Co-Morbidity Of Alzheimer’s Disease And Stroke: Cognitive Deficits And Cellular Pathologies In Two Co-Morbid Animal Models

Jennifer L. Au  
*The University of Western Ontario*

Supervisor  
Dr. David Cechetto  
*The University of Western Ontario*

Joint Supervisor  
Dr. Shawn Whitehead  
*The University of Western Ontario*

Graduate Program in Anatomy and Cell Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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CO-MORBIDITY OF ALZHEIMER’S DISEASE AND STROKE: COGNITIVE DEFICITS AND CELLULAR PATHOLOGIES IN TWO CO-MORBID ANIMAL MODELS

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by

Jennifer Lindsay Au

Graduate Program in Anatomy and Cell Biology

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

Prior to beta-amyloid (Aβ) protein accumulation into plaques in Alzheimer’s disease (AD), neuroinflammation and oxidative stress have been shown to contribute to early cognitive decline. These cellular pathologies are coincident in stroke, which is considered a risk factor for AD. This study investigated the co-morbid effects of AD and stroke on behavioural and cellular pathology in two rodent models. Motor function, memory and microglial neuroinflammation were investigated in a stroke and Aβ injection model and mutant human amyloid precursor protein (APP) transgenic model with stroke. Injections of endothelin-1 into the right striatum were used to model stroke and AD was modelled through either intracerebroventricular Aβ<sub>25-35</sub> injections or a transgenic rat that overproduces a mutated form of human APP. Furthermore, the effectiveness of a targeted antioxidant therapy (CAT-SKL) was investigated in the stroke and Aβ injection model. Memory deficits were present in both co-morbid conditions and CAT-SKL was able to ameliorate the memory deficit in the stroke and Aβ injection model. In the transgenic model, the co-morbid condition resulted in gait alterations. Levels of activated microglia in the infarct region were increased in the transgenic co-morbid condition. Exacerbation of activated microglia in the basal forebrain of the co-morbid stroke and Aβ injection model was observed and was attenuated by CAT-SKL treatment. The findings of this study demonstrate the co-morbid effects in the pathogenesis of AD pathologies. This study suggests that neuroinflammation plays a crucial role in AD and use of targeted therapies, such as CAT-SKL, should be the focus of future research on therapeutic strategies for AD.

**Keywords:** Alzheimer’s disease, stroke, co-morbid, Aβ, neuroinflammation, oxidative stress, catalase
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<tbody>
<tr>
<td>Aβ</td>
<td>Beta-amyloid protein</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADDL</td>
<td>Amyloid-derived diffusible ligand</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ApoE</td>
<td>ApolipoproteinE</td>
</tr>
<tr>
<td>ApoE4</td>
<td>ApolipoproteinE with ε4 Allele</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>hAPP</td>
<td>Human amyloid precursor protein</td>
</tr>
<tr>
<td>BACE1</td>
<td>Beta-site amyloid precursor protein-cleaving enzyme</td>
</tr>
<tr>
<td>CCAO</td>
<td>Common carotid artery occlusion</td>
</tr>
<tr>
<td>CD36</td>
<td>Cluster differentiation 36</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>F344</td>
<td>Fischer 344</td>
</tr>
<tr>
<td>FAD</td>
<td>Familial Alzheimer’s disease</td>
</tr>
<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Ind</td>
<td>Indiana single missense mutation</td>
</tr>
<tr>
<td>KANL</td>
<td>Lysine-alanine-asparagine-leucine</td>
</tr>
<tr>
<td>MCAO</td>
<td>Middle cerebral artery occlusion</td>
</tr>
<tr>
<td>MHCII</td>
<td>Major histocompatibility complex II</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>MST</td>
<td>Modified sticky tape</td>
</tr>
<tr>
<td>MWM</td>
<td>Morris water maze</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NFTs</td>
<td>Neurofibrillary tangles</td>
</tr>
<tr>
<td>•OH</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>O$_2^-$</td>
<td>Superoxide radical</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>PBST</td>
<td>Phosphate-buffered saline with Triton X-100</td>
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<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
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<tr>
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<td>Presenilin-1</td>
</tr>
<tr>
<td>PS2</td>
<td>Presenilin-2</td>
</tr>
<tr>
<td>RAGE</td>
<td>Receptor for advanced glycation end products</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RP</td>
<td>Reverse peptide</td>
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<tr>
<td>SKL</td>
<td>Serine-lysine-leucine</td>
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<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>Swe</td>
<td>Swedish double missense mutation</td>
</tr>
<tr>
<td>TIA</td>
<td>Transient ischemic attacks</td>
</tr>
<tr>
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<td>Transgenic</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like receptor 4</td>
</tr>
<tr>
<td>TLR6</td>
<td>Toll-like receptor 6</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
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Chapter 1:
Introduction
1.1 Alzheimer’s Disease

Alzheimer’s disease (AD) is a neurodegenerative disease that results in the deterioration of the brain, particularly forebrain areas associated with cognition. On a cellular level, amyloid plaques and neurofibrillary tangles (NFTs) in the brain are classically thought to accompany the behavioural impairments associated with AD. These characteristic behavioural impairments include memory loss, mood instability and learning impairments (Auld et al., 2002; Selkoe, 2001; Querfurth and LaFerla, 2010). AD is the most prevalent form of dementia in the elderly population (65+ years of age) affecting approximately 747,000 Canadians. By the year 2031, it is estimated that there will be approximately 1.4 million people in Canada living with AD and experiencing the life-changing symptoms involved (Alzheimer Society Canada, 2012). This drastic increase is partially due to the increasing number of people over 65 years of age and an increase in the lifespan of humans (Norris, 2007). The number of people living with AD and the severity of the disease emphasizes the importance of fully understanding the pathogenesis of AD and developing treatment and prevention strategies for those affected and at risk for developing the disease.

While there has been no consensus on the cause of AD, there has been major speculation about the stages and progression of the disease. The hallmark histopathology of the disease is the presence of insoluble extracellular amyloid plaques in the brain. The amyloid-beta protein (Aβ) that deposits as plaques is a product of the amyloid precursor protein (APP), a single transmembrane polypeptide (Citron, 2010; De Strooper et al., 2003; Selkoe, 2001; Querfurth and LaFerla, 2010). APP has been implicated in cell-to-cell interaction and adhesion, but its main physiological function is widely unknown (Nalivaeva and Turner, 2013; Schubert et al., 1989; Qiu et al., 1995). As APP is translocated to the endoplasmic reticulum for secretion after synthesis, the protein is subject to various post-translational modifications. In particular, APP can be processed through an amyloidogenic or nonamyloidogenic pathway (Selkoe, 2001; Querfurth and LaFerla, 2010).
In the amyloidogenic pathway, APP is sequentially cleaved by β-secretase and γ-secretase to create Aβ (Selkoe, 2001; Querfurth and LaFerla, 2010). Beta-site amyloid precursor protein-cleaving enzyme (BACE1) is the active component of β-secretase (Cai et al., 2001), cleaving APP into a smaller β-APPs fragment and the C99 transmembrane carboxyl-terminal domain of APP (Seubert et al., 1993). Following cleavage by β-secretase, the membrane-bound C99 is cleaved by γ-secretase, which contains presenilin-1 (PS1) or presenilin-2 (PS2) at its catalytic core (De Strooper et al., 2003; Xia et al., 1997; Xia et al., 1998). Cleavage by γ-secretase can occur at multiple sites, releasing a 37-43 Aβ peptide (Haass and Selkoe, 1993; Shoji et al., 1992).

The predominant form of Aβ produced in the brain is Aβ40, but Aβ42 is considered the most susceptible to aggregation and plaque formation (Jarrett et al., 1993; Walsh and Selkoe, 2007; Querfurth and LaFerla, 2010). Once Aβ is produced, it can be cleared from the brain through the glymphatic system, which consists of cerebrospinal fluid influx around arteries and exit of interstitial fluid along veins in the brain (Xie et al., 2013). An imbalance between production and clearance of Aβ in the brain has been proposed as a cause for increased Aβ levels (Hardy and Selkoe, 2002; Mawuenyega et al., 2010). If it is not cleared, Aβ can self-aggregate creating soluble oligomeric fibrils that eventually become insoluble aggregates or plaques (Selkoe, 2001; Querfurth and LaFerla, 2010). These plaques can lead to neuroinflammation, oxidative stress and synaptic dysfunction that result in the characteristic memory loss and learning impairments in AD (Hsiao et al., 1996; Palop and Mucke, 2010; Selkoe, 2002). This entire process is termed the “amyloid cascade hypothesis” and is considered an initiating factor of AD (Hardy and Higgins, 1992; Karran et al., 2011).

The inextricable link between AD and Aβ has lead to a substantial amount of research on the involvement of Aβ in the disease process. This research has resulted in an alteration to the original amyloid cascade hypothesis, since evidence has suggested that plaques are not always indicative of AD. There is clinical evidence of cognitively intact elderly individuals with Aβ accumulation, questioning the role of Aβ and plaques in the disease process (Aizenstein et al., 2008; Dickson et al., 1992). Alternatively, cognitive deficits indicative of AD can present prior to deposition of Aβ into plaques (LaFerla et
Further investigation uncovered that the level of soluble Aβ in the brain correlated to the level of cognitive decline (Lue et al., 1999; McLean et al., 1999; Oda et al., 1995). The contradictory results lead to the amyloid cascade hypothesis expanding to include soluble Aβ and amyloid-derived diffusible ligands (ADDL), while also decreasing confidence in Aβ plaques being the sole cause of AD (Krafft and Klein, 2010).

Although Aβ still remains a fundamental feature in the pathophysiology of AD, another proteinopathy implicated in the disease process involves tau protein. Tau is involved in microtubule stabilization, but can aggregate and become toxic when it is abnormally phosphorylated (Kosik et al., 1986; Trojanowski and Lee, 1995). These intracellular aggregates of abnormally phosphorylated tau are referred to as NFTs and are indicative of a progressed disease state. Previously, it has been shown that AD patients with NFTs tended to display a more severe cognitive decline compared to their counterparts devoid of NFTs (Terry et al., 1987). Furthermore, NFTs are hypothesized to be a response to the accumulation of Aβ in the brain and studies propose that without the presence of Aβ, NFTs do not result in AD (Götz et al., 2001; Selkoe, 2001; Terry et al., 1987; Querfurth and LaFerla, 2010). This is highly suggestive that tau is not the leading cause of AD and rather these two proteins interact in progressed disease states.

While proteinopathies are important in disease progression, other cellular pathologies have also been implicated in the pathogenesis of AD. There is a growing body of evidence supporting a major link between inflammation and AD (Akiyama et al., 2000; Rojo et al., 2008; Wyss-Coray and Mucke, 2002). Furthermore, oxidative stress caused by increased levels of reactive oxygen species (ROS) has also been implicated (Butterfield et al., 2001; Markesbery and Carney, 1999; Sagara et al., 1998). These cellular pathologies have both been involved in the aberrant synaptic transmission involving dysfunction in neurotransmitter release and alterations in receptor distribution in AD (Butterfield et al., 2001; Masliah et al., 2001; Shankar et al., 2007; Wang et al., 2000; Wyss-Coray and Mucke, 2002). Newly emerging evidence investigating the effect of other diseases on the pathogenesis of AD has shown a link between insulin and the development of AD, suggesting there is a relationship between diabetes and AD.
(Arvanitakis et al., 2004; Craft et al., 1998; Messier et al., 2005; Stewart and Liolitsa, 1999). More importantly, there are a substantial number of clinical studies demonstrating a synergism between vascular insults (i.e. stroke, hypertension) and AD (Kokmen et al., 1996; Snowdon et al., 1997; Snyder et al., 2015). While evidence converges at certain aspects, no consensus on the crucial factors involved in the early stages of the disease has been achieved and thus research should continue to investigate all aspects proposed thus far, including risk factors, Aβ, tau, inflammation, oxidative stress and synaptic dysfunction (Butterfield et al., 2001; Akiyama et al., 2000; Selkoe 2001; Querfurth and LaFerla, 2010).

1.2 Risk Factors for AD

AD is classified into two different forms, sporadic late-onset (> 60 years old) AD or familial early-onset (< 60 years old) AD (FAD). Sporadic AD accounts for the majority of individuals diagnosed with the disease with roughly 1-10% of cases presenting as FAD (Campion et al., 1999; Selkoe, 2001). Extensive research has uncovered the most common autosomal dominant genetic predispositions to FAD. These include missense mutations in APP and presenilin (Bertram et al., 2010; Goate et al., 1991; Sherrington et al., 1995). Direct alterations in the APP gene are linked to AD in very few cases, but there is strong evidence that APP genetic variations are the driving force in the interaction between Down syndrome and AD. Patients with Down syndrome invariably develop early-onset AD from the overexpression of APP and subsequent elevated levels of Aβ40 and Aβ42 due to the duplication of chromosome 21, which contains the APP gene (Walsh and Selkoe, 2007; Tokuda et al., 1997). Contrary to mutations in APP, mutations in presenilin are considered the most predominant genetic predisposition in FAD (Bertram et al., 2010; Selkoe, 2001). Mutations in the presenilin proteins result in a drastic increase in Aβ42 plaques in comparison to sporadic forms of AD (Lemere et al., 1996; Mann et al., 1996). The development of this phenotype is attributable to differential γ-secretase activity (Xia et al. 1997). As with Down syndrome, individuals with autosomal dominant mutations in presenilin invariably develop FAD (Bertram et al., 2010; Selkoe, 2001).
Whilst only FAD is considered genetically driven, it has been suggested that sporadic forms may originate from genetic differences. Apolipoprotein E (ApoE) can occur in three different isoforms depending on the allele in the genome and each isoform has distinct functions in the brain (Huang and Mucke, 2012). The presence of an ε4 allele in ApoE over an ε2 or ε3 allele increases the probability of developing AD (Corder et al., 1993; Saunders et al., 1993; Strittmatter et al., 1993). ApoE4 (ε4 allele) has been largely implicated with increased Aβ accumulation through decreased clearance of Aβ (Castellano et al., 2011; Kim et al., 2009). However, the presence of the ε4 allele does not guarantee an eventual AD diagnosis and thus it is not considered the singular genetic cause for sporadic forms of the disease (Ringman et al., 2014; Selkoe, 2001).

