6-16-2017 12:00 AM

Cognitive and Non-Cognitive Dysfunction in a Mouse Model of Alzheimer's Disease

Wai-Jane V. Lee, *The University of Western Ontario*

Supervisor: Dr. Marco Prado, *The University of Western Ontario*
Joint Supervisor: Dr. Vania Prado, *The University of Western Ontario*
A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

Abstract

Sensitive and translational tasks that efficiently and accurately assess cognitive function during pre-clinical trials would be useful in developing novel treatments for Alzheimer's disease (AD) patients. The Bussey-Saksida touchscreens employ various tasks similar to those used in humans to effectively evaluate high-level cognitive and executive functions in mice. This face validity provides the best chance of successful cognitive translation across species.

In our study, donepezil had minor effects on the performance of 5xFAD mice in the 5-CSRTT, a touchscreen task evaluating attention. Additionally, 5xFAD mice do not demonstrate impairments in the PVD task, which assesses visual discrimination/ cognitive flexibility. However, the parameters recorded by the touchscreen apparatus found latency differences in task response and reward collection – which led us to uncover severe gait impairments in old 5xFAD mice. In summary, our findings suggest that the 5xFAD mouse model can be used as an animal model of non-cognitive function in AD.

Keywords

Alzheimer's Disease

Amyloid-Beta (Aβ) Plaques

Acetylcholine

Acetylcholinesterase Inhibitors

Attention

Cognitive Flexibility

Donepezil

Gait

Locomotion

Pairwise Visual Discrimination (PVD)

Pathology

Reversal Learning

Touchscreens

Visual Discrimination

Vigilance

5x Familial Alzheimer's Disease (5xFAD) Mouse Model

5-Choice Serial Reaction Time Task (5-CSRTT)

Co-Authorship Statement

Wai-Jane Virginia Lee performed all the experiments and analyses in this thesis except: Talal Massod collected data for the pairwise visual discrimination touchscreen task in male 5xFAD mice at 4 months (5xFAD: n=7, WT: n=7) and 7 months (5xFAD: n=8, WT: n=7) in Figures 4-7 and 9 under the supervision of Dr. Marco A.M. Prado and Dr. Vania F. Prado. Pump implants, some locomotion and gait experiments for male 5xFAD mice (n=15) and their WT controls (n=11) were also performed by Matthew Cowan.

Acknowledgements

First and foremost, I would like to thank my supervisors, Dr. Marco Prado and Dr. Vania Prado. I am grateful for your endless support, encouragement and the time you invested in me the last two years. Thank you both for supporting my ideas while pushing me to think and evaluate all my data critically. Words cannot describe how blessed I feel to have had you both as my supervisors. Your passion for research has truly been an inspiration.

I would like to thank my advisory committee: Dr. Brian Allman (Neuroscience Program committee representative), Dr. Julio Martinez-Trujillo, and Dr. Stan Leung for their guidance and support throughout my masters.

I would also like to acknowledge and Dr. Arthur Brown for helping me to refine my scientific writing, presentation and communication skills, while providing support and advice along the way. I admire the genuine concern you have for all your students. I would also like to thank Dr. Robert Gros for his expertise and for teaching me how to perform osmotic pump implant surgeries on mice.

I would like to extend my gratitude all the members of the Prado Lab. Thank you all for your patience in answering my questions and helping me to troubleshoot my protocols. You all played an instrumental role in what I learned here. In particular, I would like to thank Flavio Beraldo. Thank you for taking me in as a summer student when I was in the second year of my undergrad – and for giving me the opportunity to follow your every step. That was the first time I was exposed to research. I am also grateful that you were so patient and willing to answer my questions – both then and now. I may not have

decided to pursue research if it weren't for you. I would also like to thank Ornela Kljakic and Rachel Lackie. You have both made the last two years (both in and outside the lab) that much more enjoyable. Also, many thanks to Jacky Lou and Hanein Madlol, my undergraduate volunteers for their assistance. Lastly, special thanks to Chris Fodor, Jue Fan for sharing her genotyping expertise and Matthew Cowan for showing me how to perform all the behavioural tasks, helping me with all the mice, the surgeries and, of course, for the multitude of insightful conversations we had.

Many thanks to my brother Vincent, my mother and my father for their unconditional love and support. I couldn't have done this without you by my side. Last but not least, I would like to thank Joshua Lee for taking care of me and providing the moral support I needed during this time. Thank you for being so understanding.

Table of Contents

Abstract……..i

Keywords……..ii

Co-authorship Statement……iii

Acknowledgements……..……... iv

Table of Contents.....…… vi

List of Tables.....…….....x

List of Figures.…… xi

List of Appendices.…… xiv

List of Abbreviations…….. xv

1 Introduction

1.1 Alzheimer's Disease.....1
- 1.1.1 *The neuropathology of AD*.....2
- 1.1.2 *Genetic risk factors and Familial Alzheimer's Disease*.....4
- 1.1.3 *The amyloid cascade hypothesis of AD*..... 5
- 1.1.4 *The cholinergic hypothesis of AD*..... 6
- 1.1.5 *Treating AD*..... 7
  - *1.1.5.1 Donepezil* 8
- 1.1.6 *Visuospatial function and attention in AD*..... 9
- 1.1.7 *Gait impairments in patients with AD*.....10
  - *1.1.7.1 The association between gait and attention.*.....11
- 1.1.8 *Mouse models of AD*..... 11
  - *1.1.8.1 The 5xFAD mouse model of AD*..... 12
    - *1.1.8.1.1 Retinal degeneration in the 5xFAD mouse model*..... 13

1.2 The Bussey-Saksida Touchscreen System..... 15
- 1.2.1 *The Pairwise Visual Discrimination Task*.....16
- 1.2.2 *The 5-Choice Serial-Reaction Time Task*.....18
- 1.2.3 *Using touchscreen tasks to identify cognitive deficits in mouse models of AD*.....19

1.3 Rationale and Hypothesis..... 21

2 Materials and Methods

2.1 5x Familial Alzheimer's Disease Mouse Model.....22

2.2 Housing and Diet..... 22

2.3 Genotyping *Pdeb*^{rd1}..... 23

2.4 Administration of Donepezil..... 24

2.4.1 *Subcutaneous Osmotic Pumps*.....24

2.4.2 *Intraperitoneal Injection* 26

2.5 Touchscreens.....27

2.5.1 *Touchscreen Pre-training* 27

2.5.2 *Pairwise Visual Discrimination*..... 31

2.5.3 *5-Choice Serial Reaction Time Task*.....34

2.6 Open-Field Locomotion.....37

2.7 Gait Analysis..... 38

2.8 Forelimb Grip Strength.....39

2.9 Transcardial Mouse Perfusion, Tissue Preservation and Slicing.....39

2.10 Thioflavin-S Stain.....40

2.11 Amyloid-Beta Immunohistochemistry.....40

2.12 To-Pro-3 Iodide Nuclear Stain.....41

2.13 Microscopy and Quantification.....41

2.14 Statistical Analysis..... 41

3 Results

3.1 Visual Discrimination and Reversal Learning in 5xFAD Mice..... 42

3.2 Genotyping *Pdeb*^{rd1}..... 50

3.2.1 *The effect of carrying the Pdeb*^{rd1} *allele on performance in PVD at the 10-month time-point*.....51

3.3 The Effects of Donepezil on Attention in 5xFAD mice..... 53

3.3.1 *Infusion of donepezil affects vigilance in the 5-CSRTT*..... 53

3.3.2 *Performance of 5xFAD mice at the 0.6 stimulus length in the 5-CSRTT following donepezil injection*..... 59

3.4 Spontaneous Locomotor Activity in Male 5xFAD Mice..... 62

3.4.1 *Effect of donepezil on spontaneous locomotor activity in 5xFAD mice*..... 64

3.4.2 *Locomotor activity in old 5xFAD mice*..... 66

3.4.3 *Locomotor activity in 15-month old 5xFAD mice*..... 68

3.5 Gait Analyses in 5xFAD Mice.....69

3.5.1 *Gait is not impaired in 10-month old 5xFAD mice*.....70

3.5.2 *Gait is impaired in 14-month 5xFAD mice*.....74

3.5.3 *The gait of 5xFAD mice at 14-months of age is mildly improved by intraperitoneal injection of donepezil*..... 79

3.5.4 *Gait remains altered in 15-month 5xFAD mice*..... 83

3.6 Neuromuscular Strength is not Affected in 5xFAD Mice at 14-months of Age 88

3.7 Mild Food-Restriction Does Not Affect Amyloid Pathology in Male and Female 5xFAD Mice..... 89

4 Discussion

4.1 5xFAD Mice Do Not Show Retinal Degeneration Due to the *Pdeb*^{rd1} Allele 93

4.2 Visual Discrimination and Cognitive Flexibility is not Impaired in 5xFAD mice..... 93

4.3 The Sustained Attention of 5xFAD Mice is Affected by the Injection of Donepezil..... 95

4.4 Open-Field Locomotion in 5xFAD Mice..... 98

4.4.1 *Donepezil reduces anxiety in 5xFAD mice*.....99

4.4.2 *The effect of food-restriction on locomotion and gait*..... 100

4.5 Gait Impairments in 5xFAD Mice..... 101

4.6 Food-restriction Had No Effect On Amyloid Pathology in 5xFAD Mice.....105

4.7 Conclusions, Significance and Future Directions.....106

5 References.....109

6 Appendices.....122

7 Curriculum Vitae..... 143

List of Tables

Table 1. Order of probe trial sessions at 0.6s, 0.8s, 1.0s and 1.5s for individual subgroups in the 5-CSRTT..... 37

Table 2. Static gait parameters in 10-month-old 5xFAD mice..... 72

Table 3. Dynamic gait parameters in 10-month-old 5xFAD mice..... 72

Table 4. Static gait parameters in 14-month-old 5xFAD mice..... 76

Table 5. Dynamic gait parameters in 14-month-old 5xFAD mice..... 77

Table 6. Static gait parameters in 14-month 5xFAD mice following donepezil treatment..... 81

Table 7. Dynamic gait parameters in 14-month 5xFAD mice following donepezil treatment..... 81

Table 8. Static gait parameters in 15-month 5xFAD mice..... 86

Table 9. Dynamic gait parameters in 15-month 5xFAD mice..... 86

List of Figures

Figure 1. Summary of touchscreen pre-training, PVD and 5-CSRTT schedules..... 30

Figure 2. S+ and S- stimulus presentation and stimuli used in pairwise visual discrimination..... 33

Figure 3. Illustration of the possible responses to the stimulus in 5-CSRTT..... 36

Figure 4. 5-CSRTT experiment timeline..... 36

Figure 5. Learning phase of the PVD Task..... 46

Figure 6. Reversal learning phase of the PVD task at the 4-month time-point..... 47

Figure 7. Reversal learning phase of the PVD task at the 7-month time-point..... 48

Figure 8. Reversal learning phase of the PVD task at the 10-month time-point..... 49

Figure 9. Representative *Pdeb*^{rd1} genotyping results..... 50

Figure 10. Reversal learning phase of the PVD task at the 10-month time-point separated by Pdeb^{rd1} genotype..... 52

Figure 11. Number of trials completed by 5xFAD mice in the 5-CSRTT..... 56

Figure 12. Performance and response measures of 5xFAD mice during the 5-CSRTT probe trial..... 58

Figure 13. The vigilance of 5xFAD mice during the 5-CSRTT probe trial..... 59

Figure 14. The vigilance of 5xFAD mice during the 0.6s stimulus length of the 5-CSRTT..... 60

Figure 15. Performance and response measures of 5xFAD mice in the 0.6s stimulus length of the 5-CSRTT..... 61

Figure 16. Spontaneous locomotor activity of 10-month old 5xFAD mice..... 63

Figure 17. Spontaneous locomotor activity of 5xFAD mice treated with donepezil..... 65

Figure 18. Spontaneous locomotor activity of 14-month-old 5xFAD mice..... 67

Figure 19. Spontaneous locomotor activity of 15-month-old 5xFAD mice..... 69

Figure 20. Step pattern and limb support measures in 10-month old 5xFAD mice..... 73

Figure 21. Base of support and weights in 10-month-old 5xFAD mice..... 74

Figure 22. Step pattern and limb support measures in 14-month-old 5xFAD mice..... 78

Figure 23. Base of support and weights in 14-month-old 5xFAD mice..... 75

Figure 24. Step pattern and limb support measures in 14-month 5xFAD mice following donepezil treatment..... 82

Figure 25. Base of support and weights in 14-month 5xFAD mice following donepezil treatment..... 83

Figure 26. Step pattern and limb support measures in 15-month old male 5xFAD mice..... 87

Figure 27. Base of support and weights in 15-month 5xFAD mice..... 88

Figure 28. Forelimb grip force in 14-month-old 5xFAD mice..... 88

Figure 29. The effect of mild caloric restriction on 6E10 amyloid pathology in male 5xFAD mice at 6 months of age..... 90

Figure 27. The effect of mild caloric restriction on 6E10 amyloid pathology in male 5xFAD mice at 6 months of age..... 91

Figure 28. The effect of mild caloric restriction on Thioflavin-S amyloid pathology in male 5xFAD mice at 6 months of age.....92

List of Appendices

Appendix 1. SOP Food restriction for adult mice (12 weeks or older)..... 122

Appendix 2. SOP 2-Choice Pairwise Visual Discrimination Task Standard Operating Procedure..... 126

Appendix 3. SOP 5-Choice Serial Reaction Time Task Standard Operating Procedure..... 133

List of Abbreviations

Abbreviation	Full Name
Aβ	Amyloid Beta
AD	Alzheimer's Disease
ANOVA	Analysis of Variance
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
BACE	β-APP cleaving enzyme
CANTAB	Cambridge Neurospsychological Test Automated Battery
ChAT	Choline Acetyltransferase transporter
CTF	C-Terminal Fragment
DMSO	Dimethyl Sulfoxide
NMDA	N-methyl-D-Aspartate
$PDEB^{rd1}$	Phosphodiesterase Subunit Beta - Retinal Degeneration 1
PVD	Pairwise Visual Discrimination
ROUT	Regression Outlier Removal
VAChT	Vesicular Acetylcholine Transporter
WT	Wild-Type
5xFAD	5x Familial Alzheimer’s Disease
5-CSRTT	5-Choice Serial Reaction Time Task

1 Introduction

1.1 Alzheimer's Disease

Alzheimer's disease (AD) is a disabling and fatal chronic disorder characterised by progressive cognitive impairment – often beginning with memory loss and neuropsychiatric symptoms such as depression, apathy and aggression (Li et al., 2014b). The disease may progress gradually for several years before other cognitive domains, such as language, executive function, visuospatial function, and attention are affected (Perry and Hodges, 1999). Although the rate of decline can be extremely variable, AD is usually fatal within 7-10 years of diagnosis (Dudgeon, 2010). The most common cause of death among AD patients is pneumonia, which is hastened by the marked inability of the patient to cough and move about normally (Dudgeon, 2010).

AD currently affects over 35.6 million individuals worldwide, accounting for approximately half of the new cases of dementia diagnosed annually (Brookmeyer et al., 2007; Prince et al., 2013). This number is predicted to double within the next 20 years (Prince et al., 2013). The majority of new cases of AD are sporadic, and tend to occur in older age groups (Dudgeon, 2010). However, 5-7% of cases are early onset, and can result from mutations in one of the many genes involved in amyloid processing (Dudgeon, 2010). These cases of AD are referred to as familial Alzheimer's disease.

The clinical diagnosis of AD is currently based on clinical history, neurological examination and neurological tests. A criterion often used to diagnose AD (as stated by the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition [DSM-IV]))

requires these patients to demonstrate a loss of two or more of the following: memory, language, calculation, orientation or judgment in the absence of other probable diseases (Kawas, 2003). A more recent criterion suggested by McKhann et al. requires patients that meet the criteria for dementia to also demonstrate a deterioration in cognition along with one of the following: amnesia, impairments in language, visuospatial function or executive function (McKhann et al., 2011). Again, this probable diagnosis must be made in the absence of evidence pointing to other disease (McKhann et al., 2011). These cognitive domains can be tested using the Mini-Mental State Examination (MMSE), the Abbreviated Mental Test Score (AMTS) and objective computerized Cambridge Neuropsychological Test Automated Battery (CANTAB). Although expert clinicians correctly diagnose AD 70-90% of the time (Beach et al., 2012; Kaye, 1998), a definitive diagnosis of AD requires a post-mortem confirmation, with the presence of two histopathological features: amyloid plaques and neurofibrillary tangles (Braak and Braak, 1991).

1.1.1 *The neuropathology of AD*

Alzheimer's disease is characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain (Braak and Braak, 1991). Although these pathological features are often seen in cognitively normal age-matched controls, the density and distribution of these features differ (Bekris et al., 2010).

Amyloid plaques are composed of small Aβ peptides that are processed from the amyloid precursor protein (APP). APP is a type-I transmembrane protein that is expressed at high levels in the brain and has been suggested to be involved in a variety

of physiological functions, including neurite outgrowth, synaptogenesis, protein trafficking along the axon, transmembrane signal transduction, cell adhesion and calcium metabolism (Zhang et al., 2011; Zheng and Koo, 2006). However, APP can also have neurotoxic properties and other aversive effects (Zhang et al., 2011).

APP is processed by a series of sequential proteases into various fragments. Aβ is produced following the cleavage of APP by β-secretase (also known as β-APP cleaving enzyme or BACE), forming soluble APPβ and a β C-terminal fragment (βCTF). Soluble APPβ is thought to be involved in neuronal pruning and axonal cell death (Nikolaev et al., 2009). Cleavage of APP by α-secretase prevents Aβ generation, as its cleavage site is within the Aβ domain – between lys16 and leu17 (Zhang et al., 2011). This forms soluble APPα and an α C-terminal fragment (αCTF). Soluble APPα plays an important role in neuronal plasticity/survival and in central nervous system development, where it regulates neural stem cell proliferation (Zhang et al., 2011). The αCTF and βCTF are further processed by γ-secretase to p83 and Aβ40/42, respectively. P83 has no known function and is rapidly degraded (Zhang et al., 2011). The βCTF can be cleaved into Aβ40 or Aβ42, where the number indicates the number of amino acids in the fragment. At low levels, Aβ has positive, modulatory roles on neurotransmission and memory, while excessive levels of Aβ leads to synaptic dysfunction and synapse loss (Zhang et al., 2011). Aβ42 is more hydrophobic and thus, more prone to fibril formation and aggregation than Aβ40 (Zhang et al., 2011). Under normal conditions, only 10% of the Aβ produced is Aβ42 (Burdick et al., 1992). Alterations in this ratio has been suggested to be critical for AD pathogenesis (Burdick et al., 1992).

Neurofibrillary tangles are composed of hyperphosphorylated filaments of the microtubule-associated phosphoprotein tau. Tau projects from the surface of microtubules, allowing them to interact with other cytoskeletal elements, cytoplasmic organelles and proteins (Buée et al., 2000). Tau is regulated via phosphorylation, which modulates the affinity between tau and the microtubules to allow for microtubule assembly, affecting axonal morphology, growth and polarity (Buée et al., 2000). In AD, the hyperphosphorylation of tau leads to the formation of fibrils which can then aggregate within cells to form insoluble paired helical filaments, leading to neurodegeneration (Buée et al., 2000).

1.1.2 *Genetic risk factors and Familial Alzheimer's Disease*

A fraction of familial AD cases can be traced to mutations in proteins that affect amyloid processing. Mutations in the gene encoding APP on chromosome 21 leads to the abnormal formation of APP, affecting how it is processed (Goate et al., 1991; Mullan, 1992). To date, over 32 APP missense mutations have been identified, and the majority of these mutations are located at the secretase cleavage sites (Goate et al., 1991; Mullan, 1992). Examples include the Swedish (K670N, and M671L) and London (V717I) mutations, which lead to an increase in the production of Aβ and the development of AD (Goate et al., 1991; Mullan, 1992). These mutations have been introduced in mice and used to generate animal models of AD.

Mutations on chromosome 14 and chromosome 1 result in the partial loss of function of presenilin 1 and 2, respectively (Bekris et al., 2010). Presenilins are major components of the γ-secretase complex, and are responsible for cleaving the C-terminal fragments of

APP (De Strooper et al., 1998). Thus, mutations in these proteins affect APP processing. Defects in presenilin 1 lead to the most severe forms of AD – with complete penetrance, leading to early onset AD (Bekris et al., 2010). Meanwhile, mutations in presenilin 2 are rarer, and are of lower penetrance than mutations in presenilin 1 (Bekris et al., 2010).

The human apolipoprotein E (ApoE) gene on chromosome 19 exists as one of three isoforms: ε2, ε3 and ε4 (Wu and Zhao, 2016). In the brain, lipidated ApoE is responsible for binding and removing aggregated Aβ in an isoform dependent manner (Wu and Zhao, 2016). The ApoE ε2 polymorphism is very rare, and is considered to protect against AD, as it enhances the ability of ApoE to clear Aβ through a variety of mechanisms (Conejero-Goldberg et al., 2014; Wu and Zhao, 2016). ApoE ε3 has recently been found to have neuroprotective effects as well (de-Almada et al., 2011). However, the ApoE ε4 polymorphism has been associated with both familial and sporadic cases of late-onset AD (Bekris et al., 2010). ApoE ε4 lipoproteins bind Aβ with a lower affinity than the other polymorphisms, thus possibly impairing Aβ clearance (Liu et al., 2013a).

1.1.3 *The amyloid cascade hypothesis of AD*

The amyloid cascade hypothesis of AD suggests that the accumulation and deposition of Aβ in the brain is a crucial step that ultimately leads to the development of AD (Karran et al., 2011). This had been observed many years prior to other AD-related manifestations (Selkoe and Hardy, 2016) and is likely a result of an unbalance between

the production and clearance of Aβ peptides. This hypothesis is supported by genetic mutations in the presenilins and APP leading to early-onset or AD in humans (Bekris et al., 2010). Meanwhile, the ApoE ε4 polymorphism, which affects the clearance of Aβ, can predispose an individual to AD (Liu et al., 2013a). In addition, soluble oligomers of Aβ42 taken from AD patients can cause neurodegeneration, inhibit long-term potentiation and enhance long-term synaptic depression in the hippocampus of healthy rats (Selkoe and Hardy, 2016). These oligomers are also capable of inducing tau hyperphosphorylation and enhancing the toxicity of tau (Selkoe and Hardy, 2016). However, treatments aimed at Aβ in humans have failed – and in some cases, treatment accelerated deterioration in cognition and reduced quality of life compared to placebo controls (Karran et al., 2011). Furthermore, the amyloid cascade hypothesis fails to consider the effect of tau on the development of the disease. Tau pathology itself can cause neuronal loss as well (Karran et al., 2011). The temporal and mechanistic relationships between Aβ and tau pathology remain to be resolved (Karran et al., 2011).

1.1.4 *The cholinergic hypothesis of AD*

The cholinergic hypothesis of AD suggests that the disease is a result of the degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic transmission (Bartus et al., 1982). This degeneration is accompanied by a decrease in choline acetyltransferase (ChAT; responsible for synthesizing acetylcholine) and a reduction of acetylcholine release and reuptake in cortex and hippocampus (Francis et al., 1999). Similar observations have also been made in mouse models of AD (Boncristiano et al., 2002; Devi and Ohno, 2010; German et al., 2003). The fact that

cholinergic transmission has been implicated in a variety of cognitive functions, and that anti-cholinergic drugs have amnestic effects and can reproduce memory deficits in non-demented elderly patients add merit to this theory (Contestabile, 2011; Francis et al., 1999). In addition, cholinergic mimetics proved to be particularly useful in treating the symptoms and cognitive decline associated with AD in humans and mouse models (Contestabile, 2011; Dong et al., 2009; Romberg et al., 2011). Thus, a majority of the drugs used to treat patients with AD target the cholinergic system.

1.1.5 *Treating AD*

There is currently no cure for AD, and all pharmacological interventions are palliative. Two classes of drugs are currently available for the treatment of patients with AD in Canada: three cholinesterase inhibitors (donepezil, rivastigmine and galantamine) and an NMDA (N-methyl-D-aspartate) receptor blocker (memantine). Cholinesterase inhibitors block acetylcholinesterase, preventing the degradation of acetylcholine in the synaptic cleft and enhancing cholinergic transmission (Birks, 2006). These drugs are usually used to treat patients with mild to moderate AD, while memantine is often prescribed to patients with severe AD or to patients that cannot tolerate the side effects of cholinesterase inhibitors (Bishara et al., 2015). Memantine blocks excess NMDA receptor activity – which is thought to result in neuronal injury and death – without disturbing its normal neuroprotective attributes (Bishara et al., 2015). The pharmacological treatments for AD are not always well tolerated, as they commonly lead to side effects including: dizziness, nausea, vomiting, diarrhea and anorexia (Bishara et al., 2015). These drugs also interact with a variety of other pharmaceuticals, including those used to treat high-blood pressure and epilepsy, making treatment for

patients with other comorbidities even more difficult (Bishara et al., 2015). Thus, there is a great need for the development of new pharmacological agents to treat patients with AD. If interventions could delay the onset and progression of the disease for only 1 year, there would be nearly 9.2 million fewer cases of the disease in 2050 (Brookmeyer et al., 2007)

1.1.5.1 *Donepezil*

Donepezil (Aricept) is a selective and reversible acetylcholinesterase inhibitor that binds to the active site of acetylcholinesterase with high affinity – thus avoiding unintended interactions with butyrylcholinesterase and other receptors (Kryger et al., 1999). Donepezil preserves the levels of acetylcholine at the synaptic cleft, which has been shown to protect against ischemic damage, glutamate excitotoxicity and Aβ toxicity, while also attenuating hippocampal and cortical neurodegeneration (Akasofu et al., 2008; Cutuli et al., 2013).

