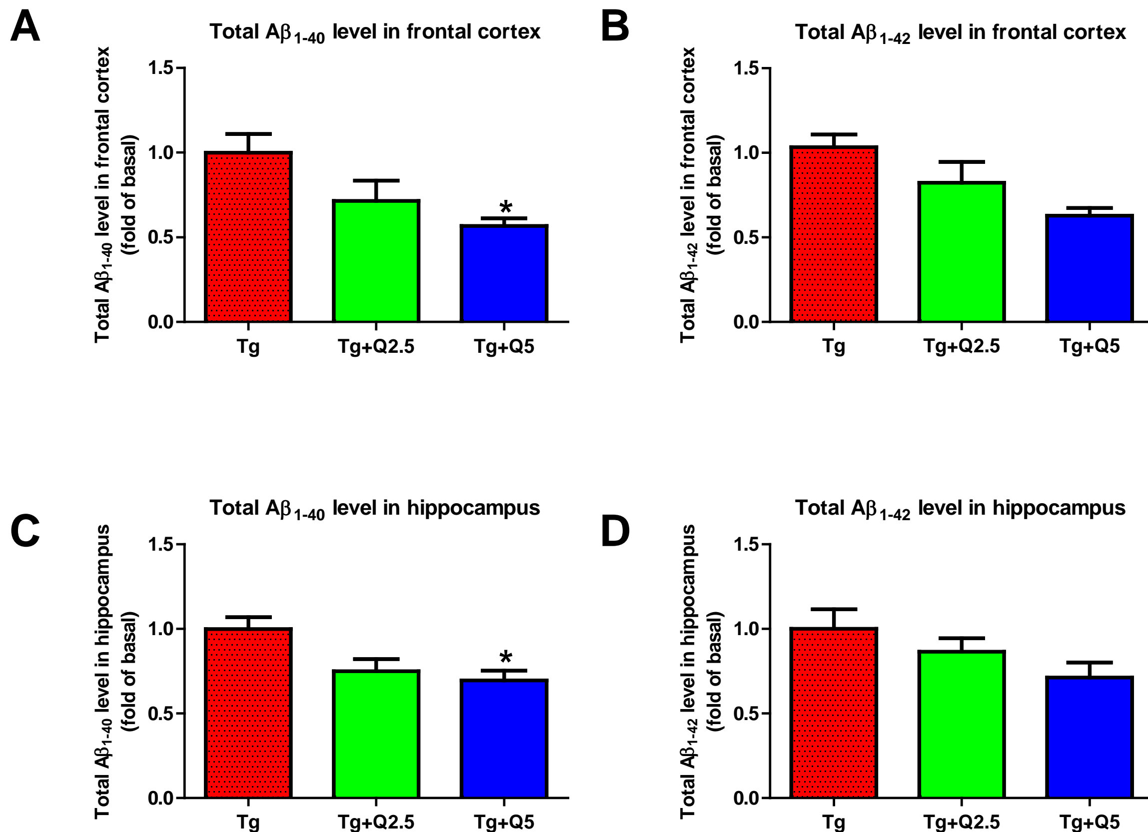


Figure 16. Effects of chronic quetiapine administration on levels of total Aβ$_{1-40}$ and Aβ$_{1-42}$ in the brains of transgenic mice
(**A-B**) Assessment of total Aβ$_{1-40}$ and Aβ$_{1-42}$ peptide contents in frontal cortical tissues of untreated or treated mice using ELISA assay. One-way ANOVA followed by a Dunnett's multiple comparisons *post hoc* analysis showed quetiapine (5 mg/kg/day) significantly decreased the total Aβ$_{1-40}$ level (*$p < 0.05$, Tg+Q5 vs. Tg). No significant differences were seen in total Aβ$_{1-42}$ level ($p > 0.05$). (**C-D**) Assessment of total Aβ$_{1-40}$ and Aβ$_{1-42}$ peptide contents in

hippocampal tissues of untreated or treated mice using ELISA assay. One-way ANOVA followed by a Turkey's multiple comparisons *post hoc* analysis showed quetiapine (5 mg/kg/day) significantly decreased the total $A\beta_{1-40}$ level (*$p < 0.05$, Tg+Q5 vs. Tg). No significant differences were seen in total $A\beta_{1-42}$ level ($p > 0.05$). Results are expressed as means ± SEM; n = 3–5 in each group.

Since soluble Aβ has been considered the center of AD pathogenesis, detergent soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ levels of brain samples and cerebrospinal fluid (CSF) in the transgenic mice were measured with Aβ ELISA kit.

In the frontal cortex, one-way ANOVA showed that quetiapine produced a significant change in the level of soluble $A\beta_{1-40}$ [$F(2,6) = 7.774$, $p = 0.0216$, Figure 17A], and a Turkey's multiple comparisons *post hoc* analysis indicated that both 2.5 mg/kg/day and 5 mg/kg/day of quetiapine significantly decreased the soluble $A\beta_{1-40}$ level (Tg+Q2.5: 39.26% ± 11.60% and Tg+Q5: 41.58% ± 3.304% relatively to Tg 100%, $p < 0.05$, Figure 17A) in the 12 months old transgenic mice. Meanwhile, one-way ANOVA showed that quetiapine produced a significant change in the level of soluble $A\beta_{1-42}$ [$F(2,6) = 6.774$, $p = 0.0289$, Figure 17B]. Turkey's multiple comparisons *post hoc* analysis indicated that both 2.5 mg/kg/day and 5 mg/kg/day of quetiapine significantly decreased the soluble $A\beta_{1-42}$ level (Tg+Q2.5: 45.39% ± 14.25% and Tg+Q5: 42.86% ± 3.395% relatively to Tg 100%, $p < 0.05$, Figure 17B)

In the hippocampus, one-way ANOVA showed that quetiapine produced a significant change on the level of soluble $A\beta_{1-40}$ [$F(2,8) = 12.31$, $p = 0.0036$, Figure 17C]. Tukey's multiple comparisons *post hoc* analysis showed both 2.5 mg/kg/day and 5 mg/kg/day of quetiapine significantly decreased the soluble $A\beta_{1-40}$ level (Tg+Q2.5: 65.34% ± 4.233% and Tg+Q5: 43.02% ± 5.442% relatively to Tg 100%, $p < 0.01$, Figure 17C) in the 12 months old transgenic mice. Meanwhile, one-way ANOVA showed that quetiapine produced a significant change on the level of soluble $A\beta_{1-42}$ [$F(2,11) = 4.252$, $p = 0.0428$, Figure 17D], and a Dunnett's multiple

comparisons *post hoc* analysis indicated that quetiapine (5 mg/kg/day) significantly decreased the soluble $A\beta_{1-42}$ level (82.30% ± 3.156% relatively to Tg 100%, $p < 0.05$, Figure 17D).

In the CSF, a two-tailed *t*-test showed that quetiapine (5 mg/kg/day) significantly decreased the level of soluble $A\beta_{1-40}$ but not $A\beta_{1-42}$ in transgenic mice (Figure 17E). The Aβ 40 level was reduced to 43.98% ± 14.22% relatively to Tg ($p < 0.05$).

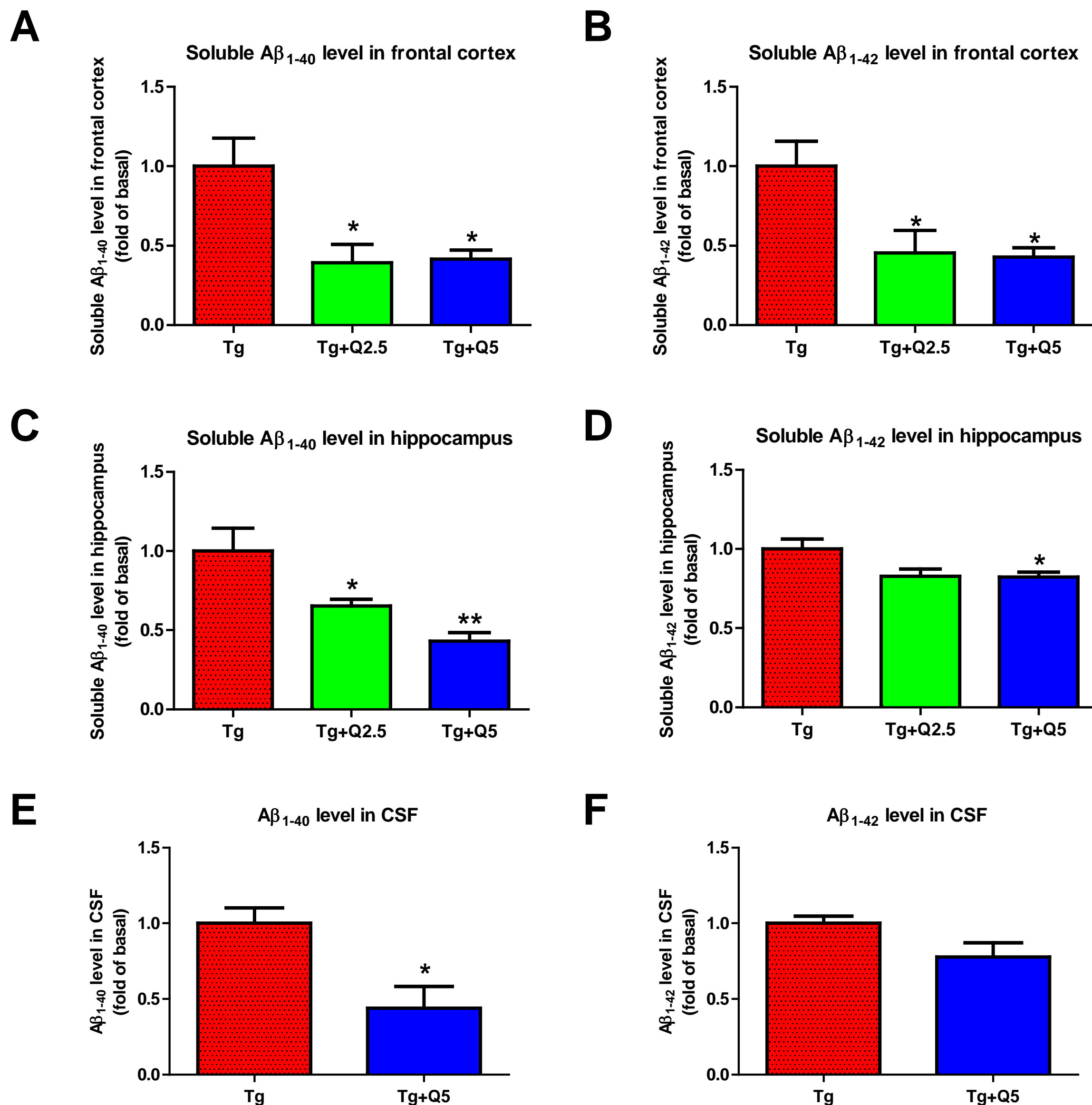


Figure 17. Effects of chronic quetiapine administration on levels of soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ in the brains and CSF of transgenic mice