Aging remains the most crucial risk factor in the development of sporadic AD. While aging alone is not necessarily responsible for disease development, cellular factors undergo changes due to age and contribute to the clinical manifestations of AD. For example, there is an increase in the levels of ROS, inflammation, protein misfolding and aberrant synaptic transmission that occur as a result of age (Godbout and Johnson, 2009; Morimoto and Cuervo, 2014; Zhang et al., 2015). Coincidently, all of these factors are inextricably linked to the pathophysiology of AD (Butterfield et al., 2001; Huang and Mucke, 2012; Wyss-coray and Mucke, 2002). In addition to age, there are also other numerous risk factors that increase an individual’s probability of developing AD. Some risk factors identified have fallen under the diagnosis of metabolic syndrome, where diabetes and obesity have been largely implicated as risk factors for AD (Arvanitakis et al., 2004; Messier et al., 2005; Stewart and Liolitsa, 1999). Furthermore, vascular conditions are considered highly important AD risk factors, as there have been numerous indications of their role in the development of the disease. The most common vascular risk factors include hypertension, atherosclerosis and stroke (Altman and Rutledge, 2010; Cechetto et al., 2008; Snyder et al., 2015) with substantial clinical evidence that implicates stroke as one of the leading vascular risk factors for AD (Kalaria, 2000; Snowdon et al., 1997; Vermeer et al., 2007).
1.3 Stroke

Stroke is defined as an alteration in blood flow to the brain resulting in a permanent loss of brain function. There are approximately 315,000 people in Canada living with the effects of stroke (Public Health Agency of Canada, 2011). Much like AD, stroke is an age-related condition most common in people over the age of 70. However, occurrences also appear in younger (≤ 65 years old) individuals (Public Health Agency of Canada, 2011). The two major categories of stroke are ischemic or haemorrhagic strokes. The latter is a result of a ruptured blood vessel that leads to uncontrollable bleeding in the brain (Lakhan et al., 2009). Arteries that supply oxygenated blood to the brain can also become blocked by debris or accumulation of lipids in the vessel. The clot formed in the arteries blocks blood supply to its downstream target in the brain resulting in an ischemic stroke. Majority of strokes manifest as ischemic strokes and range in severity (Lakhan et al., 2009). While every stroke causes tissue damage, longer-lasting ischemic blockages present differently than transient ischemic attacks (TIA) that typically only last one to five minutes. These are considered transient because the blockage dissipates and blood flow is recovered to the brain, which is not the case for other ischemic blockages that require medical intervention to recover blood flow (Easton et al., 2009). TIAs are considered “mini-strokes” in comparison to non-transient ischemic blockages, but are still an extremely serious occurrence, as it can indicate future, more severe strokes (Vermeer et al., 2002). Nevertheless, all forms of ischemic strokes are implicated as major risk factors for AD and are a very common co-morbidity with AD. (Arvanitakis et al., 2011; Cechetto et al., 2008; Kalaria, 2000).

1.4 Stroke and AD

Compared to patients without stroke, ischemic stroke patients have a higher prevalence of dementia (Tatemichi et al., 1992) and it is predicted that approximately 30% of AD patients have accompanying ischemic strokes (Kalaria, 2000). A point of interaction between stroke and AD involves ApoE4. Carriers of the ε4 allele are more likely to develop atherosclerosis and other vascular complications (Mahley and Huang, 1999; Olichney et al., 2000; Prasher et al., 2008). Moreover, incident of stroke increases the risk of developing AD five-fold for those that are ε4 allele carriers compared to non-
The overlap in cellular pathology suggests that these are potential points of interaction contributing to the relationship between stroke as a risk factor for AD.

Stroke can also exacerbate pre-existing dementias. In the breakthrough Nun study by Snowdon et al. (1997), the presence of stroke greatly increased the severity of AD in comparison to those individuals with AD alone. Moreover, these strokes were lacunar-type strokes, affecting subcortical areas of the brain (Snowdon et al., 1997). This lead to research that provided evidence that APP expression, Aβ accumulation, increased inflammation and larger infarct volumes are found in co-morbid animal models of AD and stroke (Amtul et al., 2014; Whitehead et al., 2005b; Whitehead et al., 2007). It has also been reported that APP and Aβ appears in the penumbra following stroke injury in animal models (Hiltunen et al., 2009; Shi et al., 2000). Clinical evidence that elderly patients with poor cognitive function are at an increased risk for developing stroke (Ferrucci et al, 1996; Gale et al., 1996) further supports the relationship between stroke and AD. The substantial amount of evidence available suggests that there is a crucial interaction between stroke and AD and it is vital that these two pathologies be further researched in co-morbid models to better understand the cellular mechanisms involved.

1.5 Neuroinflammation

The relationship between inflammation and AD involves propagation of pro-inflammatory mediators and increased inflammation resulting in increased Aβ production (Akiyama et al., 2000; Wyss-Coray and Mucke, 2002). Similar to AD, stroke generates a significant amount of inflammation in the brain in response to ischemic injury (Lakhan et al., 2009; Wang et al., 2007). This relationship between inflammation and AD and stroke suggests that increased inflammation may be a point of synergy between these two diseases. Neuroinflammation is mediated by microglia, the macrophages of the brain, and astrocytes, the glial cells of the brain (Ousman and Kubes, 2012; Wyss-Coray and
Each of these cell types perform different roles in the brain, but both are important with regards to neuroinflammation due to their release of pro-inflammatory molecules (Akiyama et al., 2000, Rojo et al., 2008; Wyss-Coray, 2006). Microglia are at the core of mediating the neuroinflammatory response initiated by foreign pathogens in the brain (Latta et al., 2014). Resting state microglia have extended ramified processes that can retract resulting in an “amoeboid” shape. The retraction of these processes occurs upon detection of a foreign pathogen and the resultant microglia form is deemed activated (Lynch, 2009). Activated microglia present a very different molecular and biochemical profile, where increased release of chemokines and cytokines and expression of major histocompatibility complex II (MHCII) are a few marked differences between activated and resting microglia (Akiyama et al., 2000; Luber-Narod and Rogers, 1988, Wang et al., 2007). A major role of activated microglia is to phagocytose debris and foreign pathogens in the brain to avoid further damage caused by these invaders (Ousman and Kubes, 2012). Furthermore, microglia are initiators in the post-insult repair process (Ousman and Kubes, 2012; Wang et al., 2007). While, these are highly beneficial defense mechanisms, often in disease states the attenuation of this microglial inflammation is not balanced with propagation, leading to the persistence of activated microglia and eventual development of toxic chronic inflammation (Heneka et al., 2015; Lee et al., 2010; Tuppo and Arias, 2005).

Masses of microglia have been found at sites of Aβ aggregation clinically (Haga et al., 1989; Lue et al, 1996) and experimentally (Leon et al., 2010; Liu et al., 2008), implicating a signalling interaction between Aβ and microglia. This has been attributed to various mechanisms involving direct contact and distant signalling. The latter involves microglia being attracted to Aβ-rich areas of the brain as a result of the chemotactic signalling produced by Aβ itself (Akiyama et al., 2000). The former involves Aβ directly contacting and activating microglia through binding to RAGE (receptor for advanced glycation end products), CD36 (cluster differentiation 36), TLR4 (toll-like receptor 4) and TLR6 (toll-like receptor 6) on microglia (El Khoury et al., 2003; Stewart et al., 2010; Yan et al., 1999). This interaction can initiate the release of pro-inflammatory signals, such as interleukin-1, interleukin-6 and tumour necrosis factor-α, further propagating microglial infiltration and activation (Akiyama et al., 2000; Campbell et al., 1998; Rojo
et al., 2008). There is also evidence in animal models that some of these pro-inflammatory signals precede Aβ deposition (Ferretti and Cuello, 2011; Grammas, 2011), implicating neuroinflammation in the early stages of disease progression. This prolonged activation of microglia due to sustained abnormal Aβ accumulation and increasing levels of pro-inflammatory signals is thought to give rise to the pathological chronic inflammation in AD (Heneka et al., 2015; Latta et al., 2014; Wyss-Coray, 2006).

Neuroinflammation resulting from stroke also results in microglia entering a self-propagating cycle that can become neurotoxic. Upon ischemic injury, brain tissue undergoes cell death through both apoptosis and necrosis, which in turn triggers a pronounced inflammatory response (Lakhan et al., 2009; Wang et al, 2007). Microglia infiltrate the ischemic area where they become activated due to the cellular debris and neurotoxic factors released as a result of the stroke. This leads to drastic alterations in the release patterns of interleukin-1, interleukin-6 and tumour necrosis factor-α, which have been thought to have a cytotoxic effect in stroke (Lakhan et al., 2009; Yenari et al., 2010). Furthermore, it has been demonstrated clinically (Weinstein et al., 2010) and experimentally (Ekdahl et al., 2009) that increased levels of activated microglia can persist for long periods of time after stroke. This relationship alone can become extremely damaging in stroke, but it becomes additive with the pathogenic inflammation resulting from AD. For example, increased volume of microglia within the infarct region and the hippocampus has been demonstrated in a co-morbid animal model of stroke and AD (Amtul et al., 2014; Whitehead et al., 2005b; Whitehead et al., 2007), suggesting that microglia may contribute to the exacerbated disease progression in the early stages of the co-morbid condition.

1.6 Oxidative Stress and Antioxidants

There is increasing evidence connecting oxidative stress to the pathogenesis of AD (Butterfield et al., 2001; Hensley et al., 1995; Markesbery and Carney, 1999), as well as stroke (Allen and Bayraktutan, 2009, Kontos, 2001). ROS are small oxygen-containing molecules with an unpaired electron that can be damaging to cells, such as the hydroxyl radical (•OH) and superoxide radical (O2•−). These molecules are by-products of mitochondrial and peroxisomal reactions and are released from activated microglia
While these molecules are often implicated as neurotoxic molecules, they are extremely important in microglial function, cell signalling and synaptic plasticity (Nathan, 2003; Paula-lima et al., 2014; Yenari et al., 2010). Antioxidant enzymes and molecules control the levels of ROS by converting them into neutral molecules, preventing their accumulation.

Key antioxidant mechanisms activated in the brain include the enzymes glutathione peroxidase, superoxide dismutase (SOD) and catalase. Additionally, non-enzymatic antioxidants include glutathione and nicotinamide adenine dinucleotide phosphate (NADPH) molecules (Meraz-Ríos et al., 2014; Moldovan and Moldovan, 2004). In normal cellular functioning, mitochondria produce O$_2$•$^-$ during the reduction of oxygen to water in aerobic respiration and also produce hydrogen peroxide (H$_2$O$_2$) as a by-product (Coyle and Puttfarcken, 1993; Moldovan and Moldovan, 2004). Another large source of ROS is the peroxisome, which produces H$_2$O$_2$ during the β-oxidation of fatty acids (Moldovan and Moldovan, 2004; Terlecky and Koepke, 2007). While H$_2$O$_2$ is not a considered a free radical, its oxidant properties, ability to diffuse within and between cells and yield the extremely toxic •OH through Fenton and Haber-Weiss reactions implicates it as a ROS (Christen, 2000; Dasuri et al., 2013; Markesbery and Carney, 1999). One of the main antioxidant enzymes in the mitochondria is SOD, which provides protection against increasing levels of O$_2$•$^-$ (Coyle and Puttfarcken, 1993; Moldovan and Moldovan, 2004). Furthermore, catalase is specifically targeted to the peroxisome to enzymatically breakdown H$_2$O$_2$ into water and oxygen (Terlecky et al., 2006). When the production of ROS outweighs the antioxidant activity of these enzymes, oxidative stress can occur and ROS become neurotoxic leading to oxidation of proteins, DNA, lipids and eventually cell death (Giordano and Terlecky, 2012; Zhang et al., 2015).

The brain is especially vulnerable to ROS due to its rich lipid content, lack of antioxidants compared to other organs and dependence on oxygen to produce energy (Coyle and Puttfarcken, 1993; Markesbery and Carney, 1999; Massaad, 2011). Altered ROS-producing energy metabolism in the mitochondria regularly occurs in AD brains, as well as those at high risk for developing the disease (Aliév et al., 2003; Beal, 1995;
Evidence has also suggested that ROS may be involved in the early stages of the disease, as increased oxidative damage has been demonstrated in AD animal models (Resende et al., 2008; Schuessel et al., 2006) and Down syndrome patients (Nunomura et al., 2000) prior to Aβ accumulation. Furthermore, Aβ is linked to oxidative stress, whereby Aβ directly produces ROS (Harris et al., 1995; Hensley et al., 1994; Lauderback et al.; 2002; Tabner et al., 2005). For example, there is evidence that aging and AD brains accumulate abnormal levels of iron (Fe³⁺) and Aβ can reduce Fe³⁺ to Fe²⁺ consequently producing H₂O₂ (Deibel et al., 1996; Perry et al., 2002; Rottkamp et al., 2001). This reduction contributes further to oxidative stress, as the Fenton reaction produces •OH from H₂O₂ reacting with Fe²⁺ (Jomova et al., 2010). Moreover, Aβ indirectly produces ROS through activated microglia that release ROS upon activation as part of their opsonizing methods (Akiyama et al., 2000; Lue et al., 1996). The presence of ROS further exacerbates the microglial neuroinflammation in AD, as ROS accumulation recruits activated microglia (Heneka et al., 2015). Examination of antioxidant mechanisms in human AD brains has demonstrated a decrease in SOD and catalase (Gsell et al., 1995; Venkateshappa et al., 2012), implicating alterations in antioxidant defence. The increased production of ROS and decreased antioxidant capacity suggests that the imbalance of antioxidant defense and increased production of ROS largely contributes to oxidative stress in AD.

A major factor in the pathogenesis of stroke involves oxidative stress, where the energy deprivation that occurs immediately following ischemic stroke results in ROS-induced oxidative damage (Allen and Bayraktutan, 2009; Cuzzocrea et al., 2001; Saeed et al., 2007). A release of glutamate post-stroke activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which results in downstream production of O₂⁻⁻⁻ (Allen and Bayraktutan, 2009; Kontos, 2001). Furthermore, disturbances in mitochondrial electron transport increase the production of O₂⁻⁻ during ischemia and upon reoxygenation after stroke (Allen and Bayraktutan, 2009; Sims and Anderson, 2002). The increased levels of O₂⁻⁻ can further perpetuate oxidative stress, as accumulation of lactic acid in neurons post-stroke enhances conversion of O₂⁻⁻ to H₂O₂ (Saeed et al., 2007). Increased availability of free Fe³⁺ (Selim and Ratan, 2004) and increased presence of microglia (Yenari et al., 2010) also contribute to levels of ROS in
stroke. The overlap of the importance of oxidative stress alterations in both stroke and AD coupled with the vulnerability of the brain reinforces the contribution of oxidative stress in the development and progression of neurodegeneration in the co-morbid condition of stroke and AD.

1.7 Catalase

An age-related decrease in catalase levels in the brain has been demonstrated both clinically (Venkateshappa et al., 2012) and experimentally (Alper et al., 1998), thus contributing to the oxidative stress observed in AD and stroke. Catalase is directly targeted to the peroxisome through a KANL (lysine-alanine-asparagine-leucine) protein targeting sequence (Terlecky et al., 2006). The KANL protein targeting sequence is different from other peroxisomal enzymes that have a SKL (serine-lysine-leucine) sequence (Terlecky et al., 2006). The efficiency of the carboxy-terminus KANL targeting sequence significantly decreases with increasing age resulting in a mislocalization of catalase to the cytoplasm (Koepke et al., 2007; Legakis et al., 2002). This mislocalization results in decreased digestion of H$_2$O$_2$ leading to its accumulation in cells (Koepke et al., 2007).

Due to the implications of the inefficient KANL protein targeting sequence, a genetically engineered form of catalase with the more efficient SKL (serine-lysine-leucine) protein targeting signal (CAT-SKL) was developed to better target catalase to the peroxisome (Giordano and Terlecky, 2012). Koepke et al. (2007) demonstrated increased import of CAT-SKL compared to catalase with its canonical KANL sequence in senescent cells. Furthermore, increased localization of CAT-SKL to the peroxisome and subsequent decrease in H$_2$O$_2$ and senescence marker β-galactosidase was observed in these cells (Koepke et al., 2007). The use of CAT-SKL in cultured hypocatalasemic fibroblasts demonstrated restoration of catalase levels and decreases in H$_2$O$_2$ (Wood et al., 2006). Additionally, CAT-SKL has been shown to be effective in protecting cultured myocytes against hypoxia-reoxygenation and ischemia-reperfusion injury (Undyala et al., 2011) and reducing tumour necrosis factor-α in cultured human keratinocytes (Young et al., 2008). The evidence supporting the anti-inflammatory and antioxidant properties of CAT-SKL lead to the investigation of the effect of CAT-SKL on Aβ toxicity. Giordano et
al. (2014) demonstrated that treatment of CAT-SKL prior and subsequent to ADDL administration increased cultured primary cortical and hippocampal neuron viability and decreased neurite degeneration. Additionally, treatment with CAT-SKL in a rodent model of Aβ toxicity resulted in attenuation of Aβ-induced memory deficits, neuroinflammation and cholinergic neuron loss (Nell, 2013).