Donepezil is usually prescribed to patients with mild to moderate AD (Bishara et al., 2015). However, recent studies suggest that donepezil can also be used for moderate to severe cases of AD in combination with memantine (Bishara et al., 2015; Howard et al., 2012). Donepezil must be administered to a patient daily, and administration should not be interrupted, as its effects are quickly lost and may not be fully regained when treatment is re-initiated (Bishara et al., 2015).

1.1.6 *Visuospatial function and attention in AD*

Visuospatial function and attention are among the first cognitive domains to be affected early in AD, and continue to decline as the disease progresses (Albert, 1996; Pal et al., 2016; Perry and Hodges, 1999; Quental et al., 2013). Although there are clear neuropathological correlations, the direct neurobiological correlates of these impairments have yet to be determined (Li et al., 2014a; Perry and Hodges, 1999). Visuospatial function involves the identification of a stimulus and its location, and can be assessed in humans using the Visual Object and Space Perception (VOSP) battery, which effectively evaluates visuospatial function while minimizing interference from other cognitive domains (Quental et al., 2013). Studies have shown that patients with AD score poorly on the VOSP battery when compared to controls, and that their scores continue to drop as the disease progresses (Pal et al., 2016; Quental et al., 2013). Visuospatial ability has also been found to be an important contributor to functional status in AD patients (Fukui and Lee, 2009), and requires connectivity between the prefrontal cortex and the striatum to remain intact (Brigman et al., 2013). Visual discrimination has been shown to depend on the perirhinal cortex in rodent models – the volume of which is significantly reduced in AD as well (Bussey et al., 2003; Juottonen et al., 1998).

Attention has been suggested to be the first non-memory domain to be affected after the initial amnesic stage of AD (Perry and Hodges, 1999). Cholinergic activity plays an important role in attention (Sarter et al., 2005). The cholinergic neurons that project to the cortex from the basal forebrain release acetylcholine in response to attentionally

demanding tasks (Arnold et al., 2002), while lesions to this system impair attentional function (Muir et al., 1996). These neurons also degenerate in patients with AD, resulting in the loss of cholinergic transmission (Bartus et al., 1982).

Attention consists of three subtypes: selective, sustained and divided, and these are differentially affected in AD. Selective attention refers to the ability to filter out random stimuli, while sustained attention or vigilance refers to the ability to focus attention on a task for unbroken periods of time (Perry and Hodges, 1999). Divided attention is where an individual has to either focus their attention on a stimulus or multiple stimuli, or on two separate tasks. Divided attention and some aspects of selective attention are particularly vulnerable, while sustained attention usually remains intact for the early stages of the disease (Perry and Hodges, 1999). The various domains of attention can be tested by various tasks, including tasks in the CANTAB (Perry and Hodges, 1999).

1.1.7 *Gait impairments in patients with AD*

Patients with AD also experience gait disturbances, with cautious gait dominating in patients with mild AD, and frontal gait disorders dominating in those with severe AD (O'keeffe et al., 1996; Sala et al., 2004). These extensive gait impairments contribute to the increase in fall risk and immobility in AD patients compared to age matched controls (Amboni et al., 2013; Muir et al., 2012; Nutt, 2013). Immobility is associated with changes in social behaviour, personality and deteriorations in mental health, along with poorer outcomes and the development of more co-morbidities (McCarron et al., 2005).

Cautious gait is a slow gait, with shortened steps and en bloc turns – which are defined as turns where the individual keeps their head and trunk rigid, taking multiple steps rather than twisting the body and pivoting the toes to turn (Nutt, 2013). These individuals may also widen their base to increase stability (Nutt, 2013). Cautious gait is an appropriate adaptation to real or perceived imbalance and is also commonly observed in patients with Parkinson's Disease (Nutt, 2013; O'keeffe et al., 1996).

Frontal gait disorders, or gait apraxia includes disturbances in trunk movements, standing and walking that are not caused by any orthopedic abnormalities, muscle wasting, arteriosclerosis in the lower limbs, neuromuscular deficits, ataxia, dystonias, dyskinesias, psychiatric disease, side effects of drugs or cautious gait (Sala et al., 2004). Several forms of gait apraxia have been reported in patients with AD, including small steps, freezing of gait and disequilibrium (Nutt, 2013).

1.1.7.1 *The association between gait and attention*

Gait is increasingly considered to be more than just an automated motor activity. Rather, gait requires a combination of executive function, attention and a judgement of internal and external cues, which requires intact visuospatial function as well (Amboni et al., 2013). There is a direct relationship between cognitive impairment and gait abnormalities. When individuals are subject to a dual task paradigm, gait is detrimentally affected. In this paradigm, individuals are asked to walk while performing a concurrent cognitive or motor task, thus increasing competition for attentional resources and forcing the brain to prioritize between the tasks (Amboni et al., 2013). In healthy subjects, dual task paradigms exert an adverse effect on gait variables, with stronger

effects being observed in older adults (Lindenberger et al., 2000). However, in patients with AD, the effect of dual task paradigms are much more pronounced, and an increase in the complexity of the second task and/or the severity of cognitive impairment further worsens gait measures (Amboni et al., 2013; Muir et al., 2012).

1.1.8 *Mouse models of AD*

Our knowledge of the autosomal dominant mutations leading to early onset AD have allowed for the development of many animal models of the disease. These models have been invaluable tools for identifying and characterizing molecular, cellular and pathological changes that lead to the onset of AD (Newman et al., 2007). There are over 20 strains of AD mouse models with mutations in APP, and many others with mutations in tau and presenilins, causing these mice to develop the characteristic plaques and tangles of AD (Newman et al., 2007). In addition, many of these mice recapitulate various cellular and behavioural aspects of the disease.

1.1.8.1 *The 5xFAD mouse model of AD*

The 5xFAD mouse model of AD, developed by Oakley *et al.* (2006), co-expresses five familial AD mutations: the Swedish (K670N/M671L), Florida (I716V) and London (V717I) mutations of the amyloid precursor protein, and two mutations in presenilin 1 (M146L and L286V). These mice are B6/SJL F1 hybrids and the genes were introduced by site-directed mutagenesis and then subcloned into the mouse neuron-specific Thy1 (or cluster of differentiation 90 – CD90) transgene cassette (Oakley et al., 2006). These mutations collectively cause 5xFAD mice to accumulate high levels of intraneuronal

Aβ42 in the brain and develop amyloid plaques and gliosis as early as 2 months of age (Oakley et al., 2006), while the majority of AD mouse models take at least 6-12 months to develop amyloid plaques (Spires and Hyman, 2005). The levels of Aβ42 continue to increase linearly and plaques begin to appear in the deep layers of the cortex and the subiculum, moving throughout most of the cortex and hippocampus as the mice age (Oakley et al., 2006). Plaques also eventually appear in the thalamus, brainstem and olfactory bulb, although they are fewer in number (Oakley et al., 2006). Interestingly, the cerebellum is often spared. These amyloid plaques are surrounded by activated astrocytes and microglia (indicative of neuroinflammation), another hallmark of the AD brain (Akiyama et al., 2000; Oakley et al., 2006). Gliosis increases with age in these mice and closely follows the distribution of amyloid deposits (Oakley et al., 2006). This leads to neurodegeneration and neuronal loss in specific regions of the brain – such as cortical layer 1 and 5 – as the mouse ages, correlating with the deposition of amyloid plaques and intraneuronal Aβ (Oakley et al., 2006). Although 5xFAD mice recapitulate amyloid pathology in AD relatively quickly, they do not develop neurofibrillary tangles or appear to display hyperphosphorylated tau epitopes like some other APP transgenic mice (Maarouf et al., 2013; Oakley et al., 2006).

Like AD patients, 5xFAD mice also demonstrate a significant reduction of ChAT, a marker of cholinergic neurons (Devi and Ohno, 2010; Francis et al., 1999). Moreover, the transplantation of ChAT+ basal forebrain cholinergic neurons derived from human embryonic stem cells to the basal forebrain of 4-month-old 5xFAD mice was capable of improving learning and memory in the Morris Water Maze (Yue et al., 2015). Also, Devi and Ohno were able to reduce cholinergic neuron degeneration and improve memory in

the Y-maze with the partial knockdown of BACE1 (β-APP cleaving enzyme 1) at 6 months but not at 15-18 months of age (Devi and Ohno, 2010).

With age, 5xFAD mice develop deficits in spatial memory on the Y-maze at approximately 4-5 months, disturbances in learning in the Morris Water Maze beginning at 9 months and severe motor impairments in the rota-rod at approximately 12 months of age (Macdonald et al., 2013; Oakley et al., 2006; Schneider et al., 2014). In addition, 5xFAD mice also demonstrate changes in brain glucose metabolism, anxiety and electroencephalogram (EEG) disturbances at 6 months of age (Macdonald et al., 2013; Schneider et al., 2014). EEG recordings revealed that 5xFAD mice spend less time in rapid-eye-movement sleep in relation to the total amount of sleep when compared to wild-type controls (Schneider et al., 2014). In summary, 5xFAD mice develop amyloid pathology, functional disturbances and behavioural deficits that make them a good model for studying many aspects of AD.

1.1.8.1.1 *Retinal degeneration in the 5xFAD mou*se model

5xFAD mice have a mixed background: C57Bl6 and Swiss Jim Lambert (SJL). SJL mice are homozygous for the recessive *Pdeb*^{rd1} allele, which codes for the β-subunit of cGMP phosphodiesterase on mouse chromosome 5 (Clapcote et al., 2005; Giménez and Montoliu, 2001). Thus, F1 5xFAD mice should be heterozygous for the mutation. The mutated allele is a nonsense mutation that decreases the transcription of the phosphodiesterase, leading to retinal degeneration and blindness by wean age at approximately 3 weeks and rendering mice homozygous for the *Pdeb*^{rd1} allele unsuitable for use in some experiments (Giménez and Montoliu, 2001). This same

mutation is seen in FVB/NJ (Friend Virus B/ National Health Institute Jackson) mice as well (Giménez and Montoliu, 2001).

1.2 The Bussey-Saksida Touchscreen System

High-level cognitive and executive functions in mouse models can be effectively assessed using the Bussey-Saksida touchscreen system. This touchscreen system can be used to study both impairments and enhancements in visual discrimination, extinction, attention, impulsivity, compulsivity and a variety of other cognitive domains (Bussey et al., 2012). The tasks can be identical to those used in humans – like those in the CANTAB (Brigman et al., 2005; Bussey et al., 2001; Downes et al., 1989; Robbins et al., 1994; Sahakian and Coull, 1993). This face validity provides the best chance of successful cognitive translation across species. These tasks can be used to evaluate various cognitive perturbations in mouse and rat models of disease, including those of Alzheimer's Disease, Parkinson's disease, Schizophrenia, Attention Deficit Hyperactive Disorder (ADHD), Obsessive Compulsive Disorder and addiction (Bussey et al., 2012).

This touchscreen system is automated, reducing variability and scope for error (Bussey et al., 2001). The entire task – from stimulus presentation to reward provision – is completely controlled by a computer program, minimizing experimenter interference, inconsistencies and any other confounds. To switch between tasks, the experimenter only needs to switch to the correct mask (which has task-specific windows allowing for the mouse to see and respond to the task while reducing unintended responses) and schedule. Automation also allows for the "in parallel" testing of many animals simultaneously, thus increasing statistical power and throughput.

Several parameters, such as accuracy (responding to the correct location), omissions (failing to respond), perseverative responses (responding repeatedly to a previously correct location; a measure of compulsivity), premature responses (responding before the stimulus appears; also a measure of impulsivity), time to make a correct response (correct response latency) and time to collect reward (reward collection latency) are automatically recorded by the equipment during each task. The results can then be exported to common data formats allowing for the creation of expandable databases to facilitate increased data access and reproducibility.

Lastly, the tasks employed by these touchscreens are non-aversive and low stress, providing a food reward for a correct response rather than punishing the animal for an incorrect response (Bussey et al., 2012). The touchscreen chambers are also isolated from the experimenter and its surroundings, minimizing distractions and other stress-inducing stimuli as well.

1.2.1 *The Pairwise Visual Discrimination Task*

Visual discrimination and cognitive flexibility can be assessed using the pairwise visual discrimination (PVD) touchscreen task (Brigman et al., 2008; Bussey et al., 2008). Impairments in both visual discrimination and cognitive flexibility have been reported in the early stages of AD and have been suggested to serve as specific and accurate prognostic markers of the disease (Albert, 1996; Pal et al., 2016; Quental et al., 2013). In the PVD task, the mouse is presented with two stimuli and has to learn that one of the stimuli (S+) leads to a reward while the other (S-) does not. The spatial location of the stimuli is irrelevant to the completion of the task. Once the task is learned, the

stimulus-reward association is reversed. The mouse then has to inhibit the response acquired and associate the previously unrewarded stimulus with the reward (reversal learning) and learn a new rule, providing an assessment of cognitive flexibility. Performance of this task has been shown to be dependent on the prefrontal cortex in rats and mice (Brigman and Rothblat, 2008; Bussey et al., 1997; Chudasama and Robbins, 2003). Inactivation of the perirhinal cortex with either muscimol (a gamma-Aminobutyric acid-A receptor blocker), AP5 ((2*R*)-amino-5-phosphonopentanoate, a NMDA receptor antagonist) or scopolamine (a muscarinic receptor antagonist) impairs performance in this task as well (Winters et al., 2010). Deficits in visual discrimination learning and reversal in the PVD task have also been observed in NMDA receptor 2A knockout mice (Brigman et al., 2008). AD patients also demonstrate a selective and differential reduction of NMDA receptor levels in the brain (including receptor 2A), and the levels of these receptors correlate with cognitive performance (Maragos et al., 1987; Sze et al., 2001). In addition, the reduction of forebrain cholinergic tone in VAChT$^{Six3\text{-}Cre\text{-}flox/flox}$ (vesicular acetylcholine transporter (VAChT) deficient) mice impairs cognitive flexibility in the PVD task (Kolisnyk et al., 2013a). The expression of VAChT is significantly reduced in AD patients, correlating with the severity of dementia (Efange et al., 1997; Gilmor et al., 1999). Visual discrimination acquisition is impaired in 11-month old rTg4510 mice as well (Harper et al., 2013). These mice express a human tau mutation (Harper et al., 2013).

The PVD task can be administered multiple times, each with a different set of S+ and S- stimuli. This allows for the longitudinal testing of animal models, allowing determination of when deficits in the task (if any) begin to appear.

1.2.2 *The 5-Choice Serial Reaction Time Task*

The 5-choice serial reaction time task (5-CSRTT), is mainly used to study attention, which is impaired in AD patients (Bussey et al., 2012; Carli et al., 1983; Leonard, 1959; Perry and Hodges, 1999). The task also gives information about impulsivity (premature responses) and compulsivity (preservative responses). In the 5-CSRTT, mice are required to scan a horizontal array of five screens for the presence of a brief, randomized light stimulus and respond appropriately with a nose poke. The shorter the duration of the stimulus, the higher the attentional demand required to successfully perform the task (Bari et al., 2008).

Performance in the 5-CSRTT is impaired in mouse models of cholinergic dysfunction, including M_1 muscarinic receptor deficient mice (Bartko, 2011), ChAT-ChR2-EYFP mice who express channel rhodopsin 2 (ChR2) under control of the choline acetyltransferase (ChAT) (Kolisnyk et al., 2013b) and $VAChT^{Six3\text{-}Cre\text{-}flox/flox}$ mice (Kolisnyk et al., 2013a). The accuracy of mice in the 5-CSRTT task is also significantly reduced by damage to the prefrontal cortex and/or striatum (Muir et al., 2012; Passetti et al., 2003; Rogers et al., 2001). However, performance in the task can be improved with drug treatment. For example, the attentional impairments observed in triple transgenic (3xTG) mouse model of AD, which is likely a result of pathological changes in the prefrontal cortex, was reduced following treatment with the cholinesterase inhibitor donepezil (Romberg et al., 2011). Galantamine has also been shown to improve performance of WT mice in this task (Kolisnyk et al., 2013a). Other mouse models of AD demonstrate deficits in this task as well. Response accuracy is significantly reduced in TgCRND8 mice at 4-4.5

months of age (Romberg et al., 2013). These mice have the Swedish K670N/M671L and the Indiana V717F APP mutations, leading to plaque formation by 3 months of age (Chishti et al., 2001). Deficits in the 5-CSRT task have also been observed in male 5xFAD mice at 10 months of age (Masood, 2015). Masood (2015) observed a significant decrease in percentage accuracy and the number of preservative responses, along with delays in reward collection and task response.

1.2.3 *Using touchscreen tasks to identify cognitive deficits in mouse models of AD*

Given the high prevalence and poor prognosis of AD, the development of novel treatments for patients with AD is imperative. The drugs currently given to these patients do not cure or prevent the progression of the disease, interact with drugs commonly used to treat other comorbidities and are often extremely difficult to tolerate (Bishara et al., 2015). Up to 85% of patients experience the adverse side effects of these treatments and some even opt to discontinue treatment for this reason (Burns et al., 2007). Unfortunately, the drug development process is tedious and costly; clinical trials take many years and require several million dollars to complete. Between 2002 and 2012, 24 phase 1, 206 phase 2 trials and 83 phase 3 clinical trials were attempted, with an overall success rate of 0.4% (Cummings et al., 2014). Most of these registered trials aimed to improve cognition in AD patients, but failed to do so (Cummings et al., 2014). Thus, the ability to predict whether a novel treatment can improve cognitive function in humans during pre-clinical development would be greatly beneficial. This requires a sensitive and translational task that can efficiently and accurately assess

cognitive function. Therefore, touchscreens have the potential to become a powerful new platform for the pre-clinical evaluation of new pharmacological treatments directed at cognitive function in AD patients.

Attention, visual discrimination and cognitive flexibility are commonly affected in patients with AD (Albert, 1996; Pal et al., 2016; Perry and Hodges, 1999; Quental et al., 2013), and all these parameters can be assessed in mice using touchscreen tasks. Impairments in visual discrimination and attention have been observed in various mouse models of cholinergic and glutamatergic dysfunction (Bartko, 2011; Brigman et al., 2008; Kolisnyk et al., 2013a; Romberg et al., 2013; Winters et al., 2010), and a few mouse models of AD (Harper et al., 2013; Romberg et al., 2011, 2013). However, little remains known about the onset and presence of deficits in executive function on the different mouse models of AD.

Romberg and colleagues were able to rescue attentional deficits in 3xTG mice following treatment with donepezil (Romberg et al., 2011). Since our lab has also observed attentional deficits in male and female 5xFAD mice at 10 months of age (Masood, 2015), the question remains whether donepezil can rescue the attentional impairments in these mice as well.

1.3 Rationale and Hypothesis

We hypothesize that male 5xFAD mice develop reproducible age-dependent deficits in the PVD and that deficits in the 5-CSRTT that can be ameliorated by treatment with donepezil.

The overall objective of this thesis is to perform a longitudinal evaluation of visual discrimination and cognitive flexibility in male 5xFAD mice, as the presence of cognitive dysfunction is an important proof of the face validity of the 5xFAD mouse model. We also want to determine if specific cognitive deficits can be rescued by donepezil as a proof of principle. To address these objectives, the specific aims of this thesis are:

1) To perform a longitudinal evaluation of visual discrimination and reversal learning in male 5xFAD mice at 4, 7, and 10 months of age.
2) To assess the effects of donepezil on cognition in male 5xFAD mice at 10 and 13 months of age using the 5-CSRTT.
3) To genotype the 5xFAD mice that have been subject to touchscreen evaluation for the recessive $PDEB^{rd1}$ allele to ensure that task performance is not affected.
4) To determine whether the mild food-restriction used to motivate mice to perform touchscreen tasks affected amyloid pathology in male and female 5xFAD mice.
5) To evaluate gait, locomotion and grip force in male 5xFAD mice.

2 Materials and Methods

2.1 5x Familial Alzheimer's Disease Mouse Model

The 5xFAD mice (B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax, Jax stock #006554) and age-matched wild-type controls (B6SJLF1/J, Jax stock #100012) used in the PVD experiments were purchased from the Jackson Laboratory (Bar Harbor, Maine) and delivered to the university. The 5xFAD mice used in the 5-CSRTT experiments were bred at Western University. All mice used for the experiments were tattooed in their tails at least a week prior to testing for identification purposes. Male 5xFAD mice and their age-matched wild-type controls (wild-type littermates were only used for the 5-CSRTT) were used for all behavioral tasks, whereas male and female 5xFAD mice and their age-matched wild-type controls were used for pathological analyses. All procedures were performed in accordance with the Canadian Council of Animal Care guidelines at the University of Western Ontario with an approved animal protocol (2008-127).

2.2 Housing and Diet

Mice that underwent behavioural tasks were singly housed without environmental enrichment in a temperature and pressure controlled room with a 12-hour light/dark cycle. Lights would turn on at 7:00am and shut off at 7:00pm daily. All behavioural tests were conducted during the light phase of this cycle. Cages were changed biweekly.

Mice that were to be tested on the touchscreens were mildly food restricted to 85%

percent of their original baseline adult weight (according to the Adult Mouse Food Restriction Standard Operating Procedure – Appendix 1) to ensure that they would be motivated to complete the task. The mice were put on food restriction prior to the start of behavioural testing and the body weights were gradually lowered to 85% of their original weight. All mice were weighed daily. Water was provided *ad libitum.*

For the pathology experiments, male and female 5xFAD mice were either food-restricted to 85% percent of their original baseline adult weight (according to the Adult Mouse Food Restriction Standard Operating Procedure – Appendix 1) or provided food *ad libitum* for 2-3 months – until they were euthanized at 6 months of age. Water was provided *ad libitum* in both cases. All mice were fed with Teklad Laboratory Animal Chow (Envigo).

2.3 Genotyping *Pdeb*^{rd1}

DNA was extracted from mouse ear tissue and amplified using the REDExtract-N-Amp Tissue PCR Kit Protocol (Sigma-Aldrich, Oakville, Ontario). Polymerase chain reaction (PCR) was done using the Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, California) with a 500bp x 40 cycle schedule (94°C x 3 minutes followed by 40 x [94°C x 30 seconds] + 60°C x 30 seconds + 72°C x 30 seconds then 72°C x 2 minutes). The tubes were held at 10°C until use. The following reagents were used for each sample: 5μl of 2x premix, 0.5μl of retinal degeneration (RD) 3 oligonucleotide primer (concentration: 0.5 μM; 28-mer, 5'-TGACAATTACTCCTTTTCCCTCAGTCTG-3', accession number L02109, nucleotides 84 to 111), 0.1μl of RD4 oligonucleotide primer

(concentration: 0.02 μM; 28-mer, 5'-GTAAACAGCAAGAGGCTTTATTGGGAAC-3', accession number L02109, nucleotides 644 to 617) and 2.9μl of RD6 oligonucleotide primer (concentration: 14.5 μM; 28-mer, 5'-TACCCACCCTTCCTAATTTTTCTCAGC-3', accession number L02110, nucleotides 2539 to 2512). RD3 and RD4 amplifies a 0.55kb PCR product from the *Pdeb*^{rd1} mutant allele, while RD3 and RD6 amplifies a 0.40kb PCR product from the WT allele (Giménez and Montoliu, 2001). The PCR products are then run on an agarose gel along with a 100bp ladder (Gene DireX, Frogga Bio, Toronto, Ontario) and imaged with FluorChem Q (Alpha Innotec Corp., San Leandro, California).

The positive control for *Pdeb*^{rd1} was ear tissue from a Friend Virus B NIH Jackson mouse (FVB/NJ; Jax stock #001800), an inbred strain of mouse known to be homozygous for the *Pdeb*^{rd1} mutation. This mouse was purchased from the Jackson Laboratory (Bar Harbor, Maine). The control for the WT allele of *Pdeb* for this gel was ear tissue obtained from a B6SJLF1/J mouse.

2.4 Administration of Donepezil

Donepezil hydrochloride (C6821-50mg) monohydrate was obtained from Sigma-Aldrich (Oakville, Ontario) and rehydrated using saline and 10% dimethyl sulfoxide (DMSO) to a dose of 2.5 mg/kg (for the osmotic pumps) or 1.0mg/kg for the intraperitoneal injections.

2.4.1 *Subcutaneous Osmotic Pumps*

The implantation of subcutaneous osmotic mini-pumps allows for the continuous, systemic infusion of a soluble substance over an intermediate duration of time (up to 6

weeks). Relative to bolus dosing (injection), the infusion of a modulating agent has the potential to widen the therapeutic index of the agent, increase its efficacy and/or reduce any side effects (Fara and Urquhart, 1984). This is especially true for substances with short-half lives. The infusion of an agent also reduces the possibility of injuring or subjecting laboratory animals to the additional stress of daily injections, possibly confounding the results of subsequent behavioral analyses. Thus, osmotic mini-pumps can be an effective alternative to daily injections when executing a moderately long-term study.

Osmotic mini-pumps purchased from Alzet (Durect Cooperation, Cupertino, California) are composed of 3 concentric layers: a semipermeable, rate controlling membrane, an osmotic layer that contains a high concentration of sodium chloride and an impermeable but flexible drug reservoir (http://www.alzet.com/products/guide_to_use/implantation_and_explantation.html). Water enters the pump across the semipermeable membrane due to the high concentration of sodium chloride in the osmotic layer. The entry of water causes the osmotic chamber to expand, compressing the flexible reservoir and delivering the drug solution through the flow moderator (http://www.alzet.com/products/guide_to_use/implantation_and_explantation.html).
These pumps have been used in the evaluation of a variety of different modulating agents in laboratory animals, including the effect donepezil in AD mouse models (Dam et al., 2008; Spilman et al., 2014).

