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Contribution of TRPM2 to Memory Loss in an Alzheimer's Mouse Model

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

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CONTRIBUTION OF TRPM2 TO MEMORY LOSS IN AN ALZHEIMER’S MOUSE MODEL

(Thesis format: Monograph)

by

Megan Meng Chen

Graduate Program in Physiology and Pharmacology

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

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

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ABSTRACT

Alzheimer’s disease (AD) is a neurodegenerative disease characterized by the progressive deterioration of memory and other intellectual abilities. Accumulation of amyloid-β (Aβ) peptide, the major contributor to the senile plaques central to AD, is thought to mediate neurotoxicity by inducing oxidative stress and calcium dysregulation. Transient Receptor Potential Melastatin type 2 (TRPM2) is a calcium permeable, non-selective cation channel activated under oxidative stress and ultimately induces cell death. The APPSWE/PSEN1ΔE9 double transgenic mouse model carries the human APPswe (Swedish mutations K594N/M595L) and PS1 mutations with a deletion in exon 9 (PS1-dE9), and is one of the most commonly used AD model. Both mutations have been shown to be linked to Familial (early onset) AD as well as an increased production in Aβ peptides. We propose that TRPM2 contributes to pathological deficits in AD. To test this, we crossed TRPM2 knock-out with APPSWE/PSEN1ΔE9 mice to generate four mouse genotypes (WT, TRPM2 knock-out, Alzheimer’s and Alzheimer’s TRPM2 knock-out). We hypothesize that Alzheimer’s TRPM2 knock-out mice will have improved memory performance when compared to Alzheimer’s mice. To test this hypothesis, anxiety, motor activity, recognition memory, as well as spatial learning and memory were tested in these mutant mice at 3, 6, 9, 12, and 15 months of age. For these assessments we use open field locomotor activity (OFT), elevated plus maze (Elev+), object recognition (OR), Barnes (BM) and Morris water maze (MWM) respectively. Our data showed that no genotype difference was observed in OFT, Elev+ and OR in terms of hyperactivity, anxiety, and recognition memory. On the other hand, our data indicate that Alzheimer’s mice show cognitive impairment (spatial memory deficit) compared to WT controls while Alzheimer’s mice without TRPM2 do not, suggesting that elimination of TRPM2 activity prevents the spatial learning and memory deficits observed in APPSWE/PSEN1ΔE9 double transgenic mice.

Key words: Alzheimer’s disease, TRPM2, memory, cognitive impairment, elevated plus maze, open field, object recognition, Barnes maze, Morris water maze
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Megan Chen
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>α-secretase</td>
<td>alpha secretase</td>
</tr>
<tr>
<td>β-secretase</td>
<td>beta secretase</td>
</tr>
<tr>
<td>β-cells</td>
<td>beta cells</td>
</tr>
<tr>
<td>γ-secretase</td>
<td>gamma secretase</td>
</tr>
<tr>
<td>[Ca(^{2+})](_i)</td>
<td>intracellular calcium concentration</td>
</tr>
<tr>
<td>Aβ</td>
<td>amyloid beta</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ADPR</td>
<td>adenosine diphosphate ribose</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>APP</td>
<td>amyloid precursor protein</td>
</tr>
<tr>
<td>APP(^+)</td>
<td>amyloid precursor protein positive</td>
</tr>
<tr>
<td>APP(^-)</td>
<td>amyloid precursor protein negative</td>
</tr>
<tr>
<td>APPSWE/PSEN1ΔE9</td>
<td>Alzheimer’s transgenic double mutant: transgenic mouse model expressing the Swedish mutation of K594N/M595L and presenilin-1 mutations with an exon-9 deletion</td>
</tr>
<tr>
<td>BM</td>
<td>Barnes maze</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>calcium</td>
</tr>
<tr>
<td>cADPR</td>
<td>cyclic adenosine diphosphate ribose</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CTFα</td>
<td>C-terminal fragment</td>
</tr>
<tr>
<td>Elev+</td>
<td>Elevated plus maze</td>
</tr>
<tr>
<td>FAD</td>
<td>Familial Alzheimer’s disease</td>
</tr>
<tr>
<td>H(_2)O(_2)</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>LTD/LTP</td>
<td>long-term depression/potentiation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Mg&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>magnesium</td>
</tr>
<tr>
<td>MWM</td>
<td>Morris water maze</td>
</tr>
<tr>
<td>NMDA/NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>OFT</td>
<td>Open field locomotor test</td>
</tr>
<tr>
<td>OR</td>
<td>Object recognition test</td>
</tr>
<tr>
<td>PrP</td>
<td>prion protein promoter</td>
</tr>
<tr>
<td>PS1</td>
<td>presenilin 1</td>
</tr>
<tr>
<td>PS2</td>
<td>presenilin 2</td>
</tr>
<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>sAPP&lt;sub&gt;α&lt;/sub&gt;</td>
<td>soluble N-terminal fragment</td>
</tr>
<tr>
<td>TRP</td>
<td>Transient Receptor Potential Channel</td>
</tr>
<tr>
<td>TRPA</td>
<td>Transient Receptor Potential Ankyrin Channel</td>
</tr>
<tr>
<td>TRPC</td>
<td>Transient Receptor Potential Canonical Channel</td>
</tr>
<tr>
<td>TRPM</td>
<td>Transient Receptor Potential Melastatin Channel</td>
</tr>
<tr>
<td>TRPM2</td>
<td>Transient Receptor Potential Melastatin Type 2 Channel</td>
</tr>
<tr>
<td>TRPM2&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>TRPM2 homozygous single mutant</td>
</tr>
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<td>TRPM2&lt;sup&gt;+&lt;/sup&gt;&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>TRPM2 heterozygote</td>
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<td>TRPM2-KO</td>
<td>TRPM2 knock-out</td>
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<td>TRPM2-KO-APPSWE/PSEN1ΔE9</td>
<td>APPSWE/PSEN1ΔE9 triple mutants</td>
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<tr>
<td>TRPML</td>
<td>Transient Receptor Potential Mucolipin Channel</td>
</tr>
<tr>
<td>TRPP</td>
<td>Transient Receptor Potential Polycystin Channel</td>
</tr>
<tr>
<td>TRPV</td>
<td>Transient Receptor Potential Vanilloid Channel</td>
</tr>
<tr>
<td>WT</td>
<td>wildtype</td>
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</table>
Section 1

INTRODUCTION
1.1 Alzheimer’s Disease

Alzheimer’s disease (AD) is a progressive, degenerative and fatal disease characterized by deterioration of cognitive functions such as memory and the ability to use judgment and reasoning (Coyle, Price, & Delong, 1983; Cummings, 2004). AD is the most common neurodegenerative disease worldwide and is the leading cause of dementia (Billings, Oddo, Green, McGaugh, & LaFeria, 2005; Cvetkovic-dozić, Skender-gazibara, & Dozić, 2001; Goedert & Spillantini, 2006). At present, AD is prevalent in approximately 500,000 Canadians over the age of 65, representing 1.5% of the total population in Canada (Alzheimer Society of Canada, 2010). A deterioration of the hippocampus and prefrontal cortex, entorhinal cortex, and the basal forebrain cholinergic neurons are found to be present in individuals affected by AD (Coyle et al., 1983; Gomez-Isla et al., 1996) and the degree of deterioration and the loss of synaptic function is shown to be strongly correlated with the severity of dementia (Scheff & Price, 2003). There is currently no cure for AD; however medications such as memantine, donepezil, galantamine, and rivastigmine can be used to temporarily alleviate the symptoms of dementia (Cummings, 2004).

The main pathological hallmark of AD is amyloid-beta (Aβ) plaques (Cvetkovic-dozić et al., 2001; Yankner, Lu, & Loerch, 2008). Aβ plaques are extracellular and are composed of Aβ peptides that are 39 to 42 amino acids in length, with Aβ40 and Aβ42 being the two most common lengths of Aβ found in the brain of AD patients (Oddo et al., 2003; Selkoe, 2008). These filaments are created by the proteolytic cleavage of the transmembrane glycoprotein, amyloid precursor protein (APP). There are two proteolytic pathways under which APP is processed: the amyloidogenic pathway which leads to
amyloid plaque production and the non-amyloidogenic pathway which does not. In the non-amyloidogenic pathway, APP is cleaved by α-secretase and γ-secretase within the Aβ domain yielding a soluble N-terminal fragment (sAPPα) and a C-terminal fragment (CTFα). These fragments do not accumulate in plaques. However, in the amyloidogenic pathway, the cleavage of APP by β-secretase and γ-secretase results in the generation of aggregation-prone Aβ peptides, eventually leading to amyloid plaque buildup (Figure 1.1) (Selcoe, 2001; Selkoe & Schenk, 2003; Xiong, 2011). Mutations in the APP gene were found to influence Aβ peptide levels by altering the normal processing of the protein, resulting in an elevation in both Aβ40 and 42 peptides (Citron et al., 1992; Haass, Hung, Selkoe, & Teplow, 1994; Suzuki et al., 1994) with the latter more likely to accumulate in the brain increasing the ratio of Aβ42/Aβ40 (Burdick et al., 1992; Haass & Selkoe, 2007; Jarrett & Lansbury, 1993). Presenilin 1 (PS1) and 2 (PS2) were also found to play a role in Aβ peptide production. Mutations in these genes were believed to further increase Aβ42/ Aβ40 ratio as a result from a reduced γ-secretase activity, which in turn is a critical protein involved in the within domain cleavage of the APP gene (Goedert & Spillantini, 2006; Hardy & Higgins, 1992; Jankowsky et al., 2004; Xiong et al., 2011).

Brains affected by AD contain both insoluble and soluble oligomeric assemblies (Hardy & Selkoe, 2002; Yu et al., 2009). Aβ peptides have the ability to self-aggregate from smaller monomers into readily diffusible oligomers including, dimers, trimers, higher order oligomers, as well as fibrils that ultimately accumulate into Aβ plaques. This ability to self-aggregate was demonstrated by Pike, Walenczewicz, Glabe, & Cotman (1991) who identified aggregation in cultured cells that were pre-incubated by the Aβ peptide solution for 2-4 days. In addition, Aβ peptides of different lengths were noted to
have different physical and biological properties (Haass & Selkoe, 2007; Selkoe, 2008; Shankar et al., 2008; Townsend et al., 2006). Various studies have examined the relationship between Aβ peptide length and its toxicity to cells, with the common consensus that soluble forms of oligomers; including dimers and trimers, can cause long-term potentiation (LTP) deficit and memory impairments in rodents while soluble monomers and fibrils cannot (Cleary et al., 2005; Demuro et al., 2005; Shankar et al., 2008). Dimers however, were found to be more damaging to the cells than trimers (Selkoe, 2008; Townsend, Shankar, Mehta, Walsh, & Selkoe, 2006). In keeping with this, it is the soluble form of Aβ present in the individual with AD that is most toxic and correlate strongly with the disease (Hardy & Selkoe, 2002; Yu et al., 2009). Numerous studies have examined the relationship between insoluble Aβ, memory impairment and dementia associated with AD. These studies suggest that insoluble Aβ deposition results in only minimal neuronal loss (Irizarry, McNamara, Fedorchak, Hsiao, & Hyman, 1997a; Irizarry et al., 1997b) and has a weak correlation with memory impairment (Dickson et al., 1995; Terry et al., 1991). The latter observation was further confirmed by the existence of non-cognitively impaired individuals with high amounts of insoluble Aβ deposits in the brain (Delaere et al., 1990; Dickson et al., 1995; Katzman et al., 1988). In contrast, soluble Aβ oligomers induce a number of AD-related phenotypes including a decrease in dendritic spine density and the disruption of LTP in the hippocampus of rodents. These phenomenon correlates to learning and memory as well as impairing memory in rats as measured by behavioural tests (Shankar et al., 2008; Townsend et al, 2006).
Figure 1.1. APP processing under amyloidogenic and non-amyloidogenic pathways. α-secretase and γ-secretase goes under the non-amyloidogenic pathway to produce non-plaque forming Aβ, while β-secretase and γ-secretase goes under the amyloidogenic pathway to produce amyloid plaque forming Aβ [Image source: http://www.ebi.ac.uk/interpro/potm/2006_7/Page2.htm].
The accumulation of Aβ peptides has been found by numerous studies to mediate neurotoxicity by inducing oxidative stress in the brain (Mattson, 1998; Takahashi, Kozai, Kobayashi, Ebert, & Mori, 2011). This oxidative stress is formed when an imbalance is present between reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), reactive nitrogen species (RNS), and antioxidants (Butterfield, 2003; Mattson, 1998; Takahashi et al., 2011). This relationship between Aβ peptide accumulation and oxidative stress was demonstrated by Misonou, Morishima-Kawashima, & Ihara (2000) using human neuroblastoma SH-SY5Y cells. These scientists used H₂O₂ to induce oxidative stress into neuroblastoma cells and observed an increased level of intracellular Aβ peptide. In addition, Aβ deposition was also found to take place in the striatum (Klunk et al., 2004; Wolfer, Mohajeri, Lipp, & Thal et al., 1998) where oxidative stress may act as the key factor of cell death (Lipton, 1999) under states of ischaemia and reperfusion (Hawker & Lang, 1990). However, the relationship between Aβ, oxidative stress, and the striatum is not well understood.