It has been previously demonstrated that there is a relationship between endogenous catalase and Aβ. For example, there has been a direct interaction of Aβ and catalase observed in cell cultures, whereby catalase directly binds Aβ resulting in the deactivation of catalase and subsequent increase in H$_2$O$_2$ (Habib et al., 2010; Milton, 1999) and the treatment of cell cultures with catalase has been demonstrated to protect cells against Aβ toxicity (Behl et al., 1994; Manelli and Puttfarcken, 1995). Furthermore, investigation of brains from AD patients demonstrated the presence of catalase around senile plaques (Pappolla et al., 1992). These studies coupled with the pathogenic role of ROS in stroke suggests that exacerbation of oxidative stress by age- and Aβ-induced changes in catalase may be involved in the early stages of the co-morbid condition of stroke and AD and that the use of the targeted antioxidant CAT-SKL may be beneficial as a therapeutic agent in the co-morbid condition.

### 1.8 Therapeutic Challenges in AD

A couple of therapeutic approaches for AD have been approved and implemented into the treatment regimens for patients. One commonly prescribed treatment for patients in all stages of the disease is acetylcholinesterase inhibitors (Donev et al., 2009; Huang and Mucke 2012; Selkoe, 2001). These directly target the cholinergic system of the brain, which is one of the brain regions largely affected in AD, by decreasing the degradation of acetylcholine, subsequently increasing cholinergic transmission (Auld et al., 2002). For patients in more advanced stages of AD, the N-methyl-D-aspartate receptor blocker memantine is prescribed to reduce glutamate-induced excitotoxicity (Parsons et al., 1999; Reisberg et al., 2003). All of these treatments result in improved or stabilized memory (Auld et al., 2002; Reisberg et al., 2003), but they are modest at best due to their transient effectiveness past one year of repeated use and do not treat the causes of AD, only the symptoms (Auld et al., 2002; Petersen et al., 2005).
Other therapies being investigated target various aspects of the complex pathophysiology of AD, including anti-inflammatories, antioxidants and drugs targeting the degradation, production and clearance of Aβ (Cummings, 2004; Heneka et al., 2015; Huang and Mucke, 2012). While some anti-Aβ drugs have shown promise, most do not transcend further than the first phase of clinical trials (Golde et al., 2011; Huang and Mucke, 2012; Mangialasche et al., 2010). Often anti-inflammatory and antioxidant treatments are widespread, non-specific mechanisms, which is the case with non-steroidal anti-inflammatories (Hensley, 2010; Moore et al., 2010). While these treatments have been deemed insufficient in treating AD likely due to their indirect mechanisms, it is also highly possible that treatment with antioxidants and anti-inflammatories failed to be effective because trials were initiated in patients with progressed AD (Herrup, 2010). It is conceivable that treatment in earlier stages of the disease and a more direct or targeted approach will lead to successful development of therapies in these categories.

1.9 Animals Models of Stroke

Various models have been utilized for researching mechanisms and therapeutic approaches for stroke. A standard stroke model in rodents is the middle cerebral artery occlusion (MCAO), whereby the middle cerebral artery in the brain is temporarily or permanently blocked (Borlongan et al., 2005; Gupta et al., 2002; Wahl et al., 1992). Both methods produce different results, with temporary occlusion resulting in reperfusion injury in addition to injury from the lack of blood supply (Choi et al., 2012; Li et al., 2013; Zhang et al, 2013). MCAO usually damages a large portion of the brain hemisphere supplied by that artery, causing significant neurodegeneration and inflammation in the cortex and a portion of subcortical structures. Another model that results in significant brain injury is the common carotid artery occlusion (CCAO), which can be accomplished unilaterally or bilaterally in the neck (Choi et al., 2011; Yan et al., 2007; Zhao et al., 2014).

While occlusion models are valid stroke models, there is accumulating evidence for the synergism of small lacunar or subcortical strokes and AD (Schneider et al., 2007; Snowdon et al., 1997; Vermeer et al., 2002). The occlusion stroke models do not mimic these small strokes and this lead to the development of a stroke model that targeted
subcortical structures. Endothelin-1 (ET-1) is a potent vasoconstrictor capable of depleting blood supply through constriction of arteries. While ET-1 occurs naturally in the body as a regulator of the cardiovascular system (Yanagisawa et al., 1988), injecting various concentrations of ET-1 can produce small strokes in any area in the brain (Clarke et al., 2009; Soleman et al., 2010; Weishaupt et al., 2015; Whitehead et al., 2007). This ET-1 stroke model has been utilized in various capacities, but most notable have been striatal and hippocampal stroke models (Amtul et al., 2014; Cardoso et al., 2013; Clarke et al., 2009; Driscoll et al., 2008; McDonald et al., 2008; Whitehead et al., 2005a). The ability to target a specific area of the brain using the directed injection of ET-1 renders it a strong method for modelling the lacunar infarcts in AD patients.

1.10 Animals Models of AD

The challenge with modeling AD is a direct result of its complex pathophysiology and the overall lack of understanding of the precise mechanisms involved. The majority of the rodent models available strongly simulate specific pathologies of AD demonstrated in patients, such as plaques and tangles. There are numerous commonly used transgenic mouse models exploiting the human APP and PS1 genetic mutations implicated in early-onset FAD (Holcomb et al., 1998; Hsiao et al., 1995; Neha et al., 2014; Yamada and Nabeshima, 2000). Furthermore, there are 3x transgenic mouse models that combine mutations in human APP, PS1 and tau genes and models integrating ApoE4 genetic mutations (Jucker, 2010; Medeiros et al., 2014; Oddo et al., 2003; Neha et al., 2014). These models often invariably develop amyloid plaques or NFTs, which are not always indicative of AD or represent a progressed disease state (Aizenstein et al., 2008; Dickson et al., 1992; Terry et al., 1987). Furthermore, the ApoE4 genetic background does not guarantee an eventual diagnosis of AD (Ringman et al., 2014; Selkoe 2001). Despite their shortcomings, all of these models have significantly advanced our understanding of the role of dysfunctional synaptic transmission and abnormal protein aggregation in AD. However, these models often fail to fully model the complex network of pathologies involved in the early stages of the disease.

Transgenic rat models using the same genetic manipulations have recently been developed. Two rat models implement a mutant form of human APP (hAPP) with dual
Swedish and Indiana genetic mutations, which have been implicated in FAD. One model develops significant Aβ plaque accumulation, increased levels of neuroinflammation and ROS accompanied by cognitive deficits with age (Leon et al., 2010). The other model developed by Agca et al. (2008) produces increased levels of mutant hAPP<sup>Swe/Ind</sup>, but does not develop Aβ plaques with age. However, this model has been demonstrated to accumulate Aβ aggregates upon injection of human AD brain extracts (Rosen et al., 2012). Additionally, kaolin-induced hydrocephaly increased oligomeric Aβ levels in the brain of this transgenic rat model (Silverberg et al., 2015). There are also rat models with genetic manipulations of both human APP and PS1 much like the mouse models (Cohen et al., 2013; Flood et al., 2009; Liu et al., 2008; Teng et al., 2011). The advancement into rat models is due to their closer relationship to humans genetically, morphologically and physiologically compared to mice (Do Carmo and Cuello, 2013; Jacob and Kwitek, 2002), providing a strong rationale for the future use of rats to experimentally investigate the effects of Aβ <i>in vivo</i>.

While the pathogenesis of AD in transgenic rodent models with FAD genetic backgrounds develops similar to sporadic AD and are commonly used in research approaches, other non-transgenic models of AD are available. These non-transgenic models can be used to investigate the behavioural and pathological impairments of AD. Through injection of Aβ<sub>25-35</sub>, a truncated form of Aβ<sub>42</sub>, into the intracerebral ventricles of the brain, a rat model was developed that exhibited a combination of AD pathologies (Amtul et al., 2014; Nell, 2013; Whitehead et al., 2005b; Whitehead et al., 2007). Aβ<sub>25-35</sub> is considered the core neurotoxic fragment of Aβ<sub>42</sub> with better solubility, while maintaining the aggregation properties of Aβ<sub>42</sub> (Kowall et al., 1992; Millucci et al., 2009; Pike et al., 1995; Yankner et al., 1989). Injection of this peptide into the brain has been shown to induce neuroinflammation, neurodegeneration, oxidative stress and cognitive deficits, creating a model with a complex neurotoxic pathological response to Aβ administration and demonstrating the early pathologies involved in the pathogenesis of AD (Amtul et al., 2014; Cheng et al., 2006; Kowall et al., 1992; Nell et al., 2014; Whitehead et al., 2005a, Whitehead et al., 2007; Zussy et al., 2013). All of these models have limitations in addition to their strengths, but still allow for a comprehensive approach to studying the complex nature of AD alone and in combination with other
diseases. These models can all be combined with stroke models to create co-morbid models, providing the ideal setting to elucidate the pathogenic impact of various mechanisms and direct future therapeutic approaches to battling AD in humans.

1.11 Rationale

There is an accumulation of clinical and experimental evidence demonstrating an interaction between stroke and AD, implicating stroke as both a risk factor for AD (Vermeer et al., 2007) and an exacerbating factor for pre-existing AD (Snowdon et al., 1997). Both stroke and AD are becoming increasingly prevalent in the overall Canadian population, providing the impetus for a more comprehensive understanding of the synergism of these diseases and development of more effective therapeutic approaches. While there have been several studies exploring the co-morbid condition of stroke and AD, very few have utilized an animal stroke model relevant to the human condition. Several studies implement large MCAO infarcts or smaller infarcts localized to the hippocampus (Craig et al., 2009; Driscoll et al., 2008; McDonald et al., 2008). While these studies are valuable and sometimes relevant to a subset of human cases, clinical evidence has associated small striatal strokes in the pathogenesis of AD in humans (Snowdon et al., 1997; Vermeer et al., 2007). In order to simulate these clinically relevant strokes, localized striatal infarcts would be better suited for translational research.

Several cellular factors have been identified in AD that are also implicated in stroke. Investigation of each disease independently has highlighted these commonalities and provided groundwork for the essential co-morbid studies required to better understand the synergism of stroke and AD. It has been well described that neuroinflammation plays a crucial role in the development stroke (Lakhan et al., 2009) and the early stages of cognitive decline in AD (Latta et al., 2014; Wyss-Coray and Mucke, 2002). Furthermore, oxidative stress is highly implicated in the development of stroke (Allen and Bayraktutan, 2009) and AD (Butterfield et al., 2001; Zhang et al., 2015) and is a major consequence of normal aging, which is a major risk factor for developing AD. Oxidative stress is also largely associated with neuroinflammation and neurodegeneration. Treatment with non-steroidal anti-inflammatories often does not produce the desired outcome in regards to the improvement of cognitive function.
However, it is possible that treatment with these anti-inflammatories failed due to the timing of administration in regards to disease progression (Herrup, 2010) and their non-specific, widespread mechanisms (Hensley, 2010). It has been previously demonstrated that treatment with the targeted antioxidant CAT-SKL is effective in the attenuation of Aβ-induced neuroinflammation, cholinergic loss and memory deficit in vivo (Nell, 2013). Furthermore, it has been effective in delaying the progression of cellular aging (Koepke et al., 2007) and attenuating ADDLs toxicity in vitro (Giordano et al., 2014).

To further understand the complex pathophysiology of AD and stroke, this study focuses on the effect of striatal stroke in two different animal models of AD to better understand the role neuroinflammation has in cognitive decline in both instances. One model makes use of stroke as an exacerbating factor in combination with exogenous administration of Aβ, while stroke is used as an injury in the hAPPswe/Ind transgenic rat for the second model. Moreover, this study is completed in six-month-old rats to better recapitulate the effect of age on the pathogenesis of both diseases (Nell et al., 2014). With age being the greatest risk factor for AD, this modification in age from the commonly used three-month animal model to a six-month model is highly beneficial, as it further parallels the aging factor of the clinical condition. To best assess the effects of the early pathological changes in the disease progression on the behavioural outcomes of the co-morbid condition, a twenty-one day time point was chosen that results in microglial inflammation, neuronal death and cognitive deficits without the presence of amyloid plaques (Nell et al., 2014; Whitehead et al., 2007). The effectiveness of a targeted antioxidant therapeutic approach (CAT-SKL) is also investigated in the stroke and exogenous Aβ co-morbid model, with a focus on its influence on cognitive decline and microglial inflammation.

1.12 Objectives and Hypotheses

Objective 1: Determine the cellular pathologies and corresponding behavioural responses in a six-month-old rat model of co-morbid striatal stroke and intracerebroventricular injection of Aβ.
**Hypothesis:** The combination of stroke and Aβ toxicity in a rat model will exacerbate cognitive decline and neuroinflammation in comparison to stroke alone.

**Objective 2:** Investigate the antioxidant and neuroprotective potential of CAT-SKL in rats subjected to co-morbid striatal stroke and intracerebroventricular injection of Aβ.

**Hypothesis:** Treatment with CAT-SKL will decrease the behavioural and cellular pathological consequences of the combination of stroke and Aβ toxicity.

**Objective 3:** Identify the behavioural responses and cellular pathologies of striatal stroke and Aβ toxicity in a mutant hAPP_Swe/Ind transgenic rat model.

**Hypothesis:** The combination of stroke and high levels of mutant hAPP_Swe/Ind in a transgenic rat model will exacerbate cognitive decline and neuroinflammation compared to stroke in age-matched wild type rats.
Chapter 2: Methods
2.1 Animal Husbandry

All animal procedures were performed in accordance with the guidelines from the Canadian Council on Animal Care and were approved by the Western University Animal Use Subcommittee. Male wistar rats obtained from Charles River Laboratories (Montreal, QC) and in-house bred male Fisher 344 rats were aged to 6 months of age. Wistar rats were housed separately and Fisher 344 rats were housed in a colony prior to surgery. Post-surgery, Wistar rats were housed separately and Fisher 344 rats were housed separately for 24 hours and then housed in pairs for the remainder of the study. All rats were provided with food and water ad libitum and housed in standard conditions at 22-24°C and on a 12 hr:12 hr light:dark cycle. Rats were randomly assigned to surgical and treatment groups prior to beginning the study.

2.2 Transgenic Rat Model

The transgenic rat model was developed on a Fisher 344 (F344) rat background using lentiviral gene therapy (Agca et al., 2008). The mutated hAPP_{Swe/Ind} gene delivered contained both Swedish double missense (Swe) and Indiana single missense mutations (Ind), both of which have been associated clinically with familial AD and demonstrate AD pathology in animal models (Do Carmo and Cuello, 2013; Selkoe, 2001; Yamada and Yamada, 2000). The gene was under the ubiquitin-c promoter, which effectively delivered a single copy of the mutated hAPP_{Swe/Ind} gene into the brain, kidney, lung and heart of F344 rats (Agca et al., 2008). This F344 transgenic rat model has been shown to endogenously express high levels of mutant hAPP_{Swe/Ind}, in addition to endogenous production of APP, but do not develop amyloid plaques with age. However, significant Aβ accumulation is present after external intervention, such as injection of AD patient brain extract into the hippocampus (Rosen et al., 2012) or kaolin-induced hydrocephaly (Silverberg et al., 2015). Both F344 wild type and F344 transgenic rats were obtained directly from the Agca laboratory (Colombia, MO) and homozygous bred in our animal facility. PCR gene analysis was completed to ensure only F344 wild type rats and F344 rats homozygous for the hAPP_{Swe/Ind} gene were included in this study.
2.3 ET-1 and Aβ Preparation

ET-1 is a protein that acts as a vasoconstrictor in the cardiovascular system (Yanagisawa et al., 1988). Using a targeted injection of human ET-1 induces transient focal ischemia approximately 1 hour in duration in rodents and can be used to effectively model the small striatal strokes observed in human AD patients (Amtul et al., 2014; Whitehead et al., 2005a). Human ET-1 (Sigma-Aldrich Canada Co., Oakville, ON) was dissolved in sterile saline achieving a final concentration of 60 pmol. ET-1 was then aliquoted into eppendorf tubes at a 10 µL volume and stored at −80°C until required for use. Sterile saline was used as a control for the ET-1 injections.