Twenty male 5xFAD mice were implanted with subcutaneous osmotic pumps (model 1004, Alzet, Durect Cooperation, Cupertino, California) designed to deliver the drug at a fixed rate of 0.11µL/hr for 28 days. Ten 5xFAD mice were randomly chosen to receive

pumps loaded with saline + 10% DMSO or donepezil. Donepezil was diluted and prepared based on calculations done using the drug concentration calculator on the Alzet website (http://www.alzet.com/products/guide_to_use/formulating.html). This was based on the pump model, flow rate, weight of the mouse and the desired dose. The pumps were loaded with a 1mL syringe (the appropriate needle was provided with the pumps), capped with a pin, and then soaked in saline until they were implanted within one hour. Mice were first anesthetized with 4% isoflurane at 1L/minute until unconscious. Maintenance was done with 1.5% isoflurane. The dorsal side of the mouse was sprayed with ethanol and then small incision was made on the right side (near the shoulder of the mouse). The pump was inserted and the incision was sealed with a wound clip. Mice were allowed to recover in their home cage under a heat lamp. The mice were given two days to recover from pump insertion before beginning a 5-CSRTT probe trial. Any mice that pulled out their own pump were euthanized. One mouse was dropped from the experiment for this reason. All surviving mice had their pumps removed after 28 days.

2.4.2 *Intraperitoneal Injection*

At approximately 14 months of age, 5xFAD mice were randomly chosen to receive either donepezil or saline + 10% DMSO for 5 consecutive days. After a two-day washout, the cohort that received saline received donepezil and vice-versa. Donepezil was administered via intraperitoneal injection at the lower right or left quadrant of the abdomen to avoid damage to the urinary bladder and other abdominal organs. Mice were then placed back in their home cage for 30 minutes before beginning any

behavioural tests.

2.5 Touchscreens

The pairwise visual discrimination (PVD) and 5-choice serial reaction time tasks (5-CSRTT) were conducted using the automated Bussey-Saksida Touchscreen System for mice (Model 81426, Campden Instruments, Lafayette, Indiana), which consists of a testing chamber housed within a ventilated sound and light-attenuating box. The chamber has a house light, a tone generator, and stimuli are displayed on a LCD monitor that is equipped with infrared sensors. The mouse views the stimuli through a black mask that contains windows through which each stimulus can be seen. This also prevents unintended responses by other body parts of the mouse. The mask used depends on task being administered. The entire apparatus is controlled by the Abet II Touch Software Version 2.20 (Lafayette Instrument Company, Lafayette, Indiana). The schedules were designed and the data was collected using this software as well. Each mouse was only run on one schedule at approximately the same time each day.

2.5.1 *Touchscreen Pre-training*

Prior to the commencement of either the PVD or the 5-CSRT task, mice are subject to a basic training schedule (Figure 1A). Mice are habituated to the touchscreen chambers for the first four days. Habituation 1 (day 1) lasts 10 minutes – all lights are tuned off and no stimulus or reward is presented. Habituation 2a (day 2 and 3) lasts 20 minutes. The food tray light will be on at the beginning of each trial and the mouse can complete an unlimited number of trials within the 20-minute session. When the mouse enters the food tray, a tone is played and a strawberry milkshake reward (Saputo Inc., Montreal,

Quebec) is dispensed. When the mouse leaves the food tray, the tray light turns off. The tray light will turn back on after 10 seconds and another trial begins. Habituation 2b (day 4) is the exact same as habituation 2a, but it lasts 40 minutes. The mouse must be removed from the cabinet once a habituation schedule is complete.

Following habituation, mice are subject to initial touch, where a white square (for 5-CSRTT) or image (for PVD; can be any image not designated for use in discrimination/reversal) is displayed in one window pseudo-randomly (such that the stimulus is not shown in the same position more than three times in a row) while the other window(s) are left blank. The stimulus disappears after 30 seconds and reward is delivered. If the mouse touches the screen where the white square is displayed while it is still being displayed (within 30 seconds), a tone will be played and 3x the reward will be delivered immediately (this is accompanied by a tone and the illumination of the tray light). The mouse then has to collect the reward, turning the tray light off, and initiating the inter-trial interval (20 seconds for PD, 5 seconds for 5-CSRTT) – after which another trial begins. Collection of the reward starts the next inter-trial interval. The mouse must complete 30 of these trials within 60 minutes and this usually only takes one session. Otherwise, this schedule is repeated until criterion is achieved.

Initial touch is followed by must touch. In PVD, the stimulus is an image selected pseudo-randomly (no image shown more than three times in a row) from a list of images that do not include any of the images that will be used in the discrimination and reversal trials. In 5-CSRTT, the stimulus remains as a white square. The position of the stimulus is also chosen pseudo-randomly. The mouse must touch the stimulus to receive a reward. This is accompanied by a tone and the illumination of the tray light. There is no

response if the mouse touches the blank screen(s). Collection of the reward starts the next trial after the inter-trial period (20 seconds for PD, 5 seconds for 5-CSRTT). The mouse must complete 30 of these trials within 60 minutes to reach criterion. If the mouse does not reach criterion after 7 sessions for PVD or 5 sessions for the 5-CSRTT, the mouse is retrained on initial touch until it reaches criterion. If the mouse does not reach criterion after 7 sessions (PVD) or 5 sessions (5-CSRTT) of the second attempt of must touch, it is removed from the study.

Must touch is followed by must initiate. For each trial, a free delivery of food is made, and the tray light is illuminated. The mouse must nose poke and exit the reward tray before a stimulus (same stimuli with the same criterion as those used in must touch; task dependent) appears. The other window(s) remain blank and there is no response if the mouse touches it. The mouse must touch the stimulus to receive a reward. This is accompanied by a tone and the illumination of the tray light. After the mouse collects the reward, the inter-trial interval (20 seconds for PD, 5 seconds for 5-CSRTT) begins. The mouse must nose-poke and exit the reward tray before the next stimulus is displayed. Thirty of these trials must be completed within 60 minutes to reach criterion. If the mouse does not reach criterion after 5 sessions, the mouse is retrained on must touch until it reaches criterion. For the second attempt, if the mouse does not reach the criterion for must initiate within 5 sessions, it is removed from the study.

The last pre-training phase is punish incorrect, which is similar to must initiate – except when the mouse touches a blank window, the house light will be turned on for 5 seconds and no reward will be given. After the light turns off, the mouse will not be able to initiate another trial until the inter-trial interval ends. To reach criterion, mice have to

complete ≥ 24/30 trials correctly within 60 minutes two days in a row. If the mouse cannot reach criterion after 30 days, it is removed from the study.

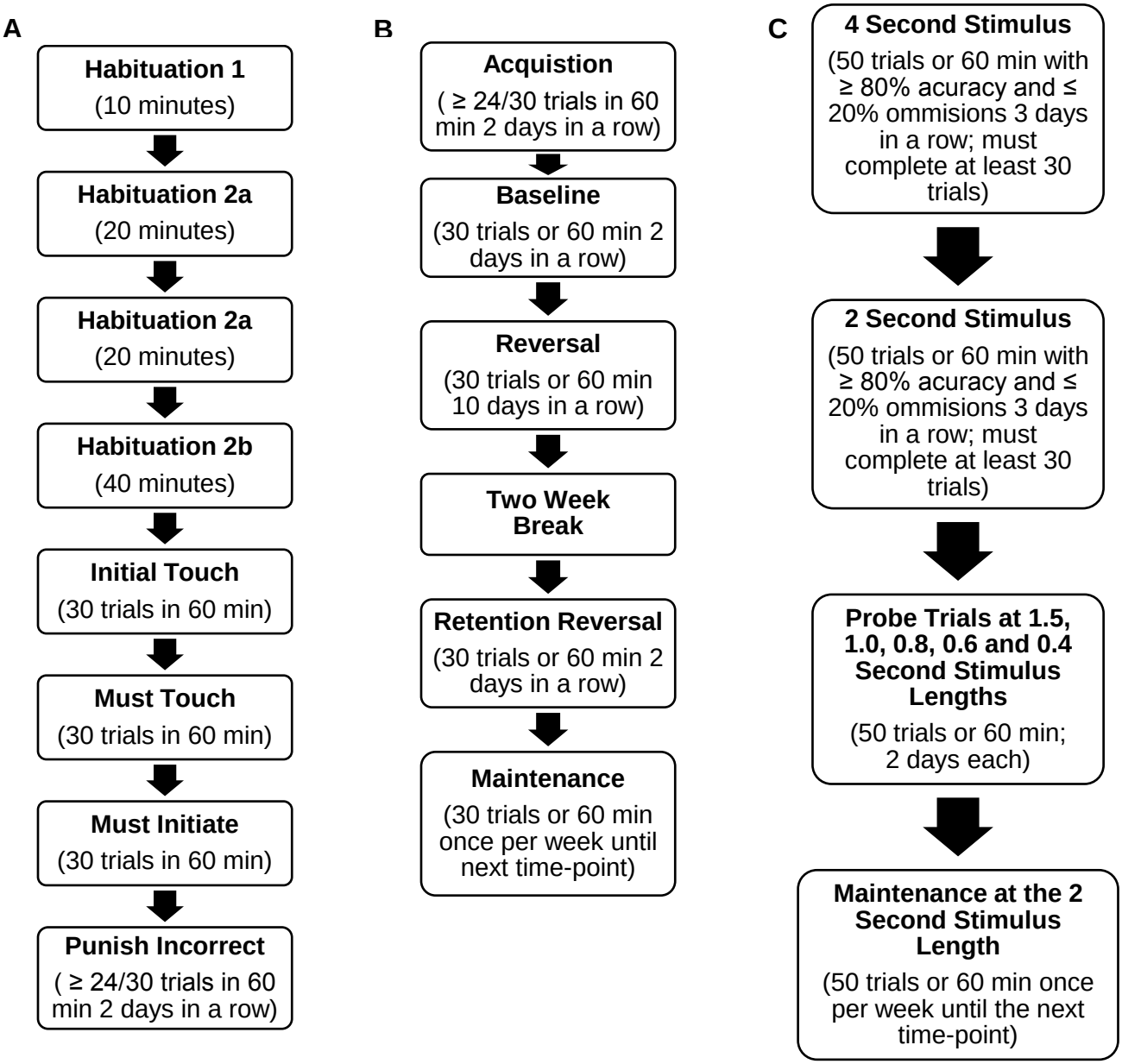


Figure 1. Summary of touchscreen pre-training, PVD and 5-CSRTT schedules. A) Flow chart of the touchscreen pre-training schedules, their duration and criterion. B) Flow chart of pairwise visual discrimination acquisition, baseline, reversal and retention reversal (7 and 10-month time-point only) which is repeated with a different pair of images at each time point. C) Flow chart of the 5-CSRTT training and probe trials at 1.5, 1.0, 0.8, 0.6 second stimulus lengths.

2.5.2 *Pairwise Visual Discrimination*

Pairwise visual discrimination involves two general phases: visual discrimination (acquisition and baseline; Figure 1B and Figure 2A) and reversal (Figure 1B and Figure 2B). A black mask with two squares is placed in front of the screen. The PVD task begins with acquisition, where the reward tray is first primed with the reward. As the mouse exits the food tray, the first trial begins and a S+ and S- image is displayed. The location (i.e., left or right side of the mask) of the stimulus is pseudo-random. If the mouse touches the location where the S+ image is displayed, a reward will be delivered, along with illumination of the tray light and a tone. The inter-trial period of 20 seconds begins after the mouse exits the food tray, after which the tray light will turn on, and the mouse must enter and exit the tray to initiate the next trial. If the mouse touches the location where the S- stimulus is displayed, the house light will turn on for 5 seconds and the inter-trial period will follow. Afterwards, the tray light will come on and the mouse must enter and exit the food tray to begin a correction trial, where the left/right locations of the stimuli remain the same as the previous trial and is repeated with each subsequent correction trial until the correct choice is made. The results of the correction trials do not count toward the criteria for completion of the session (≥24/30 trials within 60 minutes, two days in a row).

Baseline runs for two sessions immediately after reaching the criteria for acquisition, and is the same as acquisition, except that there is no score required to pass. The purpose of baseline is to take a baseline measurement of performance. The session ends after 30 trials or 60 minutes. After completing baseline, reversal is run for the next

10 sessions. For reversal, trials are initiated and correction trials are run the same way. The only difference is that the stimulus reward association is reversed (i.e., the previous S+ image will now be the S- image and vice versa). There is no score required to pass, and the session ends after 30 trials or 60 minutes.

Beginning at the 7-month time-point, we added the retention reversal schedule, which was run after a two week (maintenance-free) break from the task. Retention reversal was the same as reversal, and the purpose of this schedule was to evaluate how well the mice remembered what they learned in reversal.

Different images were used as S+ and S- stimuli for each time-point (Figure 2C). This was necessary for each time-point to include a visual discrimination phase and a subsequent reversal phase.

There are a total of three time-points: a 4-month, 7-month and 10-month time-point. Each time-point usually takes approximately 2-2.5 months to complete, although some mice can take up to 3.5 months to complete the last time-point. Thus, during the 4-month time-point, the mice are usually between 4-6.5 months of age. During the 7-month time-point, the mice usually between 7-9.5 months of age, and during the 10-month time-point, they are usually between 10-13.5 months of age. Between each time-point, the maintenance schedule was run once a week until the next time-point begins. This schedule was identical to punish incorrect, except there was no criterion that needed to be met. A summary of the PVD schedules can be found in Figure 1B. For the detailed PVD standard operating procedure see Appendix B.

The data collected was submitted to a quality control filtering program (Baycrest, Toronto, Ontario) before analysis. This program removes any duplicate sessions and sessions that were ended prematurely (the mouse neither completed 30 trials and the session was not 60 minutes long). This data will also be added to a new branch of the Extensible Neuroimaging Archive Toolkit (XNAT), with the ultimate goal of providing an open-sourced database of mouse touchscreen data.

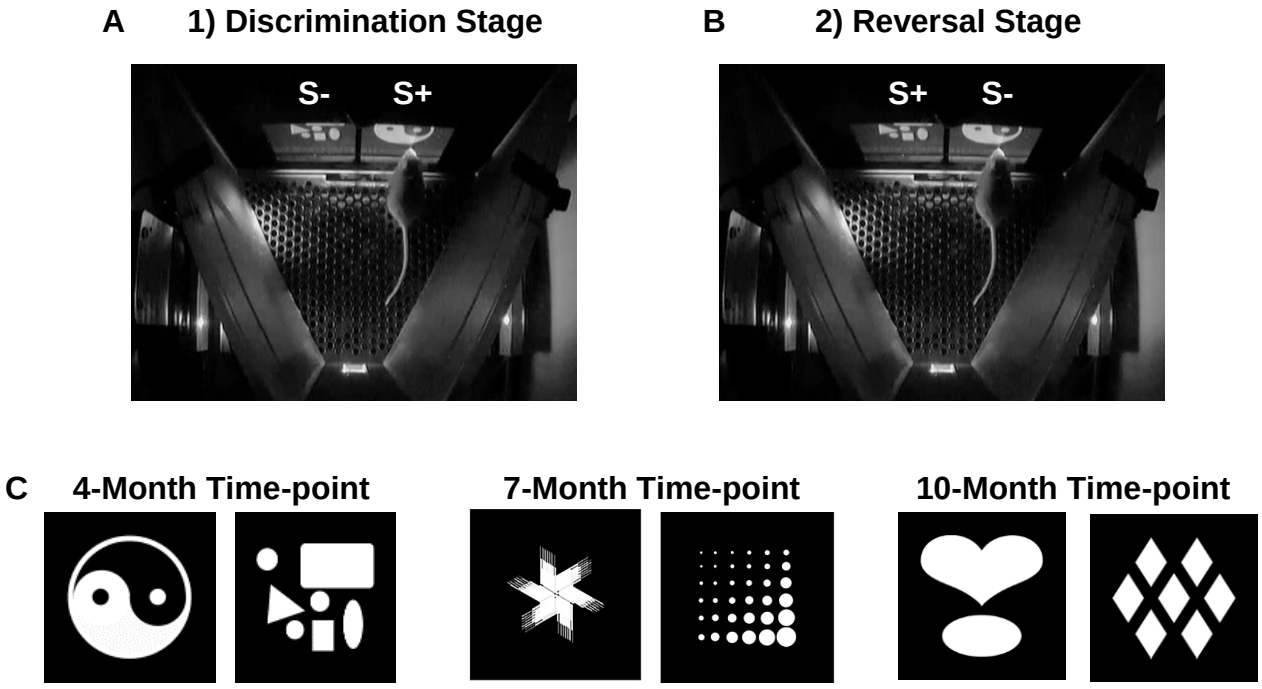


Figure 2. S+ and S- stimulus presentation and stimuli used in pairwise visual discrimination.

A) Illustration of stimuli presentation during discrimination (acquisition schedule), where the yin-yang is S+ and the array of shapes is S-. B) Illustration of stimuli presentation during reversal, where the yin-yang is now S- and the array of shapes becomes S+. C) Images used as stimuli in PVD across time-points.

2.5.3 *5-Choice Serial Reaction Time Task*

For the 5-CSRTT, a mask with a grid of 5 small squares arranged horizontally (Figure 3A) is placed in front of the screen. The 5-CSRTT begins with a 4 second (s) stimulus length. Each session begins when the mouse enters and exits the primed reward tray. Five seconds (delay interval) after the mouse exits the food tray, a stimulus (a small white square) is presented in one of the 5 grid spaces in a pseudorandom fashion, where there are 4 presentations at each spatial location within each block of 20 trials. The mouse must respond by touching the location where the stimulus was presented within 5 seconds after the stimulus disappears to receive a reward. The delivery of the reward is accompanied by a tone and illumination of the tray light. If the mouse touches the incorrect location, this is considered an incorrect response. However, if the mouse makes no response this is considered an omission. If the mouse responds during the 5 second delay interval, this is categorized as a premature response. Both incorrect responses, premature responses and omissions will cause the house light to turn on for 5 seconds. Regardless of the response (Figure 3), after the 5 second inter-interval trial, the tray light is lit once again and the mouse must enter and exit the tray to begin the next trial. Each session lasts 50 trials or 60 minutes. To reach criterion, mice must complete at least 30 trials and perform with an accuracy of ≥ 80% with ≤ 20% omissions 3 days in a row. Accuracy and omissions were calculated as follows:

$$Accuracy = \frac{Number\ of\ correct\ trials}{Total\ number\ of\ trials\ responded\ to\ (both\ correct\ and\ incorrect)}$$

$$Omissions = \frac{Number\ of\ trials\ missed}{Total\ number\ of\ trials}$$

The 4s stimulus length is followed by a 2s stimulus length. Other than the length of the stimulus, the rest of the schedule remains the same. To reach criterion the mice must once again complete at least 30 trials and perform with an accuracy of ≥ 80% with ≤ 20% omissions 3 days in a row. After the mice meet the 2s stimulus performance criteria, the probe trial evaluations begin. There is no minimum performance criterion required to advance through the probe trials. Probe trial schedules are also the same as the 4s and 2s schedules, except that the stimulus length is different. Probe trials consists of two days of 1.5, 1.0, 0.8 and 0.6s stimulus lengths with two days of 2s stimulus length sessions run in between each stimulus duration. Mice are randomly assigned to one of four subgroups and the order with which each subgroup of mice are subject to in probe trial is counterbalanced as indicated in Table 1. For the detailed 5-CSRTT standard operating procedure see Appendix C and Figure 1C.

Subcutaneous osmotic pumps were implanted into 5xFAD mice and their WT littermates at approximately 10 months of age as described in section 2.4.1. Two days after pump implantation, mice began a probe trial, starting with five days of 2s sessions to re-baseline. Each mouse is assigned to a subgroup (Table 1). After completion of this probe trial, mice were subject to one session of the 2s stimulus length once a week until the next time-point began.

At approximately 14-months of age, the 5xFAD mice were subjected to a probe trial consisting of only the 0.6s stimulus length following five 2s sessions of re-baseline. Mice repeated 8 sessions of 5-CSRTT at the 0.6s stimulus length. Thirty minutes prior to the task, mice were injected with either donepezil or saline 10% DMSO for four consecutive sessions. After a two-day washout, the cohort of mice that received donepezil received

saline + 10% DMSO and vice versa. The timeline of this experiment is outlined in Figure 4.

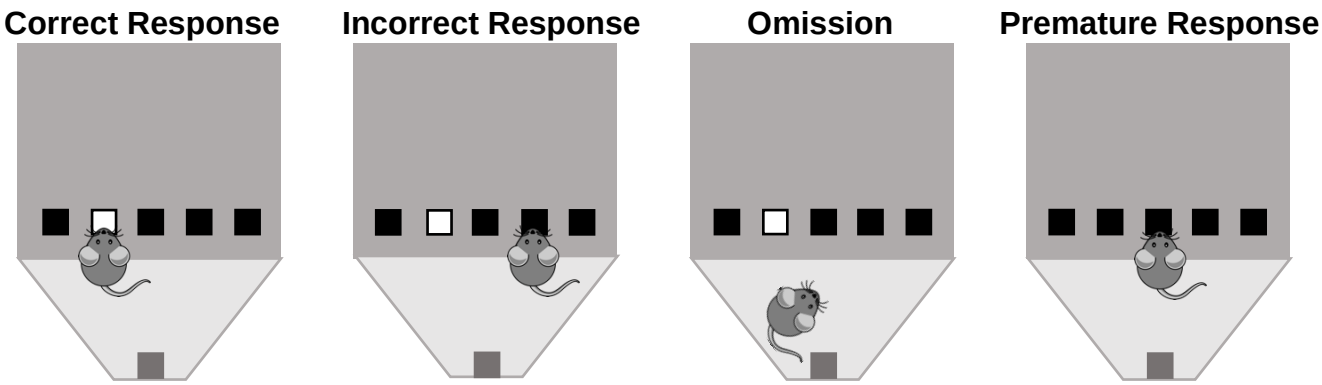


Figure 3. Illustration of the possible responses to the stimulus in 5-CSRTT. Illustration of a correct response, an incorrect response, an omission and a premature response in the 5-CSRTT.

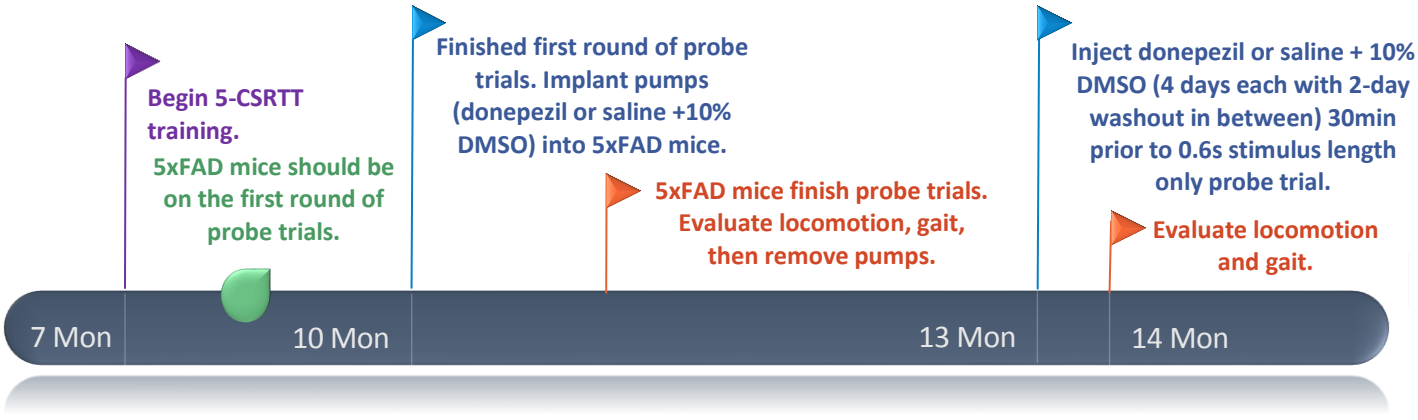


Figure 4. 5-CSRTT experiment timeline.
Illustration of the procedures and behavioural experiments performed in the cohort of mice used for the 5-CSRTT experiments.

Table 1. Order of probe trial sessions at 0.6s, 0.8s, 1.0s and 1.5s for individual subgroups in the 5-CSRTT.

	Order of Probe Trial Stimulus Duration			
# of Consecutive Sessions	**Sub-group A**	**Sub-group B**	**Sub-group C**	**Sub-group D**
2	0.6s	0.8s	1.0s	1.5s
2	2.0s	2.0s	2.0s	2.0s
2	1.5s	0.6s	0.8s	1.0s
2	2.0s	2.0s	2.0s	2.0s
2	1.0s	1.5s	0.6s	0.8s
2	2.0s	2.0s	2.0s	2.0s
2	0.8s	1.0s	1.5s	0.6s

2.6 Open-Field Locomotion

5xFAD mice and their WT controls that underwent touchscreen evaluation were all subject to locomotor assessments using open-field locomotor boxes (Omnitech Electronics Inc., Columbus, Ohio) while still on food-restriction. One group of mice was then taken off food-restriction for a month and assessed again.

Mice were first habituated to the room where the locomotor assessments were held for at least 30 minutes prior to the assessment. The mice were habituated in their home cages. The mice were placed in the open-field locomotor boxes for 2 hours, and measurements were taken every 5 minutes.

2.7 Gait Analysis

5xFAD mice and their WT controls were subject to gait analyses after completing locomotor assessments. Gait analyses were conducted using the CatWalk 7.1 automated gait analysis system (Noldus Information Technology, Wageningen, The Netherlands). The system uses a video camera to capture the reflection of light as a mouse moves across an illuminated glass plate. Mice were habituated to the room and the darkness prior to the experiment. For the first 5 minutes, the lights were dimmed and the door remained open. The lights were then turned off for another 5 minutes. The door was then closed for another 5 minutes before the first run. The mouse is placed on one side of the CatWalk and was allowed to walk across and explore for 25 seconds. The mouse was then removed, and the video file saved. The glass walkway was cleaned with Windex and/or 70% ethanol between each run. Three videos were taken for each mouse, and the two best walks across the glass were chosen. A good walk was defined as one where the mouse walks at a regular pace across the glass without pausing. The runs were subsequently analyzed using the CatWalk 7.1 program. The pixel threshold was set at 65, and each of the paws in a run were labelled by hand. The data was then exported into an excel file. This included static parameters that were not affected by the movement of the mouse, and dynamic parameters – parameters that change as the mouse moved across the glass plate. The types of limb support and step patterns used by the mice – in addition to the regularity of those step patterns (regularity index) were also recorded.