(**A-B**) Assessment of soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ in frontal cortex using ELISA assay. One-way ANOVA followed by a Turkey's multiple comparisons *post hoc* analysis showed quetiapine (2.5 and 5 mg/kg/day) significantly decreased the soluble $A\beta_{1-40/42}$ level (*$p < 0.05$, Tg+Q2.5 vs. Tg; Tg+Q5 vs. Tg). (**C-D**) Assessment of soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ in hippocampal tissues using ELISA assay. One-way ANOVA followed by a Turkey's multiple comparisons *post hoc* analysis showed quetiapine (2.5 and 5 mg/kg/day) significantly decreased the soluble $A\beta_{1-40}$ level (*$p < 0.05$, Tg+Q2.5 vs. Tg; **$p < 0.01$, Tg+Q5 vs. Tg). One-way ANOVA followed by a Dunnett's multiple comparisons *post hoc* analysis showed quetiapine (5 mg/kg/day) significantly decreased the soluble $A\beta_{1-42}$ level (*$p < 0.05$, Tg+Q5 vs. Tg). (**E-F**) Assessment of soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ in CSF using ELISA assay. A two-tailed *t*-test showed that quetiapine (5 mg/kg/day) significantly decreased the level of soluble $A\beta_{1-40}$ but not $A\beta_{1-42}$ in transgenic mice CSF. Results are expressed as means ± SEM; n = 3–5 in each group.

3.5. Quetiapine inhibits GSK3β activity in transgenic mice

The involvement of APP in the mechanism of Aβ deposition is well documented (Neve, Rogers, & Higgins, 1990). APP can be processed through the amyloidogenic pathway cleaved by BACE and γ-secretase that liberates the Aβ peptide. To determine whether quetiapine influences the production of full length APP, brain tissues of frontal cortex and hippocampus from quetiapine-treated and non-treated transgenic mice were subjected to a Western blot analysis using an anti-APP full length antibody. As shown in Figure 18A (row 1), no apparent decrease in the production of full length APP was observed in the quetiapine treated transgenic mice compared with the transgenic control mice. One-way ANOVA analysis showed no significant difference in the cortex of transgenic female and male mice, $F(2, 11) = 1.247$, $p = 0.3249$ and hippocampus, $F(2, 11) = 0.1272$, $p = 0.8818$, of 12 months old transgenic mice.

Given the important role of PS1 on Aβ production in APP processing (Duff, et al., 1996), we determined PS1 in the brain tissues by Western blotting using an antibody against PS1. As shown in Figure 18A (row 2), no apparent decrease in the production of PS1 was observed in the quetiapine treated transgenic mice compared with the transgenic control mice. Quantitative analysis showed no significant change in both frontal cortex and hippocampus in the transgenic

mice at 12 months of old. These findings suggest that quetiapine influences Aβ production via a mechanism beyond modulating the expression of APP and PS1.

Tau is the major microtubule associated protein (MAP) of a mature neuron and stabilizes microtubules. Hyperphosphorylation of tau plays an important role in the pathogenesis of AD. Given the beneficial role of quetiapine in Aβ deposition/production, we attempted to determine a possible role of quetiapine in tau expression in transgenic mice. Tau-1 expression was assessed by Western blotting with a tau-1 antibody, recognizing the nonphosphorylated tau at Ser198/Ser199/Ser202. As shown in Figure 18A (row 3), no significant difference in the tau-1 levels was observed between groups.

GSK3β links many of the major neuropathological mechanisms in AD. Increased levels of GSK3β have been found in AD brains, and neurotoxic Aβ in turn activates GSK3β. Given the inhibitory role of quetiapine in the Aβ production, we attempted to determine whether quetiapine plays a role in regulating GSK3β. As described in the introduction (Section 1.4), GSK3β activity is inhibited by its phosphorylation at the serine 9 (S^9) or activated through the phosphorylation at tyrosine 216 sites (Y^{216}) (Ding, et al., 2008; Qing, et al., 2008). Therefore, we examined the levels of phosphorylated GSK3β using specific antibodies. Thus, we examined the levels of total and phosphorylated GSK3β using specific antibodies, respectively. Brain tissues of transgenic mice were subjected to a Western blot analysis. As shown in Figure 18A (row 6), a robust enhancement of GSK3β phosphorylation at Ser9 was observed in both the frontal cortex and hippocampus in the quetiapine treated transgenic mice relative to the non-treated transgenic control mice. Non significant difference in the total GSK3β was found between quetiapine treated and non-treated transgenic mice (Figure 18A, row 5). Qantitative analysis of the Western blot bands of the phosphorylated GSK3β at Ser9 indicated an ~70% increase in the frontal cortex

and an ~65% increase in the hippocampus in the quetiapine treated transgenic mice relative to the non-treated transgenic control mice. These results suggest that quetiapine treatment downregulates GSK3β acitivity.

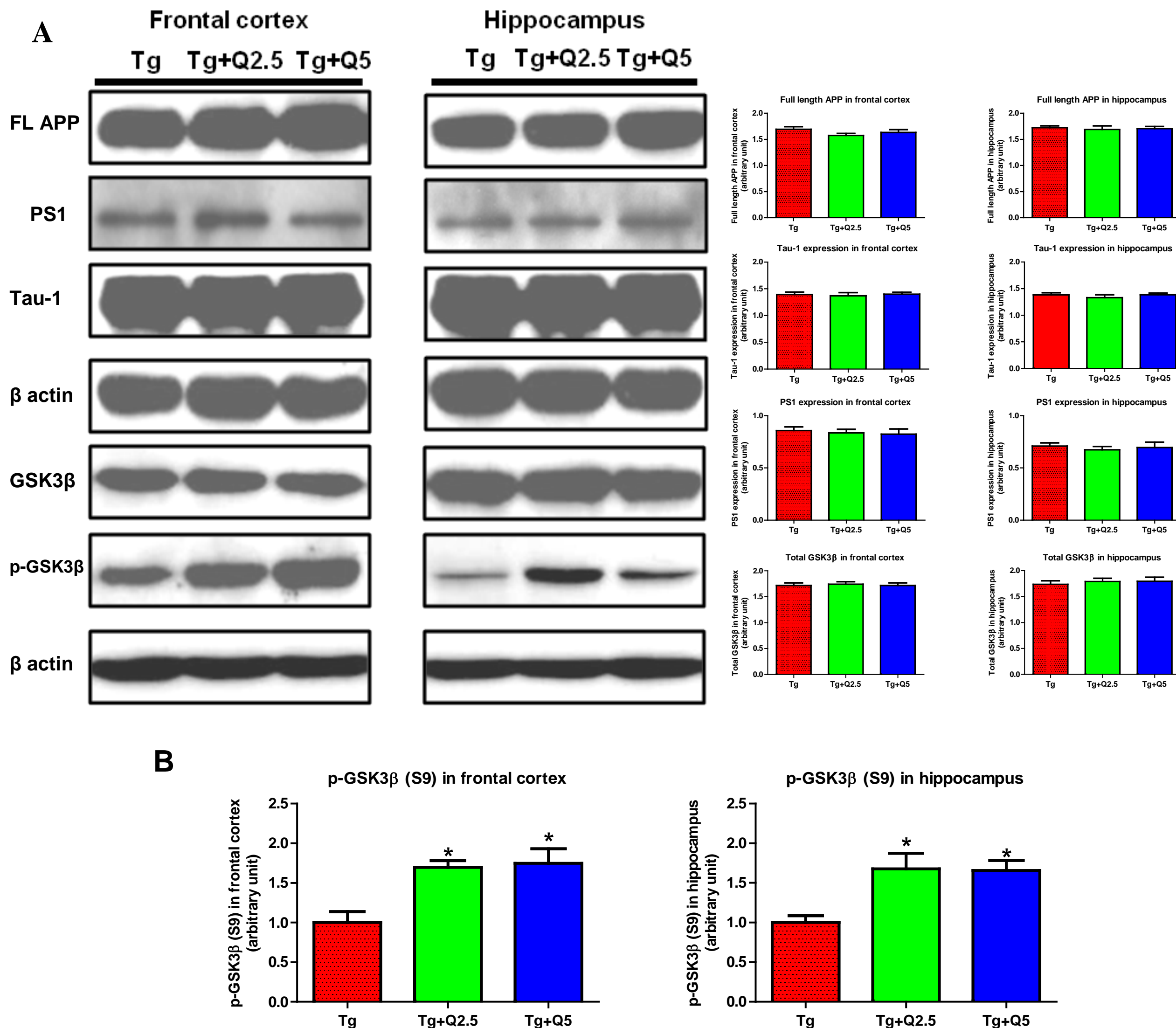


Figure 18. Chronic quetiapine administration inhibits GSK3β activity in transgenic mice (**A**) Western blots of brain extracts from the Tg, Tg+Q2.5 and Tg+Q5 mice were probed with antibodies to full length APP, PS1, Tau-1, phospho-S^9-GSK3β, total GSK3β and β-actin (as a loading control). The left panel is representative of Western blot bands made from frontal cortex and hippocampus tissues of mice. The right panel is a bar graph showing the quantification of corresponding immunoreactive bands in Western blot. (**B**) Quantification of phospho-S^9-GSK3β immunoreactive bands in Western blot. Results are expressed as means ± SEM; n = 4-6 mice per

group; *$p < 0.05$ vs. Tg.

3.6. Quetiapine inhibits activation of astrocytes in transgenic mice

In brains of both AD patients (Mancardi, Liwnicz, & Mandybur, 1983) and transgenic AD mice (Wirths et al., 2010), activated astrocytes are often seen in and around the area of amyloid plaques (Itagaki, McGeer, Akiyama, Zhu, & Selkoe, 1989; Matsuoka, et al., 2001). We wondered whether the appearance of biological markers associated with AD-like disease in mice was also delayed by quetiapine. Initially, we evaluated the reactivity of astrocytes in the transgenic mouse model of AD. Utilizing the glial fibrillary acidic protein (GFAP) antibody, which recognizes activated astrocytes (Cohen et al., 2009; Mancardi, et al., 1983), there was a notably increase in activated astrocytes positive cells in the frontal cortex and hippocampus of transgenic mice compared to age-match wild type countparts (Figure 19 A and B). In addition, consistent with the immunohistochemistry results, Western blot results also demonstrated that the protein level of GFAP was significantly increased in transgenic mice compared to wild-type mice (Figure 19D).