In recent studies, there has been evidence supporting that TRPM2 channels are important in the pathogenesis of AD. TRPM2 is a ROS/RNS gated channel that has been implicated in cell death. ROS/RNS is found to promote TRPM2 activation, leading to a disruption in Ca²⁺ homeostasis and a toxic influx of Ca²⁺, ultimately inducing cell death (Goedert & Spillantini, 2006; Mattson, 2007; Takahashi et al., 2011). Activation by ROS/RNS has been attributed to the generation of ADP-ribose (ADPR) which plays a key role in the gating of TRPM2 (Perraud et al., 2001).
1.2 TRP channels and TRPM2

Transient receptor potential (TRP) channels are a superfamily of ion channels that are non-selective cation channels, most of which have substantial permeability to Ca\(^{2+}\) (Nilius & Owsianik, 2011). The first TRP gene described was from the fruit fly *Drosophila melanogaster* where TRP is used for phototransduction purposes (Montell & Rubin, 1989). To present day, 28 mammalian TRP channels have been discovered and can be subdivided into six main subfamilies including TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin). TRP channels are expressed in almost all cell types in most tissues of the human body. These channels are usually localized in the plasma membrane where they aid in the transport of Ca\(^{2+}\), Mg\(^{2+}\) and other ions. TRP channels play a critical role in regulating various cell functions including immune response and vasomotor functions, cold and thermal pain sensing, organ development, and oxidative stress sensing in various cells of the body (Nilius, Owsianik, Voets, & Peters, 2007; Nilius & Owsianik, 2011).

TRPM1 (melastatin) was the first member of the TRPM subfamily and was first discovered from melanomas where it served as a tumor suppressor (Duncan et al., 1998). There are currently eight family members (TRPM1-TRPM8) present in the TRPM subfamily (Jiang, Yang, Zou, & Beech, 2010; Kraft & Harteneck, 2005; Nilius & Owsianik, 2011). TRPM may induce cell death under certain conditions given that they mediate cellular responses to a range of physiological stimuli and within this family, two members (TRPM2 and TRPM7) have been identified to play key roles in cell death pathways (McNulty & Fonfria, 2005).
Transient Receptor Potential Melastatin type 2 (TRPM2) is a member of the TRPM subfamily and is a calcium permeable, non-selective cation channel. It is highly expressed in the CNS including the hippocampus, cerebral cortex, thalamus and the midbrain (Fonfria et al., 2005; Fonfria et al., 2006; Hara et al., 2002; Kraft et al., 2004; Nagamine et al., 1998; Olah et al., 2009; Perraud et al., 2001) and in many tissues outside of the CNS including pancreatic β-cells (Qian et al., 2002), bone marrow, spleen, heart, as well as in immune cells of the monocytic lineage including monocytes and neutrophils (Hara et al., 2002; Perraud et al., 2001; Sano et al., 2001).

1.3 Mouse models of Alzheimer’s disease

In order to investigate neurodegenerative diseases such as AD, animal models have become a common tool used for the better understanding of the pathology, underlying mechanisms, and investigation into potential treatments of the disease. While invertebrate and primate models are used in some research, rodents are the most common and widely used animal models. Due to the fact that the mouse genome can be easily manipulated at relatively lower costs, the use of genetically modified mice strains to model neurodegenerative diseases such as AD has become extensive (Ghorayeb, Page, Gaillard, & Jaber, 2011).

Pharmacological models and transgenic mouse models have been used for AD. Pharmacological models are generated by the direct injection of Aβ peptide into the brain of the rodent and can be particularly useful for the understanding of amyloid toxicity. However, not all neuropathology are observed in these mouse models of AD, including
the loss of neurons and synapses in the cortex and hippocampus, which leads to cognitive impairments (Ghorayeb et al., 2011). Unlike pharmacological models, transgenic mouse models of AD do not involve injections of Aβ peptides. Instead, they are made from the expression of different variants of the APP gene (located on human chromosome 21 (Xiong et al., 2011)), PS1 or PS2, tau or apolipoprotein E (Ashe, 2001). One of the mouse models most commonly used to identify potential therapeutic interventions for AD is the APPSWE/PSEN1ΔE9 double transgenic mouse line. These mice show both Aβ deposition and memory loss (Ashe, 2001; Esh et al., 2005).

A number of other mouse transgenic lines have been generated using variants of the APP and PS1 genes including, the NSE: β-APP751, homozygous for the transgene array expressing wild-type human APP751 (Moran, Higgins, Cordell, & Moser, 1995); PDAPP, carrying the transgene APP717 expressing the Indiana mutation V717F with a portion of APP introns 6-8 (Chen et al., 2000; Duyckaerts, Potier, & Delatour, 2008; Esh et al., 2005); TgCRND8, carrying the transgene APP695 expressing both Swedish double mutation and the Indiana mutation K670N/M671L and V717F (Duyckaerts et al., 2008; Janus et al., 2000); Tg2576, carries the transgene APP695 that expresses the Swedish mutation K670N/M671L (Chapman et al., 1999; Duyckaerts et al., 2008; Hsiao et al., 1996; Kawarabayashi et al., 2001) and APPSWE/PSEN1ΔE9, expressing the Swedish mutation of K594N/M595L and presenilin-1 mutations with an exon-9 deletion (Bonardi, Pulford, Jennings, & Pardon, 2011; Duyckaerts et al., 2008; Malm, Koistinaho, & Kanninen, 2011). All of which produce many biochemical, physiological, pathological and behavioural characteristics that simulate AD (Ashe, 2001).
1.4 APPSWE/PSEN1ΔE9 double transgenic mouse model

The APPSWE/PSEN1ΔE9 double transgenic mouse model carries the human APPswe (Swedish mutations K594N/M595L) and PS1 mutations with a deletion in exon 9 (PS1-dE9) (Bonardi et al., 2011; Duyckaerts et al., 2008; Jackson Laboratory, 2013; Malm et al., 2011). It was created by coinjection, where each vector containing the specific transgene has its own promoter component. In this case, the two specific transgenes were Mo/HuAPP695swe and PS1-dE9. Each of these transgenes was then inserted into a plasmid at a single locus and each of these plasmids was designed to be controlled by an independent mouse prion protein (PrP) promoter. The Mo/HuAPP695swe transgene expresses a “humanized” mouse amyloid beta (A4) precursor protein gene modified to contain the K595N/M596L mutations that are linked to familial Alzheimer’s disease (FAD). The PS1-dE9 transgene expresses the human presenilin 1 mutant with the exon-9-deleted also associated with FAD. These two plasmids were then co-injected into the B6C3HF2 pronuclei and have co-integrated in the genome. Founder line 85 was obtained and by crossing transgenic mice to B6C3F1/J, the resulting colony maintained as hemizygotes. Transgenic mice were then backcrossed to the background strain C57BL/6J for at least 8 generations (Garcia-Alloza et al., 2006; Jackson Laboratory, 2013; Jankowsky et al., 2001).

APPSWE/PSEN1ΔE9 double transgenic mice have been noted to be the best characterized AD model to date (Bonardi et al., 2011); both mutations have been shown to be linked to FAD, as well as increased production of Aβ40 and Aβ42 peptides (Garcia-Alloza et al., 2006; Malm et al., 2011; O’Leary and Brown, 2009; Reiserer, Harrison, Syverud, & McDonald, 2007; Savonenko et al., 2005; Zhang et al., 2011); Increased
production of Aβ42 is one of the triggers of FAD (Savonenko et al., 2005). This increase in Aβ42 subsequently results in large senile plaque buildup in the hippocampus, cortex and subcortical nuclei (Duyckaerts et al., 2008). Moreover, various transgenic studies have shown a direct correlation between the level of Aβ42 and the age at which amyloid plaques appear (Jankowsky et al., 2002), with some studies presenting early development of amyloid plaques in the hippocampus and cortex observed as early as 4-6 months of age (Garcia-Alloza et al., 2006; Ghorayeb et al., 2011; Jackson Laboratory, 2013; Malm et al., 2011; Minkeviciene et al., 2008; O’Leary and Brown, 2009; Savonenko et al., 2005). Jankowsky et al. (2004), Jordanhazi-Kurutz et al. (2010) and Lalonde, Kim, & Fukuchi (2004) have also observed a rapid accumulation of plaques within the cortex and hippocampus of these mice at 6 months of age and even greater amounts at 9 (Lalonde et al., 2004) and 12 months (Jordanhazi-Kurutz et al., 2010). Likewise, Reiserer et al. (2007) found that Aβ plaques start to develop at 6-7 months of age.

APPSWE/PSEN1ΔE9 bigenic mice have been used extensively for the study of cognitive deficits, such as memory impairment observed in humans with AD. A variety of tests including open field test (OFT), Elevated plus maze (Elev+ maze), Object Recognition (OR), Barnes maze (BM) and Morris Water Maze (MWM) have been conducted in these mice to measure their exploratory tendencies, anxiety and memory ability. Park et al. (2010) compared the locomotor activity of aged WT with APPSWE/PSEN1ΔE9 mice and found no difference between the two genotypes. The specific age of the mice were not indicated. Likewise, Bonardi et al. (2011) also did not see a difference in locomotor activity in 4 months old WT and APPSWE/PSEN1ΔE9
mice suggesting that at least at young age these mutants do not show changes in locomotion behavior.

Elev+ maze is used to examine greater or lesser anxiety experienced by mice through assessing an individual’s exploratory tendencies in walled vs. open arms (Cryan & Holmes, 2005; File, 2001). Studies with 7-month-old APPSWE/PSEN1ΔE9 mice showed that these mutants spend less time in the closed arms than WT mice, suggesting that the bigenic mice might be less anxious than WT controls (Lalonde et al., 2004; Reiserer et al., 2007).

To test recognition memory, Bonardi et al. (2011) conducted an object recognition test on 4-month-old mice and found no difference between WT and APPSWE/PSEN1ΔE9 mice. Similar results were observed in a study by Jardanhazi-Kurutz et al. (2010) using mice at 4 and 6 months of age. Interestingly, APPSWE/PSEN1ΔE9 mice were found to show severe object recognition memory impairment at the age of 9-13 months (Donkin et al., 2010; Yoshiike et al., 2008).

Spatial learning and memory in rodents were examined in the BM (Sunyer, Patil, Hoger, & Lubec, 2007). Studies using 6-7 month old APPSWE/PSEN1ΔE9 mice showed cognitive impairment in the BM (Reiserer et al., 2007). O’Leary and Brown (2009) also observed spatial learning and memory deficits in 16-month-old APPSWE/PSEN1ΔE9 mice. MWM is another test that measures spatial learning and memory. BM and MWM are similar because both tests measures spatial learning and memory, however BM is conducted on land and MWM in water. Some studies have observed no cognitive deficit in APPSWE/PSEN1ΔE9 mice at 6 months of age as measured by MWM (Minkeviciene
et al., 2008; Savonenko et al., 2005). However, cognitive deficit was observed at 8-12 months of age (Butovsky et al., 2006; Cao, Lu, Lewis, & Li, 2007; Lalonde et al., 2004; Minkeviciene et al., 2008; Oksman et al., 2006), 12-13 months of age (Malm et al., 2011; Zhang et al., 2011) and 16-18 months of age (Savonenko et al., 2005). Thus, although there is still controversy about when spatial deficits are first observed in this mouse line, a number of studies seem to indicate that by 12 months of age APPSWE/PSEN1ΔE9 mice show spatial learning and memory deficits.