2.8 Forelimb Grip Strength

Forelimb grip strength was assessed using a grip strength meter (Columbus Instruments, Columbus, Ohio). Each mouse grasped the pull bar of the apparatus with their forelimbs and the tail of the mouse was gently pulled back horizontally, such that the body of the mouse was parallel to the bar. The peak force in Newton's (N) applied to the bar was recorded. Each mouse underwent 5 separate trials, and the average of these trials were taken.

2.9 Transcardial Mouse Perfusion, Tissue Preservation and Slicing

Mice were anesthetized using a 10% ketamine and 5% xylazine mixture diluted in 0.9% sodium chloride and subsequently euthanized by transcardiac perfusion with 1x phosphate buffered saline (PBS, pH 7.4). For each mouse, one hemibrain was stored at -80°C for biochemical analyses, while the other hemibrain was post-fixed in 4% paraformeldahyde overnight at 4°C and then stored in 1x PBS and 0.02% azide at 4°C until use. Two pieces of ear tissue were collected from each mouse as well.

Male 5xFAD hemibrains were cryopreserved using increasing concentrations of sucrose (15%, 20%, 30%), embedded in optimal cutting temperature (OCT) compound and frozen at -80°C. Sagittal sections (10μm) were cut using a cryostat (Leica Biosystems, Weltzar, Germany), directly mounted and frozen. All slides were immersed in 70% ethanol for 1 minute followed by distilled water for another minute before they were stained as described below.

Female 5xFAD hemibrains were sliced (30μm) using a vibratome (Leica Biosystems, Weltzar Germany) and free-floating sections were placed into cold 1x PBS in a 24-well plate.

2.10 Thioflavin-S Stain

Slices were washed twice with distilled water, stained with a filtered 1.25% Thioflavin-S solution in 50% ethanol for 8 minutes at room temperature (Sun et al., 2002). Slices were then washed twice with 80% ethanol, once with 95% ethanol, and then three times with distilled water (Sun et al., 2002). Slices were then stained with To-Pro-3 (Life Technologies, Bibco, Carlsbad, CA)

2.11 Amyloid-Beta Immunohistochemistry

Slices were washed in 1x Tris buffered saline (TBS), and then permeabilized with 1% Triton X-100 (Tx) in 1x TBS for 15 minutes. Non-specific binding was blocked using 2% horse serum (HS), 2% bovine serum albumin (BSA) and 0.3% Tx in TBS1x. Slices were then incubated overnight at 4°C with 6E10 primary antibody (Biolegend, San Diego, CA) diluted in TBS 1x (1:300). The next day, following two TBS 1x washes, slices were incubated at 4°C in 488 goat-anti-mouse secondary antibody (Life Technologies, Bibco, Carlsbad, CA) diluted in TBS 1x (1:1000), 1% HS and 1% BSA. Slices were then rinsed three times with TBS 1x before being stained with To-Pro (Life Technologies, Bibco, Carlsbad, CA).

2.12 To-Pro-3 Iodide Nuclear Stain

Slices were washed three times in PBS 1x, and then incubated with To-Pro-3-Iodide (Life Technologies, Bibco, Carlsbad, CA) diluted in PBS 1x (1:1000) for 15 minutes at room temperature. Slices were then washed three more times in PBS 1x before being mounted onto slides and/or cover-slipped.

2.13 Microscopy and Quantification

Mounted slices were imaged using the Leica-TSC SP8 or SP5 (Leica Biosystems, Weltzar, Germany) using the 20x/0.75 objective and quantified using ImageJ (National Institutes of Health). For each mouse, the cortex and hippocampus (dentate gyrus, CA3, CA1b and CA1a) of 3-4 slices were imaged and quantified in terms of percentage area. The experimenter was blind to genotype during image acquisition and quantification.

2.14 Statistical Analysis

All data are expressed as mean ± SEM. Analyses were done using GraphPad Prism 7.0a. Comparisons between two experimental groups were done with the Student's t-test. When several experimental groups or treatments were analyzed, a two-way analysis of variance (ANOVA) or a repeated measures (RM) two-way ANOVA was used. When ANOVA results were significant, a Sidak's multiple comparisons post-hoc analysis test was used. Outliers were removed using the regression outlier removal (ROUT) method (Motulsky and Brown, 2006) with a maximum false discovery rate (Q) of 1%. Mice that did not complete 10 sessions of reversal in PVD were also excluded.

3 Results

3.1 Visual Discrimination and Reversal Learning in 5xFAD Mice

Patients with AD begin to demonstrate deficits in visual discrimination and cognitive flexibility in the early stages of the disease (Albert, 1996; Pal et al., 2016; Quental et al., 2013). Visuospatial function is an important contributor to functional status in AD patients, accurately predicting the prognosis of the patient (Fukui and Lee, 2009). Visuospatial function involves the prefrontal and perirhinal corticies (Brigman and Rothblat, 2008; Bussey et al., 1997; Chudasama and Robbins, 2003; Winters et al., 2010) and the striatum (Brigman et al., 2013). It is important to determine whether 5xFAD mice also replicate these aspects of the disease, as it would add to the validity and versatility of this AD mouse model.

In order to evaluate visual discrimination and cognitive flexibility in 5xFAD mice, we subjected the mice to the PVD touchscreen task beginning at 4, 7 and 10 months of age – each with a different set of S+ and S- stimuli. Analysis of male 5xFAD mice on the learning phase of the visual discrimination task showed that the number of sessions taken by 5xFAD and control mice to achieve initial touch, must initiate and must touch criteria at the 4-month time-point did not differ (all mice took 1 session to reach each criterion; Figure 5A). In addition, the number of sessions taken by 5xFAD mice to achieve acquisition criteria did not differ from WT mice at the 4-month ($t_{(18)} = 0.8865$, $p = 0.3781$; Figure 5B), 7-month ($t_{(18)} = 0.587$, $p = 0.5645$; Figure 5C), or the 10-month time-point ($t_{(24)} = 1.565$, $p = 0.1312$; Figure 5D). Likewise, the total number of trials (out of 30) completed by 5xFAD and WT for each session in baseline and reversal were not

significantly different at the 4-month time-point ($F_{(1,18)}$ = 1.968, p=0.1777; Figure 6E). The total number of trials completed by 5xFAD and WT for each session in baseline, reversal and retention reversal were not significantly different at the 7-month: $F_{(1, 18)}$ = 0.3517, p=0.5605; Figure 7E) and 10-month time-point ($F_{(1, 23)}$ = 1.354, p=0.1386, Figure 8E) as well. The number of sessions completed by the mice increased as reversal progressed at the 4-month ($F_{(11,198)}$ = 18.93, p<0.0001, no significant interaction with genotype: $F_{(11,198)}$ = 1.234, p=0.2662; Figure 6E), 7-month ($F_{(13, 324)}$ = 19.78, p<0.0001, no significant interaction with genotype: $F_{(13,324)}$ = 0.8073, p=0.6521; Figure 7E) and 10-month time-point ($F_{(13, 299)}$ = 11.99, p<0.0001, no significant interaction with genotype: $F_{(13, 299)}$ = 1.475, p=0.1255; Figure 8E).

During reversal learning, the accuracy of male 5xFAD mice and WT controls improved over 10 sessions at the 4-month ($F_{(11, 198)}$ = 54.17, p<0.0001, no significant interaction with genotype: $F_{(11, 198)}$ = 1.032, p=0.4195; Figure 6A), 7-month ($F_{(13, 324)}$ = 58.83, p<0.0001, significant interaction with genotype: $F_{(13,324)}$ = 2.192, p=0.0106; Figure 7A) and 10-month time-point ($F_{(13, 299)}$ = 41.18, p<0.000, no significant interaction with genotype: $F_{(13, 299)}$ = 1.523, p=0.1079; Figure 8A). Accuracy did not differ between 5xFAD mice and WT controls during baseline or reversal learning at the 4-month time-point ($F_{(1,18)}$ = 0.06227, p=0.8058; Figure 6A), or during baseline, reversal learning and retention reversal at the 7-month ($F_{(1, 18)}$ = 0.6589, p=0.4275; Figure 7A) and 10-month time-point ($F_{(1, 23)}$ = 1.077, p=0.3102; Figure 8A).

The number of correction trials done by male 5xFAD mice and the WT controls decreased over the 10 sessions of reversal at the 4-month ($F_{(11, 198)}$ = 51.33, p<0.0001, significant interaction with genotype: $F_{(11, 198)}$ = 1.032, p=0.4195; Figure 6B), 7-month

($F_{(13, 324)}$ = 58.3, p<0.0001, no significant interaction with genotype: $F_{(13,324)}$ = 0.5936, p=0.8662; Figure 7B) and 10-month time-point ($F_{(13, 299)}$ = 77.15, p<0.0001, no significant interaction with genotype: $F_{(13,299)}$ = 1.066, p=0.3886; Figure 8B). The number of correction trials also did not differ between 5xFAD mice and WT controls during baseline or reversal at the 4-month time-point ($F_{(1,18)}$ = 3.571, p=0.0750, Figure 6B), or during baseline, reversal and retention reversal at the 7-month ($F_{(1, 18)}$ = 0.01309, p=0.9102, Figure 7B) and 10-month time-point ($F_{(1, 23)}$ = 0.07534, p=0.7862; Figure 8B).

The time taken by male 5xFAD mice and the WT controls to correctly respond to the task decreased as they progressed through baseline and reversal at the 4-month ($F_{(11, 198)}$ = 7.941, p<0.0001, no significant interaction with genotype: $F_{(11, 198)}$ = 0.4088, p=0.9511; Figure 6C), 7-month ($F_{(13, 324)}$ = 20.05, p<0.0001, no significant interaction with genotype: $F_{(13,324)}$ = 1.147, p=0.3205; Figure 7C) and 10-month time-point ($F_{(13, 299)}$ = 11.59, p<0.0001, no significant interaction with genotype: $F_{(13, 299)}$ = 1.244, p=0.2470; Figure 8C). The correct response latency did not differ between 5xFAD mice and WT controls during baseline or reversal at the 4-month time-point ($F_{(1,18)}$ = 4.012, p=0.0605; Figure 6C), or during baseline, reversal and retention reversal at the 7-month time-point ($F_{(1, 18)}$ = 0.703, p=0.4128; Figure 7C). However, at the 10-month time-point, 5xFAD mice take significantly longer than their WT controls to correctly respond to the task during baseline, reversal and retention reversal ($F_{(1, 23)}$ = 16.89, p=0.0004; Figure 8C).

The time taken by male 5xFAD mice and the WT controls to collect the reward decreased as the mice progressed through baseline and reversal at the 4-month ($F_{(11, 198)}$ = 3.095, p=0.0007, no significant interaction with genotype: $F_{(11, 198)}$ = 0.7969,

p=0.6431; Figure 6D), but not at the 7-month ($F_{(13, 324)}$ = 1.023, *p*=0.4296, no significant interaction with genotype: $F_{(13,324)}$ = 0.8913, *p*=0.5630; Figure 7D) and 10-month time-point ($F_{(13, 299)}$ = 1.3, *p*=0.2116, no significant interaction with genotype: $F_{(13, 299)}$ = 0.3533, *p*=0.9823; Figure 8D). The reward collection latency did not differ between 5xFAD mice and WT controls during baseline or reversal at the 4-month time-point ($F_{(1,18)}$ = 4.094, *p*=0.0581; Figure 6D), or during baseline, reversal and retention reversal at the 7-month time-point ($F_{(1, 18)}$ = 3.496, *p*=0.0779; Figure 7D). However at the 10-month time-point, 5xFAD mice take significantly longer than their WT controls to correctly respond to the task during baseline, reversal and retention reversal ($F_{(1, 23)}$ = 57.12, *p*<0.0001; Figure 8D).

There were two WT outliers (as calculated using the ROUT method and a G of 1%) at the 4-month time-point.

In summary, male 5xFAD mice did not demonstrate significant differences in task acquisition or performance during reversal when compared to their WT controls at all time-points. However, at the 10-month time-point, 5xFAD mice take significantly longer than their WT controls to correctly respond to the the task and collect the reward during baseline, reversal and retention reversal.

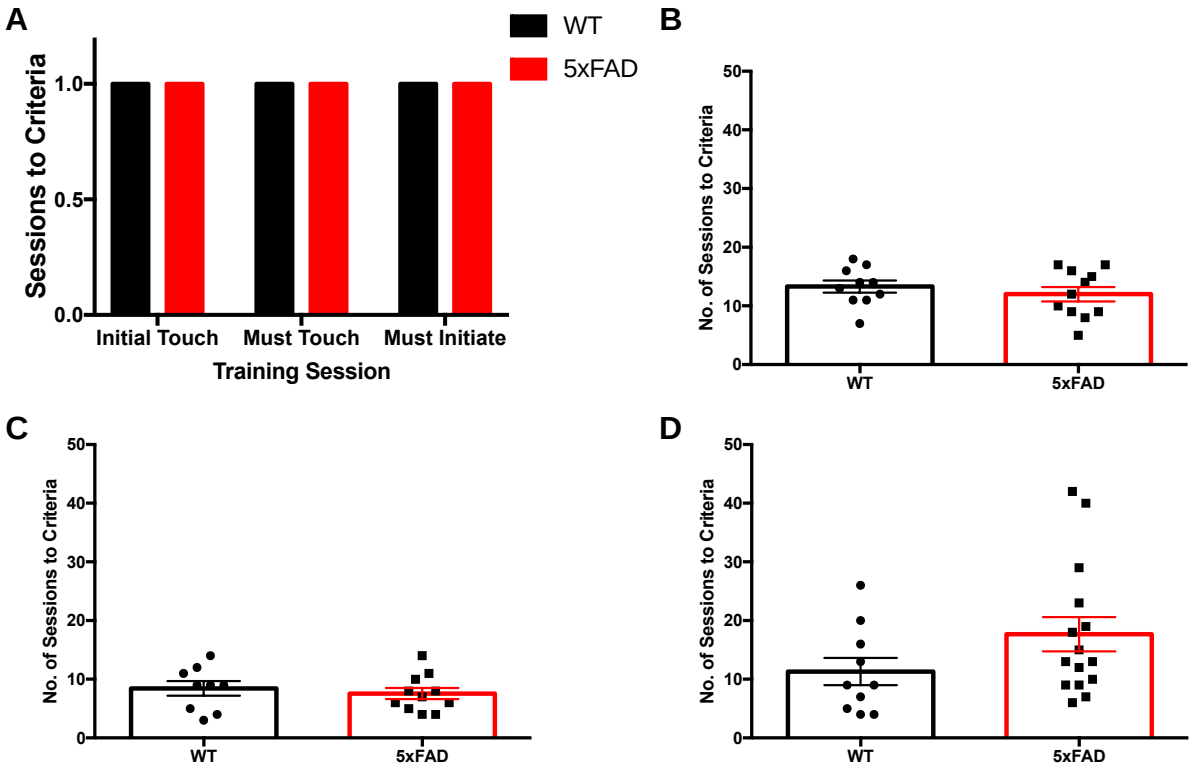


Figure 5. Learning phase of the PVD task.

Number of sessions taken to achieve A) initial touch, must initiate and must touch criterion at 4-months of age (5xFAD: n=11, WT: n=10) (Mean ± SEM of the number of sessions taken by 5xFAD mice and their WT controls to reach aquisiton criterion at B) 4 month (5xFAD: n=11, WT: n=10), C) 7 month (5xFAD: n=12, WT: n=9) and D) 10 month time-point (5xFAD: n=15, WT: n=10). Repeated measures two-way ANOVA (A) or unpaired two-tailed t-test (B-D).

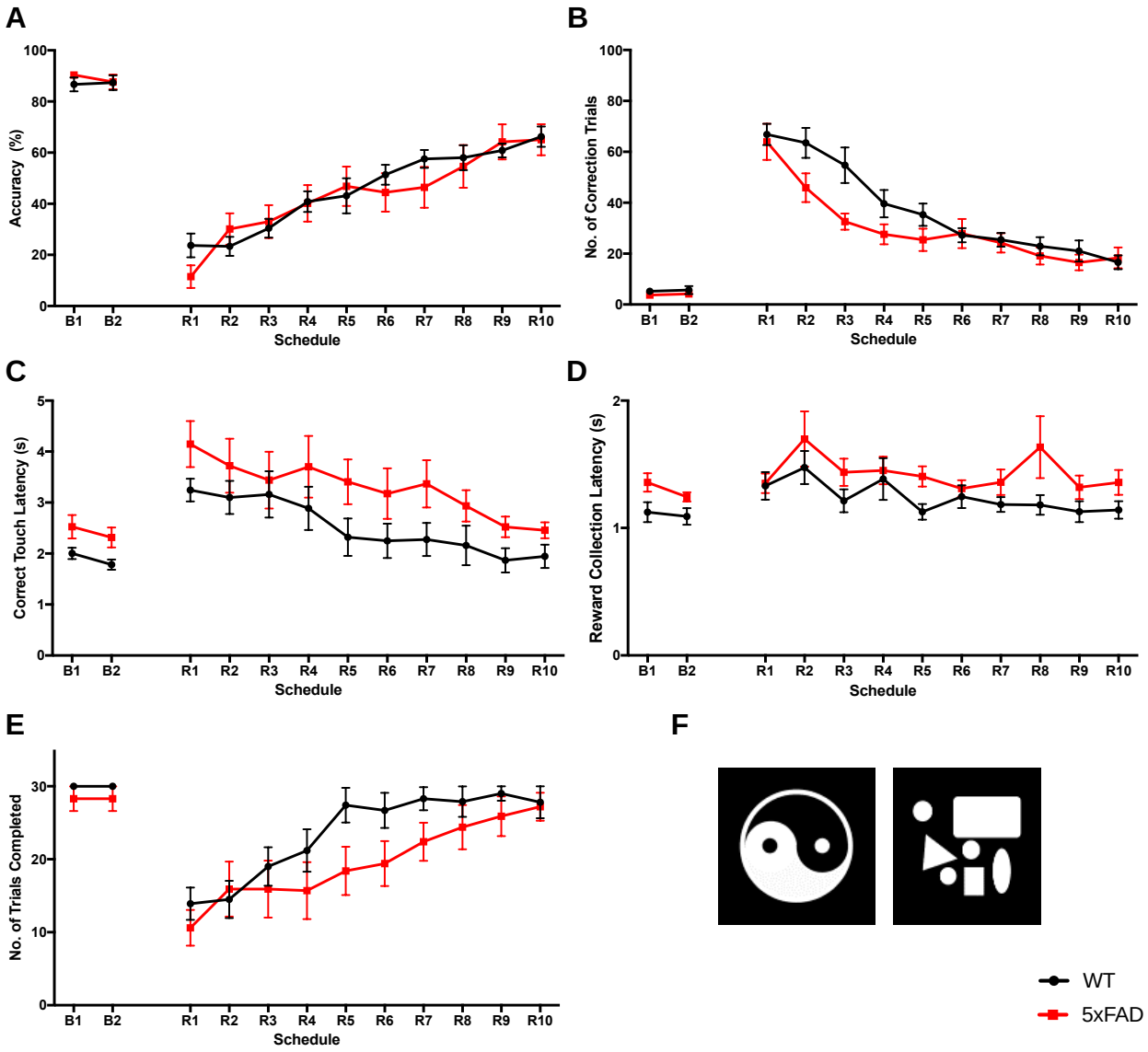


Figure 6. Reversal learning phase of the PVD task at the 4-month time-point.

A) Mean ± SEM accuracy (%), B) number of correction trials, C) correct response latency (s), D) reward collection latency (s), E) number of trials completed by 5xFAD mice (n=11) and their WT (n=10) controls and F) the stimuli used at the 4-month time-point. Parameters were measured across baseline (B) days 1 and 2 (B1, B2) and reversal (R) days 1 to 10 (R1-R10). Repeated-measures two-way ANOVA.

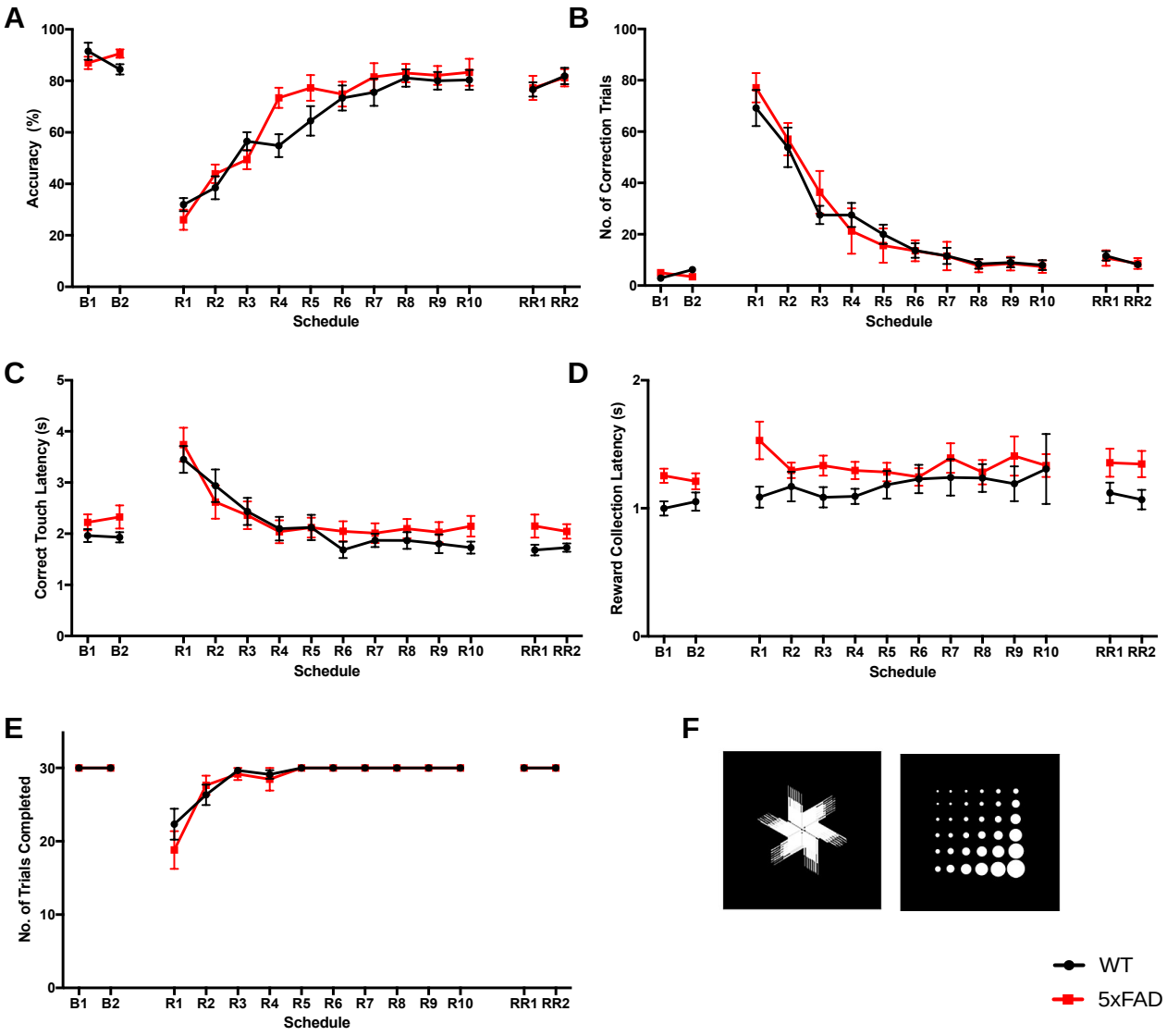


Figure 7. Reversal learning phase of the PVD task at the 7-month time-point.

A) Mean ± SEM accuracy (%), B) number of correction trials, C) correct response latency (s), D) reward collection latency (s), E) number of trials completed by 5xFAD mice (n=12) and their WT (n=9) controls and F) stimuli used at the 7-month time-point. Parameters were measured across baseline days 1 and 2 (B1, B2), reversal days 1 to 10 (R1-R10) and retention reversal (RR) days 1 and 2 (RR1, RR2). Repeated measures two-way ANOVA.