In contrast, the GFAP immunoreactivity was remarkably decreased in the quetiapine treated transgenic mice compared with the non-treated counterparts (Figure 19 B, C and D). This reduction was apparent both in the frontal cortex and hippocampus, indicating that neuroinflammation is reduced after quetiapine treatment. More interestingly, while GFAP positive cells in the frontal cortices of transgenic mice was largely diffuse, cortical GFAP staining of quetiapine treated transgenic mice appeared to be focal (compare Figure 19 B and D), which may suggest that neuroinflammation within quetiapine treated brains is confined to smaller areas than in the brains of non-treated transgenic animals. In addition, this reduction was apparent in our Western blot results. As shown in Figure 19E, quetiapine treatment prevented the

up-regulation of GFAP protein content in transgenic mouse brains. Taken together, these observations confirm the finding that quetiapine treatment suppresses the prolonged inflammation associated with AD progression.

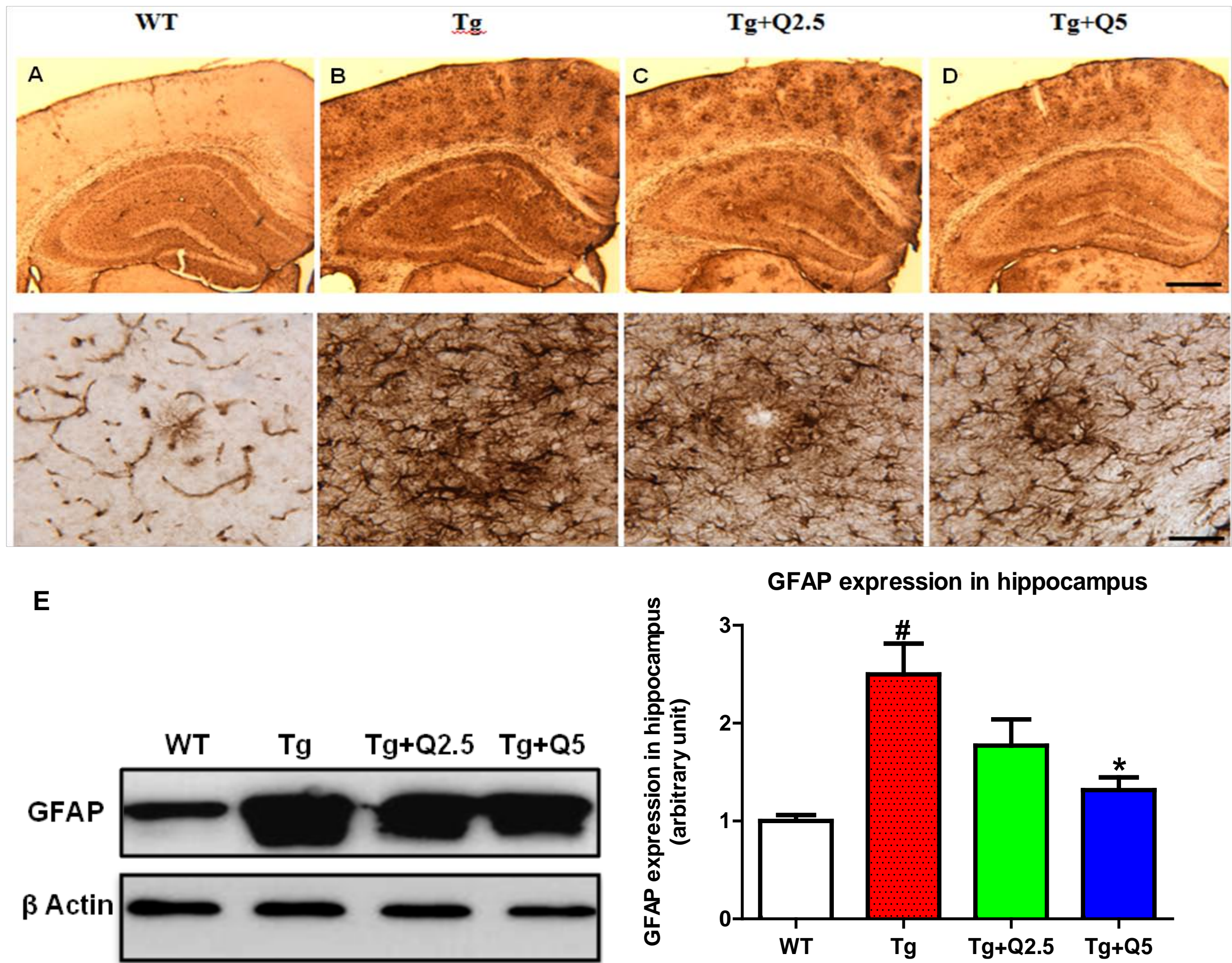


Figure 19. Chronic quetiapine administration alleviates astrogliosis in transgenic mice
(**A-D**) Immunohistochemistry using GFAP antibody indicated reduced astroglial cell densities in brain sections. Lower panel shows the higher magnification of GFAP positive cells. The scale bars represent 500µm (upper pannel) and 50µm (lower panel) (**E**) Representatives of Western blot bands made from hippocampus tissues of mice. Bar graph showing the quantification of GFAP immunoreactive bands in Western blot. One-way ANOVA followed by a Turkey's multiple comparisons *post hoc* analysis showed quetiapine (5 mg/kg/day) significantly attenuated the increased GFAP expression level in transgenic mice. Results are expressed as means ± SEM; n = 4-6 mice per group; $^{*}p < 0.05$ Tg+Q5 vs. Tg; $^{\#}p < 0.01$, Tg vs. WT.

3.7. Quetiapine attenuates synaptic protein loss in transgenic mice

Neuronal degeneration is an additional feature of AD (Hamos, et al., 1989). Given the inhibitory effect of quetiapine on glial activation in the CNS, we attempted to determine a possible role of quetiapine on neuronal integrity in transgenic mice using a synaptic marker synaptophysin (SYP) expressed on the presynaptic vesicles. Brain tissues of hippocampus from quetiapine treated and non-treated transgenic mice were subjected to a Western blot analysis using an anti-SYP antibody. As shown in Figure 19, an apparent increase in the SYP expression was observed in the quetiapine treated transgenic mice compared with the transgenic control mice. One-way ANOVA analysis showed a significant difference in the hippocampus of transgenic female and male mice, $F(2, 11) = 5.175$, $p = 0.0261$, of 12 months old transgenic mice. Since differences were found, a Dunnett's multiple comparisons *post hoc* analysis was conducted. Quetiapine (5 mg/kg/day) significantly prevented the decrease of SYP in the transgenic mice (155.9% ± 4.154% relative to Tg 100%, $p < 0.05$, Tg+Q5 vs. Tg, Figure 20). Similarly, the lower dosage of quetiapine (2.5 mg/kg/day) had a significantly increase SYP level as compared to transgenic mice (162.3% ± 19.57% relative to Tg 100%, $p < 0.05$, Tg+Q2.5 vs. Tg, Figure 20). Together, these results suggest quetiapine might beneficially influence AD pathogenesis through decreasing the rate of neuronal degeneration in the transgenic mice.

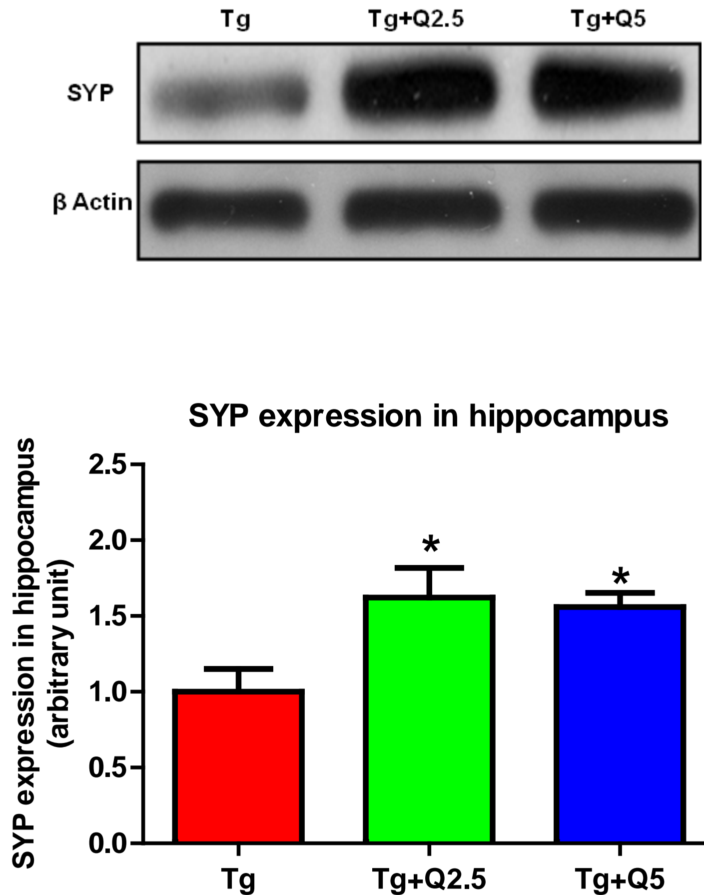


Figure 20. Chronic quetiapine administration prevents synaptic protein loss in transgenic mice

Western blots of brain extracts from the transgenic mice and quetiapine-treated transgenic mice were probed with antibodies to SYP and β actin (as a loading control). The upper panel is representative of Western blot bands made from hippocampus of mice. The bottom panel is a bar graph showing the quantification of SYP immunoreactive bands in Western blot. One-way ANOVA followed by a Dunnett's multiple comparisons *post hoc* analysis showed quetiapine (2.5 and 5 mg/kg/day) treatment significantly attenuated the decreased SYP expression level in transgenic mice. Results are expressed as means ± SEM; n = 4-6 mice per group; $*p < 0.05$ vs. Tg.

CHAPTER 4: DISCUSSION

4.1. General comments

There is increasing evidence that quetiapine has been suggested as a potential therapeutic approach to prevent or decrease Aβ associated neurodegeneration (He, et al., 2009; Scharre & Chang, 2002; Yu et al., 2009). However, the actual therapeutic role of quetiapine in AD pathology has not yet been ascertained. This thesis demonstrates that chronic quetiapine treatment, starting at 3.5 months of age till 12 months old, reduces and possibly reverses Aβ plaques formation in the frontal cortex and hippocampus. More importantly, quetiapine inhibits soluble Aβ as well in APP/PS1 transgenic mice. The quetiapine-treated transgenic mice showed a significantly decreased level of activated astroglial marker, elevated levels of neuronal markers in cortical and/or hippocampal regions, and improved spatial learning and memory, when compared with the non-treated transgnic mice. This preclinical animal study demonstrated that quetiapine can be applied as a novel treatment of memory impairment and brain pathology at the early stage of AD. This project is unique in contributing to the understanding of the pathophysiology of AD, establishing the efficacy of quetiapine in AD treatment, and suggesting a new therapeutic use for quetiapine.