1.5 Cell death by oxidative stress and TRPM2-KO mice

Calcium is an intracellular messenger that mediates many physiological responses of neurons. The concentration of intracellular Ca$^{2+}$ ([Ca$^{2+}$]$_i$) fluctuates with time under normal physiological conditions and do not result in adverse effects for neurons. However, in pathological conditions, the concentration of intracellular Ca$^{2+}$ may rise significantly as H$_2$O$_2$ and Cyclic ADP-ribose (ADPR) activates TRPM2, compromising calcium homeostasis in neurons (Mattson, 2007). A loss of calcium homeostasis leads to sequential alterations in neuronal function, including disrupting the structure and function of synapses, impairment of synaptic plasticity and culminating with cell death. TRPM2 channels have been shown to play a critical role in cell death (Hara et al., 2002). When activated by ADPR and oxidative stress such as H$_2$O$_2$, high levels of Ca$^{2+}$ will move through the channel into the cell resulting in a continued increase in [Ca$^{2+}$]$_i$, ultimately inducing cell death (Fonfria et al., 2004; Hara et al., 2002; Wehage et al., 2002). This link between H$_2$O$_2$-induced cell death and TRPM2 channels were investigated by Kaneko et al. (2006), who demonstrated that a severe deterioration of primary cultured neurons was
present after \( \text{H}_2\text{O}_2 \) exposure. Similarly, Zhang et al. (2006) demonstrated that activation of TRPM2 by oxidative stress triggers apoptotic cell death in hematopoietic cells. Recent work by Fonfria et al. (2005) has shown that the inhibition of TRPM2 function reduces the increase in \([\text{Ca}^{2+}]_i\) and toxicity induced by A\(\beta\), and therefore provides concrete evidence that TRPM2 may contribute to neuronal cell death under circumstances in which oxidative stress are generated.

Synaptic plasticity is the ability of synapses to either strengthen or weaken with time, in response to increases or decreases in their activity and has been established to be \(\text{Ca}^{2+}\)-dependent (Cavazzini, Bliss, & Emptage, 2005). NMDA (N-methyl-D-aspartate) and AMPA (\(\alpha\)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptors are the two main receptors for the excitatory transmitter, glutamate. The opening of NMDA receptors leads to an influx of \(\text{Ca}^{2+}\) leading to long-term potentiation (LTP) while a more modest influx of \(\text{Ca}^{2+}\) into the post-synaptic neuron leads to long-term depression (LTD). LTP and LTD are thought to represent the cellular basis for learning and memory (Bear & Malenka, 1994; Cavazzini et al., 2005). Using hippocampal cultures and slices from TRPM2 knockout mice, a recent study by Xie et al., (2011) demonstrated that the loss of TRPM2 channels impairs LTD while having no effect on LTP. These findings suggest that TRPM2 contributes to synaptic plasticity. Moreover, as TRPM2 is oxidative stress responsive, it is anticipated that oxidative stress associated with elevated A\(\beta\) in Alzheimer’s mouse models will provoke aberrant TRPM2 channel activation leading to altered synaptic plasticity. Therefore, eliminating TRPM2 may be beneficial to learning and memory in mice.
Our hypothesis is that when TRPM2 expression is genetically ablated, there will be reduced $[\text{Ca}^{2+}]_i$ elevation in response to Aβ and therefore reduced toxicity. We believe that the loss of TRPM2 expression will better preserve neuronal function and cognition in mice. To test that, we will investigate whether knocking out the TRPM2 gene influences behavioral deficits observed in the APPSWE/PSEN1ΔE9 mouse model of AD. We will also investigate whether TRPM2 knockout (TRPM2-KO) mice show any behavioral changes. This thesis focuses on 4 genotypes of mice: wild-type mice (WT), TRPM2-KO (TRPM2$^{-/-}$), Alzheimer transgenic (APPSWE/PSEN1ΔE9$^+$) and APPSWE/PSEN1ΔE9 TRPM2 knock-out (TRPM2-KO-APPSWE/PSEN1ΔE9) mice.

1.6 Hypothesis and Objectives

TRPM2 is a calcium permeable, non-selective cation channel activated by ADPR and oxidants such as hydrogen peroxide (H$_2$O$_2$). Activated TRPM2 causes a continued increase in the intracellular Ca$^{2+}$ concentration and ultimately disrupts neuronal function. Given the fact that oxidative stress can promote TRPM2 activation and calcium dysregulation, we propose that abnormal TRPM2 activation may contribute to the Aβ-induced neurotoxicity with consequences for cognitive dysfunction.

Therefore, we propose that TRPM2 contributes to memory deficits in Alzheimer’s disease. To test the hypothesis, we crossed the TRPM2-KO mice with APPSWE/PSEN1ΔE9 mice in order to conduct cognitive behavioral testing.
Section 2

MATERIALS AND METHODS
2.1 Animals

All animal experiments were conducted in accordance to the policies indicated on the animal use protocol at Western University. TRPM2 knockout (TRPM2-KO) mice were provided by Dr. Yasuo Mori from Kyoto University, Kyoto, Japan. APPSWE/PSEN1ΔE9 double transgenic mice were provided by Dr. Marco Prado at Western University. All mice were housed in the animal facility at Robarts Research Institute, Western University. Both mouse strains were on a mixed C57Bl/6J-C3H/HeJ background.

TRPM2 homozygous (TRPM2+/+) mice were crossed with the Alzheimer’s transgenic (APPSWE/PSEN1ΔE9) bigenic mice to produce TRPM2+/+;APP+ and TRPM2+/+;APP-. Heterozygous littermates were then interbred (TRPM2+/+;APP+ x TRPM2+/+;APP-) to generate the four genotypes in our study: Wild-type (WT); TRPM2 knock-out (TRPM2-KO) mice; APPSWE/PSEN1ΔE9, and TRPM2 and APPSWE/PSEN1ΔE9 triple mutants (TRPM2-KO-APPSWE/PSEN1ΔE9).

Due to the fact that this is a longitudinal study and that death is common at an older age, we added another group of mice to the original cohort at the 6 month time point and continued experimentation as to make sure the N value will not be too low.

2.2 General materials and methods

This study was done longitudinally using the same cohort of mice. Only male mice were used. We did not use females since their estrous cycle may interfere with our experiments.
Anxiety, motor activity, recognition memory, spatial learning and memory were tested in mice at 3, 6, 9, 12, and 15 month time points, as measured by the open field locomotor activity (OFT), elevated plus (Elev+), object recognition (OR), Barnes (BM) and Morris water maze (MWM) respectively.

For all behaviour tests, 70% ethanol was used prior to each mouse trial to obliterate any odour cues left from the previous individual, thus preventing bias in our results. In order to reduce the level of stress experienced by these mice so that behavioural responses will not be influenced, all individuals were placed in the test room before experimentation was to begin and were given a thirty minute habituation session.

In order to minimize any alteration in these rodents’ behaviour while the observer is in the room, all experimental apparatus were placed at the back of the testing room with a curtain hanging from the ceiling, separating the area where the maze and observer are located (Vorhees & Williams, 2006; Walf & Frye, 2007). All tests were conducted in the same animal facility located at Robarts Research Institute between 0900 and 1700 hours.

### 2.3 Open field locomotor activity

Locomotor activity was measured using an automated system (Figure 2.1) (Versamax animal activity monitor; Accuscan Instruments, Inc., Columbus, OH, USA). Vertical light beam holes are positioned at the base of the monitors to record activities of mice. When mice move about the activity chamber, these light beams are interrupted, and these light beam breaks are then converted to distance (cm) for simple data analysis of activity (Sotnikova et al., 2004; de Castro et al., 2009a; de Castro et al., 2009b).
Mice were placed into several 20 cm x 20 cm locomotor activity chambers made out of plexiglass with 30.5 cm high walls and left alone for two hours, while the automated activity monitor recorded the discrete movements of mice including total distance travelled during exploration, total number of rear movements, and the amount of time spent in the center which is a measure of anxiety. All activities were measured in blocks of five minute intervals.
Figure 2.1. Automated Locomotor activity chamber used to measure locomotor activity. [Image source: http://phenome.jax.org].
2.4 Elevated plus maze

A standard elevated plus maze (Med Associates Inc., St. Albans, VT, USA) was used in this test, as seen in Figure 2.2. This maze is composed of opaque white acrylic material and consists of four arms, each being 38 cm long and 6 cm wide. Two of the arms are open without walls and the other two arms were enclosed by black polypropylene walls of 20 cm in height. The maze is elevated 75 cm off the floor with metal legs supporting each arm. A camera mounted overhead on the ceiling is used to record the animals’ movements on the maze, while this behaviour data are automatically collected with the ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA).

Each mouse is placed in the center of the maze facing the open arm and is then allowed to explore the maze for 5 minutes while the video tracking system automatically records the animals’ movements. During this time, different variables were recorded including the total distance travelled, latency to enter each arm, the number of entries into and the amount of time individual mice spent in each arm.
Figure 2.2. A standard elevated plus maze used to measure anxiety responses of rodents. [Image source: http://iobs.fudan.edu.cn/En/techs_view.asp?id=27&EnBigclassname=Animal%20Model%20and%20Behavior%20Facility&EnSmallclassname=Equipments].
2.5 Object recognition

Object recognition test was performed over the course of 2d and consisted of a 1d learning trial and a 1d testing trial. Each mouse was placed in a shoebox cage (homecage) (Figure 2.3) in the test room and given a 30 minute habituation session before the test was to begin. Training was conducted immediately following habituation on day 1, where two identical objects were positioned on the opposite ends of the homecage at a fixed distance. The mouse was then left to explore its surroundings for 5 minutes. Each mouse underwent three trials on training day. After a latency of 24 hours, mice were presented with one familiar object and one novel object in the same homecage. Exploration was again recorded for 5 minutes. Exploration was defined as either sniffing or touching the object with the nose and/or forepaw (de Lima et al. 2005). All objects had similar colours, textures, and sizes, but distinctive shapes.

A counterbalance measure was created prior to experimentation in order to randomly assign the mouse with their familiar and novel object. The ANY-maze video system (Stoelting Co., Wood Dale, IL, USA) was used to record the animals’ movements.

While the video records, the observer uses stopwatches to manually record the time each mouse spends exploring either the familiar or novel object.
Figure 2.3. Homecage used during training and test days for object recognition. Red circles represent familiar objects, while purple square represent the novel object. [Image adapted from http://www.nature.com/ncomms/journal/v1/n6/images_article/ncomms1064-f1.jpg].
2.6 Barnes maze

Barnes maze is made up of a white opaque circular platform (91.5 cm in diameter) made of a plastic material, elevated 105 cm above the floor, with 20 holes equally spaced around the perimeter (5 cm in diameter; 7.5 cm between holes) (Figure 2.4) (San Diego Instruments, San Diego, CA, USA). An escape box or target hole (5 cm x 11 cm x 5 cm) acts as positive reinforcement since they provide the mice with an escape from the brightly lit and exposed platform. The location of the escape box depends on the counterbalance made prior to experimentation. All holes need to look identical to avoid any potential behavioural bias that could arise.

In order to motivate mice so they will escape from the platform and into the escape box, a weak aversive stimulus (sound of a buzzer) is applied; the same parameters of the sound were used throughout the experiment. Visual cues of different shapes and colors were placed on the walls surrounding the maze. These cues act as the animal’s reference points for locating the target hole (Sunyer et al., 2007).

The Barnes maze test was performed over the course of 6 d and consisted of a 4 d acquisition trial (4 trials per day) and a 2 d (1 trial per day) testing trial. For the adaptation period on the first trial of the first day of training, the mouse was placed in a cylindrical white start chamber at the center of the maze. After 10 seconds, the chamber was lifted and the mouse guided to the escape box, again with the sound of a buzzer acting as a weak aversive stimulus to provide increased motivation for the mouse to escape from the platform and into the escape box.

Spatial acquisition follows the adaptation period, where each mouse was given 3 minutes on the maze to locate the escape box. A camera mounted overhead on the ceiling is used to record the animals’ movements on the maze, while this behaviour data are
automatically collected with the ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA). The variables collected include the primary and total latency to get to target, primary and total number of errors, as well as total distance travelled.

To test reference memory, either short or long-term retention in these mice, two probe trials are conducted after training, with the first trial performed on day 5 and the second trial on day 12. In these trials, the number of times a mouse pokes its nose into or hovers its head above the escape box are recorded, also with the automated video-tracking system.
Figure 2.4. Barnes maze used to measure spatial learning and memory, including distal visual cues used during experimentation.
2.7 Morris Water maze and reversal

The Morris water maze (MWM) (Med Associates Inc., St. Albans, VT, USA) is made up of a smooth white opaque plastic pool 120cm in diameter and 40cm in height for the walls. The pool was filled approximately half way with clear water kept at temperatures ranging from 24-26°C with a circular escape platform 10cm in diameter submerged 1cm below the surface of the water. Two axes were designated for the maze, creating an imaginary “+” in the pool leading to the formation of four equal quadrants numbered 1-4 with the platform positioned in the middle of one quadrant. The location of the quadrant depends on the counterbalance made prior to experimentation where at least one-quarter of the animals are tested with the platform in each individual quadrants.