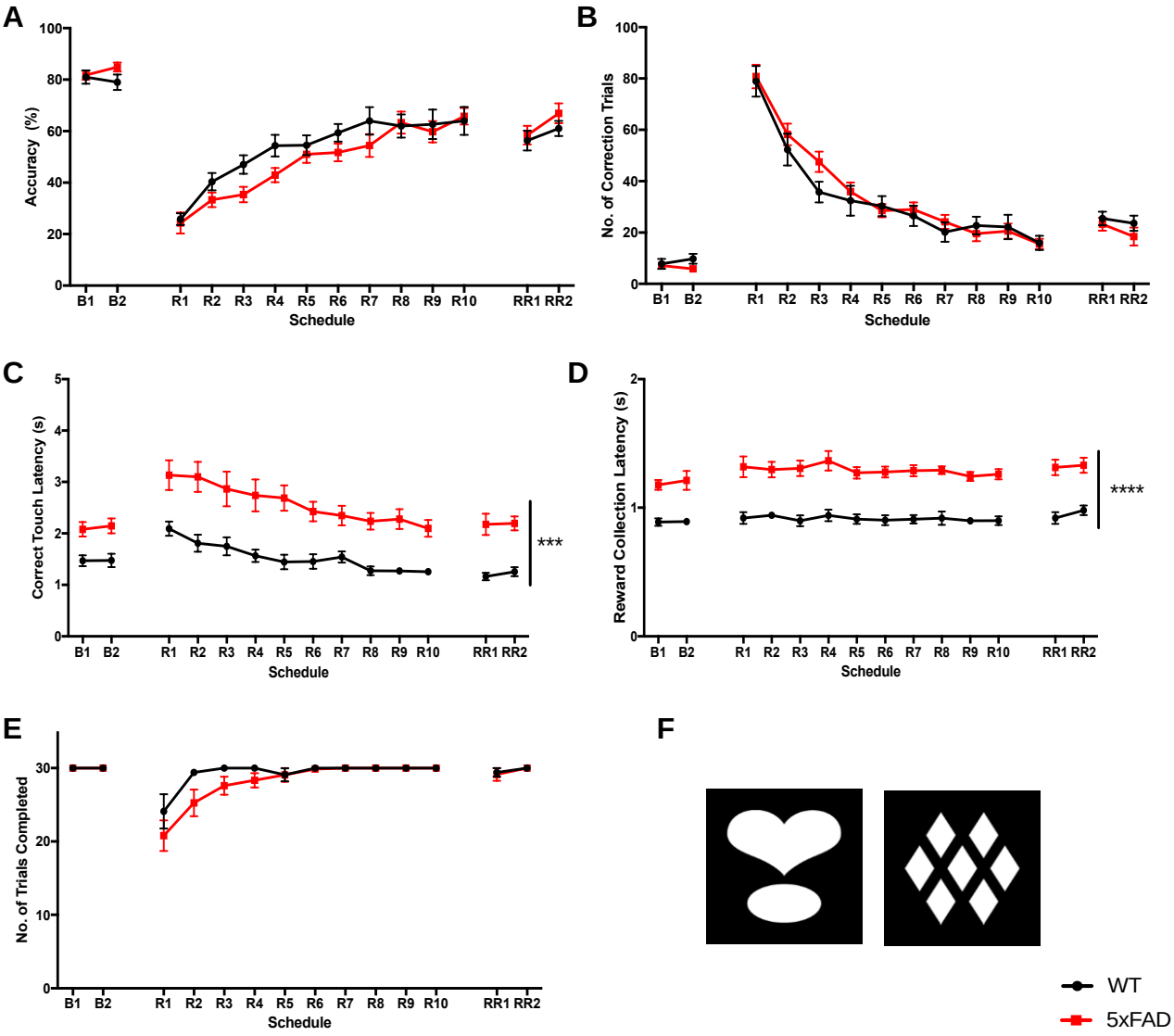


Figure 8. Reversal learning phase of the PVD task at the 10-month time-point.

A) Mean ± SEM accuracy (%), B) number of correction trials, C) correct response latency (s), D) reward collection latency (s), E) number of trials completed by 5xFAD mice (n=15) and their WT (n=10) controls and F) stimuli used at the 10-month time-point. Parameters were measured across baseline days 1 and 2 (B1, B2), reversal days 1 to 10 (R1-R10) and retention reversal days 1 and 2 (RR1, RR2). Repeated measures two-way ANOVA (****p<0.0005, ****p<0.0001)*.

3.2 Genotyping *Pdeb*^{rd1}

Fifteen 5xFAD mice and twelve of their WT controls were genotyped for the recessive retinal denegation 1 mutation of the β-subunit of cGMP phosphodiesterase (Figure 9). It is important to ensure that none of the mice are affected by this mutation as performance on the touchscreen tasks require the mouse to see the images/stimuli. Out of the 15 5xFAD mice that were genotyped, 10 of the mice (67%) were homozygous for the WT *Pdeb* allele, while 5 (33%) were heterozygous for the *Pdeb*^{rd1} allele. Out of the 12 WT mice, 2 mice (17%) were homozygous for the WT *Pdeb* allele, while 10 (83%) of the WT mice were heterozygous for the *Pdeb*^{rd1} allele. None of the mice genotyped were homozygous for the *Pdeb*^{rd1} allele. Thus, vision in these 5xFAD mice should not be affected by this mutation.

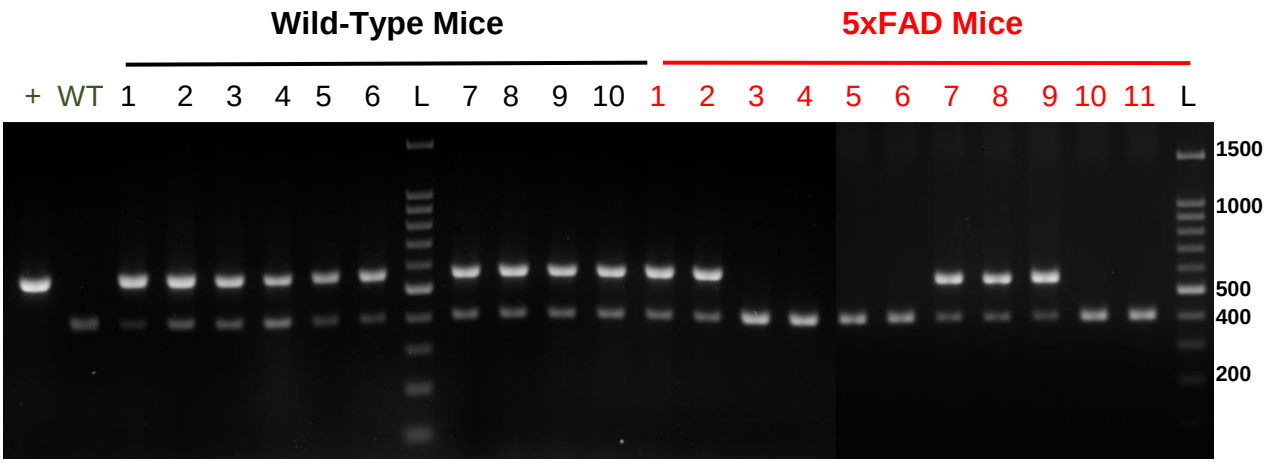


Figure 9. Representative PDEB^{rd1} genotyping results.
5xFAD mice (red) and WT controls (black) were genotyped as described. In the first lane, there is a positive control (+) for the 550bp *Pdeb*^{rd1} mutant allele obtained from FVB/NJ mice, followed by a WT control for the 400bp wild-type PDEB allele (WT). Ladder (L).

3.2.1 *The effect of carrying the Pdeb^{rd1} allele on performance in PVD at the 10-month time-point*

The 5xFAD mice that were genotyped for *Pdeb^{rd1}* were used to evaluate whether carrying one *Pdeb^{rd1}* allele affected performance in the touchscreens. Although none of the mice were homozygous for this allele, it is important to evaluate whether the presence of the allele played any role in task performance.

At the 10-month time-point, the accuracy of male 5xFAD mice that carried the *Pdeb^{rd1}* allele did not differ significantly from those who did not ($F_{(1, 13)} = 0.862$, $p=0.3701$; Figure 10A), although accuracy significantly increased across the sessions ($F_{(13, 169)} = 21.48$, $p<0.0001$, no interaction with group: $F_{(13, 169)} = 0.9362$, $p=0.5171$; Figure 10A). The number of correction trials done by both groups decreased over the sessions of reversal and retention reversal ($F_{(13, 169)} = 31.07$, $p<0.0001$, no interaction with group: $F_{(13, 169)} = 0.5835$, $p=0.8651$; Figure 10B) and did not differ between groups ($F_{(1, 13)} = 2.109$, $p=0.1701$; Figure 10B). The amount of time taken to correctly respond to the task did not significantly differ between groups ($F_{(1, 13)} = 3.031$, $p=0.1053$; Figure 10C), but significantly decreased with session ($F_{(13, 169)} = 6.252$, $p<0.0001$, no interaction with group: $F_{(13, 169)} = 1.203$, $p=0.2805$; Figure 10C). The time taken to collect the reward did not differ between groups ($F_{(1, 13)} = 1.189$, $p=0.2952$; Figure 10D) and was not affected by session ($F_{(13, 169)} = 0.6317$, $p=0.8251$, no interaction with group: $F_{(13, 169)} = 1.106$, $p=0.3573$; Figure 10D). Both groups completed the same number of trials ($F_{(1, 13)} = 0.7796$, $p=0.3933$; Figure 10E), which increased across the sessions of reversal ($F_{(13, 169)} = 7.119$, $p>0.0001$, no interaction with group: $F_{(13, 169)} = 1.237$, $p=0.2568$; Figure

10E).

In summary, carrying the *Pdeb*^{rd1} allele did not affect the performance of male 5xFAD mice in the PVD task.

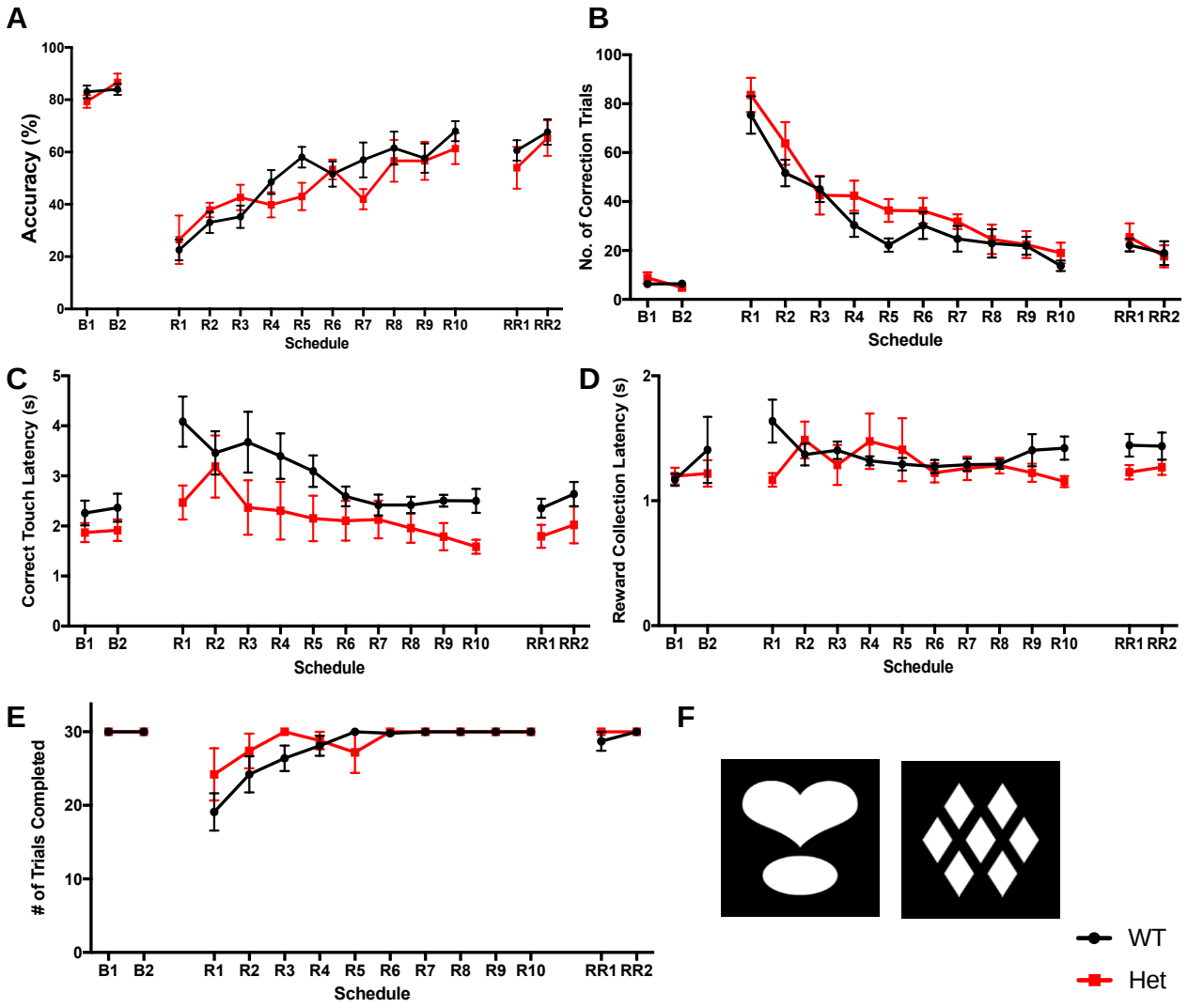


Figure 10. Reversal learning phase of the PVD task at the 10-month time-point separated by *Pdeb*^{rd1} genotype.

A) Mean ± SEM accuracy (%), B) number of correction trials, C) correct response latency (s), D) reward collection latency (s), E) number of trials completed by 5xFAD mice that did (n=5) and did not (n=10) carry the *Pdeb*^{rd1} allele and F) stimuli used at the 10-month time-point. Parameters were measured across baseline days 1 and 2 (B1, B2) reversal days 1 to 10 (R1-R10) and retention reversalndays 1 and 2 (RR1, RR2). Repeated measures two-way ANOVA.

3.3 The Effect of Donepezil on Attention in 5xFAD mice

Deficits in attention have been observed in the 3xTG mouse model of AD (Romberg et al., 2011) and linked to cholinergic dysfunction (Klinkenberg et al., 2011; Proulx et al., 2015; Sahakian et al., 1993). Romberg and colleagues were also able to rescue deficits in these mice following treatment with intraperitoneal injections of donepezil. Our lab has also observed attentional deficits in male and female 5xFAD mice between 7-10 months of age. The question remains whether donepezil can rescue these impairments in 5xFAD mice as well.

3.3.1 *Infusion of donepezil affects vigilance in the 5-CSRTT*

In addition to reducing stress and chances of injury, the infusion of donepezil has the potential to increase its therapeutic index, efficacy and/or reduce any side effects. Thus we choose initially to infuse donepezil into the male 5xFAD mice rather than performing daily injections for the duration of the task. At approximately 10 months of age, male 5xFAD mice were implanted with subcutaneous osmotic pumps that would release donepezil or saline + 10% DMSO for 28 days. The 5xFAD mice treated with donepezil completed as many trials as those treated with saline + 10% DMSO ($F_{(1, 14)} = 1.138$, p=0.3402; Figure 11). Stimulus length had no effect on the number of trials completed by 5xFAD mice ($F_{(3, 42)} = 0.922$, p=0.4059, no significant interaction with treatment: $F_{(3, 42)} = 0.8001$, p=0.5008; Figure 11) after treatment with donepezil.

Donepezil had no significant effect on the percentage accuracy ($F_{(1, 14)} = 0.7788$, p=0.3895; Figure 12A) or omission ($F_{(1, 14)} = 0.5874$, p=0.4595; Figure 12B) of 5xFAD mice in the 5-CSRTT. As previously observed, accuracy decreased ($F_{(3, 42)} = 21.83$,

p<0.0001, no significant interaction with treatment: $F_{(3, 42)}$ = 0.9016, p=0.4484; Figure 12A) and the percentage of omissions increased ($F_{(3, 42)}$ = 34.62, p<0.0001, no significant interaction with treatment: $F_{(3, 42)}$ = 0.1138, p=0.9515; Figure 12B) as stimulus length decreased.

The accuracy of 5xFAD mice generally increased across bins at the 1.5s ($F_{(4, 68)}$ = 5.484, p=0.0007, no significant interaction with treatment: $F_{(4, 68)}$ = 0.2494, p=0.9091; Figure 13A), 1.0s ($F_{(4, 67)}$ = 6.372, p=0.0002, no significant interaction with treatment: $F_{(4, 67)}$ = 0.2116, p=0.9311; Figure 13C), 0.8s ($F_{(4, 66)}$ = 3.868, p=0.0070, no significant interaction with treatment: $F_{(4, 66)}$ = 0.02601, p=0.9987; Figure 13E), or 0.6s ($F_{(4, 67)}$ = 2.787, p=0.0333, no significant interaction with treatment: $F_{(4, 67)}$ = 0.02416, p=0.9988; Figure 13G) stimulus length. Meanwhile the percentage of omissions was significantly affected across the bins at the 1.5s stimulus length ($F_{(4, 68)}$ = 3.455, p=0.0125, no significant interaction with treatment: $F_{(4, 68)}$ = 0.1124, p=0.9778; Figure 13B), but not at the 1.0s ($F_{(4, 67)}$ = 0.1299, p=0.9710, no significant interaction with treatment: $F_{(4, 67)}$ = 0.2629, p=0.9007; Figure 13D), 0.8s ($F_{(4, 66)}$ = 0.1031, p=0.9810, no significant interaction with treatment: $F_{(4, 66)}$ = 0.2421, p=0.9134; Figure 13F), or 0.6s ($F_{(4, 67)}$ = 0.1367, p=0.9682, no significant interaction with treatment: $F_{(4, 67)}$ = 0.8046, p=0.5266; Figure 13H) stimulus length. Treatment with donepezil did not affect the accuracy or omissions of 5xFAD mice across bins at the 1.5s (accuracy: $F_{(1, 68)}$ = 0.2879, p=0.5933; Figure 13A, omissions: $F_{(1, 68)}$ = 3.746, p=0.0571; Figure 13B), 0.8s (accuracy: $F_{(1, 66)}$ = 0.3762, p=0.5418; Figure 13E, omissions: $F_{(1, 66)}$ = 0.689, p=0.4095; Figure 13F) or at the 0.6s stimulus length (accuracy: $F_{(1, 67)}$ = 0.3393, p=0.5622; Figure 13G, omissions: $F_{(1, 67)}$ = 2.133, p=0.1489; Figure 13H). However, at the 1.0s stimulus length, donepezil

treatment led to a significant increase in the percentage of omissions ($F_{(1, 67)}$ = 7.488, p=0.0079; Figure 13D). Accuracy was not affected at this stimulus length ($F_{(1, 67)}$ = 0.4167, p=0.5208; Figure 13C).

Correct response latency decreased as stimulus length decreased ($F_{(3, 42)}$ = 5.07, p=0.0044, no significant interaction with treatment: $F_{(3, 42)}$ = 0.6235, p=0.6038; Figure 12C) and was not affected by donepezil treatment ($F_{(1, 14)}$ = 0.2169, p=0.6486; Figure 12C). Reward collection latency remained unaffected by stimulus length ($F_{(3, 42)}$ = 0.008512, p=0.9678, no significant interaction with treatment: ($F_{(3, 42)}$ = 0.6874, p=0.5664; Figure 12D), and was also not affected by donepezil treatment ($F_{(1, 14)}$ = 0.2302, p=0.6388; Figure 12D).

The number of premature responses did not differ in 5xFAD mice treated with donepezil ($F_{(1, 14)}$ = 3.197, p=0.0954; Figure 12E). This was not affected by the stimulus length ($F_{(3, 42)}$ = 0.04979, p=0.9851, no significant interaction with treatment: $F_{(3, 42)}$ = 0.1322, p=0.9403; Figure 12E). The number of preservative responses did not differ between 5xFAD mice that were treated with donepezil and 5xFAD mice that were treated with saline + 10% DMSO ($F_{(1, 14)}$ = 1.08, p=0.3163; Figure 12F), although for both groups, the number of preservative responses increased as stimulus length decreased ($F_{(3, 42)}$ = 3.074, p=0.0379, no significant interaction with treatment: $F_{(3, 42)}$ = 0.9088 p=0.4449; Figure 12F).

In summary, other than leading to a significant increase in the percentage of omissions at the 1.0s stimulus length, administration of donepezil using the ALZET pump has no significant effect on the performance of 5xFAD mice in the 5-CSRTT.

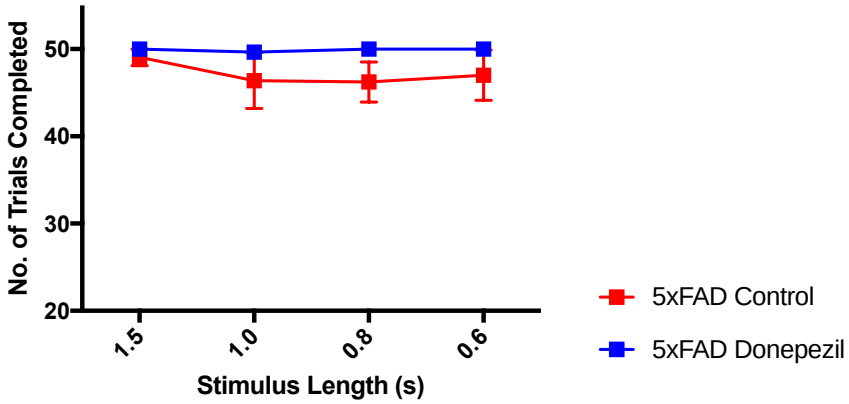


Figure 11. Number of trials completed by 5xFAD mice in the 5-CSRTT.

Mean ± SEM of the number of trials (maximum 50) completed by 5xFAD mice infused with saline + 10% DMSO (n=9) and donepezil (n=7) at each stimulus length. Repeated measures two-way ANOVA.

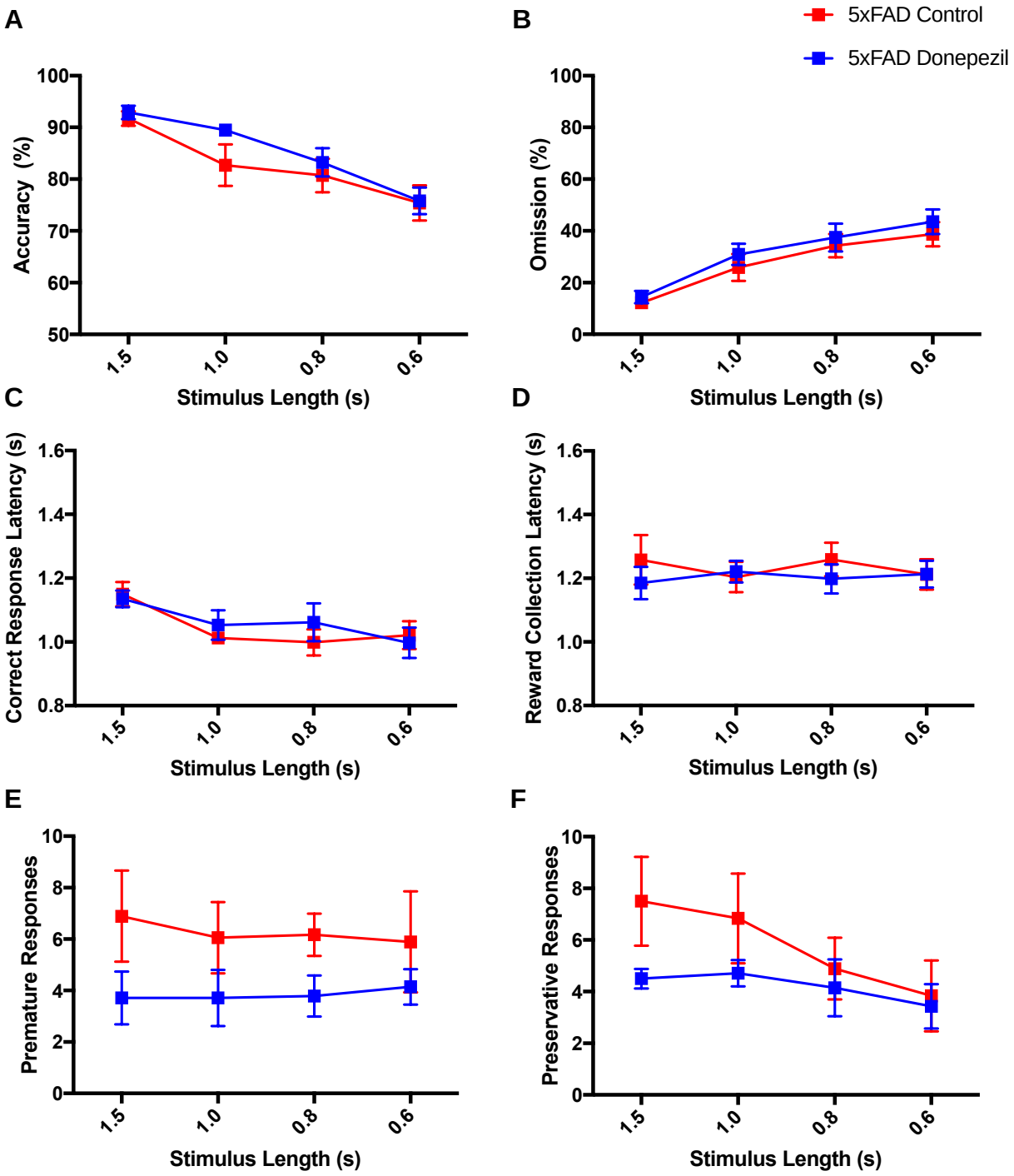
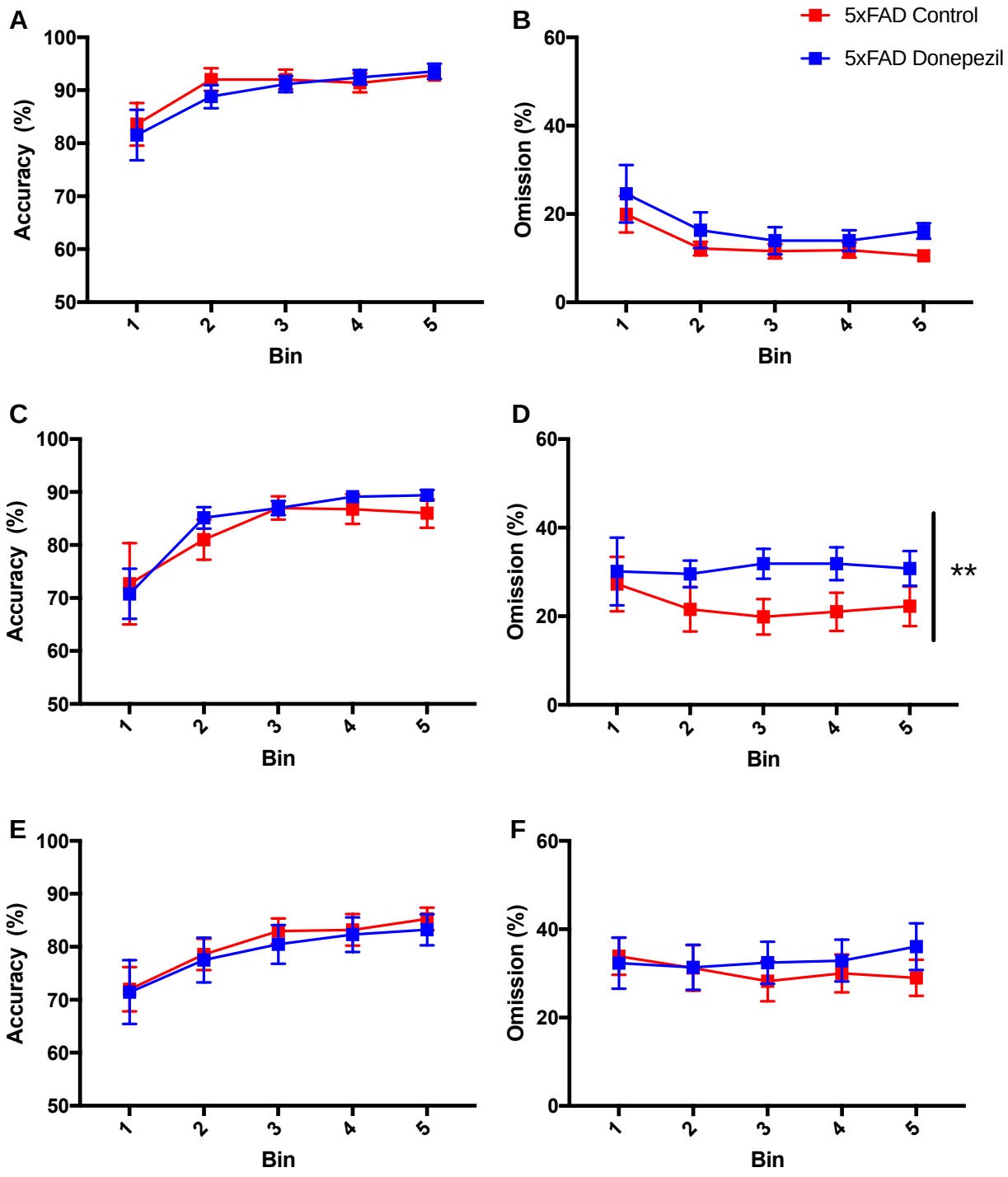


Figure 12. Performance and response measures of 5xFAD mice during the 5-CSRTT probe trial.