The discussion in this thesis is comprised of five main sections. First, the beneficial effects of quetiapine treatment on AD pathology and associated impaired memory/anxiety-like behavioural change in the APP/PS1 transgenic mice are discussed. The second section will discuss the role of GSK3β on inhibiting Aβ production in the APP/PS1 transgenic mice. The third section presents a discussion pertaining to the effect of chronic quetiapine administration on neuroinflammation in the APP/PS1 transgenic mice. In the fourth section, a discussion of the effects of quetiapine treatment on the neuronal integrity in CNS of the transgenic mice is

presented. Last, some concerns will be raised regarding the validity and limitations of the APP/PS1 double transgenic mouse model of AD.

4.2. Effects of quetiapine on behavioural changes and AD pathology

Consistent with previous studies (Arendash, et al., 2001; Holcomb, et al., 1998; Matsuoka, et al., 2001), the APP/PS1 double transgenic mice in this study demonstrated anxiety-like behavioural changes, memory impairment and high Aβ levels and Aβ plaques compared with wild type mice at the age of 12 months. This study also demonstrated that chronic quetiapine treatment protects mice from Aβ associated behavioural impairments using several behavioural assays.

Anxiety The open field test was used to assess anxiety in APP/PS1 transgenic mice. Interestingly, without treatment, genotype significantly affected mouse performance in the open field test. Double transgenic mice showed higher anxiety levels than non-transgenic wild type controls, as seen in decreased time spent in the Inner zone overall (section 3.2.1). Chronic quetiapine administration reduced heightened anxiety level in transgenic mice with no general locomotor activity significantly affected. This suggests that quetiapine may have some level of anxiolytic effect, which affected mouse performance.

Overall, when comparing anxiety measures between different genotypes, there was evidence to suggest that a high level of Aβ in APP/PS1 transgenic mice could affect their anxiety-like behaviours (He, et al., 2009). Further anxiety-specific tests, such as the elevated plus maze (EPM) and light/dark box, could potentially help support the open field findings.

Memory The APP/PS1 transgenic mice demonstrated significant cognitive impairments in hippocampal-dependent learning and memory tasks such as the Y-maze task (section 3.2.2) and the Morris water maze task (section 3.2.3). As reported, excessive Aβ deposition in the cortex and hippocampus was associated with impaired cognitive function in this APP/PS1 transgenic

mice model (Chen, et al., 2000; He, et al., 2009), with soluble Aβ oligomers leading to synaptic dysfunction or neuronal death (J. Hardy & Selkoe, 2002). In this present study, after 8 months of quetiapine administration, impaired alternation and acquisition/retention impairment of spatial memory were prevented or possible reversed in the APP/PS1 transgenic mice. This could most likely be attributed to quetiapine's effects on decreasing the increased soluble Aβ levels and/or Aβ plaques specifically in hippocampal regions. However, it is reasonable to consider the possibility that quetiapine improved learning and memory in some way that was independent of decreasing Aβ accumulation. Because quetiapine also attenuated anxiety-like behavioural changes in the double transgenic mice, its effects on anxiety-like changes or otherwise may also contribute to the amelioration of memory and/or pathological changes.

Quetiapine may also act as a neuroprotective agent in AD. As reported in earlier studies, chronic administration of quetiapine may upregulate Bcl-2 protein level (Bai, Zhang, & Li, 2004). Bcl-2, a neuroprotective protein, plays a very important role against a variety of insults in the CNS cells. The mechanism by which quetiapine regulates spatial memory has not been delineated. A previous study has shown that quetiapine counteracts the phencyclidine induced spatial memory impairment by modulating Bcl-X_L / Bax ratio in the posterior cingulated cortex of rats (He, Xu, et al., 2006). Thus, further studies are necessary to elucidate the most probable mechanism of quetiapine on alleviating memory impairment.

4.3. Quetiapine on Aβ inhibition through GSK3β

In this thesis, it was systematically demonstrated that in the APP/PS1 transgenic mouse model, chronic quetiapine administration significantly reduced Aβ production in term of amyloid plaques and soluble Aβ and alleviated memory impairment in AD animals. The inhibitory effect of quetiapine on Aβ production may likely come from its inhibition of APP processing, because

chronic quetiapine treatment decreased the production of APP-CTFs, the direct precursor of Aβ (He, et al., 2009). In addition to the reduction of APP-CTFs after quetiapine treatment *in vivo*, a previous study from this lab has shown that quetiapine inhibited Aβ(25-35) aggregation in cell-free aqueous solutions and blocked the fibrillar aggregation of Aβ(25-35) under an electron microscope (Xu, et al., 2008). This evidence suggests that quetiapine may be involved in multiple steps of Aβ deposition. Many studies have shown that the pathogenic APP processing and metabolism/Aβ production are highly regulated by a variety of signal transduction pathway, such as, protein kinase and phosphatase (Buxbaum, Ruefli, Parker, Cypess, & Greengard, 1994), expression/function of BACE1 that is a major β-secretase involved in APP process, expression/function of PS1 that is a major component of γ-secretase, but not just limited to an altered APP expression. However, surprisingly, we did not observe a significant difference in the expression of APP or PS1 between the groups. This was in line with an earlier report showing that quetiapine had no effects on α- and γ-secretase activities (He, et al., 2009), and suggested that quetiapine modulates APP processing by other mechanisms not yet elucidated.

Several studies have provided evidence that GSK3β was highly associated with the amyloid plaque neuropathology of AD through its interactions with APP. GSK3β is believed to be a key kinase responsible for APP phosphorylation in neuronal cells (Aplin, et al., 1996; Frame & Cohen, 2001), and it was believed that phosphorylation at APP Thr 668 may promote the BACE1 cleavage to increase Aβ production (Lee, et al., 2003). In turn, neurotoxic Aβ activates GSK3β. Unlike most protein kinases, GSK3β is constitutively active in all cells and can be inactivated by the phosphorylation of single serine residue, serine 9 (GSK3β), in its respective regulatory amino-terminal domain. This study found that quetiapine inhibited GSK3β activity by altering its phosphorylation state. Downregulation of GSK3β activity by quetiapine may prevent

APP processing by inhibiting its phosphorylation in the neuronal cells of the APP/PS1 transgenic mice, which results in inhibiting Aβ production. However, we could not exclude other possible pathways regulated by GSK3β on the Aβ inhibition of quetiapine treatment. For example, GSK3β is regulated by the Wnt signalling pathway and controlling β-catenin levels and transcriptional responses (Qing, et al., 2008). Qing et al. have shown that Aβ reduction may be regulated by GSK3β/β-catenin mediated γ-secretase cleavage. Unexpectedly, quetiapine treatment did not affect the γ-secretase activity and PS1 expression, although GSK3β was negatively regulated. This discrepancy may be attributable to different animal models used.

4.4. Effects of quetiapine on neuroinflammation

Prominent activation of inflammatory processes are observed in AD patients (Wyss-Coray & Mucke, 2002). In general, it is inconclusive whether neuroinflammation is the cause of AD pathology or the result of the AD ongoing process. There are many studies showing the important role of Aβ in the activation of this inflammation process. For instance, the activation of microglia and astrocytes occurs in AD brains and in AD transgenic mice models (Frautschy et al., 1998; Matsuoka, et al., 2001; Rozemuller, van Gool, & Eikelenboom, 2005). It was speculated that the significant decrease in activated astrocytes, an indicator of decreased neuroinflammation in quetiapine-treated APP/PS1 double transgenic mice, could most likely be attributed to the inhibitory effects of quetiapine on Aβ production in the CNS.

However, quetiapine also exhibits anti-inflammatory properties that are independent of Aβ production (Baune & Eyre, 2010). Although the underlying mechanism is not clear, quetiapine-mediated neuroprotective effects may be involved in this process and attenuate the activation of astrocytes (He, Xu, et al., 2006). Although there is still no convincing data to support the role of inflammation in AD, the effects of NSAIDs in AD transgenic mice, at least in

some part, point to the role of inflammation in this disease. The anti-inflammatory effects of quetiapine observed in this study provided supporting evidence for both the significance of neuroinflammation in AD, as well as the utilization of quetiapine as a therapeutic agent for AD.

4.5. Effects of quetiapine on neuronal integrity

Increased brain soluble Aβ levels may be the primary influence resulting in neuronal degeneration in AD (J. Hardy & Selkoe, 2002). Reduced synaptic density is one of AD key features and probably causative of memory deficits in AD. Thus, it was postulated that decreased soluble Aβ levels after quetiapine treatment may result in attenuation of neuronal integrity impairment. It was observed that quetiapine treatment of the APP/PS1 mice significantly prevented the synaptic protein loss compared with the untreated transgenic controls.

SYP, a synaptic protein localized in the neuronal presynaptic vesicles, has been shown to be significantly reduced in AD patients' brains. This decrease is well correlated with the severity of cognitive deficits (Masliah, Miller, & Terry, 1993; Terry et al., 1991). However, the change of SYP levels in transgenic mice is not quite conclusive, with conflicting reports showing SYP either being reduced or unchanged in APP transgenic mouse models (Hsia et al., 1999; Irizarry, McNamara, Fedorchak, Hsiao, & Hyman, 1997). This may be due to different levels of transgenic APP or different stages of the degenerative progress. In this study, a significant decrease in SYP expression levels was observed in the hippocampus of the APP/PS1 transgenic mice compared with the wild type mice (data not shown here). This decrease was significantly reversed by quetiapine treatment in APP/PS1 transgenic mice. Quetiapine mediated prevention of synaptic protein loss in the hippocampus may play an important role in rescuing the impairments of learning and memory and other cognitive functions. This alternation would be able to improve the impaired long-term potentiation and neuronal plasticity in APP/PS1

transgenic mice (Chapman et al., 1999). In order to support the results with SYP, studies measuring synaptic protein loss with further neuronal markers, such as MAP2, could potentially help support the findings regarding neuronal integrity.

4.6. Limitations of the APP/PS1 transgenic mouse model of AD

This study utilized transgenic mice overexpressing the genes, APP (Hsiao, et al., 1996) and PS1 (Duff, et al., 1996), whose mutations are associated with FAD. As described in the introduction (Section 1.3.1), APP/PS1 double transgenic mice show elevation of Aβ level and plaques in the cerebral cortex and hippocampus as well as memory impairment (Games, et al., 1995; Holcomb, et al., 1998; Hsiao, et al., 1996; Sturchler-Pierrat, et al., 1997). Compared with APP single transgenic mice, the double transgenic mice of AD show accelerated Alzheimer phenotype (Holcomb, et al., 1998). Therefore, this APP/PS1 double transgenic mouse model is useful in the study of AD and provides us a very good platform for testing possible drug therapeutics of AD.