This behaviour data were automatically collected with the ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA) (Figure 2.5).

Visual cues of different colours and shapes (gift wrap bows, large decorative flower, and plastic Hawaiian leis) were used as a guide to the escape box and were placed on the walls surrounding the maze. These cues act as the animal’s reference points for locating the target quadrant (Vorhees & Williams, 2006).

The MWM requires 4d of acquisition (4 trials per day) and 1d of testing (1 trial per day). In the acquisition phase (days 1-4), the mouse was released into the water at the desired start position of the maze, facing the pool wall. The mouse was then required to locate the hidden platform with the guidance of visual cues on the walls surrounding the maze. Once the mouse reaches the platform, the video-tracking system is stopped by the experimenter and variables such as the total distance travelled, the escape latency, and speed are recorded. In order to assess the retention of spatial memory, specifically short-term memory, a probe trial was performed 24 hours after the last training trial. The
platform was removed in the test trial and mice were allowed to swim freely for 60 seconds. During this session, the amount of time spent in each of the quadrants was measured (Faivre, Hamilton, & Holscher, 2012; Vorhees & Williams, 2006).

MWM reversal was conducted the week after MWM, under identical conditions and methodology with the only difference being the location of the platform. In this reversal learning period, the platform was relocated to the quadrant opposite of its previous location.
Figure 2.5. Morris water maze used to measure spatial learning and memory, including distal visual cues used during experimentation.
2.8 Statistical Analysis

All data are expressed as mean ± SEM. Statistical analyses were carried out using SigmaStat® (Aspire Software International, Ashburn, VA). Graphs were constructed using GraphPad Prism® (GraphPad Software, San Diego, CA). Repeated measures one-way and two-way ANOVA followed by the Holm-Sidak post hoc comparison test were used (where appropriate) to compare multiple groups. Results were considered significant when $p < 0.05$. 
Section 3

RESULTS
3.1 Open field locomotor activity

An open field activity test was conducted to measure locomotor activity in mice of all genotypes. In addition to measuring locomotion, the open field test can also measure anxiety levels in rodents. Mice have a natural tendency to avoid open spaces by travelling along the walls of enclosures as a means of protection and easy escape (Lalonde et al., 2004). Therefore, the amount of time individuals spent in the center area of the open field test provides a measure of anxiety.

No statistical differences in locomotor activity were observed between genotypes at three months (Fig. 3.1A,B; $F_{(3,920)} = 1.105, p = 0.358$; there was no interaction between genotype x time - Fig. 3.1 A,B; $F_{(69,920)} = 0.669, p = 0.982$; and habituation to the novel environment was found to occur for mice from all four genotypes, indicated by a continued decrease in distance with time - Fig. 3.1A; $F_{(69,920)} = 0.669, p = 0.982$; six months (Fig. 3.2A,B; $F_{(3,1081)} = 0.369, p = 0.775$; there was no interaction between genotype x time - Fig. 3.2A,B; $F_{(69,1081)} = 0.850, p = 0.803$; and habituation to the novel environment was found to occur for mice from all four genotypes - Fig. 3.2A; $F_{(23,1081)} = 51.910, p < 0.001$); or nine months (Fig. 3.3A,B; $F_{(3,897)} = 0.701, p = 0.557$; there was no interaction between genotype x time - Fig. 3.3A,B; $F_{(69,897)} = 1.031, p = 0.412$; and habituation to the novel environment was found to occur for mice from all four genotypes - Fig. 3.3A; $F_{(23,897)} = 44.401, p < 0.001$). Furthermore, no change was observed in terms of the amount of time spent in the center of the chamber at these different time points (three months: Fig. 3.1C; $F_{(3,920)} = 2.016, p = 0.127$; six months: Fig. 3.2C; $F_{(3,1081)} = 0.755, p = 0.525$; and nine months: Fig. 3.3C; $F_{(3,897)} = 0.370, p = 0.775$).
At **twelve months**, no difference in locomotor activity was observed between TRPM-KO and WT mice. However, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice showed significantly decreased horizontal activity (Fig.3.4A,B; $F_{(3,1035)} = 3.745$, $p = 0.017$; post hoc analysis show $p<0.05$). In addition, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent less time in the center than the control mice (Fig.3.4C; $p < 0.01$ and $p < 0.01$ respectively, in the Holm-Sidak post-hoc test). However, this difference in center time might reflect the fact that these mutant mice are hypoactive rather than any difference in anxiety.

Unfortunately, for the **fifteen months** analysis we had a smaller number of mice due to death or fight injury. Our analysis did not show any significant difference in locomotor activity between genotypes (Fig.3.5A,B; $F_{(3,374)} = 1.622$, $p = 0.202$). A statistical significance in center time was observed between genotypes as measured by two-way repeated measures ANOVA. Post hoc analysis indicates that APPSWE/PSEN1ΔE9 mice spent significantly less time in the center than WT, TRPM2-KO and TRPM2-KO-APPSWE/PSEN1ΔE9 mice (Fig.3.5C).
Figure 3.1. Locomotor activity at 3 months of age

(A) Horizontal activity in an open field for WT, TRPM2-KO, APPSWE/PSEN1\(\Delta\)E9 and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice was measured over time \((F_{(23,920)} = 61.052, p < 0.001\) in a two-way repeated measures ANOVA) and (B) cumulatively over 2 h and (C) amount of time spent in the center. All data were plotted as Mean ± SEM.
Figure 3.2. Locomotor activity at 6 months of age

(A) Horizontal activity in an open field for WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice was measured over time ($F_{(23,1081)} = 51.910$, $p < 0.001$ in a two-way repeated measures ANOVA) and (B) cumulatively over 2 h and (C) amount of time spent in the center. All data were plotted as Mean ± SEM.
Figure 3.3. Locomotor activity at 9 months of age

(A) Horizontal activity in an open field for WT, TRPM2-KO, APPSWE/PSEN1∆E9 and TRPM2-KO-APPSWE/PSEN1∆E9 mice was measured over time ($F_{(23,897)} = 44.401$, $p < 0.001$ in a two-way repeated measures ANOVA) and (B) cumulatively over 2 h and (C) amount of time spent in the center. All data were plotted as Mean ± SEM.
Figure 3.4. Locomotor activity at 12 months of age

(A) Horizontal activity in an open field for WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice was measured over time \( F_{(23,1035)} = 50.049, p < 0.001 \) in a two-way repeated measures ANOVA and (B) cumulatively over 2 h and (C) amount of time spent in the center. All data were plotted as Mean ± SEM. *\( p = 0.05 \) in a Holm-Sidak post hoc test, **\( p < 0.01 \) in a Holm-Sidak post hoc test.
Figure 3.5. Locomotor activity at 15 months of age

(A) Horizontal activity in an open field for WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice was measured over time ($F_{(11,374)} = 26.461$, $p < 0.001$ in a two-way repeated measures ANOVA) and (B) cumulatively over 2 h and (C) amount of time spent in the center. All data were plotted as Mean ± SEM. **$p < 0.01$ in a Holm-Sidak post hoc test; ***$p < 0.001$ in a Holm-Sidak post hoc test.
3.2 Elevated Plus maze

The elevated plus maze task was used to examine whether anxiety levels differed between the four genotypes of mice (WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9) at five age points (3, 6, 9, 12, and 15 months).

No statistical differences in anxiety were observed between genotypes at three months (Fig.3.6A; $F_{(3,28)} = 0.152, p = 0.928$ in terms of the percentage of time spent in open arms; there was no statistical difference in terms of the number of arm entries to the open arms – Fig.3.6B; $F_{(3,28)} = 0.121, p = 0.947$); nine months (Fig.3.6E; $F_{(3,32)} = 0.551, p = 0.651$ in terms of the percentage of time spent in open arms; there was no statistical difference in terms of the number of arm entries to the open arms – Fig.3.6F; $F_{(3,32)} = 1.248, p = 0.309$); or fifteen months (Fig.3.6I; $F_{(3,30)} = 1.241, p = 0.312$ in terms of the percentage of time spent in open arms; there was no statistical difference in terms of the number of arm entries to the open arms – Fig.3.6J; $F_{(3,30)} = 1.296, p = 0.294$).

At six months, while TRPM2-KO and APPSWE/PSEN1ΔE9 mice did not differ significantly from WT in the time spent in the open arms; TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent significantly longer time in the open arms than the control mice (Fig.3.6C; $F_{(3,33)} = 3.756, p = 0.020$; post hoc analysis show $p<0.05$ and $p<0.01$). However, no statistical difference was observed between genotypes in terms of the number of arm entries into the open arms (Fig.3.6D; $F_{(3,33)} = 2.023, p = 0.130$). These results might suggest that TRPM2-KO-APPSWE/PSEN1ΔE9 mice were less anxious than WT, TRPM2-KO and APPSWE/PSEN1ΔE9 at this age.

No statistical difference was observed in the percentage of time spent in the open arms at twelve months of age (Fig.3.6G; $F_{(3,31)} = 0.417, p = 0.742$). On the other hand,
APPswe/PSEN1ΔE9 and TRPM2-KO-APPswe/PSEN1ΔE9 mice visited the open arms significantly fewer times than the control mice (Fig. 3.6H; $F_{(3,31)} = 5.799$, $p = 0.003$; post hoc analysis show $p<0.05$ and $p<0.01$), suggesting that at this age these mutant mice might be more anxious than WT and TRPM2-KO. However, this may also be a consequence of their reduced activity when aged.
Figure 3.6. Assessment of anxiety at 3, 6, 9, 12, and 15 months of age

(A) Percentage of time WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-
APPSWE/PSEN1ΔE9 mice spent in the open arms measured at 3 months (B) Number of
arm entries measured at 3 months and (C) Percentage of time spent in open arms
measured at 6 months ($F_{(3,33)} = 3.756, p < 0.05$ in a one-way repeated measures ANOVA)

(D) Number of arm entries measured at 6 months and (E) Percentage of time spent in
open arms measured at 9 months and (F) Number of arm entries measured at 9 months

(G) Percentage of time spent in open arms measured at 12 months and (H) Number of
arm entries measured at 12 months ($F_{(3,31)} = 5.799, p < 0.01$ in a one-way repeated
measures ANOVA) (I) Percentage of time spent in open arms measured at 15 months of
age and (J) Number of arm entries measured at 15 months of age. All data were plotted as
Mean ± SEM. *$p < 0.05$ in a Holm-Sidak post hoc test; **$p < 0.01$ in a Holm-Sidak post
hoc test. 
3.3 Object recognition

To test for recognition memory, which is the ability of mice to discriminate between familiar and novel objects, we conducted an object recognition test at 9 and 12 months. Mice have a spontaneous tendency to spend more time exploring a novel object than a familiar one. Therefore, the choice to explore the novel object will reflect the use of learning and recognition memory (Otalora et al., 2012; Prado et al., 2006). This task requires that the individuals be able to distinguish between the two familiar objects on training day and recognize the novel object on test day where both familiar and novel objects are present. Although we are showing only the 9 and 12 months data here, we did conduct this test at 3 and 6 months of age. However, our protocol was flawed and therefore we could not use those results. The protocol was modified in order to conduct the test at 9 and 12 months of age. We decided not to conduct object recognition test at 15 months of age because we assumed no deficit will be seen; that mice of all genotypes will be able to discriminate between the familiar and novel objects, just like the data shown at 9 and 12 months.