A) Mean ± SEM accuracy (%), B) omission (%), C) correct response latency (s), D) reward collection latency (s), E) number of premature responses and F) number of preservative responses in 5xFAD mice that were implanted with osmotic pumps infusing donepezil (n=7) or saline + 10% DMSO (n=9). Repeated measures two-way ANOVA.


A
Accuracy (%)
100
90
80
70
60
50
1
2
3
4
5
Bin
B
5xFAD Control
5xFAD Donepezil
Omission (%)
60
40
20
0
Bin
C
Accuracy (%)
Bin
D
Omission (%)
**
Bin
E
Accuracy (%)
Bin
F
Omission (%)
Bin

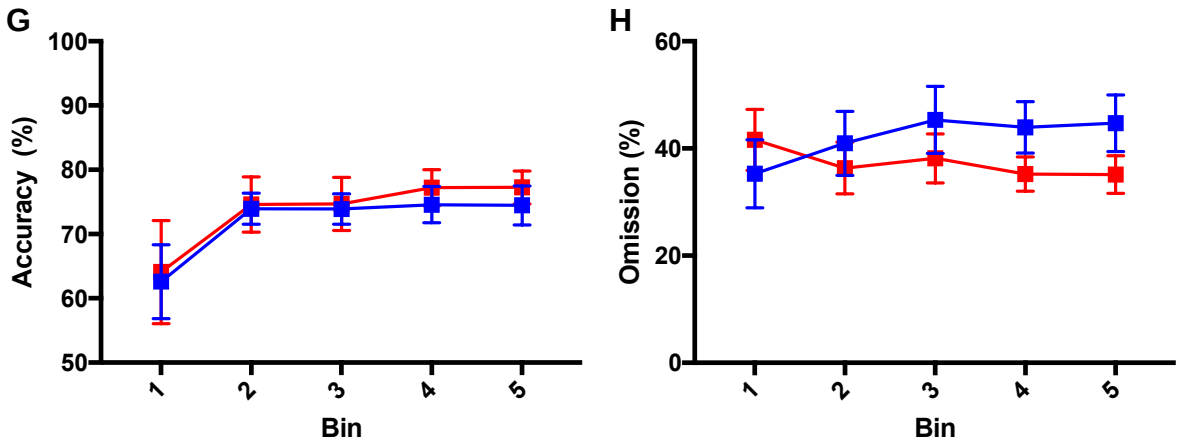


Figure 13. The vigilance of 5xFAD mice during the 5-CSRTT probe trial.

Mean ± SEM accuracy (%) and omission (%) of 5xFAD mice that were implanted with osmotic pumps infusing donepezil (n=7) or saline + 10% DMSO (n=9) at 1.5s (A, B), 1.0s (C, D), 0.8s (E, F), and 0.6s (G, H) stimulus lengths. Trials are divided into bins of 10 trials each. Two-way ANOVA (**p<0.005*).

3.3.2 *Performance of 5xFAD mice at the 0.6s stimulus length in the 5-CSRTT following Donepezil injection*

As the infusion of donepezil in 5xFAD mice did not have any effect on the performance of these mice in the 5-CSRTT, we chose to do daily injections of donepezil in order to replicate the method of administration used by Romberg et. al (2011). Male 5xFAD mice were tested in the most difficult stimulus length (0.6s) of the 5-CSRTT at approximately 13 months of age. 5xFAD mice were injected with 1mg/kg of donepezil 30 minutes prior to beginning the task. Injection of donepezil does not affect percentage accuracy ($t_{(7)}$ = 1.373, p=0.2112; Figure 15A), omission ($t_{(7)}$ = 1.296, p=0.2361; Figure 15B), correct response latency ($t_{(7)}$ = 1.094, p=0.3100; Figure 15C), reward collection latency ($t_{(7)}$ = 0.08446, p=0.9531; Figure 15D), the number of preservative responses ($t_{(7)}$ = 0.4729, p=0.6507; Figure 15E) or the number of premature responses ($t_{(7)}$ = 0.092, p=0.9293;

Figure 15F) in male 5xFAD mice. Donepezil had no significant effect on the number of trials completed ($t_{(7)}$ = 1.366, p=0.2141; Figure 15G). However, the injection of donepezil reduced the percentage of omissions in 5xFAD mice across bins of 10 trials each ($F_{(1, 66)}$ = 5.396, p=0.0233; Figure 14B). Percentage omission was not significantly affected across the bins ($F_{(4, 66)}$ = 2.431, p=0.0561, no significant interaction with treatment: $F_{(4, 66)}$ = 0.1748, p=0.9506; Figure 14B). Percentage accuracy was not affected by treatment ($F_{(1, 66)}$ = 1.744, p=0.1913; Figure 14A), but increased across bins ($F_{(4, 66)}$ = 5.988, p=0.0004, no significant interaction with treatment: $F_{(4, 66)}$ = 0.175, p=0.9505; Figure 14A).

In summary, injection of donepezil reduces the omission percentage in male 5xFAD mice across bins of ten trials each in the 0.6s second stimulus length of the 5-CSRTT at 13-months of age.

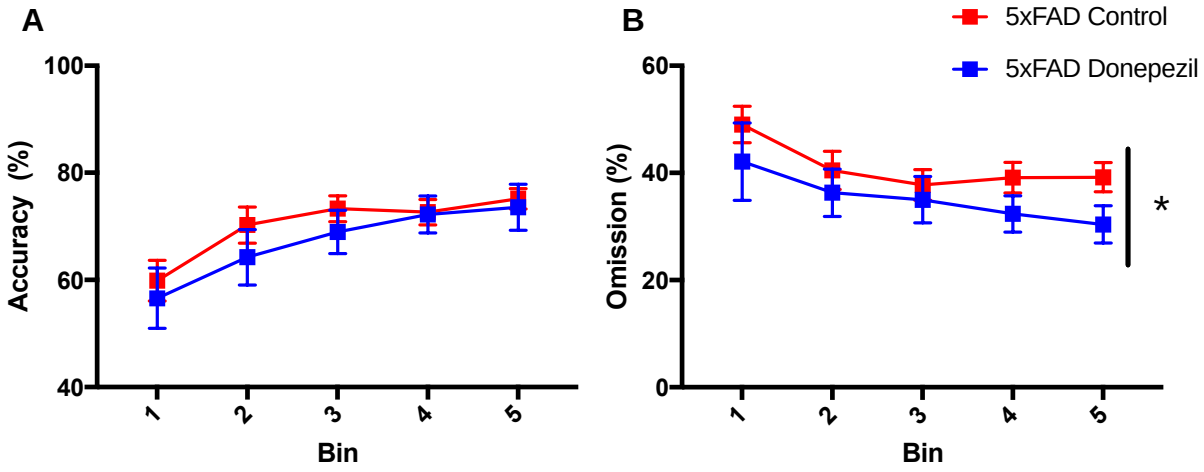


Figure 14. The vigilance of 5xFAD mice during the 0.6s stimulus length of the 5-CSRTT.

A) Mean ± SEM accuracy (%) and B) omission (%) of 5xFAD mice that were implanted with osmotic pumps infusing donepezil (n=7) or saline + 10% DMSO (n=9) at 1.5s Trials are divided into bins of 10 trials each. Two-way ANOVA (*$p<0.05$).

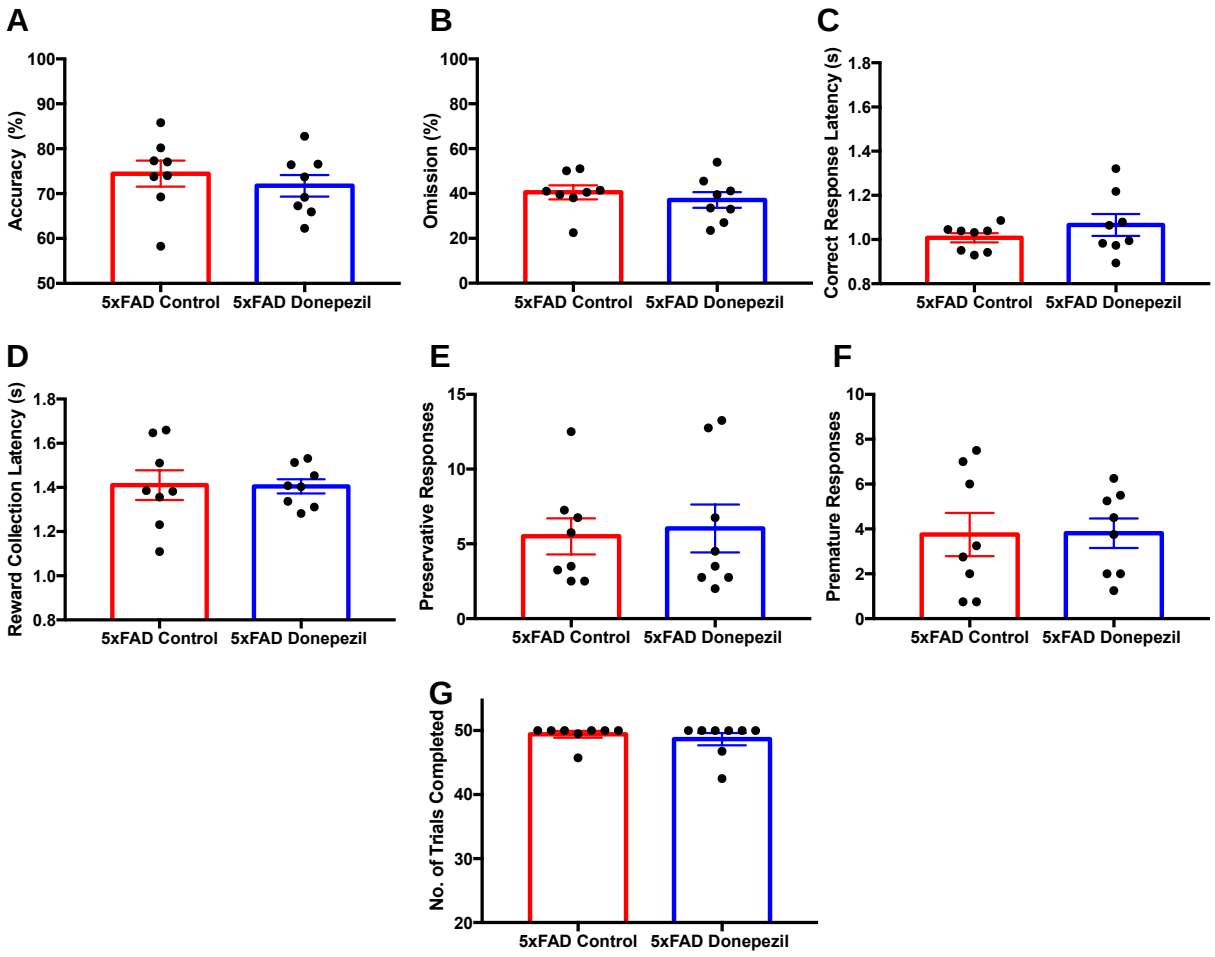


Figure 15. Performance and response measures of 5xFAD mice in the 0.6s stimulus length of the 5-CSRTT.

A) Mean ± SEM accuracy (%), B) omission (%), C) correct response latency (s), D) reward collection latency (s), E) number of premature responses and F) number of preservative responses and number of trials completed by male 5xFAD mice (n=8) following administration of donepezil (paired two-tailed t-test).

3.4 Spontaneous Locomotor Activity in Male 5xFAD Mice

Activity in open-field locomotor boxes allows us to evaluate a group of non-cognitive domains that could be important to touchscreen performance. With open-field, we can evaluate locomotion, exploratory, rearing and anxiety-like activity – some of which have been shown to be affected in 5xFAD mice (O'Leary, 2013; O'Leary et al., 2013; Schneider et al., 2014). To assess whether any of these non-cognitive parameters were affected in male 5xFAD mice at 10-months of age, 5xFAD mice and their WT controls were evaluated in open field-locomotor boxes. There was no significant difference in distance travelled between WT and 5xFAD mice every 5 minutes ($F_{(23,391)}$ = 0.000501, p=0.9824; Figure 16A) or the total distance travelled across the 2 hour testing period ($t_{(17)}$= 0.3648, p=0.7204; Figure 16B). The distance travelled by the mice decreased as the test progressed ($F_{(23, 391)}$ = 13.05, p<0.0001, no significant interaction with treatment $F_{(23, 391)}$ = 1.08 p=0.3648; Figure 16A). Genotype had no effect on rearing time ($t_{(17)}$ = 1.105 p=0.2844; Figure 16D) or center time ($t_{(17)}$ = 0.3427, p=0.7360; Figure 16C). One WT mouse and one 5xFAD mouse as they were calculated to be outliers according to the ROUT method.

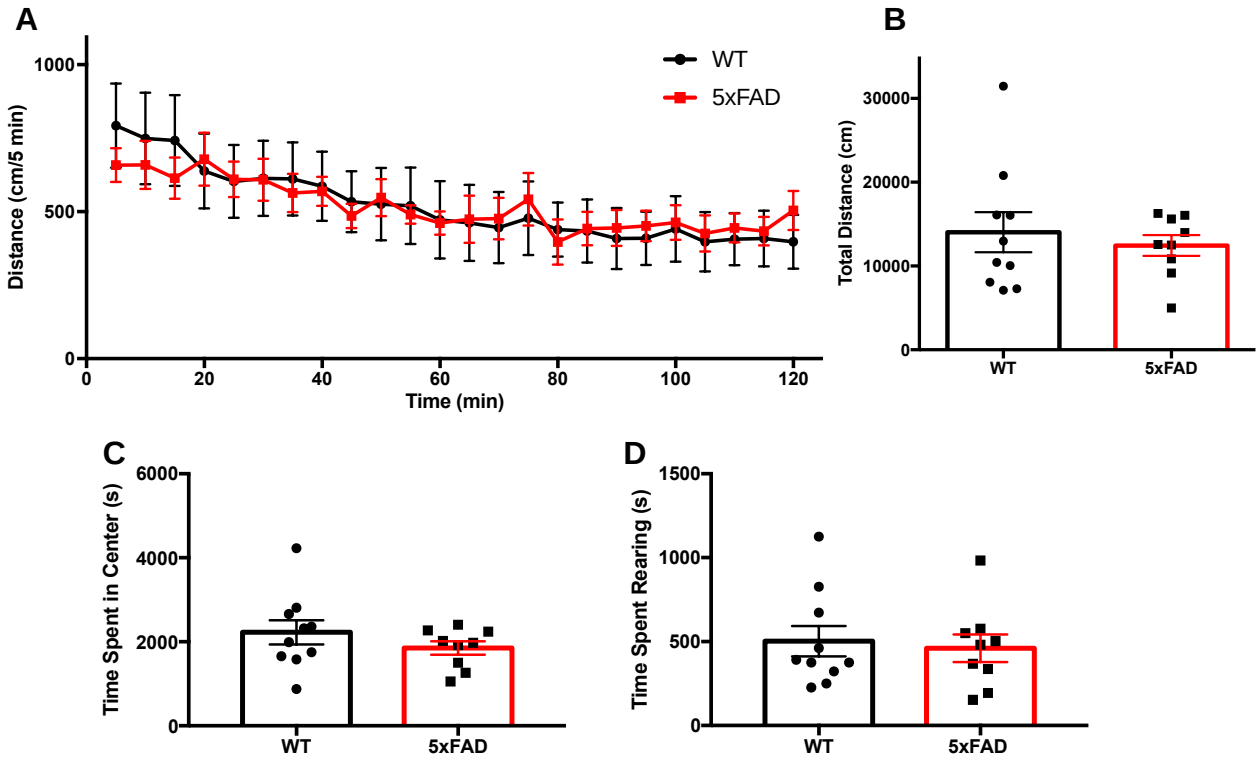


Figure 16. Spontaneous locomotor activity of 10-month old 5xFAD mice.

A) Mean ± SEM distance (cm) travelled by 10-month old male 5xFAD mice (n=9) and their WT controls (n=9) every 5 minutes for a total of 2 hours (repeated measures two-way ANOVA). B) Mean ± SEM of the total distance (cm) travelled, C) time spent in the centre (s) and D) time spent rearing (s). Unpaired two-tailed t-test.

3.4.1 *Effect of Donepezil on spontaneous locomotor activity in male 5xFAD mice*

To investigate the effect of donepezil on locomotion, anxiety and rearing activity in 5xFAD mice, following the completion of the 5-CSRTT, the locomotor activity of these mice was assessed using open-field locomotor boxes. The pumps were still implanted and releasing donepezil or saline + 10% DMSO at this time. Donepezil has previously been shown to have effects on locomotion in mice (Kim et al., 2014), and improvements in motor ability could augment touchscreen performance – specifically in terms of latencies. Infusion with donepezil had no effect on the distance travelled by 5xFAD mice every 5 minutes ($F_{(1, 15)}$ = 0.1331, p=0.7204; Figure 17A) or the total distance travelled across the 2 hour testing period ($t_{(15)}$= 0.3648, p=0.7204; Figure 17B). The distance travelled by the mice decreased as the test progressed ($F_{(23, 345)}$ = 8.706, p<0.0001, no significant interaction with treatment $F_{(23, 345)}$ = 0.9339 p=0.5528; Figure 17A). Donepezil also had no effect on rearing time ($t_{(15)}$ = 0.6816, p=05059; Figure 17D). However, 5xFAD mice that were implanted with an donepezil infusing osmotic pump spent more time in the center of the locomotor box than mice that were given saline + 10% DMSO osmotic pumps ($t_{(15)}$ = 3.403, p=0.0039; Figure 17C). Three 5xFAD mice were removed (1 control and 2 donepezil), as they were calculated to be outliers according to the ROUT method.

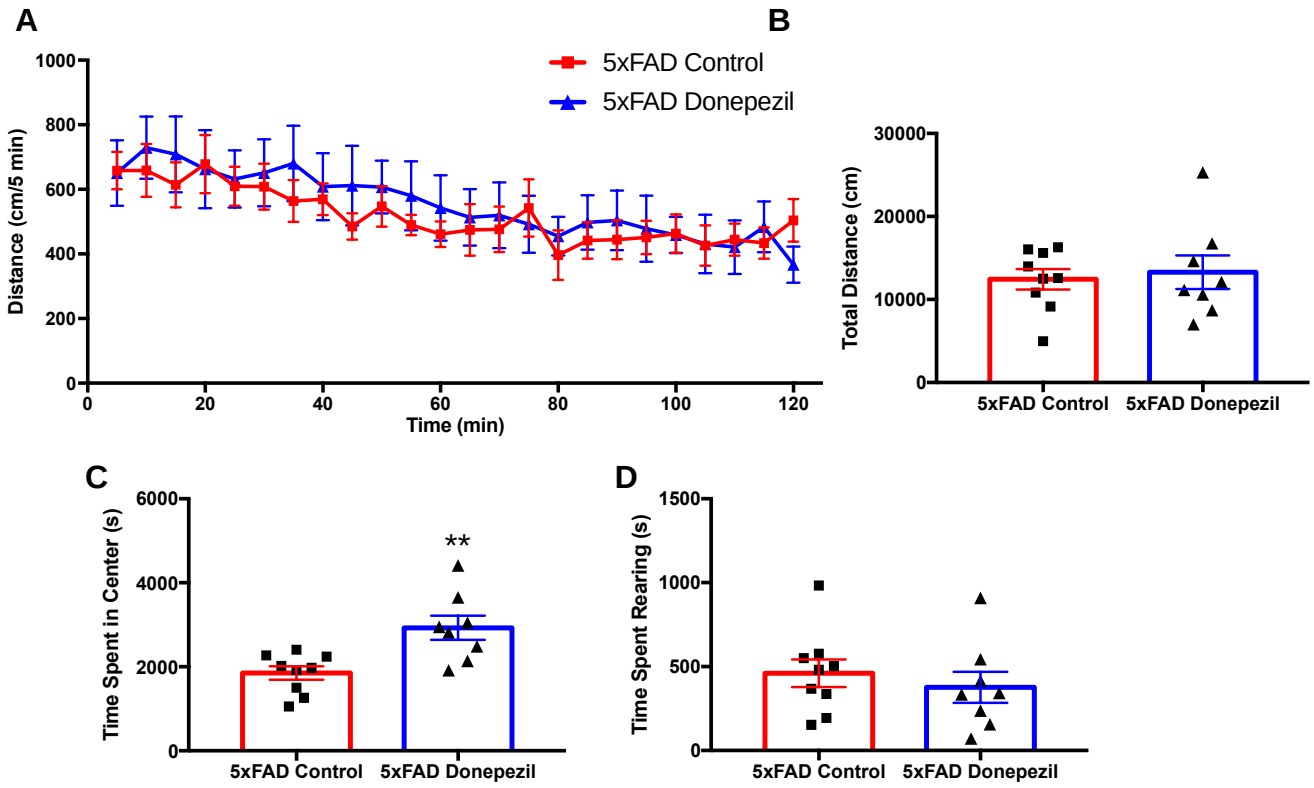


Figure 17. Spontaneous locomotor activity of 5xFAD mice treated with donepezil.

A) Mean ± SEM distance (cm) travelled by 10-month old male touchscreen 5xFAD mice every 5 minutes for a total of 2 hours (repeated measures two-way ANOVA). B) Mean ± SEM of the total distance (cm) travelled, C) time spent in the centre (s) and D) time spent rearing (s) by male 5xFAD mice treated with donepezil (n=8) or saline + 10% DMSO (n=9). Unpaired two-tailed t-test; $^{**}p<0.005$.

3.4.2 *Locomotor activity in old 5xFAD male mice*

Age-related changes in motor ability and locomotion have been observed in old 5xFAD mice (O'Leary, 2013; O'Leary et al., 2013; Schneider et al., 2014). Schneider et al. observed a significant reduction in locomotion and rearing activity at 9 months of age in male 5xFAD mice compared to WT littermates (Schneider et al., 2014). This was accompanied by a reduction in anxiety, where the 5xFAD mice spent significantly more time in the center of the open-field (Schneider et al., 2014). Meanwhile, O'Leary found that male 5xFAD mice only travel significantly less than their WT controls at 12 and 15 months of age and reared significantly less at 9, 12 and 15 months (O'Leary, 2013). However, anxiety did not appear to be affected (O'Leary, 2013). Another study found 5xFAD mice to be hyperactive at both 9 and 12 months of age (Yang et al., 2014). To determine whether 5xFAD mice used in our experiments undergoing food-restriction present any locomotor deficits when aged, we tested them in locomotor boxes at 14 months. Male 5xFAD mice do not demonstrate differences in locomotion when compared to their WT controls at 14 months of age – both in terms of distance travelled in 2 hours measured in intervals of 5 min ($F_{(1, 35)} = 1.793$, p=0.1892; Figure 18A) or total distance travelled in 2 hours ($t_{(35)} = 1.339$, p=0.1892; Figure 18B). The distance travelled by the mice decreased with time ($F_{(23, 828)} = 20.16$, p<0.0001, no significant interaction with genotype: $F_{(23, 828)} = 0.4807$, p=0.9819; Figure 18A).

On average, the 5xFAD mice spend approximately the same amount of time as their WT controls in the center of the locomotor box ($t_{(35)} = 0.1126$, p=0.9110; Figure 18C).

However, 5xFAD mice spend significantly less time rearing at 14-months of age ($t_{(35)}$ = 4.633, p<0.0001; Figure 18D).