The neurotoxic core of the Aβ_{42} peptide, Aβ_{25-35} (Bachem Americas Inc., Torrence, CA), was dissolved in sterile saline creating a 500 nmol concentration and aliquoted into eppendorf tubes. 30 µL aliquots were stored at −80°C until required for use. The non-toxic reverse peptide Aβ_{35-25} (Bachem Americas Inc., Torrence CA) was dissolved in sterile saline achieving a 500 nmol concentration providing the control for surgical procedures. Aβ_{35-25} was aliquoted and stored in the same manner as the Aβ_{25-35}. The shorter, neurotoxic Aβ_{25-35} conserves the biological properties of the canonical full-length Aβ_{42} peptide, while allowing for better diffusion (Kowall et al., 1992; Millucci et al., 2009; Pike et al., 1995; Yankner et al., 1989). The use of Aβ_{25-35} to model AD-like pathophysiology has been verified in numerous rodent models (Amtul et al., 2014; Cheng et al., 2006; Kowall et al., 1992; Nell et al., 2014; Whitehead et al., 2005a, Whitehead et al., 2007; Zussy et al., 2013).

2.4 Surgical Procedures

All rats were anesthetised with 3% isofluorane (Baxter Corporation, Mississauga, ON) at 2% of total airflow on 2 L/min oxygen supply in a Harvard anaesthesia box (Harvard Apparatus, Holliston, MA) attached to a tabletop anesthesia machine (VetEquip Inc., Livermore, CA). Once anesthetised, the head of the rat was shaved and rats were secured in a David Kopf stereotaxic surgical apparatus (David Kopf Instruments, Tujunga, CA) using ear and incisor bars. The temperature of the rats was maintained at 37°C using a heating pad throughout the surgical procedure. Rats remained anesthetised
on gas anesthesia for the duration of the surgery through a nosepiece. The anesthesia was decreased to 1.75% of total airflow after skin incision was complete. Soap, ethanol and iodine were used to sterilize the exposed skin at the surgery site. The skull was then exposed and the landmarks Lambda and Bregma were located. The ear and incisor bars were adjusted accordingly to ensure that these landmarks were aligned at the same height. Bregma was located and marked and its coordinates were used to identify the brain regions for injection. Once all brain regions were located and marked, small burr holes in the skull were made for insertion of an injection syringe.

All surgical reagents (ET-1, Aβ) were kept on dry ice prior to injection during surgery. AD was modeled through bilateral intracerebroventricular injections of 25 µL of 500 nmol Aβ25-35 using a 25 µL Hamilton® syringe (Hamilton Company, Reno, NV). Coordinates of the ventricles with respect to Bregma were ±1.4 mm medial-lateral (ML), -0.8 mm anterior-posterior (AP) and -4.0 mm dorsal-ventral (DV). Injections were completed over twenty minutes per ventricle (1.78 µL/min) with two in situ periods of 3 minutes. Control surgical procedures were completed with Aβ35-25 using the same injection paradigm.

To induce transient focal ischemia, small lacunar strokes were induced through unilateral right striatal injection of 3 µL of 60pmol ET-1 using a 5 µL Hamilton® syringe (Hamilton Company, Reno, NV). Coordinates of the striatum with respect to Bregma were -3.0 mm ML, +0.5 mm AP and -5.0 mm DV. Injections were completed over 5 minutes (0.6 µL/min) with an in situ period of 3 minutes following the injection. Sterile saline was injected in the same manner as a control.

Following completion of injections, rats were administered the analgesic buprenorphine (0.03 mg/mL, Champion Alstoe Animal Health, Whitby, ON) at a dose of 1 mg/kg subcutaneously. Additionally, rats were administered 0.03 mL of the antibiotic Baytril® (22.7 mg/mL enrofloxacin, Bayer HealthCare Animal Health, Mississauga, ON). The surgery site was sutured closed and rats remained in the surgical apparatus with pure oxygen at 2 L/min for 1 minute. Following oxygen administration, rats were closely monitored under a heat lamp during recovery until they regained sternal recumbency.
2.5 CAT-SKL Administration

CAT-SKL is an engineered enzyme different from the canonical catalase due to its altered protein-targeting signal. The sequence of the core catalase enzyme is conserved in the recombinant protein, but CAT-SKL has a serine-lysine-leucine (SKL) targeting sequence. Furthermore, the enzyme has a cell penetrating peptide attached to assist with cellular and tissue delivery (Giordano and Terlecky, 2012; Koepke et al., 2007). CAT-SKL was obtained from Dr. Paul Walton and Dr. Stanley Terlecky (United States patent 7601366 and related patents pending) and stored at 4°C. Rats were randomly assigned to either CAT-SKL or vehicle treatment groups. Stock CAT-SKL (9 mg/mL) was diluted in sterile saline to 1 mg/mL. Wistar rats were administered 1 mg/kg of CAT-SKL or the equivalent volume of saline intraperitoneally. Treatment was administered weekly for four weeks starting one week prior to their scheduled surgery date.

2.6 Surgical Groups

The stroke model procedure was implemented the same in both the Wistar and hAPP<sub>Swe/Ind</sub> transgenic and wild type Fisher 344 rats to model small lacunar stokes in the striatum. The Aβ surgical intervention was only completed in the Wistar rats to model AD. The Aβ paradigm was combined with the stroke procedure to model a co-morbid stroke/Aβ rat. Control rats underwent the same surgical procedures with the injection combination of saline and reverse peptide Aβ<sub>35-25</sub> (RP). Table 1 and Table 2 provide a summary of surgical interventions along with the corresponding animal numbers for each animal model.

2.7 Behaviour: Cylinder Task

The cylinder task assesses the use of forelimbs when contacting the wall of the cylinder apparatus during spontaneous rearing. This task is commonly used to measure forelimb gross motor function and symmetry between forelimbs (Schallert et al., 2002). The cylinder task testing was completed on day -3, 3, 7 and 20 for Wistar rats and on day -3, 7 and 20 for hAPP<sub>Swe/Ind</sub> transgenic rats in regards to surgery day (day 0). Rats were placed in a plexiglass cylinder apparatus (23 cm diameter x 40 cm height) with a perforated lid. A mirror was placed at a 45° angle below the cylinder and a video camera
was positioned to record the full width of the cylinder from the mirror. Each trial had a duration of 5 min and three trials were recorded for each testing day. A ring of coloured tape was placed along the wall three-quarters from the bottom of the cylinder and banana and almond extract (Goodas Food Products Co. LTD., Concord, ON) were applied to the tape to motivate spontaneous rearing during testing.

All videos were compiled and rendered using iMovie for quantitative analysis. Two individuals, blinded to the rat’s surgical identity, analyzed the use of the rat’s forelimbs during each trial using the compiled videos. The percent of affected forelimb use was calculated with the following equation: \([\frac{(\text{affected contacts} + \frac{1}{2} \text{ bilateral contacts})}{\text{total number contacts}} \times 100]\). Each individual rat’s post-surgery values were then standardized to their pre-surgery values as follows: \([(\text{post-surgery percent of affected forelimb use/pre-surgery percent of affected forelimb use})\]. The criterion for each trial was set at six or more forelimb contacts and trials where animals failed to meet six forelimb contacts were excluded. If an animal did not meet the criterion in all three trials, the animal was excluded entirely.

2.8 **Behaviour: Modified Sticky Tape Task**

Sughrue et al. (2006) adapted the modified sticky tape (MST) task from the conventional sticky tape task. Assessment of fine motor function and somatosensory function in the forelimbs was measured through MST analysis. Wistar rats completed the MST on day -1, 3 and 21 in regards to surgery day (day 0). A small piece of tape was wrapped around the rat’s forelimb and the rat was placed into a clean cage for 45 sec. Two individuals, blinded to the rat’s surgical identity, analyzed the amount of time the rat spent attending to the tape during the trial. Three trials for each forelimb were completed on each testing day giving a total of 6 trials per testing day.

The ratio of the time spent attending to the affected forelimb in comparison to the unaffected forelimb was calculated and post-surgery values were standardized to pre-surgery values using the following equation: \([(\text{post-surgery ratio of affected forelimb use/pre-surgery ratio of affected forelimb use})\]. If an animal completely neglected to attend to the tape on either forelimb on any testing day, the animal was excluded entirely.
Table 1. **Stroke and Aβ injection rat model summary of surgical procedures and treatment groups with corresponding animal numbers.** Only animals that underwent successful surgeries and were included in behavioural and immunohistochemical analysis are listed. Abbreviations are as follows: RP = reverse peptide Aβ_{35-25}, Aβ = neurotoxic Aβ_{25-35} and CAT-SKL = catalase with serine-lysine-leucine targeting sequence.

<table>
<thead>
<tr>
<th>Striatal Injection</th>
<th>Bilateral Ventricular Injection</th>
<th>Treatment</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>RP</td>
<td>Saline</td>
<td>6</td>
</tr>
<tr>
<td>Stroke (ET-1)</td>
<td>None</td>
<td>Saline</td>
<td>7</td>
</tr>
<tr>
<td>Stroke (ET-1)</td>
<td>None</td>
<td>CAT-SKL</td>
<td>8</td>
</tr>
<tr>
<td>Stroke (ET-1)</td>
<td>Aβ</td>
<td>Saline</td>
<td>8</td>
</tr>
<tr>
<td>Stroke (ET-1)</td>
<td>Aβ</td>
<td>CAT-SKL</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2. **Summary of surgical procedures with corresponding animal numbers for the hAPP\textsubscript{Swe/Ind} transgenic rat model.** The number of animals with successful surgeries included in behavioural and immunohistochemical analysis and are listed. Abbreviations are as follows: WT = wild type animal and TG = transgenic animal.

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>Surgical Procedure</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Saline</td>
<td>8</td>
</tr>
<tr>
<td>WT</td>
<td>Stroke</td>
<td>9</td>
</tr>
<tr>
<td>TG</td>
<td>Saline</td>
<td>8</td>
</tr>
<tr>
<td>TG</td>
<td>Stroke</td>
<td>8</td>
</tr>
</tbody>
</table>
2.9 **Behaviour: Beam-walk Task**

The beam-walk task was implemented to measure motor function in the hindlimbs when completing a skilled movement requiring accurate foot placement and balance. hAPP\textsubscript{Swe/Ind} transgenic and wild type rats were trained to walk across a 1.9 cm wide wooden beam secured approximately 40 cm above the ground. The environment enrichment tubing from the rat’s home cage was placed at the opposite side of the beam as motivation to walk across the beam. During training, rats were guided to walk across the beam. Once rats crossed the beam without assistance, the training session was ceased. A video camera was positioned to view the full length of the beam and six trials (three in each direction) were recorded on day -7 and day 21 with respect to surgery day. All videos were compiled and rendered using iMovie for quantitative analysis. Two individuals, blinded to the rat’s surgical identity, quantified the number of hindlimb errors and total steps required to cross the beam using the compiled videos. Hindlimb errors were standardized to the number of total steps and the difference between pre-surgery and post-surgery errors was calculated (post-surgery errors – pre-surgery errors).

2.10 **Behaviour: Morris Water Maze**

Morris water maze (MWM) testing is a commonly used paradigm for assessment of learning and memory, specifically focusing on spatial learning and memory (Vorhees and Williams, 2006). The MWM consisted of a circular pool (148 cm diameter x 58 cm depth) filled with 36 cm deep water. The water was made opaque using non-toxic black paint and the temperature was kept at approximately 21°C. The pool was divided into four equal quadrants designated as northeast (NE), southeast (SE), southwest (SW) and northwest (NW). A room divider was placed on one side of the pool to create an enclosed pool area that allowed experimenters to be out of sight during testing. All trials were recorded through a webcam (Logitech, Newark, CA) positioned above the center of the pool and connected to a laptop. Behavioural data (i.e. swimming speed, distance and latency) was compiled in ANY-maze® video-tracking software (Stoelting CO, Wood Dale, IL). Outside the NW quadrant, a radio at low volume was on during all testing to provide background noise. This was used to avoid sudden startle responses to unexpected
loud noises during testing. Wistar and Fisher hAPP<sub>swe/Ind</sub> transgenic and wild type rats completed three phases of testing: spatial learning, probe testing and cued learning. Furthermore, two additional groups (Aβ + saline and Aβ + CAT-SKL) from a previous investigation for which the original MWM data was available were analyzed in the manner in this study and included with the MWM data for the stroke/Aβ injection model.

During spatial learning, rats were trained to locate a hidden stationary platform in the SW quadrant. External cues were placed along the walls and the divider to assist rats in locating the platform. 4 trials per day were completed over 4 days of training for a total of 16 trials starting on day 8 after surgery (day 0). An inter-trial interval time of 20 min was allotted for each rat. Rats were placed in the pool at one of the four designated start positions (N, E, SE, NW) assigned randomly prior to testing and given 90 sec to find the location of the platform. If a rat did not find the platform, they were guided to its location. If a rat was successful in finding the platform, the tracking software was stopped and the trial ended. During the first two days of training, rats were required to remain on the platform for 30 sec upon finding the platform. A rat was not removed from the water until they had remained on the platform for 30 sec consecutively. After two days of training, rats were only required to sit on the platform for 15 sec. Distance and latency to reach the platform were measured as an indication of learning.

Following completion of spatial learning, a single probe trial was conducted on days 12 and 19 to assess short-term and long-term spatial reference memory, respectively. The platform was removed from the pool and the extra-maze cues remained in place. Each rat was released into the pool from the NE start position and allowed to swim for 30 sec. Once the probe trial was completed on day 12, the platform was returned to the SW quadrant of the pool while the rat remained in the pool. A rat was required to remain on the platform for 10 seconds before they were retrieved from the pool in order to avoid memory extinction on day 19. Spatial reference memory was measured as the latency to first enter the SW quadrant target zone. If a statistically significant differences in speed during probe testing was observed, this was corrected for using the following equation: (|[rat swim speed/average group swim speed] x latency to first enter the target zone|).
As a control, cued learning trials occurred after all probe trials were completed on day 20 and 21. Cued learning assessed any potential differences in motivation to escape the MWM and the ability to use spatial cues to locate a hidden platform. During cued learning, a cue was attached to the platform that was visible above the water and all extra-maze cues were removed. The platform (NE, SE, SW, NW) and start (N, E, S, W) position combination was varied every trial for each rat. A rat was released into the pool at a designated start position and was allotted 60 sec to locate the platform. Upon reaching the platform on day 20, rats were required to remain on the platform for 15 sec before they were retrieved from the pool. On day 21, rats were removed from the pool immediately upon finding the platform. Quantifying the distance and latency to reach the platform assessed cued learning.

2.11 Behaviour: Summary of Timelines

Each model was subjected to a different behavioural paradigm and timeline. The stroke and Aβ injection model completed the cylinder task, MST task and MWM and a summary of the experimental protocol in regards to surgery day (day 0) is provided in Figure 1. The hAPP<sub>Swe/Ind</sub> transgenic model completed the cylinder task, beam-walk task and MWM and the timeline is summarized in Figure 2.