No significant difference was observed in terms of the percentage of time spent exploring the familiar (F) and novel (N) objects. Mice from all genotypes preferred the novel object on testing day, as shown by an increased exploration of N at nine months (Fig.3.7B; WT - $F_{(1,5)} = 33.235, p = 0.002$; post hoc analysis show $p<0.01$; TRPM2-KO - $F_{(1,5)} = 17.506, p = 0.009$; post hoc analysis show $p<0.01$; APPSWE/PSEN1∆E9 - $F_{(1,9)} = 7.625, p = 0.022$; post hoc analysis show $p<0.01$; TRPM2-KO-APPSWE/PSEN1∆E9 - $F_{(1,11)} = 12.696, p = 0.004$; post hoc analysis show $p<0.01$) and twelve months (Fig.3.7D; WT - $F_{(1,7)} = 10.469, p = 0.014$; post hoc analysis show $p<0.01$; TRPM2-KO -...
\( F_{(1,7)} = 27.963, p = 0.001; \) post hoc analysis show \( p < 0.01; \) APPSWE/PSEN1ΔE9 - \( F_{(1,11)} = 6.621, p = 0.026; \) post hoc analysis show \( p < 0.05; \) TRPM2-KO-APPSWE/PSEN1ΔE9

- \( F_{(1,13)} = 20.757, p < 0.001; \) post hoc analysis show \( p < 0.01). \)
Figure 3.7. Assessment of recognition memory at 9 and 12 months of age

(A) The time that WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent exploring the homecage on training trials 1-3 and test trial 4 was measured at 9 months and (C) 12 months. (B) Percentage of time mice spent exploring the familiar (F) and novel (N) objects on both training and test days were measured at 9 months and (D) 12 months. All data were plotted as Mean ± SEM. *p < 0.05 in a Holm-Sidak post hoc test; **p < 0.01 in a Holm-Sidak post hoc test.
3.4 Barnes maze

Spatial learning and memory were examined in the Barnes maze (Sunyer et al., 2007) for all four groups of mice at 3, 6, 9, 12, and 15 months of age. Learning was observed for all ages as measured by the primary latency to find the escape box, indicated by the reduced time needed for the mice to find the escape box. Primary number of errors, the number of errors made by the mice in order to reach the target hole was also measured. Memory, or the ability to remember the location of the target hole with the guidance of visual cues, was assessed on test/probe days 5 and 12. Here, the number of times the mouse pokes its nose into, or hover its head above the escape box was measured.

No statistical differences in spatial memory was observed between genotypes at three months (there was no statistical difference between genotypes in terms of primary latency to target hole for training days 1 to 4 as well as testing days 5 and 12 - Fig.3.8A (left); $F_{(3,195)} = 1.128, p = 0.350$; there was no statistical difference between genotypes in terms of primary latency to target hole when only looking at training days – Fig.3.8A (middle); $F_{(3,117)} = 0.861, p = 0.469$; and there was no statistical difference between genotypes in terms of primary number of errors to target hole on training days – Fig.3.8A (right); $F_{(3,117)} = 1.167, p = 0.335$. There was no statistical difference between genotypes on test day 5 (Fig.3.8B (left); $F_{(3,117)} = 1.213, p = 0.318$) or day 12 (Fig.3.8C (left); $F_{(3,117)} = 2.807, p = 0.052$) in terms of number of times the mouse pokes its nose into the escape box.

At six months, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice showed longer primary latency to the target hole than control mice - Fig.3.9A (left);
$F_{(3,230)} = 4.051, \ p < 0.05$; post hoc analysis show $p \leq 0.01$ and $p < 0.05$; APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice also showed longer primary latency to the target hole than control mice over the 4 days of training - Fig.3.9A (middle); $F_{(3,138)} = 4.125, \ p < 0.05$; post hoc analysis show $p < 0.01$ and $p < 0.05$; and APPSWE/PSEN1ΔE9 mice made more errors compared to the others throughout the 4 training days – Fig.3.9A (right); $F_{(3,138)} = 2.970, \ p < 0.05$; post hoc analysis show $p < 0.05$.

On probe trial day 5, latency to find the target hole was no different between genotypes (Fig 3.9A (left)) and time spent investigating the target quadrant was significantly longer compared with the other quadrants for all genotypes (Fig.3.9B (left); $F_{(3,138)} = 3.176, \ p = 0.033$; post hoc analysis show $p < 0.05$; all mice visited the target hole location more regardless of genotypes as indicated by the percentage of time spent in each quadrant - Fig.3.9B (right); $F_{(3,138)} = 91.008, \ p < 0.001$; post hoc analysis show $p < 0.001$. On probe trial day 12, even though APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice visited the target hole significantly less than controls - Fig.3.9C (left); $F_{(3,138)} = 4.512, \ p = 0.007$; post hoc analysis show $p < 0.05$ and $p < 0.01$, the results indicate that these mutants do remember the position of the target hole.

At nine months, there was no statistical difference between genotypes in terms of primary latency to target hole for training days 1 to 4 as well as testing days 5 and 12 - Fig.3.10A (left); $F_{(3,230)} = 2.269, \ p = 0.093$; there was no statistical difference between genotypes in terms of primary latency to target hole when only looking at training days – Fig.3.10A (middle); $F_{(3,138)} = 1.631, \ p = 0.195$; and there was no statistical difference between genotypes in terms of primary number of errors to target hole on training days – Fig.3.10A (right); $F_{(3,138)} = 0.506, \ p = 0.680$. On probe trial day 5, APPSWE/PSEN1ΔE9
and TRPM2-KO-APPSWE/PSEN1ΔE9 mice visited the target hole location significantly less than controls - Fig. 3.10B (left); $F_{(9,138)} = 3.223, p = 0.001$; post hoc analysis show $p \leq 0.001$ and $p < 0.05$; TRPM2-KO-APPSWE/PSEN1ΔE9 mice also visited the left hole location significantly less than controls - Fig. 3.10B (left); $F_{(9,138)} = 3.223, p = 0.001$; post hoc analysis show $p < 0.05$; all mice visited the target hole location more regardless of genotypes on this day as indicated by the percentage of time spent in each quadrant - Fig. 3.10B (right); $F_{(3,138)} = 103.229, p < 0.001$; post hoc analysis show $p < 0.001$; and there was no statistical difference between genotypes on test day 12 – Fig. 3.10C (left); $F_{(9,138)} = 1.407, p = 0.191$; again, all mice visited the target hole location more regardless of genotypes as indicated by the percentage of time spent in each quadrant - Fig. 3.10C (right); $F_{(3,138)} = 68.221, p < 0.001$; post hoc analysis show $p < 0.001$ and $p < 0.01$.

At twelve months, there was no statistical difference between genotypes in terms of primary latency to target hole for training days 1 to 4 as well as testing days 5 and 12 (Fig. 3.11A (left); $F_{(3,185)} = 0.856, p = 0.472$); there was no statistical difference between genotypes in terms of primary latency to target hole when only looking at training days – Fig. 3.11A (middle); $F_{(3,111)} = 0.575, p = 0.635$; and there was no statistical difference between genotypes in terms of primary number of errors to target hole on training days – Fig. 3.11A (right); $F_{(3,111)} = 1.129, p = 0.350$. On probe trial days 5 and 12, while target hole location visits of TRPM2-KO and TRPM2-KO-APPSWE/PSEN1ΔE9 mice did not differ from that of WT, APPSWE/PSEN1ΔE9 mice visited the target hole location significantly less than control and TRPM2-KO-APPSWE/PSEN1ΔE9 mice - Fig. 3.11B (left); $F_{(3,111)} = 2.284, p = 0.035$; post hoc analysis show $p < 0.01$ and $p < 0.05$; and Fig. 3.11C (left); $F_{(3,111)} = 1.789, p = 0.05$; post hoc analysis show $p < 0.05$. All mice
visited the target hole location more regardless of genotypes on both day 5 (Fig.3.11B (middle); \(F_{(3,111)} = 59.827, \ p < 0.001\); post hoc analysis show \(p<0.001\) and \(p<0.01\)) and day 12 (Fig.3.11C (middle); \(F_{(3,111)} = 54.250, \ p < 0.001\); post hoc analysis show \(p<0.001\) and \(p<0.01\)) as indicated by the percentage of time spent in each quadrant.

At *fifteen months*, APPSWE/PSEN1\(\Delta\)E9 and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice had longer primary latency to target hole than control mice (Fig.3.12A (left); \(F_{(3,200)} = 7.198, \ p < 0.001\); post hoc analysis show \(p<0.001\) and \(p<0.01\)); APPSWE/PSEN1\(\Delta\)E9 and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice also had longer primary latency to target hole than control mice when only looking at the first 4 training days (Fig. 3.12A (middle); \(F_{(3,120)} = 4.763, \ p < 0.01\); post hoc analysis show \(p<0.01\) and \(p<0.05\)); and there was no statistical difference between genotypes in terms of primary number of errors to target hole on training days (Fig.3.12A (right); \(F_{(3,120)} = 0.803, \ p = 0.499\)). On probe trial day 5, APPSWE/PSEN1\(\Delta\)E9 mice visited the target hole location significantly less than control and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice (Fig.3.12B (left); \(F_{(9,120)} = 4.344, \ p < 0.001\); post hoc analysis show \(p<0.001\) and \(p<0.05\)). On probe trial day 12, APPSWE/PSEN1\(\Delta\)E9 mice visited the target hole location significantly less than control and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice (Fig.3.12C (left); \(F_{(9,120)} = 2.006, \ p = 0.044\); post hoc analysis show \(p<0.01\)). Thus, APPSWE/PSEN1\(\Delta\)E9 mice visited less and spent significantly less time in the target quadrant on both probe days compared to TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice. These results indicate that TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice perform significantly better than APPSWE/PSEN1\(\Delta\)E9 mice.
Taking all of the above findings into consideration, results from BM indicate that APPSWE/PSEN1ΔE9 mice have impaired spatial memory and that AD mouse model that lack TRPM2 (TRPM2-KO-APPSWE/PSEN1ΔE9) had their performance rescued in the Barnes maze.
Figure 3.8. Barnes Maze assessment of spatial memory at 3 months of age.
(A (left)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) and test days 5 and 12 was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 3 months. (A (middle)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 3 months. (A (right)) Primary number of error to target hole on acquisition days 1-4 was measured for all genotypes at 3 months. (B (left)) Number of nose pokes per quadrant measured on test day 5 in a 90s probe trial. (B (middle)) Percentage of nose pokes per quadrant separated by genotypes on day 5. (B (right)) Representative path traces on day 5 comparing WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. Target hole indicated by black dot. (C (left)) Number of nose pokes per quadrant measured on test day 12 in a 90s probe trial. (C (middle)) Percentage of nose pokes per quadrant separated by genotypes on day 12. (C (right)) Representative path traces on day 12 comparing WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. All data were plotted as Mean ± SEM. *p < 0.05 in a Holm-Sidak post hoc test; **p < 0.01 in a Holm-Sidak post hoc test; ***p < 0.001 in a Holm-Sidak post hoc test; ***p = 0.001 in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Figure 3.9. Barnes Maze assessment of spatial memory at 6 months of age.

(A (left)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) and test days 5 and 12 was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 6 months ($F_{(3,230)} = 4.051, p < 0.05$ in a two-way repeated measures ANOVA). (A (middle))
Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 6 months ($F_{(3,138)} = 4.125, p < 0.05$ in a two-way repeated measures ANOVA). (A (right)) Primary number of error to target hole on acquisition days 1-4 was measured for all genotypes at 6 months ($F_{(3,138)} = 2.970, p < 0.05$ in a two-way repeated measures ANOVA). (B (left)) Number of nose pokes per quadrant measured on test day 5 in a 90s probe trial ($F_{(3,138)} = 3.176, p < 0.05$ in a two-way repeated measures ANOVA). (B (right)) Percentage of nose pokes per quadrant separated by genotypes on day 5 ($F_{(3,138)} = 91.008, p < 0.001$ in a two-way repeated measures ANOVA). (C (left)) Number of nose pokes per quadrant measured on test day 12 in a 90s probe trial ($F_{(3,138)} = 4.512, p < 0.01$ in a two-way repeated measures ANOVA). (C (right)) Percentage of nose pokes per quadrant separated by genotypes on day 12 ($F_{(3,138)} = 80.773, p < 0.001$ in a two-way repeated measures ANOVA). All data were plotted as Mean ± SEM. *$p < 0.05$ in a Holm-Sidak post hoc test; **$p < 0.01$ in a Holm-Sidak post hoc test; ***$p = 0.01$ in a Holm-Sidak post hoc test; ****$p < 0.001$ in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Figure 3.10. Barnes Maze assessment of spatial memory at 9 months of age

(A (left)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) and test days 5 and 12 was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 9 months. (A (middle)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) was measured for WT, TRPM2-KO,
APPswe/PSEN1ΔE9, and TRPM2-KO-APPswe/PSEN1ΔE9 mice at 9 months. (A (right)) Primary number of error to target hole on acquisition days 1-4 was measured for all genotypes at 9 months. (B (left)) The number of nose pokes per quadrant were measured on test day 5 in a 90s probe trial \( F_{(9,138)} = 3.223, p = 0.001 \) in a two-way repeated measures ANOVA). (B (right)) Percentage of nose pokes per quadrant separated by genotypes on day 5 \( F_{(9,138)} = 103.229, p < 0.001 \) in a two-way repeated measures ANOVA). (C (left)) The number of nose pokes per quadrant were measured on test day 12 in a 90s probe trial. (C (right)) Percentage of nose pokes per quadrant separated by genotypes on day 12 \( F_{(9,138)} = 68.221, p < 0.001 \) in a two-way repeated measures ANOVA). All data were plotted as Mean ± SEM. *\( p < 0.05 \) in a Holm-Sidak post hoc test; **\( p < 0.01 \) in a Holm-Sidak post hoc test; ***\( p < 0.001 \) in a Holm-Sidak post hoc test; ***'\( p = 0.001 \) in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Figure 3.11. Barnes Maze assessment of spatial memory at 12 months of age