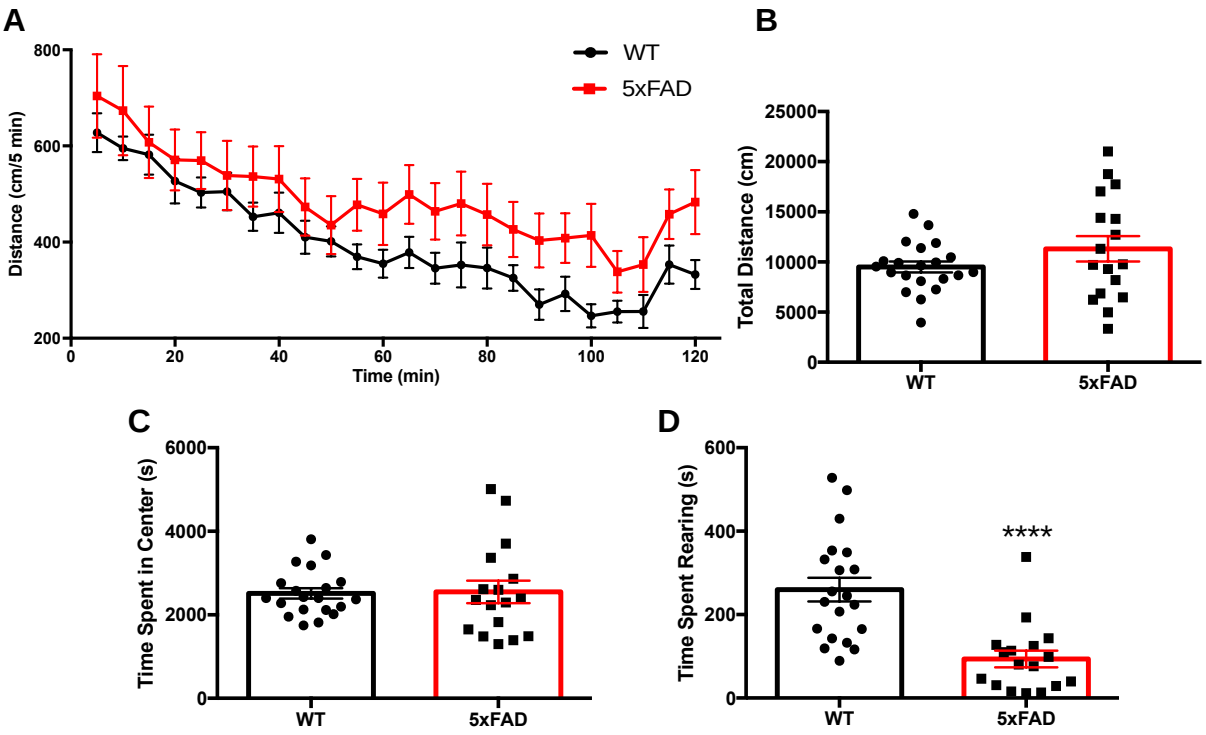


Figure 18. Spontaneous locomotor activity of 14-month old 5xFAD mice.

A) Mean ± SEM distance (cm) travelled by 14-month old 5xFAD male mice (n=17) and their WT controls (n=20) every 5 minutes for a total of 2 hours (repeated measures two-way ANOVA). B) Mean ± SEM of the total distance (cm) travelled, C) time spent in the centre (s) and D) time spent rearing (s) by 5xFAD male mice (n=17) and their WT controls (n=20). Unpaired two-tailed t-test; ****p<0.0001.

3.4.3 *Locomotor activity in 15-month old 5xFAD mice*

The same mice used for the experiments in Figure 18 were taken off food-restriction and given food *ad libitum* to evaluate whether food-restriction had an effect on locomotion in these mice, as caloric-restriction has been observed to have a significant effect on spontaneous locomotor activity in mice (Kuhla et al., 2013). After one month of free-food, 5xFAD mice and their WT controls do not travel significantly different distances every 5 minutes ($F_{(1, 20)}$ = 0.8303, p=0.3730; Figure 19A) or total distances ($t_{(20)}$ = 0.9112, p=0.3730; Figure 19B). The distance travelled by the mice decreased with time ($F_{(23, 460)}$ = 6.929, p<0.0001, no significant interaction with treatment: $F_{(23, 460)}$ = 1.428, p=0.0914; Figure 19A).

5xFAD mice and their WT controls did not spend significantly different amounts of time in the center of the locomotor box ($t_{(20)}$ = 0.8951, p=0.3814; Figure 19C). Interestingly, 5xFAD mice still spent less time rearing ($t_{(20)}$ = 4.83, p=0.0001; Figure 19D). Two 5xFAD mice were calculated to be outliers and were removed according to the ROUT method.

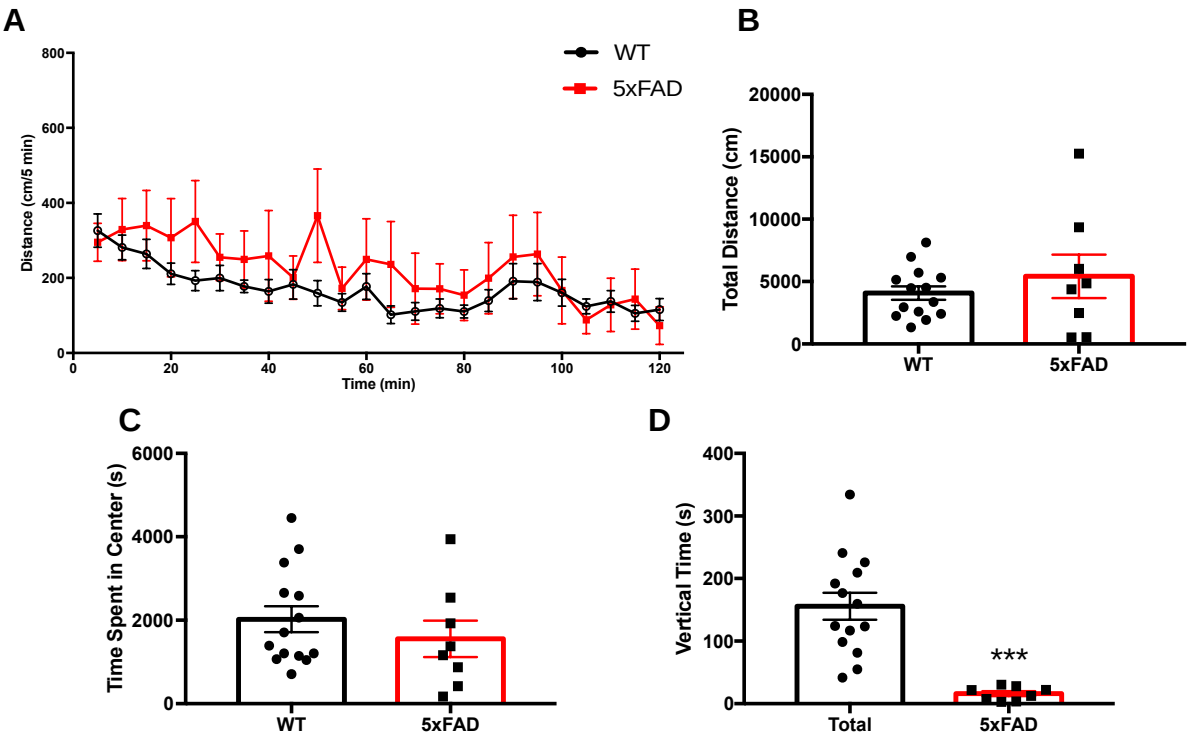


Figure 19. Spontaneous locomotor activity of 15-month old 5xFAD mice.

A) Mean ± SEM distance (cm) travelled by 15-month old 5xFAD male mice (n=8) and their WT controls (n=14). Measurements were taken every 5 minutes for a total of 2 hours (repeated measures two-way ANOVA). B) Mean ± SEM of the total distance (cm) travelled, C) time spent in the centre (s) and D) time spent rearing (s) by male 5xFAD mice (n=8) and their WT controls (n=14). Unpaired two-tailed t-test; ***p<0.0005.

3.5 Gait Analyses in Male 5xFAD Mice

Gait and the ability of mice to walk is vital to their performance in many behavioural assessments. Given touchscreen results indicating that 5xFAD mice take more time to touch the screen and collect their reward, it is important to determine if perturbations in gait are also present in the 5xFAD mouse model. The presence of gait impairments would also add to the validity of the 5xFAD mouse model as an animal model of AD. AD patients begin to develop perturbations in gait early in the disease and these

impairments continue to evolve as the disease progresses (O'keeffe et al., 1996; Sala et al., 2004).

3.5.1 *Gait is not impaired in 10-month-old 5xFAD mice*

We subjected male 5xFAD mice to gait analyses at 10-months of age to probe for any gait impairments. At 10-months of age, the print width (forelimb: $t_{(16)}$ = 0.6151, p=0.5741, hindlimb: $t_{(16)}$ = 1.647, p=0.1190) and print length (forelimb: $t_{(16)}$ = 0.07134, p=0.9440, hindlimb: $t_{(16)}$ = 0.4885, p=0.6318) of 5xFAD mice was not significantly altered when compared to WT mice. Thus, there were also no significant differences in print area (forelimb: $t_{(16)}$ = 0.7174, p=0.4835, hindlimb: $t_{(16)}$ = 1.826, p=0.0866). There were no significant differences in paw intensity as well (forelimb: $t_{(16)}$ = 1.841, p=0.0843, hindlimb: $t_{(16)}$ = 1.539, p=0.1435). 5xFAD mice also do not demonstrate significant changes in forelimb ($t_{(16)}$ = 0.052, p=0.9592) or hindlimb paw angle ($t_{(16)}$ = 1.156, p=0.2646). The values of these static gait parameters (parameters that are not affected by the mouse as its walks) are listed in Table 2.

Stride length (forelimb: $t_{(16)}$ = 0.8291, p=0.4192, hindlimb: $t_{(16)}$ = 1.074, p=0.2986) and swing speed (forelimb: $t_{(16)}$ = 0.8155, p=0.4267, hindlimb: $t_{(16)}$ = 0.3184, p=0.7543) did not differ significantly between groups. The duration of the swing phase (forelimb: $t_{(16)}$ = 0.7965, p=0.4374, hindlimb: $t_{(16)}$ = 0.6361, p=0.5337), stand/stance phase (forelimb: $t_{(16)}$ = 0.4176, p=0.6818, hindlimb: $t_{(16)}$ = 0.05118, p=0.9598), and duty cycle (forelimb: $t_{(16)}$ = 0.03817, p=0.9700, hindlimb: $t_{(16)}$ = 0.3945, p=0.6984), was not altered. The forelimb ($t_{(16)}$ = 0.1806, p=0.8589) and hindlimb ($t_{(16)}$ = 1.46, p=0.1637) stand index of 5xFAD mice also did not differ from WT mice. The values of these dynamic gait parameters (parameters that are affected by the mouse as it walks) are listed in Table 3.

5xFAD mice do not have significantly different step pattern frequencies when compared to their WT controls. ($F_{(1, 96)}$ = 1.296 x 10^{-9}, p>0.9999; Figure 20A). The frequency of each of the step patterns differed significantly within groups ($F_{(5, 96)}$ = 29.01, p<0.0001, no interaction with genotype: $F_{(5, 96)}$ = 0.3908, p=0.8541; Figure 20A). The alternate b (Ab) step pattern was the most common amongst both groups, with an average frequency of 60.95% for WT mice and 54.25% for 5xFAD mice. There were no significant differences in regularity index ($t_{(16)}$ = 1.247, p=0.2302; Figure 20B), forelimb base of support ($t_{(16)}$ = 0.186, p=0.8548; Figure 21A), hindlimb base of support ($t_{(16)}$ = 0.1946, p=0.8482; Figure 21B), or weights between the groups ($t_{(16)}$ = 0.9989, p=0.3327; Figure 21C).

The frequency of each of the support types differed significantly within groups ($F_{(6, 112)}$ = 135.9, p<0.0001, no interaction effect: $F_{(6, 112)}$ = 2.132, p=0.0551; Figure 20C). However, there was no significant difference between genotypes ($F_{(1, 112)}$ = 0.00, p>0.9999; Figure 20C). Diagonal limb support was most frequently used by WT (62.57%) and 5xFAD mice (52.61%).

In summary, male 5xFAD mice do not demonstrate gait impairments when compared to their WT controls at 10 months of age.

Table 2. Static gait parameters in 10-month 5xFAD mice.

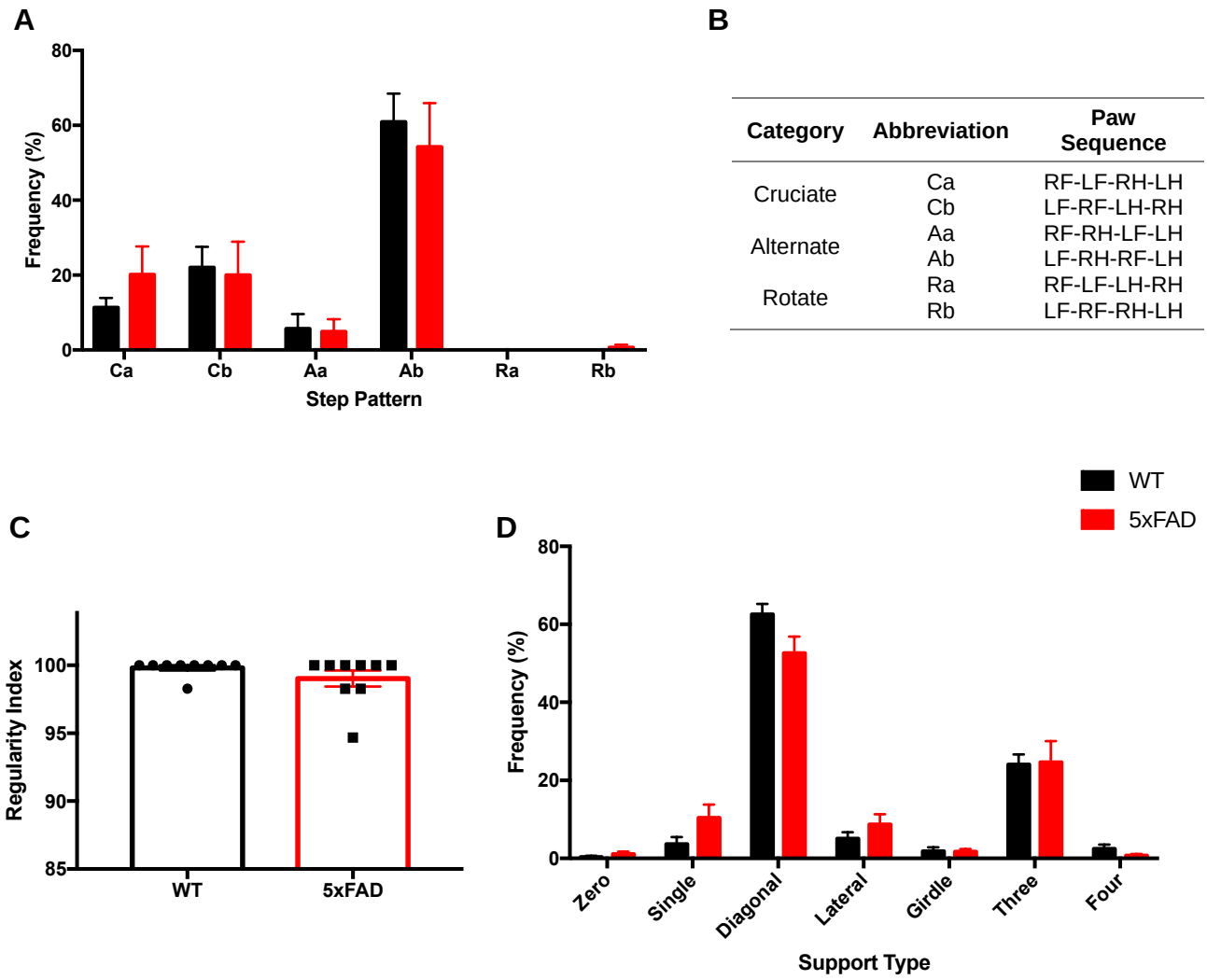
Static gait parameters were assessed in male 5xFAD mice (n=9) and their WT controls (n=9). All values shown as mean ± SEM (unpaired two-tailed t-test).

Parameter	Wild-Type	5xFAD
Paw Print Area (mm^2)		
Forelimb	10.4 ± 0.86	9.49 ± 0.92
Hindlimb	12.98 ± 1.17	9.70 ± 1.37
Paw Print Intensity (arbitrary units)		
Forelimb	84.06 ± 1.06	80.97± 1.31
Hindlimb	97.32 ± 2.36	91.67 ± 2.81
Paw Print Width (mm)		
Forelimb	4.85 ± 0.21	5.07 ± 0.28
Hindlimb	5.54 ± 0.30	4.71 ± 0.40
Paw Print Length (mm)		
Forelimb	4.67± 0.16	4.70 ± 0.20
Hindlimb	5.54 ± 0.14	5.18 ± 0.73
Paw Angle (degrees)		
Forelimb	21.33 ± 4.27	20.98 ± 5.18
Hindlimb	13.37 ± 2.52	25.16 ± 9.88

Table 3. Dynamic gait parameters in 10-month 5xFAD mice.

Dynamic gait parameters were assessed in male 5xFAD mice (n=9) and their WT controls (n=9). All values shown as mean ± SEM (unpaired two-tailed t-test).

Parameter	Wild-Type	5xFAD
Stride Length (mm)		
Forelimb	57.14 ± 2.62	54.22 ± 2.36
Hindlimb	56.60 ± 2.24	53.10 ± 2.37
Swing Speed (m/s)		
Forelimb	0.69 ± 0.05	0.64 ± 0.05
Hindlimb	0.82 ± 0.10	0.82 ± 0.10
Stand (s)		
Forelimb	0.08 ± 0.01	0.09 ± 0.01
Hindlimb	0.10 ± 0.01	0.10 ± 0.01
Swing (s)		
Forelimb	0.09 ± 0.00	0.09 ± 0.00
Hindlimb	0.07 ± 0.00	0.08 ± 0.01
Stand Index		
Forelimb	-12.27 ± 0.66	-12.53 ± 1.28
Hindlimb	-12.66 ± 0.78	-10.78 ± 1.03
Duty Cycle (s)		
Forelimb	47.62 ± 1.64	47.54 ± 1.32
Hindlimb	57.28 ± 1.53	55.5 ± 4.25



Category	Abbreviation	Paw Sequence
Cruciate	Ca	RF-LF-RH-LH
	Cb	LF-RF-LH-RH
Alternate	Aa	RF-RH-LF-LH
	Ab	LF-RH-RF-LH
Rotate	Ra	RF-LF-LH-RH
	Rb	LF-RF-RH-LH

Figure 20. Step pattern and limb support measures in 10-month old 5xFAD mice.

A) Mean ± SEM percentage occurrence of each of the step patterns indicated in B, C) regularity index (%) and D) percentage occurrence of each of the possible limb support types in male 5xFAD (n=9) and their WT controls (n=9). Unpaired two-tailed t-test (C) or repeated measures two-way ANOVA (A, D).

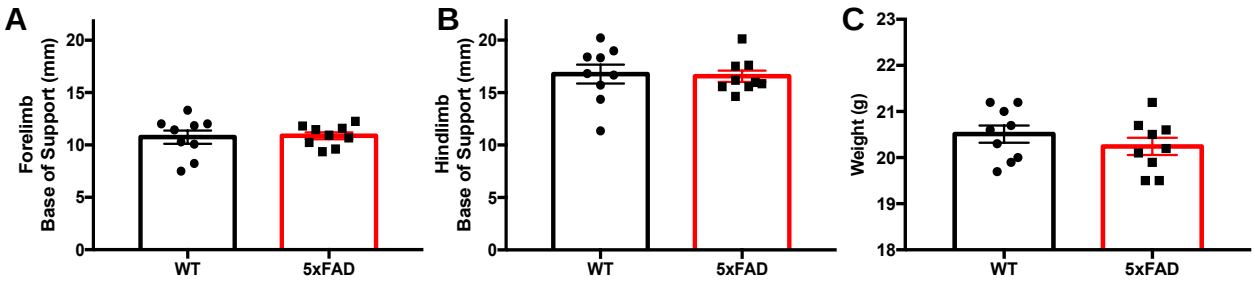


Figure 21. Base of support and weights in 10-month old 5xFAD mice.

Mean ± SEM A) forelimb base of support (mm), B) hindlimb base of support (mm) and weights of male 5xFAD (n=9) mice and their WT controls (n=9). Unpaired two-tailed t-test.

3.5.2 *Gait is impaired in 14-month 5xFAD mice*

To evaluate whether gait becomes impaired in old male 5xFAD mice, we subjected 14-month-old male 5xFAD mice to gait analyses.

Forelimb paw print length ($t_{(37)}$ = 2.497, p=0.0171) was reduced in 5xFAD mice when compared to controls, as well as forelimb ($t_{(37)}$ = 2.485, p=0.0176) and hindlimb paw print width ($t_{(37)}$ = 2.42, p=0.0205). Likewise, 5xFAD mice showed a significant decrease in forelimb print area ($t_{(37)}$ = 3.654, p=0.0008). There was no change in hindlimb paw print area ($t_{(37)}$ = 1.693, p=0.0988) or hindlimb paw print length ($t_{(37)}$ = 0.3455, p=0.7317). Forelimb paw print intensity was significantly lower in 5xFAD mice ($t_{(37)}$ = 3.563, p=0.0010), while hindlimb paw print intensity was not affected ($t_{(37)}$ = 1.284, p=0.2070). 5xFAD mice also exhibit an increase in forelimb ($t_{(37)}$ = 2.228, p=0.0321) and hindlimb paw angle ($t_{(37)}$ = 2.661, p=0.0115). These static gait parameters are listed in Table 4.

5xFAD mice showed significantly shorter stride lengths (forelimb: $t_{(37)}$ = 6.66, p<0.0001; hindlimb: $t_{(37)}$ = 6.335, p<0.0001) and slower swing speeds (forelimb: $t_{(37)}$ = 4.17, p=0.0002; hindlimb: $t_{(37)}$ = 2.156, p<0.0001) than their WT controls. There were no differences in stand/stance time duration (forelimb: $t_{(37)}$ = 2.01, p=0.0517; hindlimb: $t_{(37)}$ = 0.0618, p=0.9511), swing (forelimb: $t_{(37)}$ = 0.9667, p=0.9667; hindlimb: $t_{(37)}$ = 0.7251, p=0.4729), stand index (forelimb: $t_{(37)}$ = 1.578, p=0.1231; hindlimb: $t_{(37)}$ = 0.9501, p=0.3482), or duty cycle (forelimb: $t_{(37)}$ = 1.643, p=0.1089; hindlimb: $t_{(37)}$ = 0.5578, p=0.5669). These dynamic gait parameters are listed in Table 5.

The frequency of each of the step patterns differed significantly within the groups ($F_{(5, 222)}$ = 84.03, p<0.0001; Figure 22A), but there was no difference between genotypes ($F_{(1, 222)}$ = 2.115 x 10^{-8}, p=0.9999, no interaction effect: $F_{(5, 222)}$ = 2.734, p=0.0203; Figure 22A). The Ab step pattern was the most common among both genotypes – with an average of 64.13% for WT mice and 49.19% for 5xFAD mice. However, the regularity index of the 5xFAD mice was significantly lower than that of the WT controls ($t_{(37)}$ = 3.054, p=0.0042; Figure 22B). The frequency of each of the support types differed significantly within groups ($F_{(6, 259)}$ = 5.262, p<0.0001, interaction effect: $F_{(6, 259)}$ = 5.262, p<0.0001; Figure 22C). However, there was no significant difference between genotypes ($F_{(1, 222)}$ = 3.264 x 10^{-9}, $p>0$.9999; Figure 22C). Diagonal limb support was most frequently used by WT mice (53.08%), while a three-limb support was most frequently used by 5xFAD mice (39.06%). Diagonal limb support was used 36.32% of the time by 5xFAD mice.

5xFAD mice demonstrate a decrease in forelimb base of support ($t_{(37)}$ = 2.919, p=0.0059; Figure 23A) and an increase in hindlimb base of support when compared to

their WT controls ($t_{(37)}$ = 5.609, p<0.0001; Figure 23B). There were no significant differences in weight between the genotypes ($t_{(37)}$ = 1.463, p=0.1517; Figure 23C). A total of 6 outliers were removed – 3 from each group according to the ROUT method and a G of 1%.

In summary, male 5xFAD mice demonstrate significant impairments in gait at 14 months of age.

Table 4. Static gait parameters in 14-month 5xFAD mice.

Static gait parameters were assessed in male 5xFAD mice (n=19) and their WT littermates (n=21) at 14 months. All values shown as mean ± SEM (unpaired two-tailed t-test; *p<0.05, **p<0.005).

Parameter	Wild-Type	5xFAD
Paw Print Area (mm^2)		
Forelimb	21.22 ± 1.19	13.94 ± 1.64**
Hindlimb	22.44 ± 1.42	18.06 ± 2.25
Paw Print Intensity (arbitrary units)		
Forelimb	92.13 ± 1.21	85.24 ± 1.55**
Hindlimb	101.1 ± 1.35	97.97 ± 2.07
Paw Print Width (mm)		
Forelimb	6.93 ± 0.23	5.92 ± 0.35*
Hindlimb	7.71 ± 0.33	6.48 ± 0.38*
Paw Print Length (mm)		
Forelimb	6.00 ± 0.19	5.18 ± 0.27*
Hindlimb	6.64 ± 0.22	6.69 ± 0.41
Paw Angle (degrees)		
Forelimb	11.82 ± 1.51	20.97 ± 4.08*
Hindlimb	9.82 ± 1.44	17.21 ± 2.49*

Table 5. Dynamic gait parameters in 14-month 5xFAD mice.