There are, however, a number of limitations associated with this model. Of primary concern is the fact that AD is a human disease and the human condition is much more complex than a mouse model could depict. It has been already demonstrated that human AD brain shows extensive neuron loss, primarily in cholinergic neurons of the nucleus basalis and noncholinergic neurons throughout the cortex and hippocampus (Whitehouse et al., 1982). Studies have shown that fibrillar Aβ peptides are neurotoxic in primary neuron culture, and thus the Aβ transgenic mouse model overexpressing human Aβ should be expected to be seen extensive neurodegeneration in the brains of mice. However, the APP/PS1 double transgenic mouse did not show significant neuronal cell loss or larger ventricles as would be expected in a true model of AD, even though the amyloid burden exceeded 30% (He, et al., 2009; Yu, et al., 2009).

Second, the fact that genetic mutations can be demonstrated to play a role in pathological entities of the disease may only provide a partial perspective rather than a complete picture of the disease. This model is based on APP and PS1 mutations that are associated with FAD; it should be further determined whether these mutations offer certain similarities to sporadic AD in humans, which comprises the vast majority (>95%) of AD cases.

Third, although the conditions for behavioural testing were maintained as consistent as possible, many factors may have confounded the results from these tests. For example, the age and gender of the animals, environmental layout, changes in experimenters during the tests, handling, timing, techniques, equipments, and time of day of conducting tests are difficult to keep constant. When comparing results to previous studies, conditions in other labs may also differ from this study. Any and all of these factors could have confounded behavioural outcomes in any degree. Likewise, the effects of genetic background of different strains used for the transgenic mouse AD model design should be taken into consideration because the background strain of mouse may affect animals' performances on various behavioural tasks (Pugh, Ahmed, Smith, Upton, & Hunter, 2004).

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1. Overall conclusions

The present study provides evidence that quetiapine was able to attenuate Aβ associated neuropathology and memory deficits in an APP/PS1 transgenic AD mouse model. The inhibitory effect of quetiapine on Aβ production may likely come from its inhibition of APP processing, as indicated by decreased activity of GSK3β. There was a significant decrease in activated astrocytes seen in the quetiapine-treated transgenic mice which could be attributed to its inhibition of Aβ. Because brain inflammation is a risk factor for Alzheimer's disease, the anti-inflammatory effect of quetiapine in the AD model mouse provided additional evidence for its therapeutic potential for AD. Furthermore, it was observed that quetiapine treatment significantly attenuated impairment of synaptic integrity, specifically synaptic protein loss, in transgenic mice. Overall, it can be concluded that therapeutic potential of the atypical antipsychotics quetiapine is mechanistically linked to Aβ suppression in the CNS, and Aβ suppression should be explored as a target for AD therapeutics in the future.

5.2. Significance of the research

This thesis presented several novel findings that are significant in advancing the understanding of AD, including:

1. An *in vivo* study showing that early quetiapine intervention is effective on neuropathology changes and cognitive impairments.

2. A novel modified method of collecting cerebrospinal fluid (CSF) that can be used to monitor the effect of therapeutic interventions over time from the same mouse.

3. Evidence that quetiapine may not only affect AD pathogenesis through its modulatory role on BACE (He, et al., 2009) or its neuroprotective property, but also through its influence on GSK3β, providing a new possible target for future treatment of AD.

4. Evidence that may provide insights for the initiation of quetiapine clinical trial designs.

5.3. Potential applications for future research

This thesis has found that quetiapine administration after pathology onset slows the pathogenesis of AD and improves cognitive impairments in APP/PS1 transgenic mice, which may suggest that future clinical trials using quetiapine could show significant improvements in early or mild AD patients. However, it is unknown whether quetiapine exerts similar beneficial effects on late stage or severe AD patients. Therefore, the next step of this project will be to further investigate whether quetiapine is still effective on neuropathology changes and improving memory deficits in older mice when there is profound amyloid plaque formation and severe cognitive dysfunction.

AD is characterized by both Aβ plaque formation and neurofibrillary tangles composed of abnormal tau protein; however, the APP/PS1 transgenic mice only exhibit Aβ plaque formation. In future experiments, a triple transgenic mouse model of AD, which also overexpresses tau, would be a superior choice for an animal model of AD (Oddo, Caccamo, Kitazawa, Tseng, & LaFerla, 2003). In addition, tau was identified as a substrate of GSK3β, further confirming its role in AD and the necessity of using the triple transgenic mouse model for future studies. As chronic quetiapine treatment can inhibit GSK3β activity by altering its phosphorylation state, it would be extremely interesting to investigate the effects of quetiapine on tau protein accumulation, and tangle formation using a triple transgenic mouse model of AD. This study may also elucidate more insights on the pathogenesis of AD.

As the prevalence of AD is an ever-growing concern in today's aging population, it is of critical importance that effective treatment for this devastating disease be found. The conclusions reached in this thesis are important for considerations of future studies investigating the possible underlying pathophysiology of AD, for leading to potential therapeutic targets for the treatment of AD, and for the initiation of quetiapine's clinical trials.

BIBLIOGRAPHY

Alimohamad, H., Rajakumar, N., Seah, Y. H., & Rushlow, W. (2005). Antipsychotics alter the protein expression levels of beta-catenin and GSK-3 in the rat medial prefrontal cortex and striatum. *Biol Psychiatry, 57*(5), 533-542.

Alvarez, G., Munoz-Montano, J. R., Satrustegui, J., Avila, J., Bogonez, E., & Diaz-Nido, J. (1999). Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration. *FEBS Lett, 453*(3), 260-264.

Andjelkovic, M., Alessi, D. R., Meier, R., Fernandez, A., Lamb, N. J., Frech, M., et al. (1997). Role of translocation in the activation and function of protein kinase B. *J Biol Chem, 272*(50), 31515-31524.

Aplin, A. E., Gibb, G. M., Jacobsen, J. S., Gallo, J. M., & Anderton, B. H. (1996). In vitro phosphorylation of the cytoplasmic domain of the amyloid precursor protein by glycogen synthase kinase-3beta. *J Neurochem, 67*(2), 699-707.

Arendash, G. W., King, D. L., Gordon, M. N., Morgan, D., Hatcher, J. M., Hope, C. E., et al. (2001). Progressive, age-related behavioral impairments in transgenic mice carrying both mutant amyloid precursor protein and presenilin-1 transgenes. *Brain Res, 891*(1-2), 42-53.

Arnold, S. E., & Trojanowski, J. Q. (1996). Recent advances in defining the neuropathology of schizophrenia. *Acta Neuropathol, 92*(3), 217-231.

Ashe, K. H., & Zahs, K. R. (2010). Probing the biology of Alzheimer's disease in mice. *Neuron, 66*(5), 631-645.

Bai, O., Wei, Z., Lu, W., Bowen, R., Keegan, D., & Li, X. M. (2002). Protective effects of atypical antipsychotic drugs on PC12 cells after serum withdrawal. *J Neurosci Res, 69*(2), 278-283.

Bai, O., Zhang, H., & Li, X. M. (2004). Antipsychotic drugs clozapine and olanzapine upregulate bcl-2 mRNA and protein in rat frontal cortex and hippocampus. *Brain Res, 1010*(1-2), 81-86.

Baune, B. T., & Eyre, H. (2010). Anti-inflammatory effects of antidepressant and atypical antipsychotic medication for the treatment of major depression and comorbid arthritis: a case report. *J Med Case Reports, 4*, 6.

Beaulieu, J. M., Gainetdinov, R. R., & Caron, M. G. (2009). Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol, 49*, 327-347.

Beaulieu, J. M., Sotnikova, T. D., Marion, S., Lefkowitz, R. J., Gainetdinov, R. R., & Caron, M. G. (2005). An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell, 122*(2), 261-273.

Beaulieu, J. M., Sotnikova, T. D., Yao, W. D., Kockeritz, L., Woodgett, J. R., Gainetdinov, R. R., et al. (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc Natl Acad Sci U S A, 101*(14), 5099-5104.

Bertram, L., Blacker, D., Mullin, K., Keeney, D., Jones, J., Basu, S., et al. (2000). Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. *Science, 290*(5500), 2302-2303.

Blanchard, V., Moussaoui, S., Czech, C., Touchet, N., Bonici, B., Planche, M., et al. (2003). Time sequence of maturation of dystrophic neurites associated with Abeta deposits in APP/PS1 transgenic mice. *Exp Neurol, 184*(1), 247-263.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol, 82*(4), 239-259.

Braak, H., & Braak, E. (1998). Evolution of neuronal changes in the course of Alzheimer's disease. *J Neural Transm Suppl, 53*, 127-140.

Broadhurst, P. L. (1961). Analysis of maternal effects in the inheritance of behaviour. *Animal Behaviour, 9*(3-4), 129-141.

Buxbaum, J. D., Ruefli, A. A., Parker, C. A., Cypess, A. M., & Greengard, P. (1994). Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. *Proc Natl Acad Sci U S A, 91*(10), 4489-4493.

Caballero, J., Hitchcock, M., Scharre, D., Beversdorf, D., & Nahata, M. C. (2006). Cognitive effects of atypical antipsychotics in patients with Alzheimer's disease and comorbid psychiatric or behavioral problems: a retrospective study. *Clin Ther, 28*(10), 1695-1700.

Calhoun, M. E., Wiederhold, K. H., Abramowski, D., Phinney, A. L., Probst, A., Sturchler-Pierrat, C., et al. (1998). Neuron loss in APP transgenic mice. *Nature, 395*(6704), 755-756.

Canadian study of health and aging: study methods and prevalence of dementia. (1994). *CMAJ, 150*(6), 899-913.

Cantillon, M., Harwood, D., Barker, W., St George-Hyslop, P., Tsuda, T., Ekatarina, R., et al. (1997). No association between apolipoprotein E genotype and late-onset depression in Alzheimer's disease. *Biol Psychiatry, 41*(2), 246-248.

Chapman, P. F., White, G. L., Jones, M. W., Cooper-Blacketer, D., Marshall, V. J., Irizarry, M., et al. (1999). Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci, 2*(3), 271-276.

Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., et al. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature, 353*(6347), 844-846.

Chen, G., Chen, K. S., Knox, J., Inglis, J., Bernard, A., Martin, S. J., et al. (2000). A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature, 408*(6815), 975-979.

Chishti, M. A., Yang, D. S., Janus, C., Phinney, A. L., Horne, P., Pearson, J., et al. (2001). Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem, 276*(24), 21562-21570.

Cho, J. H., & Johnson, G. V. (2004). Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3beta (GSK3beta) plays a critical role in regulating tau's ability to bind and stabilize microtubules. *J Neurochem, 88*(2), 349-358.

Chun, W., & Johnson, G. V. (2007). The role of tau phosphorylation and cleavage in neuronal cell death. *Front Biosci, 12*, 733-756.