(A (left)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) and test days 5 and 12 was measured for WT,
TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 12 months. (A (middle)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 12 months. (A (right)) Primary number of error to target hole on acquisition days 1-4 was measured for all genotypes at 12 months. (B (left)) The number of nose pokes per quadrant were measured on test day 5 in a 90s probe trial ($F_{(3,111)} = 2.284, p = 0.035$ in a two-way repeated measures ANOVA). (B (middle)) Percentage of nose pokes per quadrant separated by genotypes on day 5 ($F_{(9,111)} = 59.827, p < 0.001$ in a two-way repeated measures ANOVA). (B (right)) Representative path traces on day 5 comparing WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. Target hole indicated by black dot. (C (left)) The number of nose pokes per quadrant were measured on test day 12 in a 90s probe trial ($F_{(3,111)} = 1.789, p = 0.05$ in a two-way repeated measures ANOVA). (C (middle)) Percentage of nose pokes per quadrant separated by genotypes on day 12 ($F_{(3,111)} = 54.250, p < 0.001$ in a two-way repeated measures ANOVA). (C (right)) Representative path traces on day 12 comparing WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. All data were plotted as Mean ± SEM. *$p < 0.05$ in a Holm-Sidak post hoc test; **$p < 0.01$ in a Holm-Sidak post hoc test; ***$p < 0.001$ in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) and test days 5 and 12 was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 15 months of age.
months \( (F_{(3,200)} = 7.198, \ p < 0.001 \) in a two-way repeated measures ANOVA). (A (middle)) Primary latency to target hole on acquisition days 1-4 (the average values of four 3min trials per day are plotted) was measured for WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at 15 months \( (F_{(3,120)} = 4.763, \ p < 0.05 \) in a two-way repeated measures ANOVA). (A (right)) Primary number of error to target hole on acquisition days 1-4 was measured for all genotypes at 15 months. (B (left)) The number of nose pokes per quadrant were measured on test day 5 in a 90s probe trial \( (F_{(9,120)} = 4.344, \ p < 0.001 \) in a two-way repeated measures ANOVA). (B (right)) Percentage of nose pokes per quadrant separated by genotypes on day 5 \( (F_{(9,120)} = 22.974, \ p < 0.001 \) in a two-way repeated measures ANOVA). (C (left)) The number of nose pokes per quadrant were measured on test day 12 in a 90s probe trial \( (F_{(9,120)} = 2.006, \ p < 0.05 \) in a two-way repeated measures ANOVA). (C (right)) Percentage of nose pokes per quadrant separated by genotypes on day 12 \( (F_{(9,120)} = 14.823, \ p < 0.001 \) in a two-way repeated measures ANOVA). All data were plotted as Mean ± SEM. *\( p < 0.05 \) in a Holm-Sidak post hoc test; **\( p < 0.01 \) in a Holm-Sidak post hoc test; ***\( p < 0.001 \) in a Holm-Sidak post hoc test; ***’\( p = 0.001 \) in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
3.5 Morris water maze hidden-platform test

Spatial learning and memory were examined by Morris water maze (MWM) at twelve and fifteen months of age. In acquisition trials, mice were trained to locate a hidden platform placed in 1 of 4 quadrants in the maze. On day 5 of the probe trial, the platform was removed and mice were allowed to swim freely for 60 seconds. During this session, the amount of time spent in each of the quadrants was measured.

No statistical differences in spatial memory were observed between WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at twelve months. There was no statistical difference between genotypes in terms of the latency to find the escape platform in the 4d training period, and all mice improved in the time it took to find the platform over training trials (Fig.3.13A; $F_{(3,105)} = 1.279$, $p = 0.297$). No statistical difference between genotypes were observed in terms of distance required to find the platform (Fig.3.13B; $F_{(3,105)} = 1.636$, $p = 0.199$). When spatial memory was investigated on probe trial day 5, the time WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent investigating the target quadrant was significantly longer than all other quadrants (Fig.3.13C; $F_{(3,105)} = 39.134$, $p<0.001$; post hoc analysis show $p<0.05$, $p<0.01$, $p=0.001$, and $p<0.001$), indicating that all mice have learned the location of the target quadrant. Closer examination of representative path traces indicate that mice of all genotype are spending more time in the target quadrant and thus are learning, as well as remembering the locations of the platform (Fig.3.13D).

At fifteen months, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice had a longer latency to find the escape platform in the 4d training period than control mice (Fig.3.14A; $F_{(3,117)} = 4.167$, $p < 0.05$; post hoc analysis
show $p<0.05$ and $p<0.01$). In addition, the distance required for APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice to find the platform is significantly longer than controls (Fig. 3.14B; $F_{(3,117)} = 9.619$, $p < 0.001$; post hoc analysis show $p<0.01$ and $p<0.001$). When spatial memory was investigated on probe trial day 5, the time WT, TRPM2-KO, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent investigating the target quadrant was significantly longer than all other quadrants (Fig. 3.14C; $F_{(3,117)} = 30.070$, $p<0.001$; post hoc analysis show $p<0.05$, $p<0.01$, and $p<0.001$), suggesting that these mice remembered where the platform was located. However, APPSWE/PSEN1ΔE9 mice did not remember the location of the platform since these mice did not spend a significantly longer time investigating the target quadrant. Representative path traces further indicate that APPSWE/PSEN1ΔE9 mice, contrary to WT, TRPM2-KO and TRPM2-KO-APPSWE/PSEN1ΔE9 mice, do not remember the position of the platform and their swim paths are in a random pattern around the pool instead of only focusing on the target quadrant (Fig. 3.14D).
Figure 3.13. MWM assessment of spatial memory at 12 months of age

(A) WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice were subject to the MWM paradigm. Latency to find the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted (B) Total distance traveled to the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted (C) Percentage of time spent in each quadrant was measured on day 5 in a 60s probe trial.
with the platform removed \(F_{(3,105)} = 39.134, p < 0.001\) in a two-way repeated measures ANOVA). (D) Representative path traces on probe day 5 comparing WT, TRPM2-KO, APPSWE/PSEN1\(\Delta E9\), and TRPM2-KO-APPSWE/PSEN1\(\Delta E9\) mice. All data were plotted as Mean ± SEM. *\(p < 0.05\) in a Holm-Sidak post hoc test; **\(p < 0.01\) in a Holm-Sidak post hoc test; ***\(p < 0.001\) in a Holm-Sidak post hoc test; ****\(p = 0.001\) in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Figure 3.14. MWM assessment of spatial memory at 15 months of age

(A) WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice were subject to the MWM paradigm. Latency to find the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted ($F_{(3,117)} = 4.167, p < 0.05$ in a two-way repeated measures ANOVA) (B) Total distance traveled to the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted ($F_{(3,117)} = 9.619, p <$
0.001 in a two-way repeated measures ANOVA) (C) Percentage of time spent in each quadrant was measured on day 5 in a 60s probe trial with the platform removed ($F_{(3,117)} = 16.466, p < 0.001$ and $F_{(3,117)} = 30.070, p < 0.001$ in a two-way repeated measures ANOVA). (D) Representative path traces on probe day 5 comparing WT, TRPM2-KO, APPSWE/PSEN1∆E9, and TRPM2-KO-APPSWE/PSEN1∆E9 mice. All data were plotted as Mean ± SEM. *p < 0.05 in a Holm-Sidak post hoc test; **p < 0.01 in a Holm-Sidak post hoc test; ***p < 0.001 in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
3.6 Morris water maze reversal learning

Reversal learning is another set of experiment conducted one week after Morris water maze, again requiring 4d of acquisition (4 trials per day) and 1d of testing (1 trial per day). In this test however, the platform is relocated to the quadrant opposite of its former location. This reversal learning reveals whether or not animals can extinguish their previously learnt platform location and acquire a direct path to the new location (Faivre et al., 2012; Vorhees & Williams, 2006). In order to re-learn the location of this newly placed platform, cognitive flexibility needs to be present in these animals (Rosczyk, Sparkman, & Johnson, 2008).

At 12 months of age, reversal learning experiments show no statistical difference between genotypes in terms of the latency to find the escape platform in the 4d training period, with all mice improved in the time it took to find the platform over training (Fig.3.15A; $F_{(3,105)} = 1.936, p = 0.142$). No statistical difference between genotypes were observed in terms of distance required to find the platform (Fig.3.15B; $F_{(3,105)} = 2.891, p = 0.059$). When spatial memory was investigated on probe trial day 5, the time WT, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice spent investigating the target quadrant was significantly longer than all other quadrants (Fig.3.15C; $F_{(3,105)} = 39.134, p<0.001$; post hoc analysis show $p<0.05$, $p<0.01$, and $p<0.001$). However, TRPM2-KO mice did not spend more time investigating the target quadrant. Instead, these mice spent similar amounts of time investigating all quadrants. This suggests that TRPM2-KO mice show impaired reversal learning. The representative path traces indicate that TRPM2-KO mice do not remember the position of the platform and their swim paths are in a random pattern around the pool instead of only focusing on the target
quadrant. On the other hand, WT, APPSWE/PSEN1\(\Delta\)E9 and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice are spending more time in the target quadrant and thus are learning, as well as remembering the locations of the platform (Fig.3.15D).

At **15 months** of age, no statistical differences was observed between genotypes in terms of the latency to find the escape platform in the 4d training period, and all mice improved in the time it took to find the platform over training (Fig.3.16A; \(F_{(3,117)} = 2.017, p=0.127\)). The distance required for APPSWE/PSEN1\(\Delta\)E9 and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice to find the platform is significantly longer than controls (Fig.3.16B; \(F_{(3,117)} = 6.069, p < 0.01\); post hoc analysis show \(p<0.05, p<0.01,\) and \(p<0.001\)). When spatial memory was investigated on probe trial day 5, the time WT and TRPM2-KO-APPSWE/PSEN1\(\Delta\)E9 mice spent investigating the target quadrant was significantly longer than all other quadrants (Fig.3.16C; \(F_{(3,117)} = 29.914, p<0.001\); post hoc analysis show \(p<0.05, p<0.01,\) and \(p<0.001\)). Both TRPM2-KO and APPSWE/PSEN1\(\Delta\)E9 mice did not learn the location of the target quadrant since these mice did not spend a significantly longer time investigating the target quadrant, indicating that at this age both TRPM2-KO and APPSWE/PSEN1\(\Delta\)E9 mice show impaired reversal learning. The representative path traces indicate that both TRPM2-KO and APPSWE/PSEN1\(\Delta\)E9 mice show swim paths that are random around the pool instead of only focusing on the target quadrant (Fig.3.16D).
Figure 3.15. MWM assessment of reversal learning at 12 months of age

(A) WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice were subject to reversal learning in MWM. Latency to find the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted. (B) Total distance traveled to the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted. (C) Percentage of time spent in each quadrant was measured on day 5 in a 60s
probe trial with the platform removed ($F_{(3,105)} = 31.318, p < 0.001$ in a two-way repeated measures ANOVA). (D) Representative path traces on probe day 5 of reversal comparing WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. All data were plotted as Mean ± SEM. *$p < 0.05$ in a Holm-Sidak post hoc test; **$p < 0.01$ in a Holm-Sidak post hoc test; ***$p < 0.001$ in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Figure 3.16. MWM assessment of reversal learning at 15 months of age

(A) WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice were subject to reversal learning in MWM. Latency to find the platform on acquisition days 1-4 (the average of four 90s trials per day) is plotted (B) Total distance traveled to the platform on acquisition days 1-4 (the average of four 90s trials per day) is
plotted \( F_{(3,117)} = 6.069, \ p < 0.01 \) in a two-way repeated measures ANOVA. (C) Percentage of time spent in each quadrant was measured on day 5 in a 60s probe trial with the platform removed \( F_{(3,117)} = 11.375, \ p < 0.001 \) and \( F_{(3,117)} = 29.914, \ p < 0.001 \) in a two-way repeated measures ANOVA. (D) Representative path traces on probe day 5 of reversal comparing WT, TRPM2-KO, APPSWE/PSEN1\( \Delta \)E9, and TRPM2-KO-APPSWE/PSEN1\( \Delta \)E9 mice. All data were plotted as Mean \( \pm \) SEM. *\( p < 0.05 \) in a Holm-Sidak post hoc test; **\( p < 0.01 \) in a Holm-Sidak post hoc test; ***\( p < 0.001 \) in a Holm-Sidak post hoc test. L, left; O, opposite; R, right; T, target.
Section 4

DISCUSSION
4.1 Summary of Key Findings

The main purpose of this thesis was to explore the relationship between TRPM2 channels and cognitive impairment observed in an Alzheimer’s mouse model. I tested the hypothesis that genetic ablation of TRPM2 would prevent cognitive deficits in APPSWE/PSEN1ΔE9 transgenic mice. Behavioral analysis suggested that:

1) TRPM2 channels are important for reversal learning in aged mice, as TRPM2-KO mice at 12 month and older showed impaired performance in the MWM reversal learning task.