2.12 Euthanasia

Twenty-one days post-surgery, rats were be administered a 2.0 mL overdose of euthanyl (240 mg/mL, Bimeda-MTC Animal Health Inc., Cambridge, ON) intraperitoneally. Following drug administration, rats were transaortically perfused with 0.01 M phosphate-buffered saline solution (PBS, pH 7.35) for 3 min. Subsequently, rats were formalin-fixed with 4% paraformaldehyde (PFA) for 7 min. Perfused brain tissue was extracted and placed in a vial for further fixation with 4% PFA for 24 hr at 4°C and then was transferred into 30% sucrose and stored at 4°C until sectioning.

2.13 Immunohistochemistry: Microglia

Formalin-fixed brain tissue was sliced into 35 µm coronal sections using the Cryostar NX50 Cryostat (Thermo Fisher Scientific Inc., Waltham, MA) and free-floating
Figure 1. Stroke and Aβ injection model behaviour timeline. All testing days are in reference to surgery day 0. (A) Pre-surgery testing for the cylinder task (C) and modified sticky tape task (MST) was completed on day -3 and -1, respectively. Post-surgery testing for C occurred on day 3, 7 and 20 and MST testing occurred on day 3 and 21. (B) MWM spatial learning was completed day 8, 9, 10 and 11 with probe trials following on day 12 and 19. Cued learning occurred on day 20 and 21.
Figure 2. hAPP<sub>Swe/Ind</sub> transgenic rat model behavioural paradigm for motor and cognitive tasks. Surgery day is assigned day 0 and all testing days are in reference to this day. (A) Pre-surgery testing for cylinder task (C) and beam traversing task (BM) was completed on day -3 and -7, respectively. Post-surgery testing occurred on day 7 and 20 for C and day 21 for BM. (B) Morris Water Maze spatial learning was completed day 8, 9, 10 and 11 with probe trials following on day 12 and 19. Cued learning occurred on day 20 and 21.
sections were divided into a series of 6 and stored in cryoprotectant (sucrose, ethylene glycol, phosphate buffer) at –20°C until required for immunohistochemical processing. Formalin-fixed brain slices were analyzed for microglial activation with a two-day immunohistochemistry protocol. A single series of a 6 series division was further divided into a series of 3 and 2 series of the 3 were immunohistochemically processed. On day 1, brain sections were washed with 0.1 M PBS (pH 7.35) six times for 10 min to remove residual cryoprotectant. To block endogenous peroxidase activity in the brain tissue, sections were then incubated in 1% H2O2 for 10 min. Sections were then washed with 0.1 M PBS (pH 7.35) three times for 5 min before being incubated in 2% horse serum blocking solution consisting of horse serum (HO146, Sigma Aldrich Canada Co., Oakville, ON) diluted in 0.2% PBST (0.1 M PBS, pH 7.35 with 0.2% Triton X-100) for 1 hr. Sections were then incubated on a shaker at 4°C overnight with a 1:1000 dilution of the OX-6 primary antibody (RT1B purified monoclonal mouse, BD Pharmingen™, Mississauga, ON) with 2% horse serum blocking solution. The OX-6 primary antibody detects MHC-II receptors on activated microglial cells.

On day 2, sections were washed with 0.1 M PBS (pH 7.35) three times for 5 min. Following washing, sections were incubated with biotinylated horse anti-mouse IgG (H + L) secondary antibody (Vector Laboratories Canada Inc., Burlington, ON) diluted to 1:500 with 2% horse serum blocking solution for 1 hr. Sections were subsequently washed with 0.1 M PBS (pH 7.35) three times for 5 min. Sections were then incubated in Avidin-Biotin Complex reagent (Vector Laboratories Canada Inc., Burlington, ON) prepared in 0.2% PBST for 1 hr. Again, sections were washed with 0.1 M PBS (pH 7.35) three times for 5 min. Sections were then incubated in 3,3’-diaminobenzidine tetrahydrochloride (Sigma Aldrich Canada Co., Oakville, ON) diluted to 0.05% in 0.1 M PBS (pH 7.35) with 1% H2O2 for 2 – 3 min. Sections were washed in 0.1 M PBS (pH 7.35) three times for 5 min and mounted on VWR VistaVision™ microscope slides (VWR International LLC., Mississauga, ON) with 0.3% gelatin and left to air dry overnight. After drying, the slides were dehydrated through a series of increasing ethanol concentrations (50%, 70%, 95%, 100%) for 5 minutes at each concentration. Slides were then immersed in a 50% ethanol/50% xylene solution for 5 minutes and subsequently immersed in 100% xylene for 10 minutes. The slides were covered with microscope slide
coverslips using DEPEX mounting media (Electron Microscopy Sciences, Hatfield, PA, USA) immediately after immersion in xylene.

2.14 Imaging

Immunohistochemically processed brain sections were imaged with a Nikon Eclipse Ni-E microscope using NIS-Elements Imaging Software Version 4.30.02 (Nikon Instruments Inc., Melville, NY). Regions of interest for analysis included the infarct area in the striatum (between Bregma 2.28 mm and -1.44 mm) and the basal forebrain (Bregma 0.96 – 0.36 mm). Images for analysis of the striatum and the basal forebrain were taken at 2x and 10x magnification, respectively.

2.15 Immunohistochemical Quantification

OX-6 microglial inflammation analysis was completed using the 64-bit ImageJ software (Version 1.48u4, Wayne Rasband, National Institutes of Health, Bethesda, MD). Two individuals, blinded to the surgical identity of the rats, completed all immunohistochemical analysis. OX-6 inflammation volume in the striatum was calculated using the following equation: \([\Sigma \text{OX-6 immunolabelled surface area} \times \left\{\frac{n-1}{0.035 \times 0.315}\right\}]\), where \(n\) = number of immunolabelled sections, 0.035 mm is the thickness of the sections and 0.315 mm is the distance between adjacent sections. OX-6 microglial cell counts were completed on a total of four consecutive sections corresponding to the basal forebrain (Bregma 0.96 – 0.36 mm). Counts were completed by manually selecting positively immunostained microglia in the predefined basal forebrain region.

2.16 Statistical Analysis

One-way and two-way analysis of variance (ANOVA) was completed with a Tukey’s or Bonferroni multiple comparisons post hoc test using GraphPad Version 6.0 software for Mac (GraphPad Software Inc., La Jolla, CA). Data is represented as the mean ± SEM with corresponding analysis methods noted in the figure legends. A value of \(p \leq 0.05\) was considered significant and where necessary asterisks denote significance on data sets.
Chapter 3: Results
3.1 Stroke and Aβ Injection Model: Effect of Antioxidant CAT-SKL

3.1.1 Motor Function in the Cylinder Task

Stroke is often associated with phenotypic motor deficits that result due to the loss of brain function within the infarct area. Previous studies that have demonstrated exacerbated motor deficits in the co-morbid AD/stroke condition lead to the use of the cylinder task to investigate motor function in this model (Biernaskie and Corbett, 2001; Clarke et al., 2007; Soleman et al., 2010). The goal of the cylinder task was to assess the effect of stroke and the co-morbid condition on forelimb motor function and symmetry. Due to the connectivity of the brain, stroke that is constrained to one hemisphere results in one-sided motor deficits affecting the side of the body contralateral to the stroke. In the animal models used, the stroke is induced in the right striatum and motor deficits should occur in the left side of the body. Therefore, the left is considered the affected side.

The percent of affected forelimb use was standardized to individual baseline (day -3) values resulting in an affected forelimb use score. A score of 1.0 represents the equal use of the unaffected and affected forelimb when contacting the wall of the cylinder during spontaneous rearing. Scoring greater than 1.0 represents a greater use of the affected forelimb and scoring less than 1.0 signifies a greater use of the unaffected forelimb. In the cylinder task, there were no significant differences in affected forelimb use between testing days in any surgical group (Figure 3).

3.1.2 Motor Function in the Modified Sticky Tape

Other levels of motor function can also be affected by stroke and the co-morbid condition. MST task testing was aimed at assessing fine motor function and somatosensory function in the forelimb. Quantification of the time spent attending to the tape on each forelimb was recorded and a ratio of time attending to affected compared to unaffected forelimb was calculated. Subsequently, ratios were standardized to baseline (day -1) values resulting in a standardized ratio score. A ratio greater than 1.0 signifies greater attendance to the affected forelimb and less than 1.0 represents greater attendance to the unaffected forelimb. A score equal to 1.0 represents equal attending time to the affected and unaffected forelimb. For all surgical groups, no significant differences
Figure 3. Testing forelimb gross motor function using the cylinder task. Percent use of affected forelimb was calculated ({[affected contacts + 1/2 bilateral contacts]/total number contacts}x100) and averaged over 3 trials per day. The average percent of contralateral forelimb use was then normalized to individual day -3 pre-surgery values (post-surgery % affected forelimb use/pre-surgery % affected forelimb use). The red dotted line at the level of 1.0 on the y-axis represents the equal use of the affected and unaffected forelimb during spontaneous rearing. Animal numbers are as follows: saline/RP + saline (n=6), stroke + saline (n=5), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=8), stroke/Aβ + CAT-SKL (n=8). Values are presented as mean ± SEM. (Two-way ANOVA, Tukey post hoc test).
Figure 4. Fine motor or somatosensory function assessment using the modified sticky tape task. The ratio of the time spent attending to the tape on the affected forelimb compared to the unaffected forelimb was calculated and averaged over 3 trials per day. Average ratios were then normalized to individual day -3 pre-surgery values (post-surgery time attending to affected forelimb/pre-surgery time attending to affected forelimb). The red dotted line at the level of 1.0 on the y-axis represents the equal use of the affected and unaffected forelimb. Animal numbers are as follows: saline/RP + saline (n=6), stroke + saline (n=6), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=7), stroke/Aβ + CAT-SKL (n=8). Values are presented as mean ± SEM. (Two-way ANOVA, Tukey post hoc test).
were observed in time spent attending to the affected forelimb between testing days (Figure 4).

### 3.1.3 Attenuation of Cognitive Deficit in Co-morbid Condition with CAT-SKL

Spatial learning and reference memory in the MWM was a test of cognitive processes often mediated by the hippocampus, a highly vulnerable area of the brain in AD (D’Hooge and De Deyn, 2001; Gouras et al., 2000; Selkoe, 2001). While clinically it has been demonstrated that patients with stroke and AD present with worse cognitive deficits than those with AD alone (Schneider et al., 2007; Snowdon et al., 1997), there has been a lack of experimental evidence investigating this interaction. All learning and memory testing was completed after induction of stroke and Aβ toxicity to assess the effect of the co-morbid condition on learning and memory. Moreover, this testing allowed assessment of the effectiveness of CAT-SKL in attenuating cognitive deficits.

Latency and distance to reach the platform were measured as indicators of learning. Latency to reach the platform decreased significantly over 4 days of spatial learning for all surgical groups (day 8 vs. day 11, \( p < 0.05 \), Figure 5A). This effect was also observed in the distance to reach the platform, which significantly decreased over the course of learning for all surgical groups (day 8 vs. day 11, \( p < 0.05 \), Figure 5B). The decreased latency and distance to reach the platform signified that all groups successfully learned to locate the platform and the extent of learning was the same between all groups, as there were no differences between surgical groups on any testing day (Figure 5A-B). Swim speed was also monitored during learning to ensure there were no differences between groups that would account for the decreased latency and distance to reach the platform. There were no significant differences in swim speed across all surgical groups observed on any of the testing days (Figure 5C).

Spatial reference memory was measured as the latency to first entry into the target zone during a probe trial where the platform is removed. The target zone is the quadrant where the platform was located during spatial learning. Memory was tested 24 hours after
Figure 5. Spatial learning of hidden platform location in MWM. Spatial learning is represented as latency (A) and path length (B) to reach the platform across four consecutive days of learning. Swim speed (C) was monitored during spatial learning to ensure differences in latency and path length were not due to speed differences. Animal numbers are as follows: saline/RP + saline (n=4), Aβ + saline (n=9), Aβ + CAT-SKL (n=8), stroke + saline (n=7), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=8), stroke/Aβ + CAT-SKL (n=8). All values are presented as mean ± SEM. (Two-way ANOVA, Tukey post hoc).
spatial learning (day 12) and one week following spatial learning (day 19) to test short-term and long-term memory, respectively. The co-morbid group treated with saline (stroke/Aβ + saline) required a significantly greater latency to first enter the target zone on day 19 compared to day 12 ($p < 0.05$, Figure 6). This effect was not demonstrated in the control groups, Aβ or stroke alone groups (Figure 6). Furthermore, there was no difference in latency to first entry into the target zone observed in the co-morbid group treated with CAT-SKL (stroke/Aβ + CAT-SKL, Figure 6), indicating that CAT-SKL attenuated the memory deficits observed in the co-morbid condition. To account for differences in latency due to swim speed, the swim speed was also measured during memory testing. There were no differences in swim speed between surgical groups or days during probe testing (data not shown). Furthermore, percent time in the target zone during probe testing was analyzed, but was not significant between groups (data not shown).

Cued learning was completed to ensure there were no differences in the ability to use visual cues for orientation and motivation to escape the pool. Learning was measured as the latency and distance to reach the platform and swim speed was monitored. All measurements were averaged over two days of learning. There were no significant differences in latency and distance to reach the platform or swim speed across all surgical groups (Figure 7A-C). This suggests that all groups were able to use spatial cues to locate the platform and were equally motivated to escape the pool.