Cohen, E., Paulsson, J. F., Blinder, P., Burstyn-Cohen, T., Du, D., Estepa, G., et al. (2009). Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell, 139*(6), 1157-1169.

Colton, C. A., Vitek, M. P., Wink, D. A., Xu, Q., Cantillana, V., Previti, M. L., et al. (2006). NO synthase 2 (NOS2) deletion promotes multiple pathologies in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 103*(34), 12867-12872.

Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., & Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature, 378*(6559), 785-789.

Cuesta, M. J., Peralta, V., & Zarzuela, A. (2001). Effects of olanzapine and other antipsychotics on cognitive function in chronic schizophrenia: a longitudinal study. *Schizophr Res, 48*(1), 17-28.

Cummings, J. L., Vinters, H. V., Cole, G. M., & Khachaturian, Z. S. (1998). Alzheimer's disease: etiologies, pathophysiology, cognitive reserve, and treatment opportunities. *Neurology, 51*(1 Suppl 1), S2-17; discussion S65-17.

Cummings, J. L., & Zhong, K. (2006). Treatments for behavioural disorders in neurodegenerative diseases: drug development strategies. *Nat Rev Drug Discov, 5*(1), 64-74.

Davies, C. A., Mann, D. M., Sumpter, P. Q., & Yates, P. O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. *J Neurol Sci, 78*(2), 151-164.

Davis, J., Xu, F., Deane, R., Romanov, G., Previti, M. L., Zeigler, K., et al. (2004). Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor. *J Biol Chem, 279*(19), 20296-20306.

Davis, J., Xu, F., Miao, J., Previti, M. L., Romanov, G., Ziegler, K., et al. (2006). Deficient cerebral clearance of vasculotropic mutant Dutch/Iowa Double A beta in human A betaPP transgenic mice. *Neurobiol Aging, 27*(7), 946-954.

DeFries, J. C., Hegmann, J. P., & Weir, M. W. (1966). Open-field behavior in mice: evidence for a major gene effect mediated by the visual system. *Science, 154*(756), 1577-1579.

DeKosky, S. T., Scheff, S. W., & Styren, S. D. (1996). Structural correlates of cognition in dementia: quantification and assessment of synapse change. *Neurodegeneration, 5*(4), 417-421.

DeLisi, L. E., Sakuma, M., Tew, W., Kushner, M., Hoff, A. L., & Grimson, R. (1997). Schizophrenia as a chronic active brain process: a study of progressive brain structural change subsequent to the onset of schizophrenia. *Psychiatry Res, 74*(3), 129-140.

Dillon, G. M., Qu, X., Marcus, J. N., & Dodart, J. C. (2008). Excitotoxic lesions restricted to the dorsal CA1 field of the hippocampus impair spatial memory and extinction learning in C57BL/6 mice. *Neurobiol Learn Mem, 90*(2), 426-433.

Dineley, K. T., Xia, X., Bui, D., Sweatt, J. D., & Zheng, H. (2002). Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins. *J Biol Chem, 277*(25), 22768-22780.

Ding, Y., Qiao, A., Wang, Z., Goodwin, J. S., Lee, E. S., Block, M. L., et al. (2008). Retinoic acid attenuates beta-amyloid deposition and rescues memory deficits in an Alzheimer's disease transgenic mouse model. *J Neurosci, 28*(45), 11622-11634.

Doble, B. W., & Woodgett, J. R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci, 116*(Pt 7), 1175-1186.

Duff, K., Eckman, C., Zehr, C., Yu, X., Prada, C. M., Perez-tur, J., et al. (1996). Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature, 383*(6602), 710-713.

Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., & Haass, C. (2003). Reconstitution of gamma-secretase activity. *Nat Cell Biol, 5*(5), 486-488.

Emamian, E. S., Hall, D., Birnbaum, M. J., Karayiorgou, M., & Gogos, J. A. (2004). Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat Genet, 36*(2), 131-137.

Fischer, F., Molinari, M., Bodendorf, U., & Paganetti, P. (2002). The disulphide bonds in the catalytic domain of BACE are critical but not essential for amyloid precursor protein processing activity. *J Neurochem, 80*(6), 1079-1088.

Fonseca, M. I., Zhou, J., Botto, M., & Tenner, A. J. (2004). Absence of C1q leads to less neuropathology in transgenic mouse models of Alzheimer's disease. *J Neurosci, 24*(29), 6457-6465.

Forde, J. E., & Dale, T. C. (2007). Glycogen synthase kinase 3: a key regulator of cellular fate. *Cell Mol Life Sci, 64*(15), 1930-1944.

Frame, S., & Cohen, P. (2001). GSK3 takes centre stage more than 20 years after its discovery. *Biochem J, 359*(Pt 1), 1-16.

Frautschy, S. A., Horn, D. L., Sigel, J. J., Harris-White, M. E., Mendoza, J. J., Yang, F., et al. (1998). Protease inhibitor coinfusion with amyloid beta-protein results in enhanced deposition and toxicity in rat brain. *J Neurosci, 18*(20), 8311-8321.

Fukumoto, H., Rosene, D. L., Moss, M. B., Raju, S., Hyman, B. T., & Irizarry, M. C. (2004). Beta-secretase activity increases with aging in human, monkey, and mouse brain. *Am J Pathol, 164*(2), 719-725.

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., et al. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature, 373*(6514), 523-527.

Gefvert, O., Lundberg, T., Wieselgren, I. M., Bergstrom, M., Langstrom, B., Wiesel, F., et al. (2001). D(2) and 5HT(2A) receptor occupancy of different doses of quetiapine in schizophrenia: a PET study. *Eur Neuropsychopharmacol, 11*(2), 105-110.

Gozes, I. (2010). Tau pathology and future therapeutics. *Curr Alzheimer Res, 7*(8), 685-696.

Green, M. F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry, 153*(3), 321-330.

Grimes, C. A., & Jope, R. S. (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol, 65*(4), 391-426.

Hamos, J. E., DeGennaro, L. J., & Drachman, D. A. (1989). Synaptic loss in Alzheimer's disease and other dementias. *Neurology, 39*(3), 355-361.

Hanger, D. P., Betts, J. C., Loviny, T. L., Blackstock, W. P., & Anderton, B. H. (1998). New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry. *J Neurochem, 71*(6), 2465-2476.

Hanger, D. P., Hughes, K., Woodgett, J. R., Brion, J. P., & Anderton, B. H. (1992). Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett, 147*(1), 58-62.

Hardy, J. (1992). Framing beta-amyloid. *Nat Genet, 1*(4), 233-234.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353-356.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science, 256*(5054), 184-185.

He, J., Luo, H., Yan, B., Yu, Y., Wang, H., Wei, Z., et al. (2009). Beneficial effects of quetiapine in a transgenic mouse model of Alzheimer's disease. *Neurobiol Aging, 30*(8), 1205-1216.

He, J., Xu, H., Yang, Y., Rajakumar, D., Li, X., & Li, X. M. (2006). The effects of chronic administration of quetiapine on the phencyclidine-induced reference memory impairment and decrease of Bcl-XL/Bax ratio in the posterior cingulate cortex in rats. *Behav Brain Res, 168*(2), 236-242.

He, J., Xu, H., Yang, Y., Zhang, X., & Li, X. M. (2005). Chronic administration of quetiapine alleviates the anxiety-like behavioural changes induced by a neurotoxic regimen of dl-amphetamine in rats. *Behav Brain Res, 160*(1), 178-187.

He, J., Yang, Y., Yu, Y., Li, X., & Li, X. M. (2006). The effects of chronic administration of quetiapine on the methamphetamine-induced recognition memory impairment and dopaminergic terminal deficit in rats. *Behav Brain Res, 172*(1), 39-45.

Heinonen, O., Soininen, H., Sorvari, H., Kosunen, O., Paljarvi, L., Koivisto, E., et al. (1995). Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in Alzheimer's disease. *Neuroscience, 64*(2), 375-384.

Hellstrom-Lindahl, E., Viitanen, M., & Marutle, A. (2009). Comparison of Abeta levels in the brain of familial and sporadic Alzheimer's disease. *Neurochem Int, 55*(4), 243-252.

Heutink, P. (2000). Untangling tau-related dementia. *Hum Mol Genet, 9*(6), 979-986.

Hirsch, S. R., Link, C. G., Goldstein, J. M., & Arvanitis, L. A. (1996). ICI 204,636: a new atypical antipsychotic drug. *Br J Psychiatry Suppl*(29), 45-56.

Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., et al. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med, 4*(1), 97-100.

Holmes, A. (2001). Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neurosci Biobehav Rev, 25*(3), 261-273.

Hoshi, M., Takashima, A., Noguchi, K., Murayama, M., Sato, M., Kondo, S., et al. (1996). Regulation of mitochondrial pyruvate dehydrogenase activity by tau protein kinase I/glycogen synthase kinase 3beta in brain. *Proc Natl Acad Sci U S A, 93*(7), 2719-2723.

Hsia, A. Y., Masliah, E., McConlogue, L., Yu, G. Q., Tatsuno, G., Hu, K., et al. (1999). Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc Natl Acad Sci U S A, 96*(6), 3228-3233.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., et al. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science, 274*(5284), 99-102.

Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F., & Woodgett, J. R. (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J, 12*(2), 803-808.

Hughes, R. N. (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neurosci Biobehav Rev, 28*(5), 497-505.

Iijima, K., Ando, K., Takeda, S., Satoh, Y., Seki, T., Itohara, S., et al. (2000). Neuron-specific phosphorylation of Alzheimer's beta-amyloid precursor protein by cyclin-dependent kinase 5. *J Neurochem, 75*(3), 1085-1091.

Imahori, K., & Uchida, T. (1997). Physiology and pathology of tau protein kinases in relation to Alzheimer's disease. *J Biochem, 121*(2), 179-188.

Irizarry, M. C., McNamara, M., Fedorchak, K., Hsiao, K., & Hyman, B. T. (1997). APPSw transgenic mice develop age-related A beta deposits and neuropil abnormalities, but no neuronal loss in CA1. *J Neuropathol Exp Neurol, 56*(9), 965-973.

Itagaki, S., McGeer, P. L., Akiyama, H., Zhu, S., & Selkoe, D. (1989). Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol, 24*(3), 173-182.

Jacinto, E., Facchinetti, V., Liu, D., Soto, N., Wei, S., Jung, S. Y., et al. (2006). SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell, 127*(1), 125-137.

Jann, M. W. (2004). Implications for atypical antipsychotics in the treatment of schizophrenia: neurocognition effects and a neuroprotective hypothesis. *Pharmacotherapy, 24*(12), 1759-1783.

Jeste, D. V., & Dolder, C. R. (2004). Treatment of non-schizophrenic disorders: focus on atypical antipsychotics. *J Psychiatr Res, 38*(1), 73-103.