2) Genetic ablation of TRPM2 improves cognition in APPSWE/PSEN1ΔE9 transgenic mice, as TRPM2-KO-APPSWE/PSEN1ΔE9 mice showed improved performance on spatial memory, measured by BM and MWM tests and reversal learning.

4.2 Open field locomotor activity test

The primary reason of conducting an open field locomotor activity test was to test whether mice were hyperactive, which is a common characteristic associated with several neurodegenerative disease models (Arendash et al., 2001). It is expected that mice will spend more time exploring their environment at the beginning because of the novel environment; however they will gradually decrease their exploration with time, since they will become familiar with the environment, otherwise known as habituation. This change in exploratory tendency was observed in our study; WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice at all ages showed a continued decrease in distance travelled during the two hour test period. Likewise,
Daenen et al. (2001) and Bolivar et al. (2000) also observed habituation in their cohort of male APPSWE/PSEN1ΔE9 mice.

Anxiety level in these rodents can also be recorded, by measuring the amount of time spent in the center of the open field activity chamber. Mice have a natural tendency to avoid open spaces by travelling along the walls of enclosures as a means of protection and easy escape (Lalonde et al., 2004). Therefore, the amount of time individuals spent in the center area of the open field test provides a measure of anxiety.

No difference between WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice was observed in terms of total distance travelled and total time spent in the center of the chamber at 3, 6, and 9 months of age. However, after 12 months of age, while no difference was seen between control mice (WT and TRPM-KO), APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice showed significantly decreased horizontal activity. These mice also spent less time in the center of the chamber than controls. A number of studies in the literature have investigated locomotor activity in APPSWE/PSEN1ΔE9 transgenic mice. Consistent with our results, no difference in locomotor activity was observed when APPSWE/PSEN1ΔE9 transgenic mice were compared to controls at different age points, such as at 4 months (Bonardi et al., 2011), 5-7 months (Lalonde et al., 2004), and 8 months (Park et al., 2010) of age.

As mentioned above, no difference was seen between WT and TRPM2-KO mice while hypoactivity was observed in APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. Because hypoactivity was only observed in Alzheimer’s mice and not in the control mice, regardless of the presence of TRPM2, we can conclude
that TRPM2 did not play a role in causing a decrease in horizontal activity in the Alzheimer’s mice. The behaviour of TRPM2-KO-APPSWE/PSEN1ΔE9 mice was not improved when compared to the behaviour of APPSWE/PSEN1ΔE9 mice, suggesting that knocking out TRPM2 did not interfere with locomotor activity in mice.

4.3 Elevated plus maze

The elevated plus maze is used to measure anxiety responses of rodents, where mice choose to either enter the open or closed arms. Mice were placed in the maze and their activities were measured for 5 minutes. This length of recording is based on an early study by Montgomery, (1955) who demonstrated that strongest avoidance behaviour for the open arms was seen in rats in the first 5 minutes after placement. If mice were left in the maze for a longer period of time, such as 10 or 15 minutes, these individuals would no longer experience fear and avoidance toward the open arms since they would habituate to the environment.

The anxiety levels of WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice did not differ at 3, 9, and 15 months of age. All mice performed equally well when placed on the elevated plus maze in terms of number of entries onto the open arm and the time spent on the open arms. To date, there have been controversial results regarding whether anxiety occurs or are absent in the APPSWE/PSEN1ΔE9 transgenic mice. Consistent with these results, Arendash et al. (2001) studied the Tg2576 transgenic mice and found no difference between transgenic and control mice at 5-7 and 15-17 months of age in terms of performance in closed and open arm entries as well as time spent in open arms. On the other hand, there have been reports on APPSWE/PSEN1ΔE9 mice which had results opposite of those found in this
current study. Lalonde et al. (2004) and Reiserer et al. (2007) found that 7 months old transgenic mice display reduced anxiety compared with wild type controls as measured by the elevated plus maze. Lalonde et al., (2004) also observed an increase in open arm entries as well as more time spent in the open arms in 7-month-old transgenic mice, suggesting that APPSWE/PSEN1ΔE9 mice were less anxious than the control mice. The reason why results from our study and previous literatures did not match could be due to many factors such as temperature, noise levels, social interaction, the presence of enrichment and diet, which can impact anxiety levels and influence outcomes of the study. Light/dark cycle was found to be a significant factor in anxiety-like behaviour measured with the elevated plus maze (Clénet et al., 2006; Gomes et al., 2011). These scientists demonstrated that mice in the dark cycle spent more time in the open arm of the elevated plus maze, thus less anxious than mice in the light cycles. Elevated plus maze in our study was conducted when mice were in their light cycles which could be the reason why we are not seeing results consistent with others. In addition, housing conditions of mice has also been found to influence anxiety levels, as demonstrated by da Silva et al. (1996) where mice were either housed individually or in groups. They observed that mice housed individually had a reduced number of entries and spent less time in the open arms compared to mice housed in groups, coming to the conclusion that mice housed individually were more anxious than grouped mice, and vice versa. Although our mice were housed in groups and therefore should be less anxious, we did not see this result in mice at 3, 9, and 15 months of age.

At 6 months however, TRPM2-KO-APP/SWE/PSEN1ΔE9 mice spent significantly longer time in the open arms than the control mice, suggesting that these
mice may be less anxious than WT, TRPM2-KO and APPSWE/PSEN1ΔE9 mice. In contrast, at 12 months of age, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice visited the open arms significantly fewer times than control mice, suggesting that these mice are more anxious than controls. Consistent with this, Filali, Lalonde, and Rivest (2011) found that APPSWE/PSEN1ΔE9 transgenic mice spent more time in the closed arms and less time in the open arms and therefore were more anxious than controls. Overall, our results suggest that APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice do not differ in anxiety levels, in terms of hyperactivity when compared to control mice. Because there were no consistent changes in anxiety in all mice, this suggests that elimination of TRPM2 does not affect anxiety levels in the elevated plus maze.

4.4 Object recognition

The object recognition test, which is based on the ability of mice to discriminate novel objects, was used to evaluate the performance of WT, TRPM2-KO, APPSWE/PSEN1ΔE9, and TRPM2-KO-APPSWE/PSEN1ΔE9 mice. Mice have a natural tendency to explore a novel object, which can reflect the individual’s use of discriminatory short- or long-term memory (Otalora et al., 2012; Prado et al., 2006). Short-term memory would involve a test being given to the mice shortly (a few hours) after training, whereas long-term memory involves a test being given 24 hours after training. Our study tested the long-term memory of WT, TRPM2-KO, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice.
Our results demonstrated no genotype difference in terms of the percentage of time spent exploring the familiar and novel objects. All mice preferred to explore the novel object on testing day at both 9 and 12 months of age. A number of researchers have studied the APPSWE/PSEN1ΔE9 transgenic mouse model using this test and controversial results have been found regarding the age at which discriminatory memory deficits occur. Consistent with our results, Bonardi et al. (2011) and Jardanhazi-Kurutz et al. (2010) found no memory deficit in the same APPSWE/PSEN1ΔE9 transgenic mice at earlier ages (4 and 6 months). However, findings from Donkin et al. (2010) and Yoshiike et al. (2008) on the APPSWE/PSEN1ΔE9 mice were contradictory to our results since both cohorts of mice demonstrated a severe object recognition memory deficit in APPSWE/PSEN1ΔE9 mice at 9-13 months of age. Overall, our results suggest that discriminatory memory of APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice do not differ from control mice. Because all mice learnt well in this test, this allowed me to demonstrate that knocking out TRPM2 does not affect mice’s discriminatory memory in object recognition.

4.5 Barnes maze

One of the methods to assess spatial learning and memory in mice is with the Barnes maze, consisting of an open, exposed platform elevated off the ground with an escape box located somewhere along the perimeter of the platform.

At 3, 6, and 9 months of age, no cognitive impairment was present in APPSWE/PSEN1ΔE9 mice since all mice remembered the location of the escape box with the guidance of visual cues surrounding the maze. At 12 months however, when
testing memory retention on day 5, APPSWE/PSEN1ΔE9 mice visited the target hole location significantly less than control indicating spatial memory deficit in these mice. Day 12 of the probe trial also indicated a spatial memory deficit in APPSWE/PSEN1ΔE9 mice. TRPM2-KO-APPSWE/PSEN1ΔE9 mice had improved performance in the BM on both test days 5 and 12. Therefore, elimination of TRPM2 alleviates spatial memory deficit seen in mice expressing the APPSWE/PSEN1ΔE9 transgene. In addition, APPSWE/PSEN1ΔE9 mice at 15 months of age also showed spatial memory deficit as measured on probe day 5 and 12, as well as an improvement in performance when the TRPM2 gene is eliminated. These results suggest that spatial memory deficits, as measured by BM, only occur in aged APPSWE/PSEN1ΔE9 mice, at 12 and 15 months of age. Our data also support the notion that elimination of TRPM2 can reverse or prevent these alterations in spatial memory.

APPSWE/PSEN1ΔE9 transgenic mice have been shown to exhibit age-dependent spatial learning and memory impairment in the Barnes maze. To date, there have been controversial results regarding whether spatial memory deficit occurs at an earlier age or a later age. For instance, Reiserer et al. (2007) has demonstrated that 6-7 months old APPSWE/PSEN1ΔE9 mice shows cognitive impairment in BM. However, O’Leary & Brown, (2009) have shown results consistent with our current results; spatial memory deficit only occurs in aged mice. O’Leary & Brown, (2009) assessed spatial memory of APPSWE/PSEN1ΔE9 transgenic mice at 16 months of age using the Barnes maze and observed that transgenic mice took a longer time to locate the target hole than WT mice, as well as made more errors during acquisition trials. The above observations therefore
demonstrate the presence of spatial learning and memory impairment in APPSWE/PSEN1ΔE9 mice as measured by Barnes maze.

4.6 Morris water maze hidden-platform test

Morris water maze (MWM) is a test used for spatial learning and memory (Vorhees & Williams, 2006). Since mice have a natural fear of water, this test uses this fear as a motivation for these individuals to find the hidden platform with the aid of visual cues. Similarly to the Barnes maze, WT mice are expected to utilize spatial learning and memory in order to locate the hidden platform. Because of the potential spatial memory deficits we observed in the BM, we decided to determine if a second task would allow us to confirm these results.

At 12 months of age, all mice learned and retained the memory of the location of the target platform, as seen by the large amount of time spent in the target quadrant. Opposite from our findings, Zhang et al. (2011) demonstrated the presence of spatial memory deficit in the APPSWE/PSEN1ΔE9 mice at 12 months of age.

At 15 months of age, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice took a longer period of time to find the target location (Fig.3.14A) as well as travelled a longer distance (Fig.3.14B) than control mice but they learnt the test. It is interesting to note that, likely, WT and TRPM-KO mice remember that they have done this experiment multiple times and already in the first day of training they promptly find the platform, while APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice behave as if they were doing the test for the first time. However, we observe a spatial memory deficit in APPSWE/PSEN1ΔE9 mice, since these
mice do not remember the location of the target platform on the probe trial. TRPM2-KO-APPSWE/PSEN1ΔE9 mice were found to have improved performance in this test since they remember the location of the target platform and therefore spent more time in that quadrant. This demonstrates that knocking out the expression of TRPM2 led to an improved cognitive function in APPSWE/PSEN1ΔE9 mice.