3.1.4 Microglia Within the Infarct Area

Neuroinflammation has a major role in the pathophysiology of AD and stroke with emphasis on the role of microglia in the development of both diseases (Heneka et al., 2015; Latta et al., 2014; Lee et al., 2010; Tuppo and Arias, 2005). The extent of microglia within the infarct region was measured as an area of activated microglia. The antibody OX-6 labels microglia presenting the MHC-II complex, which signifies an activated phagocytic state (Akiyama et al., 2000; Luber-Narod and Rogers, 1988, Wang et al., 2007). Across all groups there were no significant differences in the volume of inflammation in the right striatum (Figure 8F).
Figure 6. CAT-SKL rescues reference memory deficits in co-morbid animals in MWM. Spatial reference memory is represented as the latency to first enter the target zone during probe testing on day 12 and 19. The target zone is the quadrant that housed the hidden platform during spatial learning. Animal numbers are as follows: saline/RP + saline (n=4), Aβ + saline (n=9), Aβ + CAT-SKL (n=8), stroke + saline (n=7), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=8), stroke/Aβ + CAT-SKL (n=8). All values are presented as mean ± SEM and significance is represented with an asterisk. (p < 0.05, Two-way ANOVA, Bonferroni post hoc).
Figure 7. Cued learning of a hidden platform with a visible cue attached in the MWM. Latency (A) and path length (B) to reach the platform are represented as an average of all eight trials across two days of cued learning. Swim speed (C) is presented as an average of all eight trials across two days of cued learning. Animal numbers are as follows: saline/RP + saline (n=4), Aβ + saline (n=9), Aβ + CAT-SKL (n=8), stroke + saline (n=7), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=8), stroke/Aβ + CAT-SKL (n=8). Values are presented as the mean ± SEM. (One-way ANOVA, Tukey post hoc).
Figure 8. Microglial expression within the right striatum infarct area. (A-E) 2X photomicrographs of representative microglial inflammation in the right striatum. (F) Volume of the right striatal inflammation based on the extent of OX-6 immunolabelled microglia. Volume was calculated using the following equation: \[ (\sum \text{OX-6 immunolabelled surface area}) \times (\{n-1\} \times 0.035 \times 0.315) \] where \( n \) = number of OX-6 immunolabelled sections. Scale bar = 1 mm. Animal numbers are as follows: saline/RP + saline (n=6), stroke + saline (n=7), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=8), stroke/Aβ + CAT-SKL (n=8). Values are presented as the mean ± SEM. (One-way ANOVA, Tukey post hoc).
3.1.5 CAT-SKL Attenuates Microglial Expression Within the Basal Forebrain

The basal forebrain is one of the earliest brain structures affected in the pathogenesis of AD (Auld et al., 2002; Stepanichev et al., 2004; Vaucher et al., 2001). Coupled with evidence of neuroinflammation present in the early stages of AD and its connectivity to the hippocampus, the basal forebrain is a major region of interest in regards to the early stages of AD. The co-morbid group treated with saline (stroke/Aβ + saline) demonstrated greater microglial numbers in the basal forebrain compared to both stroke alone groups ($p < 0.05$, Figure 9L). In comparison to the control group, the co-morbid group treated with saline was the only group with significantly increased microglias in the basal forebrain ($p < 0.05$, Figure 9L). The lack of significance between the co-morbid group treated with CAT-SKL and the control and stroke alone groups suggests that CAT-SKL was able to attenuate the level of microglia in the basal forebrain in the co-morbid condition.
Figure 9. **CAT-SKL attenuates microglial numbers within the basal forebrain of comorbid animals.** Representative 2X (A-E) and 10X (F-J) photomicrographs of microglia in the basal forebrain. Boxed areas in A-E represent the area in the 10X photomicrographs that accurately represented the mean microglial cell count for each group. (K) Atlas representation of rat brain with sections used in counting outlined with thick black lines. (L) Microglial cell counts of 10X photomicrographs of OX-6 immunolabelled basal forebrain sections. Scale bar in E = 1mm. Scale bar in J = 100 µm. Animal numbers are as follows: saline/RP + saline (n=6), stroke + saline (n=6), stroke + CAT-SKL (n=8), stroke/Aβ + saline (n=6), stroke/Aβ + CAT-SKL (n=7). Values are presented as mean ± SEM and significance is represented with an asterisk. (p < 0.05, One-way ANOVA, Tukey post hoc).
3.2 Stroke and hAPP_{Swe/Ind} Transgenic Rat Model: Effect of Stroke Injury

3.2.1 Motor Function in the Cylinder Task

Previous studies have demonstrated an exacerbation in stroke behavioural pathology in the co-morbid AD and stroke condition (Biernaskie and Corbett, 2001; Clarke et al., 2007; Soleman et al., 2010; Whitehead et al., 2005a; Whitehead et al., 2007). The cylinder task measures gross motor function in the forelimbs during spontaneous rearing and affected forelimb use was standardized to baseline values (day -3). Statistical analysis did not show significant differences in affected forelimb use between days across all surgical groups (Figure 10).

3.2.2 Motor Function in the Beam Walk Task

There is a great deal of evidence implicating subtle changes in gait and balance in AD patients in the early stages of the disease (Sheridan and Hausdorff, 2007), but the effect of the interaction between stroke and AD on gait has rarely been investigated. The task of walking across a thin wooden beam requires a greater level of coordination and skilled limb control compared to the motor function required in the cylinder task. Hindlimb function was measured as the number of errors per step across the entire length of the beam for the affected and unaffected hindlimbs. These values were standardized to baseline (day -7) errors per step resulting in a hindlimb error score. Furthermore, total steps were presented as the raw number of steps required to cross the full length of the beam. The number of errors was relatively low (less than or equal to 1 error in total) across all six trials and there were no significant differences observed for the hindlimb error score for the affected or unaffected hindlimb between groups (Figure 11A-B). However, the co-morbid condition (transgenic + stroke) was the only group that required significantly more steps to cross the beam on day 21 compared to day -7 (p < 0.05, Figure 11C). This may indicate early changes in the gait pattern of the transgenic + stroke group as a result of the co-morbid condition.
Figure 10. Assessment of gross forelimb motor function using the cylinder task.

Percent use of affected forelimb was calculated (\(\frac{\text{affected contacts} + 1/2 \ \text{bilateral contacts}}{\text{total number contacts}}\) \times 100) and averaged over 3 trials per day. Averaged values were then normalized to individual day -3 pre-surgery values (post- surgery % affected forelimb use/pre-surgery % affected forelimb use). The red dotted line at the level of 1.0 on the y-axis indicates the value that represents the equal use of the affected and unaffected forelimb during spontaneous rearing. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=7), WT + stroke (n=8), TG + saline (n=8), TG + stroke (n=6). Values are presented as mean ± SEM. (Two-way ANOVA, Tukey post hoc).
Figure 11. Co-morbid rats take an increased number of steps during the beam-walk task. Errors were counted as hindlimb slips while walking across the beam for affected (A) and unaffected (B) hindlimbs. Difference in errors was calculated to normalize to baseline (day -7) values (post-surgery errors – pre-surgery errors). Total steps (C) required to cross the length of the beam. All values are an average of 6 trials per testing day. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=6), WT + stroke (n=6), TG + saline (n=6), TG + stroke (n=5). Values are presented as mean ± SEM and significance is annotated with an asterisks. (Two-way ANOVA, Tukey post hoc for hindlimb errors and $p < 0.05$, Two-way ANOVA, Bonferroni post hoc for total steps).
3.2.3 Spatial Reference Memory Deficit Present in the Co-morbid Condition

Spatial learning was measured as the latency and distance to the reach the platform across 4 days of acquisition. For all groups there was a significant decrease in the latency to reach the platform observed (day 8 vs. day 11, \( p < 0.05 \), Figure 12A). This result was accompanied by a significant decrease in the distance to reach the platform over 4 days of spatial learning (day 8 vs. day 11, \( p < 0.05 \), Figure 12B). This suggests successful learning of the platform location by all surgical groups. Furthermore, there were no differences in learning between surgical groups, as there were no differences in the latency or distance to reach the platform between groups on each testing day (Figure 12A-B). Swim speed was monitored to ensure that differences in time and distance were not a result of different swim speeds during testing and there were no differences in swim speed across groups on each testing day (Figure 12C).

Spatial reference memory for the target zone that housed the hidden platform during spatial learning was measured as the latency to first entry into the target zone during probe testing. Short-term and long-term memory was assessed 24 hours after spatial learning (day 12) and 1 week after spatial learning (day 19), respectively. The swim speed during probe testing was monitored to ensure that differences in swim speed did not account for the difference in latency. While there were no differences across surgical groups on each testing day, the co-morbid group (transgenic + stroke) exhibited slower swim speeds on day 19 compared to day 12 (\( p < 0.05 \), data not shown). To account for the difference in speed, the latency to first enter into the target zone was standardized to a speed ratio for each individual rat on both testing days and is presented in Figure 13 as the standardized latency to first entry into the target zone. The co-morbid condition (transgenic + stroke) demonstrated a greater latency to first enter into the target zone across testing days (day 12 vs. day 19, \( p < 0.05 \), Figure 13). This effect indicates that the co-morbid condition induced long-term memory deficits, as the difference was not observed among other surgical groups representing each condition alone (Figure 13). Percent time in the target zone during probe testing was also analyzed, but was not significant between groups (data not shown).
Figure 12. Spatial learning of location of hidden platform in MWM. Spatial learning is represented as latency (A) and path length (B) to reach the platform across four consecutive days of learning. Swim speed (C) was monitored during spatial learning to ensure differences in latency and path length were not due to speed differences. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=8), WT + stroke (n=9), TG + saline (n=8), TG + stroke (n=8). All values are presented as mean ± SEM. (*p<0.05, Two-way ANOVA, Tukey post hoc).
Figure 13. Co-morbid transgenic animals with stroke demonstrate spatial reference memory deficits. Spatial reference memory is represented as the latency to first enter the target zone during probe testing on day 12 and 19. The target zone is the quadrant that housed the hidden platform during spatial learning. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=8), WT + stroke (n=9), TG + saline (n=8), TG + stroke (n=8). All values are presented as mean ± SEM and significance is represented with an asterisk. ($p < 0.05$, Two-way ANOVA, Bonferroni post hoc).
Cued learning was implemented to assess motivation to escape the pool and the ability to use spatial cues to locate a hidden platform. The latency and distance to reach the platform was measured and averaged over two days of learning. Additionally, swim speed was assessed and averaged across both testing days. The outcome indicates that there were no differences in motivation to escape the pool or the ability to use spatial cues, as the latency and distance to reach the platform was not significantly different between groups (Figure 14A-B). A potential difference in the latency and distance to reach the platform was not masked by differences in swim speed, as there were no differences in swim speed across all surgical groups (Figure 14C).

### 3.2.4 Increased Presence of Activated Microglia in the Infarct Region

Transgenic rats with stroke exhibited a significantly greater volume of activated microglia in the right striatum compared to their transgenic counterparts without stroke ($p < 0.05$, Figure 15E). Furthermore, transgenic + stroke rats tended to have a greater volume of microglia compared to wild type rats without stroke, but this effect was not significant ($p = 0.0509$, Figure 15E). Wild type rats with stroke did not demonstrate significantly greater levels of microglial inflammation within the infarct region compared to their wild type and transgenic counterparts without stroke (Figure 15E). The coupling of these results suggests that the co-morbid condition resulted in an exacerbated microglial inflammation volume in the right striatum.

### 3.2.5 Activated Microglia Neuroinflammation in the Basal Forebrain

Previously, Aβ toxicity alone induced a significant increase in activated microglia presence in the basal forebrain (Nell et al., 2014). Therefore, analysis of the effect of the co-morbidity of stroke and AD on microglia in the basal forebrain was conducted. Cell counts of activated microglia detected using the OX-6 antibody were quantified in the basal forebrain. There were no significant differences in the number of microglia present in the basal forebrain across surgical groups (Figure 16J). The co-morbid group (transgenic + stroke) tended to have greater levels of activated microglia compared to their wild type counterparts with or without stroke, but this effect was not significant ($p = 0.0515$ and $p = 0.0889$, Figure 16J).
Figure 14. MWM cued learning of a hidden platform with a visible cue attached.

Latency (A) and path length (B) to reach the platform are represented as an average of all eight trials across two days of cued learning. Swim speed (C) is presented as an average of all eight trials across two days of cued learning. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=8), WT + stroke (n=9), TG + saline (n=8), TG + stroke (n=8). All values are presented as mean ± SEM. (One-way ANOVA, Tukey post hoc).
Figure 15. Co-morbid transgenic animals with stroke demonstrate exacerbated microglial volume in the right striatum infarct area. (A-D) 2X photomicrographs of representative microglial inflammation in the right striatum. (E) Volume of right striatal inflammation based on the extent of OX-6 immunolabelled microglia. Volume was calculated using the following equation: \[ \left( \Sigma \text{OX-6 inflammation surface area} \right) \times \left( \left\{ \frac{n-1}{0.035} \right\} \times 0.315 \right) \] where \( n \) = number of OX-6 immunolabelled sections. Scale bar = 1 mm. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=7), WT + stroke (n=7), TG + saline (n=6), TG + stroke (n=6). All values are presented as mean ± SEM and significance is annotated by an asterisk. \( p < 0.05 \), One-way ANOVA, Tukey post hoc.)
Figure 16. Activated microglial presence in the basal forebrain. Representative 2X (A-D) and 10X (E-H) photomicrographs of microglia in the basal forebrain. Boxed areas in A-D represent the area in the 10X photomicrographs that accurately represented the mean microglial cell count for each group. (I) Atlas representation of rat brain with areas used in counting outlined with thick black lines. (J) Microglial cell counts of 10X photomicrographs of OX-6 immunolabelled basal forebrain sections. Scale bar in D = 1 mm. Scale bar in H = 100 µm. WT represents wild type animals and TG represents transgenic animals. Animal numbers are as follows: WT + saline (n=7), WT + stroke (n=7), TG + saline (n=6), TG + stroke (n=6). All values are presented as mean ± SEM. (One-way ANOVA, Tukey post hoc).
Chapter 4: Discussion
This study used two unique co-morbid rat models of stroke and AD to examine gross and fine motor changes, cognitive impairment and neuroinflammation. In the first study, stroke was paired with the intracerebroventricular Aβ toxicity model in six-month-old Wistar rats. Previously, this had only been implemented in three-month-old rats (Amtul et al., 2014; Whitehead et al., 2005b; Whitehead et al., 2007). While there were no observed motor deficits, there were significant cognitive deficits related to spatial reference memory exhibited in the six-month-old co-morbid model. This cognitive deficit was not apparent in the control or stroke alone groups and was ameliorated when the co-morbid group received CAT-SKL treatment. Previous studies have demonstrated the protective effect of CAT-SKL in \textit{in vitro} (Giordano et al., 2014) and \textit{in vivo} (Nell, 2013) models of Aβ toxicity. However, for the first time, this study demonstrated the effectiveness of CAT-SKL as a protective therapeutic agent against cognitive decline in a co-morbid animal model of stroke and Aβ toxicity. Previous studies investigating Aβ toxicity have suggested that neuroinflammation plays a key role in the pathogenesis of AD (Haga et al., 1989; Lue et al., 1996; Nell et al., 2014). One study observed an increased microglia neuroinflammatory response in the basal forebrain, an area of the brain implicated in the early stages of AD (Auld et al., 2002; Nell et al., 2014). In the present study, the co-morbid condition had significantly increased activated microglia in the basal forebrain that was attenuated upon treatment with CAT-SKL.

In the second series of experiments, a novel model was utilized in the investigation of the co-morbidity of stroke and AD. This is the first evidence of stroke inducing pathological cognitive decline and neuroinflammation in a hAPP\textsubscript{Swe/Ind} transgenic rat model that alone does not develop any pathological hallmarks of AD with age. The co-morbid group demonstrated early indication of potential gait disturbances in the beam-walk task, which has been considered an early and often overlooked symptom in AD (Sheridan and Hausdorff, 2007). Moreover, transgenic rats with stroke were the only group to exhibit deficits in hippocampal-dependent spatial reference memory in the MWM. Previous studies have postulated that increased levels of neuroinflammation are involved in the early stages of the pathogenesis of AD (Akiyama et al., 2000; Heneka et al., 2015; Wyss-Coray and Mucke, 2002). Furthermore, the exacerbation of microglia
within the infarct region in a co-morbid stroke and Aβ toxicity rat model has been previously demonstrated (Hepburn, 2012; Whitehead et al., 2007). Immunohistochemical investigation of activated microglia demonstrated that the co-morbidity of stroke in the transgenic group resulted in increased levels of microglial neuroinflammation in the infarct region.

4.1 Motor Assessment: Cylinder, Modified Sticky Tape and Beam-walk Tasks

In both co-morbid models, there was no evidence of forelimb gross motor deficits during spontaneous rearing in the cylinder task. This task has been widely implemented in research investigating various models of stroke. Clarke et al. (2007) demonstrated that APP transgenic rats with MCAO exhibit a significant decrease in the use of the affected forelimb for postural support during spontaneous rearing. Furthermore, a decrease in the use of the affected forelimb in an ET-1 induced cortical stroke model (Soleman et al., 2010) and bilateral CCAO model has been previously observed (Choi et al., 2011). These stroke models involve relatively large regions of the cortex and would be expected to induce relatively gross contralateral motor deficits. To our knowledge, this task has never been implemented in the striatal stroke model.