Jope, R. S. (1999). Cholinergic muscarinic receptor signaling by the phosphoinositide signal transduction system in Alzheimer's disease. *J Alzheimers Dis, 1*(4-5), 231-247.

Jope, R. S., & Johnson, G. V. (2004). The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci, 29*(2), 95-102.

Juncos, J. L., Roberts, V. J., Evatt, M. L., Jewart, R. D., Wood, C. D., Potter, L. S., et al. (2004). Quetiapine improves psychotic symptoms and cognition in Parkinson's disease. *Mov Disord, 19*(1), 29-35.

Kang, U. G., Seo, M. S., Roh, M. S., Kim, Y., Yoon, S. C., & Kim, Y. S. (2004). The effects of clozapine on the GSK-3-mediated signaling pathway. *FEBS Lett, 560*(1-3), 115-119.

Kapur, S., Zipursky, R. B., & Remington, G. (1999). Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry, 156*(2), 286-293.

Keefe, R. S., Sweeney, J. A., Gu, H., Hamer, R. M., Perkins, D. O., McEvoy, J. P., et al. (2007). Effects of olanzapine, quetiapine, and risperidone on neurocognitive function in early psychosis: a randomized, double-blind 52-week comparison. *Am J Psychiatry, 164*(7), 1061-1071.

Kim, S. H., Ikeuchi, T., Yu, C., & Sisodia, S. S. (2003). Regulated hyperaccumulation of presenilin-1 and the "gamma-secretase" complex. Evidence for differential intramembranous processing of transmembrane subatrates. *J Biol Chem, 278*(36), 33992-34002.

King, D. L., & Arendash, G. W. (2002). Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. *Physiol Behav, 75*(5), 627-642.

Kobayashi, D. T., & Chen, K. S. (2005). Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes Brain Behav, 4*(3), 173-196.

Kokkinidis, L., & Anisman, H. (1976). Dissociation of the effects of scopolamine and d-amphetamine on a spontaneous alternation task. *Pharmacol Biochem Behav, 5*(3), 293-297.

Kung, H. C., Hoyert, D. L., Xu, J., & Murphy, S. L. (2008). Deaths: final data for 2005. *Natl Vital Stat Rep, 56*(10), 1-120.

Lazarov, O., Robinson, J., Tang, Y. P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M., et al. (2005). Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell, 120*(5), 701-713.

Lee, M. S., Kao, S. C., Lemere, C. A., Xia, W., Tseng, H. C., Zhou, Y., et al. (2003). APP processing is regulated by cytoplasmic phosphorylation. *J Cell Biol, 163*(1), 83-95.

Lehmann, H. E., & Ban, T. A. (1997). The history of the psychopharmacology of schizophrenia. *Can J Psychiatry, 42*(2), 152-162.

Lewis, J., Dickson, D. W., Lin, W. L., Chisholm, L., Corral, A., Jones, G., et al. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science, 293*(5534), 1487-1491.

Li, X., Zhu, W., Roh, M. S., Friedman, A. B., Rosborough, K., & Jope, R. S. (2004). In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. *Neuropsychopharmacology, 29*(8), 1426-1431.

Liu, L., & Duff, K. (2008). A technique for serial collection of cerebrospinal fluid from the cisterna magna in mouse. *J Vis Exp*(21).

Liu, L., Herukka, S. K., Minkeviciene, R., van Groen, T., & Tanila, H. (2004). Longitudinal observation on CSF Abeta42 levels in young to middle-aged amyloid precursor protein/presenilin-1 doubly transgenic mice. *Neurobiol Dis, 17*(3), 516-523.

Lochhead, P. A., Kinstrie, R., Sibbet, G., Rawjee, T., Morrice, N., & Cleghon, V. (2006). A chaperone-dependent GSK3beta transitional intermediate mediates activation-loop autophosphorylation. *Mol Cell, 24*(4), 627-633.

Logue, S. F., Paylor, R., & Wehner, J. M. (1997). Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci, 111*(1), 104-113.

Ma, H., Lesne, S., Kotilinek, L., Steidl-Nichols, J. V., Sherman, M., Younkin, L., et al. (2007). Involvement of beta-site APP cleaving enzyme 1 (BACE1) in amyloid precursor protein-mediated enhancement of memory and activity-dependent synaptic plasticity. *Proc Natl Acad Sci U S A, 104*(19), 8167-8172.

Madhusoodanan, S., Shah, P., Brenner, R., & Gupta, S. (2007). Pharmacological treatment of the psychosis of Alzheimer's disease: what is the best approach? *CNS Drugs, 21*(2), 101-115.

Mancardi, G. L., Liwnicz, B. H., & Mandybur, T. I. (1983). Fibrous astrocytes in Alzheimer's disease and senile dementia of Alzheimer's type. *Acta Neuropathol, 61*(1), 76-80.

Mandelkow, E. M., Drewes, G., Biernat, J., Gustke, N., Van Lint, J., Vandenheede, J. R., et al. (1992). Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. *FEBS Lett, 314*(3), 315-321.

Mangialasche, F., Solomon, A., Winblad, B., Mecocci, P., & Kivipelto, M. (2010). Alzheimer's disease: clinical trials and drug development. *Lancet Neurol, 9*(7), 702-716.

Martinez, A., Castro, A., Dorronsoro, I., & Alonso, M. (2002). Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med Res Rev, 22*(4), 373-384.

Masliah, E., Miller, A., & Terry, R. D. (1993). The synaptic organization of the neocortex in Alzheimer's disease. *Med Hypotheses, 41*(4), 334-340.

Matsuoka, Y., Picciano, M., Malester, B., LaFrancois, J., Zehr, C., Daeschner, J. M., et al. (2001). Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease. *Am J Pathol, 158*(4), 1345-1354.

Mayeux, R. (2010). Clinical practice. Early Alzheimer's disease. *N Engl J Med, 362*(23), 2194-2201.

McGeer, E. G., & McGeer, P. L. (1999). Brain inflammation in Alzheimer disease and the therapeutic implications. *Curr Pharm Des, 5*(10), 821-836.

Medina, M., & Castro, A. (2008). Glycogen synthase kinase-3 (GSK-3) inhibitors reach the clinic. *Curr Opin Drug Discov Devel, 11*(4), 533-543.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods, 11*(1), 47-60.

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683.

Mudher, A., & Lovestone, S. (2002). Alzheimer's disease-do tauists and baptists finally shake hands? *Trends Neurosci, 25*(1), 22-26.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., et al. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat Genet, 1*(5), 345-347.

Nagele, R. G., Wegiel, J., Venkataraman, V., Imaki, H., & Wang, K. C. (2004). Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. *Neurobiol Aging, 25*(5), 663-674.

Nagy, Z., Esiri, M. M., Jobst, K. A., Morris, J. H., King, E. M., McDonald, B., et al. (1995). Relative roles of plaques and tangles in the dementia of Alzheimer's disease: correlations using three sets of neuropathological criteria. *Dementia, 6*(1), 21-31.

Neve, R. L., Rogers, J., & Higgins, G. A. (1990). The Alzheimer amyloid precursor-related transcript lacking the beta/A4 sequence is specifically increased in Alzheimer's disease brain. *Neuron, 5*(3), 329-338.

Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B. P., & LaFerla, F. M. (2003). Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging, 24*(8), 1063-1070.

Oyama, F., Sawamura, N., Kobayashi, K., Morishima-Kawashima, M., Kuramochi, T., Ito, M., et al. (1998). Mutant presenilin 2 transgenic mouse: effect on an age-dependent increase of amyloid beta-protein 42 in the brain. *J Neurochem, 71*(1), 313-322.

Pae, C. U., Lim, H. K., Peindl, K., Ajwani, N., Serretti, A., Patkar, A. A., et al. (2008). The atypical antipsychotics olanzapine and risperidone in the treatment of posttraumatic stress disorder: a meta-analysis of randomized, double-blind, placebo-controlled clinical trials. *Int Clin Psychopharmacol, 23*(1), 1-8.

Paradisi, S., Sacchetti, B., Balduzzi, M., Gaudi, S., & Malchiodi-Albedi, F. (2004). Astrocyte modulation of in vitro beta-amyloid neurotoxicity. *Glia, 46*(3), 252-260.

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., & Pieke-Dahl, S. (2006). The use of behavioral test batteries, II: effect of test interval. *Physiol Behav, 87*(1), 95-102.

Pei, J. J., Braak, E., Braak, H., Grundke-Iqbal, I., Iqbal, K., Winblad, B., et al. (1999). Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. *J Neuropathol Exp Neurol, 58*(9), 1010-1019.

Pei, J. J., Tanaka, T., Tung, Y. C., Braak, E., Iqbal, K., & Grundke-Iqbal, I. (1997). Distribution, levels, and activity of glycogen synthase kinase-3 in the Alzheimer disease brain. *J Neuropathol Exp Neurol, 56*(1), 70-78.

Phiel, C. J., Wilson, C. A., Lee, V. M., & Klein, P. S. (2003). GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature, 423*(6938), 435-439.

Pigino, G., Morfini, G., Pelsman, A., Mattson, M. P., Brady, S. T., & Busciglio, J. (2003). Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. *J Neurosci, 23*(11), 4499-4508.

Polvikoski, T., Sulkava, R., Haltia, M., Kainulainen, K., Vuorio, A., Verkkoniemi, A., et al. (1995). Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med, 333*(19), 1242-1247.

Pugh, P. L., Ahmed, S. F., Smith, M. I., Upton, N., & Hunter, A. J. (2004). A behavioural characterisation of the FVB/N mouse strain. *Behav Brain Res, 155*(2), 283-289.

Purdon, S. E., Malla, A., Labelle, A., & Lit, W. (2001). Neuropsychological change in patients with schizophrenia after treatment with quetiapine or haloperidol. *J Psychiatry Neurosci, 26*(2), 137-149.

Qing, H., He, G., Ly, P. T., Fox, C. J., Staufenbiel, M., Cai, F., et al. (2008). Valproic acid inhibits Abeta production, neuritic plaque formation, and behavioral deficits in Alzheimer's disease mouse models. *J Exp Med, 205*(12), 2781-2789.

Qing, H., Xu, H., Wei, Z., Gibson, K., & Li, X. M. (2003). The ability of atypical antipsychotic drugs vs. haloperidol to protect PC12 cells against MPP+-induced apoptosis. *Eur J Neurosci, 17*(8), 1563-1570.

Rapoport, M., Dawson, H. N., Binder, L. I., Vitek, M. P., & Ferreira, A. (2002). Tau is essential to beta -amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A, 99*(9), 6364-6369.

Reisel, D., Bannerman, D. M., Schmitt, W. B., Deacon, R. M., Flint, J., Borchardt, T., et al. (2002). Spatial memory dissociations in mice lacking GluR1. *Nat Neurosci, 5*(9), 868-873.