APPSWE/PSEN1ΔE9 transgenic mice have been shown to exhibit age-dependent spatial learning and memory impairment in the MWM. To date, just like BM, there have been controversial results regarding whether spatial memory deficit occurs at an earlier age or a later age in the MWM. Some studies have observed a lack of spatial memory impairment in APPSWE/PSEN1ΔE9 mice at 6 months (Holcomb et al., 1999; Minkeviciene et al., 2008; Savonenko et al., 2005) and 9 months (Holcomb et al., 1999) of age, while memory deficit have been found to present itself at 8-12 (Butovsky et al., 2006; Cao et al., 2007; Lalonde et al., 2004; Minkeviciene et al., 2008; Oksman et al., 2006), 12-13 months of age (Malm et al., 2011; Zhang et al., 2011), 14 months of age (Liu et al., 2003), and 16-18 months of age (Malm et al., 2007; Minkeviciene et al., 2008; Savonenko et al., 2005). The results from our study are consistent with numerous reports that indicate APPSWE/PSEN1ΔE9 mice show spatial learning and memory deficits at 12 and 15 months of age. Overall, our results demonstrated that elimination of TRPM2 allows for improved performance compared to APPSWE/PSEN1ΔE9 mice in MWM.

4.7 Morris water maze reversal learning

MWM reversal, or reversal learning, tests an animal’s ability to extinguish their memory of the previously learnt platform position and essentially to “re-learn” the new
platform position (Vorhees & Williams, 2010). This test was conducted a week after the original MWM, using the same materials but a slight modification to the methodology, with the location of the hidden platform now being placed in the opposite quadrant of where it used to be. In order to re-learn the location of this newly placed platform, cognitive flexibility needs to be present in these animals.

An unexpected result arose when observing MWM reversal learning. At 12 months of age, cognitive flexibility was present in WT, APPSWE/PSEN1ΔE9 and TRPM2-KO-APPSWE/PSEN1ΔE9 mice since they found the new location of the hidden platform quickly and accurately. However, TRPM2-KO mice did not find the new hidden platform location and therefore presented a deficit in cognitive flexibility. At 15 months of age, WT and TRPM2-KO-APPSWE/PSEN1ΔE9 mice were observed to remember the new location of the platform while TRPM2-KO and APPSWE/PSEN1ΔE9 mice did not; indicating a deficit in cognitive flexibility in these mice as well as a possible impairment in LTP in the APPSWE/PSEN1ΔE9 mice since these mice had trouble remembering the new location of the platform. The fact that we observe cognitive flexibility deficits in mice with elimination of TRPM2 (TRPM2-KO) at 12 months of age but no deficits in Alzheimer’s mice, were interesting yet puzzling. Moreover, 15 months results showed that mice with elimination of TRPM2 (TRPM-KO) as well as APPSWE/PSEN1ΔE9 mice had a deficit in cognitive flexibility, again suggesting a possible LTP impairment in these mice. However, TRPM2-KO-APPSWE/PSEN1ΔE9 mice had no deficit in cognitive flexibility at 12 and 15 months of age, instead they had normal performance at both age points, suggesting that at both time points, the elimination of TRPM2 channels may be beneficial and may rescue the LTP impairment.
There is no explanation for our results at this time; future *in vivo* research should further investigate TRPM2-KO mice and reversal learning as well as the role that TRPM2 has in these behaviour tests. For example, because there are numerous mouse models of AD as noted in the introduction, longitudinal research could be conducted in those mouse models to investigate whether or not knocking out TRPM2 played a role in performance. TRPM2-KO mice and reversal learning can also be further investigated using other mouse models of AD to see if the same results are obtained compared to the unexpected results we saw in reversal learning. These results observed in the MWM reversal tests in our study could be correlated to an *in vitro* experiment conducted by Xie et al., (2011), described below.

TRPM2 channels are highly expressed in the CNS and have previously been found to be expressed in the CA1 pyramidal neurons in the hippocampus (Olah et al., 2009). Based on this finding, an *in vitro* study by Xie et al., (2011) used hippocampal cultures and slices from TRPM2 knockout mice and demonstrated that a loss of TRPM2 channels selectively impairs NMDAR-dependent LTD while sparing LTP. LTD is an activity-dependent reduction in the efficacy of excitatory synaptic transmission while LTP is the opposite; an enhancement in the efficacy of transmission. In order to further explore the loss of NMDAR-dependent LTD in TRPM2 knockout mice, these scientists examined the activity of GSK-3β (glycogen synthase kinase-3β), which has been widely implicated in AD and is required in the formation of this LTD (Jo et al., 2011). Genetic deletion of TRPM2 was to be associated with decreased GSK-3β activity. However, it was noted that a rescue of LTD was observed in TRPM2 knockout mice when GSK-3β
activity was restored. Therefore, TRPM2 channels have been demonstrated to play a key role in hippocampal synaptic plasticity.

Recall that Aβ plaques are the main pathological hallmark of AD (Cvetkovic-dozić et al., 2001; Yankner, Lu, & Loerch, 2008) and that Aβ40 and Aβ42 are the two most common species of Aβ (Oddo et al., 2003; Selkoe, 2008). An increased production of oligomeric Aβ42 is one of the triggers of FAD (Savonenko et al., 2005), resulting in the accumulation of large senile plaque in the hippocampus, cortex and subcortical nuclei (Duyckaerts et al., 2008). Oligomeric Aβ42 has been shown by numerous studies to inhibit LTP, important in memory formation (Shankar et al., 2008; Walsh et al., 2002) as well as increase LTD, contributing in memory loss (Hsieh et al., 2006; Li et al., 2009; Shankar et al., 2008).

Given that the loss of TRPM2 expression leads to deficits in LTD induction, an increase in TRPM2 channel function, for example as a result of oxidative stress, may favor LTD. Accordingly, we propose that the increased LTD associated with AD is partially contributed by augmented TRPM2 function, via Aβ42 initiated oxidative stress. And therefore, it can be concluded that a loss of TRPM2 channel activity may play a beneficial role in learning and memory in AD.

4.8 Future directions

As noted in the introduction, soluble oligomers have been found by numerous studies to mediate neurotoxicity by inducing oxidative stress in the brain which promote TRPM2 activation, leading to a disruption in Ca²⁺ homeostasis and a toxic influx of Ca²⁺, ultimately inducing cell death (Goedert & Spillantini, 2006; Mattson, 2007;
Takahashi et al., 2011). An improvement in memory in the TRPM2-KO-APPSWE/PSEN1ΔE9 mice has been observed in our study and therefore knocking out TRPM2 has been found to be protective. Cognition was found to be mildly impaired in TRPM2-KO mice, as shown by impaired reversal learning. Future in vitro research could explore Aβ load in these TRPM2-KO individuals and see whether or not the load has decreased in these individuals since there was an improvement in behaviour. Presently, there are no specific antagonists of TRPM2. However, a number of pharmacological agents are able to inhibit the channel, although only non-selectively. These agents include flufenamic acid, econazole, clotrimazole, and N-(p-amycinnamoyl) (Eisfeld & Luckhoff, 2007; Olah et al., 2009). Thus, these potential TRPM2 antagonists should also be investigated in order to examine whether TRPM2 represents a target for the treatment of AD. Given our findings, we predict that a TRPM2 antagonist could potentially be effective in treating AD.

My study focuses on mice with TRPM2 absent from birth, which is different than humans who develop AD with age. To test the effectiveness of a TRPM2 blocker in patients already suffering the symptoms, longitudinal studies in which treatment is initiated at various time points (e.g. 3, 6, 9 months etc.) should be performed on mouse models of AD. In addition, biomarkers of AD may soon allow us to identify humans at risk of developing AD; treatment in these patients with a TRPM2 blocker could be considered.
4.9 Overall conclusions

Although the precise mechanism through which Aβ causes AD pathogenesis is not known at present, this mechanism can be speculated taking into consideration the large amount of research on this topic. To date, it is known that the accumulation of Aβ is the main pathological hallmarks of AD, and that Aβ induces oxidative stress and a loss of Ca²⁺ homeostasis in the brain. According to the amyloid cascade hypothesis, the accumulation of soluble Aβ peptides causes progressive synaptic and neuritic injury by reducing glutamatergic synaptic transmission strength and plasticity (Hardy, & Selkoe, 2002; Palop, & Mucke, 2010). Similarly, a loss of Ca²⁺ homeostasis is known to disrupt the structure and function of synapses, impair synaptic plasticity, and ultimately cause cell death. Although the precise mechanisms through which oxidative stress causes a loss of Ca²⁺ homeostasis is not known, we do know that oxidative stress promotes the activation of TRPM2 channels and that this activation causes a toxic influx of Ca²⁺ and ultimately induce cell death (Takahashi et al., 2011). Collectively, these findings led us to consider TRPM2 channels as a likely contributor to the neurotoxic cascades initiated by elevated Aβ levels.

This thesis was the first to explore the relationship between TRPM2 and cognitive deficits associated with a well characterized mouse model of Alzheimer’s disease. Our results suggest that knocking out the TRPM2 transgene improves the spatial learning and memory abilities of the APPSWE/PSEN1ΔE9 double transgenic mice, suggesting that TRPM2 might represent a potential target for the treatment of AD.
Section 5

REFERENCES


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Curriculum Vitae

EDUCATION

Master of Science (Physiology) 2011-2013
Western University, London, Canada

Bachelor of Science (Honours Specialization Biology) 2006-2011
Western University, London, Canada

LABORATORY AND RESEARCH EXPERIENCE

Master's Thesis Research – Dr. John MacDonald’s Laboratory 2011-2013
Department of Physiology, Robarts Research Institute, Western University
Thesis: Contributions of TRPM2 to memory deficiency in an Alzheimer’s mouse model

Summer Student Research Assistant – Dr. John MacDonald’s Laboratory 2011
Department of Physiology, Robarts Research Institute, Western University
Investigated the role of TRPM2 on the life span and memory deficit of Alzheimer’s mice by conducting behaviour experiments using elevated plus maze, object recognition, locomotor, Barnes maze and Morris water maze.

Summer Student Research Assistant – Dr. Subrata Chakrabarti’s Laboratory 2004, 2007-2009
Department of Pathology, Western University
Investigated the pathogenic mechanisms and treatment of heart failure in diabetes patients using RT-PCR, Western blot and ELISA techniques; assisted other research team members with their experiments

TEACHING EXPERIENCE

Western University Graduate Teaching Assistantship, London, Canada 2011-2013
Physiology 3130Y – 3rd year undergraduate lab course
Assisting professors with the teaching of course materials; assisting students with various experiments; evaluating lab reports and presentations; proctoring exams

PUBLICATIONS AND PRESENTATIONS

Journal articles:
Coauthored three articles in the following journals:

Presentations (with Abstracts):
Participated in group presentations at the following events and venues:
- Canadian Association for Neuroscience, Vancouver, Canada, May 20-23, 2012 2012
- Annual Meeting of the Southern Ontario Neuroscience Association, Toronto, Canada, April 30, 2012 2012
- Canadian Diabetes Association, Toronto, Canada, October 26-29, 2011 2011
**VOLUNTEER AND EXTRACURRICULAR ACTIVITIES**

**Alzheimer’s Society, London, Canada**

**Volunteer Companion**  
2012-2013  
Providing relief for caregivers by offering companionship and social stimulation for persons in the early stages of Alzheimer’s disease and related dementias

**Participation House, London, Canada**

**Friendship/Buddy Volunteer**  
2012-2013  
Assisting physically and mentally disabled individuals with handcrafts, gardening, walks, swimming, yoga, manicures, music, and sensory room activities

**Western Serves, Western University, London, Canada**

**Participation House Volunteer**  
2012  
Assisted at the annual cookout, bonfire, and games; provided support in preparing for and running games; assisted individuals with developmental disabilities and complex physical needs in engaging in conversation and participating in games

**Indian Cultural Connection Camp, London, Canada**

**Camp Counselor**  
2012  
Assisted in organizing a number of activities during camp week; supervised and assisted children in doing various activities such as sports and crafts

**Chinese Students Association, Western University, London, Canada**

**Executive and Director in the Cultural and Academics Department**  
2007-2009  
Provided information about Asian culture to the general public; organized review sessions to help students with exam preparation; organized various club activities

**University Hospital, London Health Sciences Center, London, Canada**

**Outpatient Clinic and Dietary Department**  
2007-2009  
Recorded patient data, organized charts, and kept track of patients for the Blood Taking Clinic; assisted patients in making meal choices, retrieved menus from patients, and delivered cards and flowers to patients while working in the Dietary Department

**AWARDS AND ACHIEVEMENTS**

- Western University Graduate Student Teaching Award Nominee  
  2012
- Western Graduate Research Scholarships, Western University, London, Canada  
  2011-2013
- Dean’s Honour List, Western University, London, Canada  
  2010
- Orchard Park Public School ESL Award, London, Canada  
  2002

**SKILLS**

- **Language**: Fluent in English and Mandarin (Chinese)
- **Computer**: Proficient in word processing and the use of quantitative data analysis software; conversant with the entire range of Internet functions and applications
- **Interpersonal**: excellent verbal and written communication skills; social skills; leadership skills; problem-solving and conflict resolution