The striatum is a key structure in the basal ganglia, which also includes the globus pallidus, substantia nigra, thalamus and cortex. Through a series of connections, these structures regulate each other by sending and receiving signals and ultimately provide information to control motor movement (Groenewegen, 2003). In Parkinson’s patients, dysfunction of the basal ganglia results in the phenotypic inability to control motor movement. For example, the striatum receives aberrant signalling from the damaged neurons of the substantia nigra resulting in the disruption of this pathway and contributing to the inability to control motor movement (Ena et al., 2011; Moore et al., 2010). While it is widely accepted that the striatum plays a role in motor control, the striatal lesion induced in both co-morbid models may be incapable of producing major gross motor deficits due to the relatively small size of the lesion. It is possible that with a longer timeline following stroke intervention or a larger stroke encompassing more of the striatum, significant gross motor deficits would appear during forelimb use in both the
hAPP_{Swe/Ind} transgenic model and the stroke and Aβ injection model. While the cylinder task is an effective paradigm for detecting motor deficits in previously mentioned stroke models, it is also possible that the location and size of the infarct is incapable of producing such motor deficits and no amount of time will influence the outcome.

Investigation of fine motor and somatosensory function in the MST task produced similar results to the cylinder task. The co-morbid stroke and Aβ injection model did not develop fine motor or somatosensory deficits, as was the case with all of the other surgical groups. Sughrue et al. (2006) adapted the MST from the conventional sticky tape task in order to create testing that was more specific to focal unilateral damage and did not require pre-training. It has previously been demonstrated that rats with MCAO are significantly less attentive to tape on the affected forelimb compared to the unaffected forelimb (Komotar et al., 2007; Sughrue et al., 2006). This result was not replicated using the conventional sticky tape task in the same model (Sughrue et al., 2006). Moreover, it has previously been shown that rats with an ET-1 induced striatal stroke demonstrated impaired functional recovery during assessment with MST testing (Carodoso et al., 2013).

Exacerbation of fine motor deficits has been previously reported in co-morbid models of stroke and AD (Clarke et al., 2007; Whitehead et al., 2005a), but to our knowledge few studies have investigated the effect of co-morbidity on somatosensory function. The striatum receives inputs from and provides output to the somatosensory cortex in addition to the aforementioned connections. Previous evidence would suggest that disruption of these connections through lesions in the striatum might result in abnormal neuronal processing of and response to somatosensory stimuli (Carodoso et al., 2013). Therefore, a co-morbid condition involving a striatal stroke could hypothetically result in exacerbated somatosensory deficits compared to a stroke alone condition. Often in clinical co-morbid cases of stroke and AD, patients experience infarcts that are on the level of “mini-strokes” and are essentially imperceptible (Vermeer et al., 2002; Smith et al., 2012). Upon repeat insult or over time, these strokes can eventually result in visible stroke-related symptoms in addition to exacerbated AD-related cognitive decline (Snowdon et al., Vermeer et al., 2007). As with the cylinder task, it is possible that the
infarct size induced in the stroke and Aβ injection model is not large enough to significantly impact the function of somatosensory processes. Additionally, there is no evidence from the present results suggesting that an increase in animal number will affect the overall result, but a longer timeline after induction of stroke may have a different effect.

In the co-morbid hAPP_{Swe/Ind} transgenic rat model with stroke, there were no significant motor deficits present in the affected or unaffected hindlimb when considering error rate alone. However, upon analysis of the total steps taken to cross the beam, co-morbid hAPP_{Swe/Ind} transgenic rats with stroke required a significantly greater number of steps to cross the beam post-stroke. Classically, the beam-walk task is used to evaluate motor deficit by quantifying the number of slips or falls that occur while walking across the beam (Schallert et al., 2002). Lipsanen et al. (2011) reported a significantly increased number of slips in a MCAO model compared to sham animals. Furthermore, a multi-stroke model involving focal ischemia in the sensorimotor cortex and striatum demonstrated an increased number of errors following stroke (Clarke et al., 2009, Windle et al., 2006). While the co-morbid hAPP_{Swe/Ind} transgenic model with stroke did not produce a motor deficit as defined by an increased number of errors, the significant difference in the number of steps may indicate potential gait disruption.

Alterations in gait patterns and balance have been implicated as an early clinical indication of AD (Sheridan and Hausdorff, 2007). Visser (1983) demonstrated that AD patients had significantly shorter step length, lower gait speed, lower stepping frequency, greater step-to-step variability, greater double support ratio and greater sway path compared to their age- and sex-matched controls. Furthermore, patients with AD and cerebrovascular disease have impaired gait and balance compared to their AD counterparts without cerebrovascular disease (Inzitari et al., 2013). The co-morbid hAPP_{Swe/Ind} transgenic rat model with stroke had a stroke induced in the striatum and stroke is a form of cerebrovascular disease. Specifically, the striatum and other basal ganglia structures are involved in balance and walking in addition to the aforementioned functions (Sheridan and Hausdorff, 2007). The coupling of previous evidence with the potential gait alterations highlighted in the beam-walk task, it is possible that the co-
morbid hAPP<sub>Swe/Ind</sub> transgenic rat model developed gait or balance abnormalities as a result of the co-morbidity. Additional studies using gait-specific motor tasks would be required to confirm this speculation in the current model and are highly suggested for future research investigating the early stages of AD pathogenesis.

4.2 Development of Cognitive Decline in Co-morbid Models of Stroke and AD

Spatial learning in the MWM provided no evidence for differences in the ability to learn the location of the hidden platform for either the stroke and A<beta> injection or the hAPP<sub>Swe/Ind</sub> transgenic model. In both studies, all groups learned the task to the same extent, as demonstrated by the lack of differences between groups for the latency and distance to reach the platform. However, Choi et al. (2011) previously reported the development of spatial learning deficits in a co-morbid A<beta><sub>25-35</sub> injection and bilateral CCAO model in the MWM. Deficits in spatial learning have also been demonstrated following intervention with A<beta> peptide in rats (Zussy et al., 2013) and in transgenic rodent models of AD (Kloskowska et al., 2010). The inconsistency between these studies and the current studies may be explained by various differences in the animal model and behavioural timeline. The behavioural paradigm implemented here started MWM spatial learning one week following surgical intervention, whereas the aforementioned studies used various start points that were later than one week following intervention. Furthermore, Zussy et al. (2011) reported a time-course difference in spatial learning, whereby spatial learning deficits were not present until three weeks following A<beta> surgical intervention. Therefore, the one-week timeline between surgical intervention and the beginning of MWM spatial learning may not have been an adequate amount of time to develop co-morbid or A<beta>-induced learning deficits.

Regarding the variability between animal models and learning, it has been described that the aggregation state of A<beta> upon injection influences spatial learning abilities. Delobette et al. (1997) reported that the injection of aggregated A<beta><sub>25-35</sub> significantly increased the latency to reach the platform, which is indicative of a spatial learning deficit. Moreover, injection of soluble A<beta><sub>25-35</sub> did not result in the same effect and demonstrated equal spatial learning abilities to the control group by the end of spatial
learning (Delobette et al., 1997). Spatial learning impairments demonstrated in transgenic models of AD are often present in models that inevitably develop amyloid plaques and significant cognitive deficit with age (Cohen et al., 2013; Leon et al., 2010; Nalbantoglu et al., 1997). The hAPP<sub>Swe/Ind</sub> transgenic rat model utilized in the present study does not develop amyloid plaques or cognitive deficits with age alone. This model is likely unable to produce significant spatial learning deficits on account of the lack of age-related amyloid accumulation, as demonstrated in other transgenic rodent models that develop learning deficits.

In the instance of co-morbid models with spatial learning deficits, the aforementioned bilateral CCAO model is a stroke model with extensive cortical damage (Choi et al., 2011) compared to the ET-1 striatal stroke model in the experiments described here. It is possible that due to the smaller size and location of the stroke, the co-morbid interaction is not large enough to induce learning impairments. The discrepancy between these studies and this thesis is not a cause for major concern, as differences in learning may account for differences in subsequent spatial reference memory.

While cued learning was primarily used here as a control for the ability to use spatial cues to locate the platform and motivation to escape the pool, it has been previously demonstrated that cued learning can be affected by lesions in the striatum (Lovinger, 2010). Specifically, the striatum has been shown to be involved in learning and memory processes, such as skill learning, response-based learning and goal-directed learning (Lovinger, 2010). Packard and Teather (1997) previously demonstrated that administration of an NMDA receptor antagonist in the striatum after completion of eight training trials impaired retention of the cued learning task 24 hours after the initial training. For both co-morbid models, there were no differences in cued learning based on the latency and distance to reach the platform after two days of learning. This indicates that the infarct in the striatum did not induce a memory impairment of the cued learning task 24 hours after the first day of learning. It is possible that the damage in the striatum in both of these models is not significant enough to impair learning and memory in the cued task. Furthermore, this allows us to be confident that any significant differences demonstrated in other aspects of MWM were not due to deficits in striatal participation in
learning and memory, but the result of the co-morbid condition on other areas associated with learning and memory.

The results of these experiments did demonstrate that long-term spatial reference memory following spatial learning in the MWM was impaired in both co-morbid models of stroke and AD. This effect was strictly due to the co-morbidity of the two diseases, as the striatal stroke, Aβ injection or transgenic background alone did not induce the same impairment. Previous studies have associated the co-morbid condition with exacerbated behavioural pathology and evidence of the interaction clinically has been well described (Kalaria, 2000; Levine et al., 2015; Vermeer et al., 2007; Whitehead et al., 2007). Clinically, Snowdon et al. (1997) reported that in a controlled population, the co-morbidity of small striatal strokes and AD resulted in a more progressed level of cognitive impairment compared to their AD counterparts. Furthermore, animal studies have demonstrated significant memory deficit in Aβ injection (Nell et al., 2014), transgenic rodent (Cohen et al., 2013; Hsiao et al., 1996) and co-morbid models of AD (Cechetto et al., 2008). The stroke and Aβ injection model and hAPP<sub>Swe/Ind</sub> transgenic model with stroke results are in accordance with previously described findings, but this is the first time that significant cognitive impairment has been reported in these unique models. Additionally, most behavioural studies have been conducted in three-month-old rats (Amtul et al., 2014; Leon et al., 2010; Whitehead et al., 2007) and to our knowledge this is the first evidence of exacerbated cognitive impairment in a more appropriately aged model in relation to the clinical onset of AD. The memory impairment observed in these two models further emphasizes the importance of expanding our understanding of the interaction of these two diseases and the inevitable advanced decline that accompanies the co-morbid condition.

4.3 Stroke Pathology: Activated Microglia Presence in the Striatum

The volume of activated microglia in the striatal infarct region did not differ between groups in the stroke and Aβ injection model. However, exacerbated microglia in the striatum of the co-morbid hAPP<sub>Swe/Ind</sub> transgenic with stroke group compared to its saline counterparts was observed. There is substantial evidence implicating aberrant microglial presence in the early stages of AD (Nell et al., 2014; Wyss-Coray and Mucke,
and microglia are an important factor in post-stroke repair mechanisms (Lakhan et al., 2009; Wang et al., 2007). Whitehead et al. (2007) previously reported increased volume of microglia in the striatum of rats with striatal stroke and Aβ_{25-35} injections compared to striatal stroke alone. Various other studies have also demonstrated this effect in stroke and Aβ co-morbid conditions with varied concentrations of ET-1 and Aβ (Amtul et al., 2014; Hepburn, 2012). Moreover, increased infarct volume measured as the extent of neurodegeneration in a co-morbid stroke and Aβ model further supports the notion that the co-morbid condition results in exasperated stroke pathology (Amtul et al., 2014; Caughlin et al., 2015).

The aforementioned studies investigating the neuroinflammatory and neurodegenerative outcome in the infarct region of co-morbid models were completed in three-month-old Wistar rats (Amtul et al., 2014; Caughlin et al., 2015; Whitehead et al., 2007). Inflammation is believed to undergo progressive changes with age, whereby the level of inflammatory mediators throughout the body is greater in healthy older individuals (Godbout and Johnson, 2009). The age-related increase in inflammatory mediators may render the brain vulnerable to drastic alterations in neuroinflammation. Therefore, when age-related inflammatory changes are coupled with brain injury, the brain may quickly succumb to an aberrant neuroinflammatory response. In the current study, the stroke and Aβ injection model was completed in six-month-old Wistar rats. It is feasible that the level of neuroinflammation demonstrated in this model is relatively maximized in the anatomical confines of the striatum in the six-month-old rat. However, further investigation of the time-course of the inflammatory response to stroke would need to be completed to confirm this possibility. This does not devalue the development of cognitive impairment in the co-morbid condition, as it is highly possible that the co-morbid condition is still causing exacerbated consequences in downstream targets.

While the hAPP^{Swe/Ind} transgenic rats were also six months of age, the co-morbid group demonstrated increased microglia in the striatal infarct region. The hAPP^{Swe/Ind} transgenic rat model is on a different genetic background than the stroke and Aβ injection model and thus cannot be directly compared. However, when comparing the volume of microglia within the striatum between strains, the APP transgenic rats appear to have less
microglial volume in the striatum compared to the Wistar rats. Regardless of the differences between the models, the increased volume of neuroinflammation in the striatum of hAPP<sub>Swe/Ind</sub> transgenic rats with stroke further confirms the effect of the co-morbidity on stroke pathology. Additionally, it has previously been demonstrated that co-morbid rats demonstrate increased levels of neuroinflammation and greater motor impairments in the staircase task (Whitehead et al., 2005a). Previous studies and the exacerbated striatal neuroinflammation combined with the potential motor deficits observed in the beam-walk task suggests that microglia may contribute to the development of pathological behavioural changes in the co-morbid hAPP<sub>Swe/Ind</sub> transgenic rat model.

4.4 Neuroinflammation in the Basal Forebrain

Measuring the extent of microgliosis in the basal forebrain showed a significant response to the co-morbid condition in the stroke and Aβ injection model. In response to striatal stroke and Aβ<sub>25-35</sub>, there was an exacerbated level of microglia present in the basal forebrain, which is a key brain structure involved in the pathogenesis of AD (Auld et al., 2002). A significant increase in activated microglia was not demonstrated in the hAPP<sub>Swe/Ind</sub> transgenic model with stroke. However, the co-morbid hAPP<sub>Swe/Ind</sub> transgenic with stroke group certainly showed a trend in greater levels of microglia in the basal forebrain compared to their wild type counterparts and a longer timeline or a larger stroke may have resulted in significance.

The basal forebrain is considered one of the earliest structures affected in the pathogenesis of AD (Auld et al., 2002). Structures within the basal forebrain are an integral part of the cholinergic system and send projections to the hippocampus and neocortex, two brain structures largely implicated in learning and memory (Auld et al., 2002; D’Hooge and Deyn, 2001; Schliebs, 2005). The damaging properties of aberrant neuroinflammation in neurodegenerative diseases are evident throughout the literature (Akiyama et al., 2000; Latta et al., 2014; Wyss-Coray and Mucke, 2002). In the basal forebrain specifically, it is possible that the extensive microglial presence contributes to the chronic hypofunction of cholinergic neurons in the basal forebrain in AD patients (Auld et al., 2002; Schliebs and Arendt, 2011). Previously, administration of Aβ<sub>1-40</sub>
induced a significant increase in microglia in the basal forebrain (Scali et al., 1999). Nell et al. (2014) also reported significant microgliosis along with a corresponding decrease in the number of cholinergic neurons in the basal forebrain in six-month-old rats receiving Aβ25-35. Combined with previous evidence, the increased level of microgliosis demonstrated in the stroke and Aβ injection model suggests that neuroinflammation plays a major role in the damaging interaction of this co-morbid condition.