Rizzu, P., Joosse, M., Ravid, R., Hoogeveen, A., Kamphorst, W., van Swieten, J. C., et al. (2000). Mutation-dependent aggregation of tau protein and its selective depletion from the soluble fraction in brain of P301L FTDP-17 patients. *Hum Mol Genet, 9*(20), 3075-3082.

Roh, M. S., Seo, M. S., Kim, Y., Kim, S. H., Jeon, W. J., Ahn, Y. M., et al. (2007). Haloperidol and clozapine differentially regulate signals upstream of glycogen synthase kinase 3 in the rat frontal cortex. *Exp Mol Med, 39*(3), 353-360.

Rozemuller, A. J., van Gool, W. A., & Eikelenboom, P. (2005). The neuroinflammatory response in plaques and amyloid angiopathy in Alzheimer's disease: therapeutic implications. *Curr Drug Targets CNS Neurol Disord, 4*(3), 223-233.

Rutten, B. P., Van der Kolk, N. M., Schafer, S., van Zandvoort, M. A., Bayer, T. A., Steinbusch, H. W., et al. (2005). Age-related loss of synaptophysin immunoreactive presynaptic boutons within the hippocampus of APP751SL, PS1M146L, and APP751SL/PS1M146L transgenic mice. *Am J Pathol, 167*(1), 161-173.

Scharre, D. W., & Chang, S. I. (2002). Cognitive and behavioral effects of quetiapine in Alzheimer disease patients. *Alzheimer Dis Assoc Disord, 16*(2), 128-130.

Scheid, M. P., & Woodgett, J. R. (2001). PKB/AKT: functional insights from genetic models. *Nat Rev Mol Cell Biol, 2*(10), 760-768.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., et al. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med, 2*(8), 864-870.

Schmitz, C., Rutten, B. P., Pielen, A., Schafer, S., Wirths, O., Tremp, G., et al. (2004). Hippocampal neuron loss exceeds amyloid plaque load in a transgenic mouse model of Alzheimer's disease. *Am J Pathol, 164*(4), 1495-1502.

Seeman, P., & Tallerico, T. (1999). Rapid release of antipsychotic drugs from dopamine D2 receptors: an explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. *Am J Psychiatry, 156*(6), 876-884.

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science, 298*(5594), 789-791.

Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J., & Tonegawa, S. (1997). Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell, 89*(4), 629-639.

Spittaels, K., Van den Haute, C., Van Dorpe, J., Geerts, H., Mercken, M., Bruynseels, K., et al. (2000). Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice. *J Biol Chem, 275*(52), 41340-41349.

Storey, E., & Cappai, R. (1999). The amyloid precursor protein of Alzheimer's disease and the Abeta peptide. *Neuropathol Appl Neurobiol, 25*(2), 81-97.

Streit, W. J., Walter, S. A., & Pennell, N. A. (1999). Reactive microgliosis. *Prog Neurobiol, 57*(6), 563-581.

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., et al. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A, 90*(5), 1977-1981.

Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K. H., Mistl, C., Rothacher, S., et al. (1997). Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A, 94*(24), 13287-13292.

Su, Y., Ryder, J., Li, B., Wu, X., Fox, N., Solenberg, P., et al. (2004). Lithium, a common drug for bipolar disorder treatment, regulates amyloid-beta precursor protein processing. *Biochemistry, 43*(22), 6899-6908.

Suh, Y. H., & Checler, F. (2002). Amyloid precursor protein, presenilins, and alpha-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. *Pharmacol Rev, 54*(3), 469-525.

Takashima, A., Honda, T., Yasutake, K., Michel, G., Murayama, O., Murayama, M., et al. (1998). Activation of tau protein kinase I/glycogen synthase kinase-3beta by amyloid beta peptide (25-35) enhances phosphorylation of tau in hippocampal neurons. *Neurosci Res, 31*(4), 317-323.

Takashima, A., Murayama, M., Murayama, O., Kohno, T., Honda, T., Yasutake, K., et al. (1998). Presenilin 1 associates with glycogen synthase kinase-3beta and its substrate tau. *Proc Natl Acad Sci U S A, 95*(16), 9637-9641.

Takashima, A., Yamaguchi, H., Noguchi, K., Michel, G., Ishiguro, K., Sato, K., et al. (1995). Amyloid beta peptide induces cytoplasmic accumulation of amyloid protein precursor via tau protein kinase I/glycogen synthase kinase-3 beta in rat hippocampal neurons. *Neurosci Lett, 198*(2), 83-86.

Tanzi, R. E., & Bertram, L. (2005). Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell, 120*(4), 545-555.

Tariot, P. N., Profenno, L. A., & Ismail, M. S. (2004). Efficacy of atypical antipsychotics in elderly patients with dementia. *J Clin Psychiatry, 65 Suppl 11*, 11-15.

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., et al. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol, 30*(4), 572-580.

Tiraboschi, P., Sabbagh, M. N., Hansen, L. A., Salmon, D. P., Merdes, A., Gamst, A., et al. (2004). Alzheimer disease without neocortical neurofibrillary tangles: "a second look". *Neurology, 62*(7), 1141-1147.

Toledo, E. M., & Inestrosa, N. C. (2010). Activation of Wnt signaling by lithium and rosiglitazone reduced spatial memory impairment and neurodegeneration in brains of an APPswe/PSEN1DeltaE9 mouse model of Alzheimer's disease. *Mol Psychiatry, 15*(3), 272-285, 228.

Tuppo, E. E., & Arias, H. R. (2005). The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol, 37*(2), 289-305.

Van Gassen, G., & Annaert, W. (2003). Amyloid, presenilins, and Alzheimer's disease. *Neuroscientist, 9*(2), 117-126.

Velligan, D. I., Newcomer, J., Pultz, J., Csernansky, J., Hoff, A. L., Mahurin, R., et al. (2002). Does cognitive function improve with quetiapine in comparison to haloperidol? *Schizophr Res, 53*(3), 239-248.

von Strauss, E., Viitanen, M., De Ronchi, D., Winblad, B., & Fratiglioni, L. (1999). Aging and the occurrence of dementia: findings from a population-based cohort with a large sample of nonagenarians. *Arch Neurol, 56*(5), 587-592.

Waddington, J. L., O'Callaghan, E., Buckley, P., Larkin, C., Redmond, O., Stack, J., et al. (1991). The age dependencies of MRI abnormalities in schizophrenia suggest early ventricular enlargement but later prominence of cortical atrophy. *Schizophr Res, 5*(3), 188-189.

Walsh, D. M., Klyubin, I., Fadeeva, J. V., Cullen, W. K., Anwyl, R., Wolfe, M. S., et al. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature, 416*(6880), 535-539.

Wang, H., Xu, H., Dyck, L. E., & Li, X. M. (2005). Olanzapine and quetiapine protect PC12 cells from beta-amyloid peptide(25-35)-induced oxidative stress and the ensuing apoptosis. *J Neurosci Res, 81*(4), 572-580.

Wei, Z., Bai, O., Richardson, J. S., Mousseau, D. D., & Li, X. M. (2003). Olanzapine protects PC12 cells from oxidative stress induced by hydrogen peroxide. *J Neurosci Res, 73*(3), 364-368.

Wei, Z., Mousseau, D. D., Richardson, J. S., Dyck, L. E., & Li, X. M. (2003). Atypical antipsychotics attenuate neurotoxicity of beta-amyloid(25-35) by modulating Bax and Bcl-X(l/s) expression and localization. *J Neurosci Res, 74*(6), 942-947.

Weihl, C. C., Ghadge, G. D., Kennedy, S. G., Hay, N., Miller, R. J., & Roos, R. P. (1999). Mutant presenilin-1 induces apoptosis and downregulates Akt/PKB. *J Neurosci, 19*(13), 5360-5369.

West, M. J., Coleman, P. D., Flood, D. G., & Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet, 344*(8925), 769-772.

Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., & Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science, 215*(4537), 1237-1239.

Wirths, O., Breyhan, H., Marcello, A., Cotel, M. C., Bruck, W., & Bayer, T. A. (2010). Inflammatory changes are tightly associated with neurodegeneration in the brain and spinal cord of the APP/PS1KI mouse model of Alzheimer's disease. *Neurobiol Aging, 31*(5), 747-757.

Wong, A. H., & Van Tol, H. H. (2003). Schizophrenia: from phenomenology to neurobiology. *Neurosci Biobehav Rev, 27*(3), 269-306.

Woods, B. T., Yurgelun-Todd, D., Benes, F. M., Frankenburg, F. R., Pope, H. G., Jr., & McSparren, J. (1990). Progressive ventricular enlargement in schizophrenia: comparison to bipolar affective disorder and correlation with clinical course. *Biol Psychiatry, 27*(3), 341-352.

Wyss-Coray, T. (2006). Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med, 12*(9), 1005-1015.

Wyss-Coray, T., Loike, J. D., Brionne, T. C., Lu, E., Anankov, R., Yan, F., et al. (2003). Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med, 9*(4), 453-457.

Wyss-Coray, T., & Mucke, L. (2002). Inflammation in neurodegenerative disease--a double-edged sword. *Neuron, 35*(3), 419-432.

Xiao, L., Xu, H., Zhang, Y., Wei, Z., He, J., Jiang, W., et al. (2008). Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. *Mol Psychiatry, 13*(7), 697-708.

Xu, H., Qing, H., Lu, W., Keegan, D., Richardson, J. S., Chlan-Fourney, J., et al. (2002). Quetiapine attenuates the immobilization stress-induced decrease of brain-derived neurotrophic factor expression in rat hippocampus. *Neurosci Lett, 321*(1-2), 65-68.

Xu, H., Wang, H., Zhuang, L., Yan, B., Yu, Y., Wei, Z., et al. (2008). Demonstration of an anti-oxidative stress mechanism of quetiapine: implications for the treatment of Alzheimer's disease. *FEBS J, 275*(14), 3718-3728.

Yamada, K., Hiramatsu, M., Noda, Y., Mamiya, T., Murai, M., Kameyama, T., et al. (1996). Role of nitric oxide and cyclic GMP in the dizocilpine-induced impairment of spontaneous alternation behavior in mice. *Neuroscience, 74*(2), 365-374.

Yamaguchi, H., Ishiguro, K., Uchida, T., Takashima, A., Lemere, C. A., & Imahori, K. (1996). Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I/glycogen synthase kinase-3 beta and cyclin-dependent kinase 5, a component of TPK II. *Acta Neuropathol, 92*(3), 232-241.

Yu, Y., He, J., Zhang, Y., Luo, H., Zhu, S., Yang, Y., et al. (2009). Increased hippocampal neurogenesis in the progressive stage of Alzheimer's disease phenotype in an APP/PS1 double transgenic mouse model. *Hippocampus, 19*(12), 1247-1253.