In addition to neuroinflammation, there are various cellular and metabolic changes and brain structures involved in the pathogenesis of AD that interact to cause the development of the characteristic symptoms. While the role of neuroinflammation in AD has been widely investigated and is considered to play a major role in the early stages of the disease, it is not the only factor involved (Butterfield et al., 2001; Huang and Mucke, 2012; Querfurth and LaFerla, 2010). The co-morbid hAPP_{Swe/Ind} transgenic with stroke model may not have demonstrated significant increases in microglia in the basal forebrain, but there was evidence that this was beginning to appear. It is possible that other factors may be mediating the behavioural deficits demonstrated, such as oxidative stress, Aβ deposition, among other elements. On the contrary, the trend in exacerbated inflammation suggests that the co-morbid condition in the hAPP_{Swe/Ind} transgenic rat model may have developed microgliosis in the basal forebrain at a later time point. Studies in other transgenic rodent models of AD have demonstrated significant microglia presence in a brain devoid of amyloid plaques (Ferretti and Cuello, 2011; Hanzel et al., 2014). Therefore, transgenic backgrounds are still able to initiate a neuroinflammatory cascade in response to insult, foreign pathogens or Aβ. Furthermore, evidence of increased levels of microglia in the infarct region of this co-morbid model also supports the role of neuroinflammation in the development of exacerbated pathologies in the co-morbid condition.

4.5 Effectiveness of CAT-SKL as a Therapeutic Mechanism

In the stroke and Aβ injection model, the use of the targeted antioxidant CAT-SKL as a treatment in the co-morbid condition was investigated. In the current experiment, CAT-SKL was able to effectively ameliorate cognitive decline as a result of the co-morbid condition. Furthermore, treatment with CAT-SKL decreased the levels of
microglia in the basal forebrain to control levels suggesting that inflammation was involved in mediating the cognitive decline in the co-morbid condition. This is the first evidence of CAT-SKL reducing the behavioural and cellular pathologies resulting from the toxic interaction that occurs in the co-morbid condition. Coupled with studies investigating the protective effects of CAT-SKL in vitro and in vivo, these results further support the role of oxidative stress and H2O2 in mediating cellular damage and neuroinflammation in injury and neurodegeneration (Giordano et al., 2014; Nell, 2013).

Several studies investigating the effect of CAT-SKL on various injury and disease models have been completed. Undyala et al. (2011) demonstrated that pre-treatment of cultured rat cardiac myocytes with CAT-SKL resulted in decreased levels of H2O2. Furthermore, investigation of cardiac myocyte cell viability showed that CAT-SKL was protective against hypoxia and ischemia reperfusion injury (Undyala et al., 2011). Giordano et al. (2014) reported that treatment of cultured rat primary cortical and hippocampal neurons with CAT-SKL decreased ADDL-induced oxidative stress. This study also reported that treatment with CAT-SKL prior and subsequent to ADDL exposure resulted in decreased ADDL-induced neurotoxicity and neurite degeneration, implicating CAT-SKL as a preventative and therapeutic treatment. Nell (2013) reported that the cellular and behavioural pathological consequences of Aβ25-35 toxicity in vivo were attenuated by CAT-SKL treatment. This previous evidence along with the evidence presented in the co-morbid stroke and Aβ injection model in the present experiments suggests that H2O2 and oxidative stress play an important role in mediating cellular degeneration and neuroinflammation and that CAT-SKL may be an effective means of ameliorating this component leading to an improved outcome in several conditions.

In accordance with prior research demonstrating that catalase levels in the brain decrease with age (Alper et al., 1998; Venkateshappa et al., 2012), catalase activity in the rat brain has been shown to begin to decrease in three-month-old rats (Sandhu and Gurcharan, 2002). This suggests that our six-month-old rat model may be vulnerable to elevated levels of oxidative stress due to decreased breakdown of H2O2 by catalase prior to surgical intervention. Furthermore, there is a large amount of evidence demonstrating that increased ROS production occurs prior and subsequent to Aβ toxicity (Aliev et al.,
In particular, studies investigating levels of H$_2$O$_2$ specifically have shown increased levels of H$_2$O$_2$ in response to Aβ \textit{in vitro} and \textit{in vitro} (Behl et al., 1994; Kaminsky and Kosenko, 2008). Thus, the beneficial effect of CAT-SKL is likely due to its enzymatic degradation of H$_2$O$_2$ abolishing the ability for H$_2$O$_2$ to react with other molecules to produce highly toxic ROS. Furthermore, H$_2$O$_2$ and ROS can initiate a neuroinflammatory response resulting in activation of microglia and activated neuroinflammatory cells in turn release ROS (Akiyama et al., 2000; Hensley, 2010). Previously, CAT-SKL has been shown to attenuate Aβ-induced cholinergic loss and neuroinflammation in the basal forebrain with the subsequent attenuation of memory deficits (Nell, 2013). The interaction between oxidative stress and neuroinflammation contributes to a vicious cycle conducive to producing uncontrolled and damaging levels of ROS and neuroinflammation.

Considering these relationships and effectiveness of CAT-SKL, a proposed mechanism of how CAT-SKL exerts its beneficial effects on cognitive decline and cellular pathology in a co-morbid model of stroke and AD can be suggested. It is probable that CAT-SKL results in the decrease of H$_2$O$_2$ with the subsequent decrease in ROS production resulting from stroke and Aβ toxicity. This decrease in oxidative stress in turn results in a decrease in the activation of microglia, which is upregulated in the co-morbid stroke and Aβ condition. Thus, memory deficit induced by the co-morbid condition is attenuated owing to less damage to the basal forebrain due to aberrant levels of microglia and ROS. It is interesting to note that previous studies using anti-inflammatory treatments in a co-morbid stroke and AD model were successful in ameliorating some of the pathology or cognitive deficits in either stroke or AD alone, but not the interaction between the two conditions, suggesting that neuroinflammation is not the only contributing factor in the pathogenesis of the co-morbid condition (Whitehead et al., 2005a). This theory is purely speculative and requires further investigation of a myriad of other factors, but the current and previous evidence of the effectiveness of the targeted antioxidant CAT-SKL strongly suggests that the interaction of neuroinflammation and oxidative stress is contributing to the pathological consequences of the co-morbid condition.
4.6 Limitations and Future Directions

The studies presented are not without their limitations regarding models, timelines and dosages. In the stroke and Aβ injection model, the Aβ25-35 peptide is used to exert toxicity rather than the full length Aβ42. The full length Aβ42 is considered the canonical peptide in AD that has toxic and aggregative properties (Jarrett et al., 1993; Walsh and Selkoe, 2007; Querfurth and LaFerla, 2010). However, Aβ25-35 fragments have been found in brains of AD patients, but not in age-matched controls (Kubo et al., 2002). Previous studies of the effects of Aβ25-35 have demonstrated that the shorter length peptide is still capable of producing AD-like cellular and behavioural pathology in vivo (Amtul et al., 2014; Cheng et al., 2006; Nell et al., 2014; Whitehead et al., 2007). Furthermore, Aβ25-35 has been implicated as the neurotoxic core of the full-length peptide and retains the aggregation properties of Aβ42 (Pike et al., 1995; Yankner et al., 1989). In addition to its toxic properties, Aβ25-35 is more soluble than Aβ42 allowing for better diffusion into the brain upon injection (Kowall et al., 1992; Millucci et al., 2009). AD is a complicated disease with an intertwining web of cellular factors resulting in the inevitable neurodegeneration and modeling the disease, as a whole remains difficult. While this limitation is evident, the use of Aβ25-35 in rodent models is equal in its effectiveness and easier to use compared to Aβ42 and provides extremely important information regarding key aspects involved in the pathogenesis of AD-like pathologies and the stroke co-morbid condition. Future studies should seek to replicate the results of this model in a rodent model with the ability to produce the full length Aβ42 peptide or Aβ oligomers.

AD is considered a highly progressive neurodegenerative disease with various stages of cognitive decline (Karran et al., 2011; Schliebs and Arendt; 2011; Selkoe, 2001). While the goal of these studies were to investigate the early cellular and behavioural implications involved in the pathogenesis of the disease, the twenty-one day timeline only provides a snapshot of changing pathologies in a progressive disease. To enhance our understanding of the overall contribution of neuroinflammation and oxidative stress in the pathogenesis and progression of AD, future studies must be completed at earlier and later time frames. Furthermore, studies encompassing numerous
time points will provide a better understanding of the time course involved in the co-morbid condition. For example, understanding when the brain is most vulnerable to stroke injury and what downstream brain structures are particularly vulnerable to striatal strokes at different stages of the disease may be helpful in highlighting therapeutic targets in the co-morbid condition.

The present study in the stroke and Aβ injection model also effectively demonstrated the therapeutic potential of the targeted antioxidant CAT-SKL. Dosages and treatment timelines were based on successful therapeutic effect in previous in vivo studies (Nell, 2013). However, many questions remain regarding the therapeutic mechanism, pharmacological properties and its effect on other cellular outcomes in the co-morbid condition. Further investigation into the levels of ROS, oxidative damage and catalase levels are required to determine whether CAT-SKL exerts its antioxidant properties by directly abolishing ROS production or indirectly by increasing levels of endogenous catalase. Examination of these aspects will also elucidate the potential therapeutic mechanism behind CAT-SKL. This study was the first time CAT-SKL was used as a treatment in the stroke and Aβ co-morbid condition. The results of this study provided crucial information about the interaction in the co-morbid condition and are useful in guiding future use of CAT-SKL and other antioxidants as therapeutic treatments. However, continual use of CAT-SKL in future studies warrants investigation into the aforementioned aspects to gain a more comprehensive understanding of the interaction of the co-morbid condition and the mechanism of CAT-SKL.
Chapter 5:
Summary and Conclusions
This thesis has presented considerable evidence indicating that there is exacerbated memory loss in the co-morbid stroke and AD condition. It has also been previously demonstrated that motor deficits can be exacerbated by the interaction of these two diseases in a co-morbid animal model (Whitehead et al., 2005a). The present results support this idea by the presence of gait-related motor disturbances in the co-morbid hAPP\textsubscript{Swe/Ind} transgenic rats with stroke. While the focus of this study was investigating the cognitive impact of the co-morbid condition, as observed in the MWM as a memory deficit in both co-morbid models, the results reported also implicate the potential role of microglia in the early stages of cognitive decline associated with AD and stroke. Furthermore, aspects of this study have provided evidence for the use of targeted antioxidants as potential therapeutic mechanisms for further treatment of AD and its co-morbidity with stroke and possibly other vascular pathologies.

To our knowledge, this is the first time that the co-morbid condition has been demonstrated in an older animal model. Previous studies often induced A\textsubscript{β} toxicity or a co-morbid disease state in three-month-old rats (Amtul et al., 2014; Caughlin et al., 2015; Cheng et al., 2006; Whitehead et al., 2007). The leading risk factor for sporadic forms of AD is age and Nell et al. (2014) demonstrated that older six-month-old animals demonstrate higher levels of A\textsubscript{β}-induced neuroinflammation and cholinergic loss accompanied by the characteristic memory loss associated with AD. Both the stroke and A\textsubscript{β} injection model and the hAPP\textsubscript{Swe/Ind} transgenic rat model with stroke demonstrated significant memory impairment in the MWM at six months of age. This is also the first time that stroke has been shown to induce AD-related memory deficits in a hAPP\textsubscript{Swe/Ind} transgenic rat model that does not develop the AD-related memory deficits, amyloid plaques or neurofibrillary tangles with age alone.

There was an exacerbation of microglial neuroinflammation in the basal forebrain of the co-morbid stroke and A\textsubscript{β} group and in the infarct region of the hAPP\textsubscript{Swe/Ind} transgenic group with stroke. This suggests that the neuroinflammatory response may be a key component in the pathogenesis of AD behavioural pathologies and the interaction of the co-morbid condition. Neuroinflammation has been previously implicated in the disease progression of AD, as well as in post-stroke tissue repair, and it is widely
accepted as a key component in the early stages of cognitive decline in AD (Akiyama et al., 2000; Lakhan et al., 2009; Wyss-Coray and Mucke, 2002). This study further implicates the role of neuroinflammation in contributing to cognitive deficit, as increased levels of microglia were observed in the basal forebrain. This area of the brain is known to be vulnerable to AD-related neurodegeneration and contribute to learning and memory directly and indirectly through projections to the hippocampus (Auld et al., 2002; Schliebs and Arendt, 2011).

While neuroinflammation has a major role in the pathogenesis of AD and repair of damaged tissue post-stroke, it is not the only factor at play in these two diseases. This study uses the targeted antioxidant CAT-SKL as a treatment in the co-morbid condition. Its effectiveness at ameliorating memory deficits and neuroinflammation in the basal forebrain in the stroke and Aβ injection model supports the role of oxidative stress in the network of factors involved in the co-morbid condition. Although oxidative stress alone likely cannot produce the level of damage that results in AD and post-stroke injury, evidence suggests that it is a key component in the disease process of both conditions and the co-morbid condition (Allen and Bayraktutan, 2009; Akiyama et al., 2000; Butterfield et al., 2001). The self-propagating relationship of oxidative stress with neuroinflammation, in addition to the relationship of Aβ with both of these cellular processes, underlies the importance of preventing or treating aberrant levels of oxidative stress. Furthermore, evidence of the beneficial effect of CAT-SKL supports further investigation into the maintaining the balance of ROS and antioxidants in diseased states and the use of CAT-SKL or other targeted antioxidants in the treatment of stroke, AD and the co-morbid condition.

Lastly, the models used demonstrate early AD cellular and behavioural pathology and CAT-SKL demonstrated a therapeutic effect in one model, suggesting that these models can be effectively used to assess the early stages of stroke and AD pathogenesis and therapeutic agents that might be capable of interrupting the interaction between stroke and early AD pathology, subsequently preventing further decline. It is likely that it will be far easier to design therapeutic approaches that can prevent or slow the onset or reduce the frequency of conversion to the late stages of AD than it will be to treat the
fully expressed late-stage disease (i.e. amyloid plaques and neurofibrillary tangles). For that reason, these models can offer a great advantage in that they replicate the beginning of motor and cognitive decline due to the co-morbid conditions that are prevalent in the human population.
References


Curriculum Vitae
Jennifer Au

Academic Background

M.Sc Anatomy and Cell Biology 2013 – Present
Supervisors: Dr. David Cechetto and Dr. Shawn Whitehead
Schulich School of Medicine and Dentistry
University of Western Ontario
London, Ontario, Canada

B.Sc (Honours) Human Kinetics 2008 – 2012
College of Biological Science
University of Guelph
Guelph, Ontario, Canada

Awards and Scholarships

Anatomy and Cell Biology Travel Scholarship 2014
Anatomy and Cell Biology
Valued at $500 for students with an excellent CV and strong desire to attend a conference

Alzheimer Society London and Middlesex Master’s Scholarship 2014 – 2015
Alzheimer Society London-Middlesex
Valued at $15 000 for students demonstrating remarkable research capability and potential and a strong research proposal contributing to Alzheimer’s disease knowledge

Western Graduate Scholarship 2013 – 2015
Anatomy and Cell Biology
Valued at $4500 for students achieving an 80% average in each year of study

Related Work Experience

Teaching Assistantship 2013 – 2014
University of Western Ontario, Department of Anatomy and Cell Biology
Course: Mammalian Histology

Publications

Peer-reviewer Methods Article and Scientific Video Journal
Publications Continued

Peer-reviewed Research Article

Conference Proceeding

Peer-reviewed Research Article

Abstracts and Presentations


Abstracts and Presentations Continued


Extracurricular Activities

*Anatomy and Cell Biology Graduate Student Council – Social Committee Chair*
University of Western Ontario 2014 – 2015

*Alzheimer Society London-Middlesex – Social Recreation and Weldon Resource Centre Volunteer*

*Let’s Talk Science – Outreach and Teacher-Partnership Volunteer*
University of Western Ontario 2013 – 2015