Dynamic gait parameters were assessed in 5xFAD mice (n=19) and their WT littermates (n=21) at 14 months of age. All values shown as mean ± SEM (unpaired two-tailed t-test; *p<0.05, ***p<0.0005, ****p<0.0001).

Parameter	Wild-Type	5xFAD
Stride Length (mm)		
Forelimb	60.48 ± 1.14	47.50 ± 1.64****
Hindlimb	60.13 ± 1.13	48.23 ± 1.54****
Swing Speed (m/s)		
Forelimb	0.70 ± 0.03	0.53 ± 0.02***
Hindlimb	0.88 ± 0.03	0.77 ± 0.04*
Stand (s)		
Forelimb	0.12 ± 0.00	0.10 ± 0.01
Hindlimb	0.13 ± 0.01	0.13 ± 0.01
Swing (s)		
Forelimb	0.09 ± 0.00	0.09 ± 0.00
Hindlimb	0.07 ± 0.00	0.07 ± 0.00
Stand Index		
Forelimb	-9.98 ± 0.55	-11.95 ± 0.68
Hindlimb	-9.37 ± 0.59	-10.15 ± 0.55
Duty Cycle (s)		
Forelimb	55.76 ± 0.94	51.67 ± 2.47
Hindlimb	62.15 ± 1.27	63.61 ± 2.31

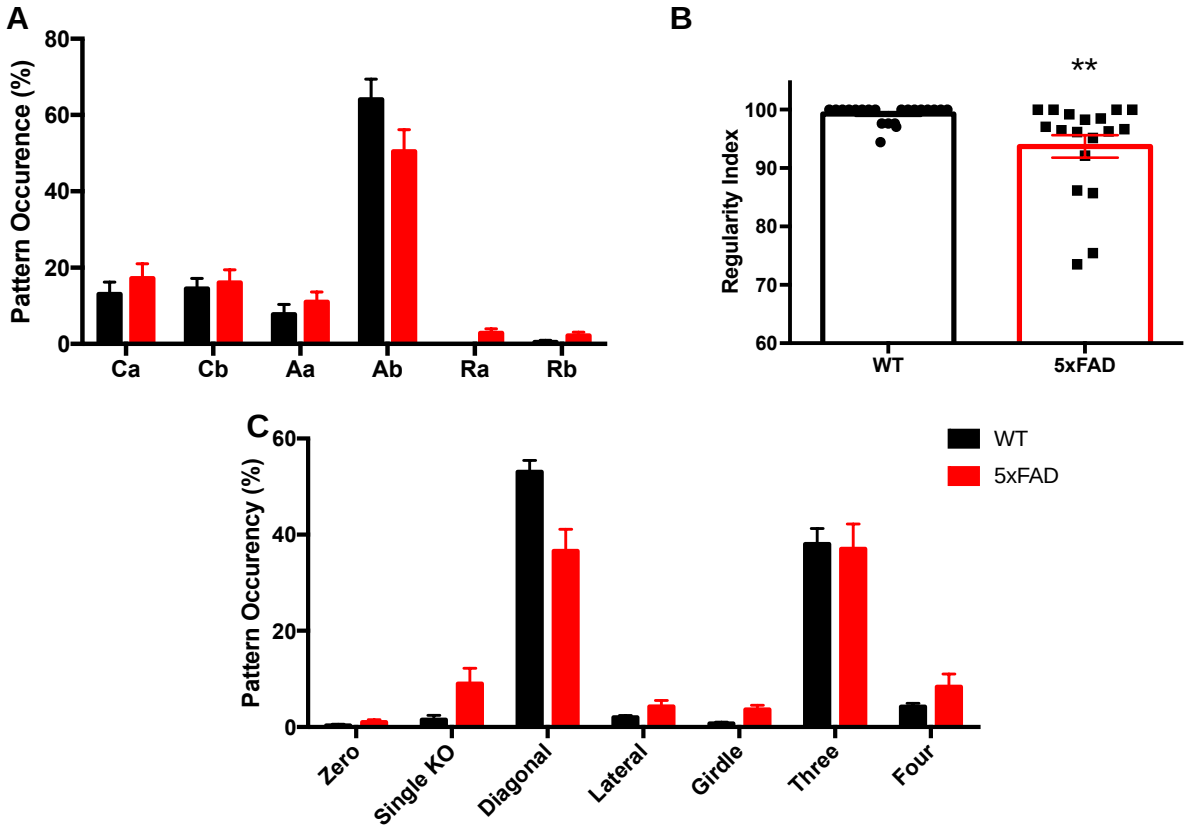


Figure 22. Step pattern and limb support measures in 14-month 5xFAD mice.

A) Mean ± SEM percentage occurrence of each of the step patterns, B) regularity index (%) and C) percentage occurrence of each of the possible limb support types in male 5xFAD mice (n=18) and their WT controls (n=21). Two-way ANOVA (A, C) or unpaired two-tailed t-test (B); **p<0.005.

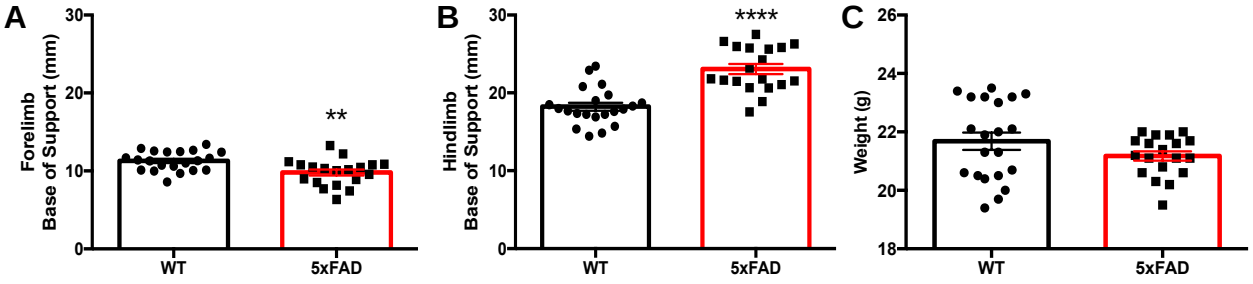


Figure 23. Base of support and weights in 14-month 5xFAD mice.

Mean ± SEM A) forelimb base of support (mm), B) hindlimb base of support (mm) and C) weights of male 5xFAD mice (n=18) and their WT controls (n=21). Unpaired two-tailed t-test; **p<0.005, ****p<0.0005.

3.5.3 *The gait of 5xFAD mice at 14-months of age is mildly improved by intraperitoneal injection of donepezil*

To determine whether intraperitoneal injection of donepezil had an effect on the previously observed gait impairments in 14-month male 5xFAD mice, the 5xFAD mice received intraperitoneal injections (replicating the Romberg et al. (2013) study) of either donepezil or saline + 10% DMSO. Gait was evaluated 30 min after the injection.

Forelimb print length ($t_{(7)}$ = 1.853, p=0.1063), hindlimb print length ($t_{(7)}$ = 0.7068, p=0.5025) and forelimb print width ($t_{(7)}$ = 2.162, p=0.0674) were not significantly affected by treatment with donepezil. However, there was a significant increase in hindlimb print width ($t_{(7)}$ = 2.721, p=0.0297), leading to a significant increase in hindlimb print area ($t_{(7)}$ = 2.457, p=0.0437). There was also a significant increase in forelimb print area ($t_{(7)}$ = 3.182, p=0.0155). Intensity (forelimb: $t_{(7)}$ = 0.304, p=0.7699, hindlimb: $t_{(7)}$ = 0.171, p=0.8691) and paw angle (forelimb: $t_{(7)}$ = 0.4448, p=0.6699, hindlimb: $t_{(7)}$ = 1.100, p=0.3078) were not significantly affected by treatment. The values of these static gait parameters are listed in Table 6.

Stride length (forelimb: $t_{(7)}$ = 2.342, p=0.0517, hindlimb: $t_{(7)}$ = 2.007, p=0.0847) and swing speed (forelimb: $t_{(7)}$ = 1.654, p=0.1422, hindlimb: $t_{(7)}$ = 1.188, p=0.2736) did not differ significantly between the groups. The duration of swing phase (forelimb: $t_{(7)}$ = 0.4286, p=0.6811, hindlimb: $t_{(7)}$ = 1.768, p=0.1204) and stand/stance phase (forelimb: $t_{(7)}$ = 1.414, p=0.2004, hindlimb: $t_{(7)}$ = 0.8443, p=0.4264) were not affected by donepezil. The forelimb stand index ($t_{(7)}$ = 1.455, p=0.1890), hindlimb stand index ($t_{(7)}$ = 1.976, p=0.0886) and forelimb duty cycle ($t_{(7)}$ = 2.138, p=0.0698) were not significantly

different. However, there was a significant increase in hindlimb duty cycle ($t_{(7)}$ = 2.53, p=0.0393) in the 5xFAD mice that were treated with donepezil. The values of these dynamic gait parameters are listed in Table 7.

Intraperitoneal injection of donepezil had no effect on the frequency of step patterns in 5xFAD mice ($F_{(1, 84)}$ = 0.00, p>0.9999; Figure 24A). The frequency of each of step pattern differed significantly within groups ($F_{(5, 84)}$ = 11.76, p<0.0001, no interaction with treatment: $F_{(5, 84)}$ = 0.2834, p=0.9210; Figure 24A). The Ab step pattern was the most common amongst both groups – 42.43% among those who were treated with saline + 10% DMSO and 50.53% among those who were treated with donepezil. Treatment had no effect on the regularity index ($t_{(7)}$ = 1.256, p=0.2493; Figure 24B), forelimb ($t_{(7)}$ = 1.107, p=0.3050; Figure 25A) or hindlimb base of support ($t_{(7)}$ = 2.328, p=0.0528; Figure 25B). There were no significant differences between the weights of the mice ($t_{(7)}$ = 0.7662, p=0.4686; Figure 25C).

Treatment did not significantly affect the frequency of each support type ($F_{(1, 98)}$ = 4.265 x 10^{-9}, p>0.9999; Figure 24C), although the frequency differed significantly within groups (($F_{(6, 98)}$ = 122.9, p<0.0001, no interaction with treatment: ($F_{(6, 98)}$ = 3.093, p=0.0081; Figure 24C). A diagonal support was most commonly used by 5xFAD mice treated with saline + 10% DMSO (47.60%), while a three-limbed support was most commonly used by 5xFAD mice treated with donepezil (49.35%).

Table 6. Static gait parameters in 14-month 5xFAD mice following donepezil treatment.

Static gait parameters were assessed male 5xFAD that have been given intraperitoneal injections of donepezil (n=8) or saline + 10% DMSO (n=8). All values shown as mean ± SEM (paired two-tailed t-test; *p<0.05).

Parameter	5xFAD Control	5xFAD Donepezil
Paw Print Area (mm^2)		
Forelimb	13.79 ± 1.11	18.47 ± 2.03*
Hindlimb	17.28 ± 2.51	29.09 ± 3.75*
Paw Print Intensity (arbitrary units)		
Forelimb	86.95 ± 1.12	86.50 ± 1.99
Hindlimb	104.7 ± 4.61	105.6± 1.52
Paw Print Width (mm)		
Forelimb	6.41 ± 0.20	7.04 ± 0.37
Hindlimb	6.18 ± 0.40	8.01 ± 0.51*
Paw Print Length (mm)		
Forelimb	4.87 ± 0.14	5.13 ± 0.20
Hindlimb	7.40 ± 0.90	8.52 ± 1.37
Paw Angle (degrees)		
Forelimb	14.34 ± 4.09	12.00 ± 2.54
Hindlimb	6.84 ± 2.06	9.99 ± 1.31

Table 7. Dynamic gait parameters in 14-month 5xFAD mice following donepezil treatment.

Dynamic gait parameters were assessed in male 5xFAD that have been given intraperitoneal injections of donepezil (n=8) or saline + 10% DMSO (n=8). All values shown as mean ± SEM (paired two-tailed t-test; *p<0.05).

Parameter	5xFAD Control	5xFAD Donepezil
Stride Length (mm)		
Forelimb	52.94 ± 2.67	45.26 ± 2.06
Hindlimb	52.8 ± 1.93	47.32 ± 2.04
Swing Speed (m/s)		
Forelimb	0.62 ± 0.02	0.55 ± 0.03
Hindlimb	0.70± 0.30	1.05 ± 0.07
Stand (s)		
Forelimb	0.09 ± 0.00	0.11 ± 0.01
Hindlimb	0.34 ± 0.22	0.15 ± 0.01
Swing (s)		
Forelimb	0.09 ± 0.00	0.09 ± 0.00
Hindlimb	0.06 ± 0.01	0.05 ± 0.00
Stand Index		
Forelimb	-13.24 ± 0.59	-11.35 ± 0.97
Hindlimb	-10.37 ± 0.66	-8.51 ± 0.75
Duty Cycle (s)		
Forelimb	50.15 ± 1.22	54.99 ± 1.52
Hindlimb	64.96 ± 0.71	73.91 ± 1.84*

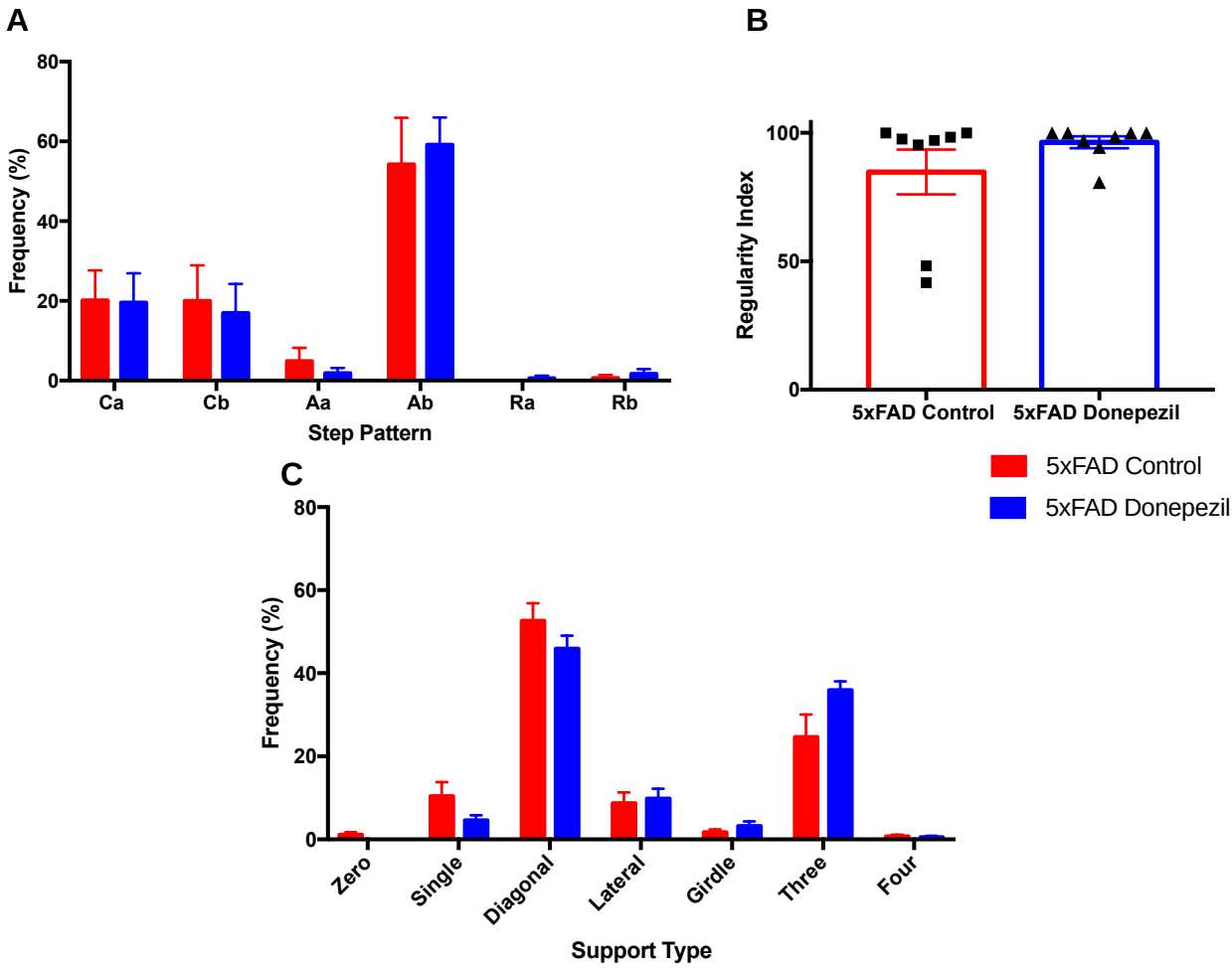


Figure 24. Step pattern and limb support measures in 14-month old 5xFAD mice following donepezil treatment.

A) Mean ± SEM percentage occurrence of each of the step patterns, B) regularity index (%) and C) percentage occurrence of each of the possible limb support types in male 5xFAD mice given intraperitoneal injections of donepezil (n=8) or saline + 10% DMSO (n=8) following the completion of the second 5-CSRTT probe trial (paired two-tailed t-test (B) or two-way ANOVA (A, C)).

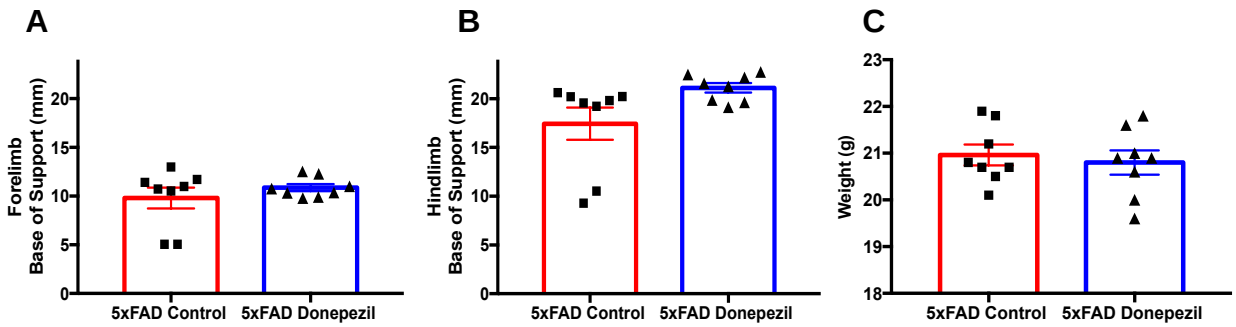


Figure 25. Base of support and weights in 14-month 5xFAD mice following donepezil treatment.

Mean ± SEM A) forelimb base of support (mm), B) hindlimb base of support (mm) and weights of the male 5xFAD that have been given intraperitoneal injections of donepezil (n=8) or saline + 10% DMSO (n=8). Paired two-tailed t-test.

3.5.4 *Gait remains altered in 15-month male 5xFAD mice*

To determine whether food-restriction during touchscreen evaluation affected the gait of mice and may have contributed for the deficits observed in 5xFAD mice, we took the cohort of mice from the 14-month experiment that did not receive donepezil, provided food *ad libitum* for one month, and then assessed gait once again.

Paw print width was not significantly altered in 5xFAD mice (forelimb: $t_{(16)}$ = 1.076, p=0.2981; hindlimb: $t_{(16)}$ = 0.5839, p=0.5675). There was also no significant difference in forelimb paw print width ($t_{(16)}$ = 3.161, p=0.0060), however hindlimb print length was significantly increased ($t_{(16)}$ = 0.5839, p=0.5675). This led to a significant increase in hindlimb print area ($t_{(16)}$ = 2.234, p=0.0401). There was no change in forelimb paw print area ($t_{(16)}$ = 1.297, p=0.2131). Forelimb ($t_{(16)}$ = 5.255, p<0.0001) and hindlimb paw print intensity was significantly lower in 5xFAD mice ($t_{(16)}$ = 7.583, p<0.0001). There were also no significant changes in forelimb ($t_{(16)}$ = 1.544, p<0.1420) and hindlimb paw angle ($t_{(16)}$ = 1.544, p=0.1420). These static gait parameters are listed in Table 8.

5xFAD mice have significantly shorter stride lengths (forelimb: $t_{(16)}$ = 2.756, p=0.0141; hindlimb: $t_{(16)}$ = 3.339 p=0.0042) than their WT controls. Although there was no difference in forelimb swing speed ($t_{(16)}$ = 1.253, p=0.2281), 5xFAD mice had a significantly faster hindlimb swing speed than WT mice ($t_{(16)}$ = 2.454, p=0.0260). There were no differences in stand/stance time duration (forelimb: $t_{(16)}$ = 0.2905, p=0.7752; hindlimb: $t_{(16)}$ = 2.081, p=0.0539), and forelimb swing ($t_{(16)}$ = 0.02621, p=0.9794). However, the length of the hindlimb swing phase was significantly lower in 5xFAD mice ($t_{(16)}$ = 5.309, p<0.0001). Forelimb ($t_{(16)}$ = 1.459, p=1.640) and hindlimb stand index ($t_{(16)}$ = 0.7853, p=0.4437) was not significantly different. Forelimb duty cycle was not affected ($t_{(16)}$ = 0.4272, p=0.6749), while there was a significant increase in the hindlimb duty cycle ($t_{(16)}$ = 5.650, p<0.0001). These dynamic gait parameters are listed in Table 9.

Once the 5xFAD mice were given food *ad libitum*, 5xFAD mice do not gain as much weight as WT mice ($t_{(16)}$ = 2.983, p<0.0125; Figure 27C). The frequency of each of the step patterns differed significantly within the groups ($F_{(5, 96)}$ = 13.94, p<0.0001; Figure 26A), but there was no difference between genotypes ($F_{(1, 96)}$ = 8.082x 10^{-9}, p>0.9999, with an interaction effect: $F_{(5, 96)}$ = 8.614, p>0.9999; Figure 26A). The Ab step pattern was most frequently used (53.87%) by 5xFAD mice, while the alternate a (Aa) pattern was most frequently used by WT mice (31.44%). This was followed closely by the cruciate b (Cb) at 28.64%. The regularity index was not significantly different between 5xFAD mice and their WT controls ($t_{(16)}$ = 0.6824, p=0.5048; Figure 26B).

The frequency of each of the support types differed significantly within groups ($F_{(6, 112)}$ = 222.5, p<0.0001, interaction effect: $F_{(6, 112)}$ = 12.18, p<0.0001; Figure 26C). However, there was no significant difference between genotypes ($F_{(1, 112)}$ = 1.843 x 10^{-7},

p=0.9997; Figure 26C). Diagonal limb support was most frequently used by WT mice (56.60%), while a three-limb support was most frequently used by 5xFAD mice (44.39%). 5xFAD mice had a significantly lower forelimb base of support compared to their WT controls ($t_{(16)}$ = 0.3.457, *p*=0.0032; Figure 27A), while their hindlimb base of support was significantly higher ($t_{(16)}$ = 2.474, *p*=0.0279; Figure 27B).

A total of 2 outliers were removed – 1 from each group according to the ROUT method and a G of 1%.

In summary, although the weights of 5xFAD mice are significantly lower than that of WT mice following one month of free-food, gait remains altered.

Table 8. Static gait parameters in 15-month 5xFAD mice.
Static gait parameters were assessed in male 5xFAD mice (n=7) and their WT littermates (n=11) following one month of free-food. All values shown as mean ± SEM (unpaired two-tailed t-test; *p<0.05, **p<0.005, ****p<0.0001).

Parameter	Wild-Type	5xFAD
Paw Print Area (mm^2)		
Forelimb	27.19 ± 1.20	24.54 ± 1.76
Hindlimb	24.12 ± 0.95	32.80 ± 4.71*
Paw Print Intensity (arbitrary units)		
Forelimb	118.40 ± 2.12	100.20 ± 2.78****
Hindlimb	126.60 ± 2.16	100.40± 2.12****
Paw Print Width (mm)		
Forelimb	7.90 ± 0.15	7.64 ± 0.20
Hindlimb	8.16 ± 0.11	8.41 ± 0.51
Paw Print Length (mm)		
Forelimb	7.40 ± 0.31	6.72 ± 0.60
Hindlimb	6.940 ± 0.15	11.16 ± 1.69**
Paw Angle (degrees)		
Forelimb	7.14 ± 1.80	14.64 ± 4.47
Hindlimb	8.18 ± 1.68	11.60 ± 3.40

Table 9. Dynamic gait parameters in 15-month 5xFAD mice.
Dynamic gait parameters were assessed in male 5xFAD mice (n=7) and their WT littermates (n=11) one month of free food. All values shown as mean ± SEM (unpaired two-tailed t-test; *p<0.05, **p<0.005, ****p<0.0001).

Parameter	Wild-Type	5xFAD
Stride Length (mm)		
Forelimb	61.36 ± 0.95	50.83 ± 4.62*
Hindlimb	60.58 ± 1.03	52.07 ± 2.79**
Swing Speed (m/s)		
Forelimb	0.75 ± 0.05	0.64 ± 0.07
Hindlimb	0.92 ± 0.06	1.164 ± 0.08*
Stand (s)		
Forelimb	0.12 ± 0.00	0.12 ± 0.01
Hindlimb	0.13 ± 0.01	0.17 ± 0.02
Swing (s)		
Forelimb	0.09 ± 0.00	0.09 ± 0.00
Hindlimb	0.07 ± 0.00	0.05 ± 0.00****
Stand Index		
Forelimb	-8.65 ± 0.47	-10.08 ± 0.99
Hindlimb	-8.74 ± 0.66	-7.95 ± 0.72
Duty Cycle (s)		
Forelimb	56.20 ± 0.70	57.11 ± 2.45
Hindlimb	63.71 ± 0.71	73.31 ± 1.84****

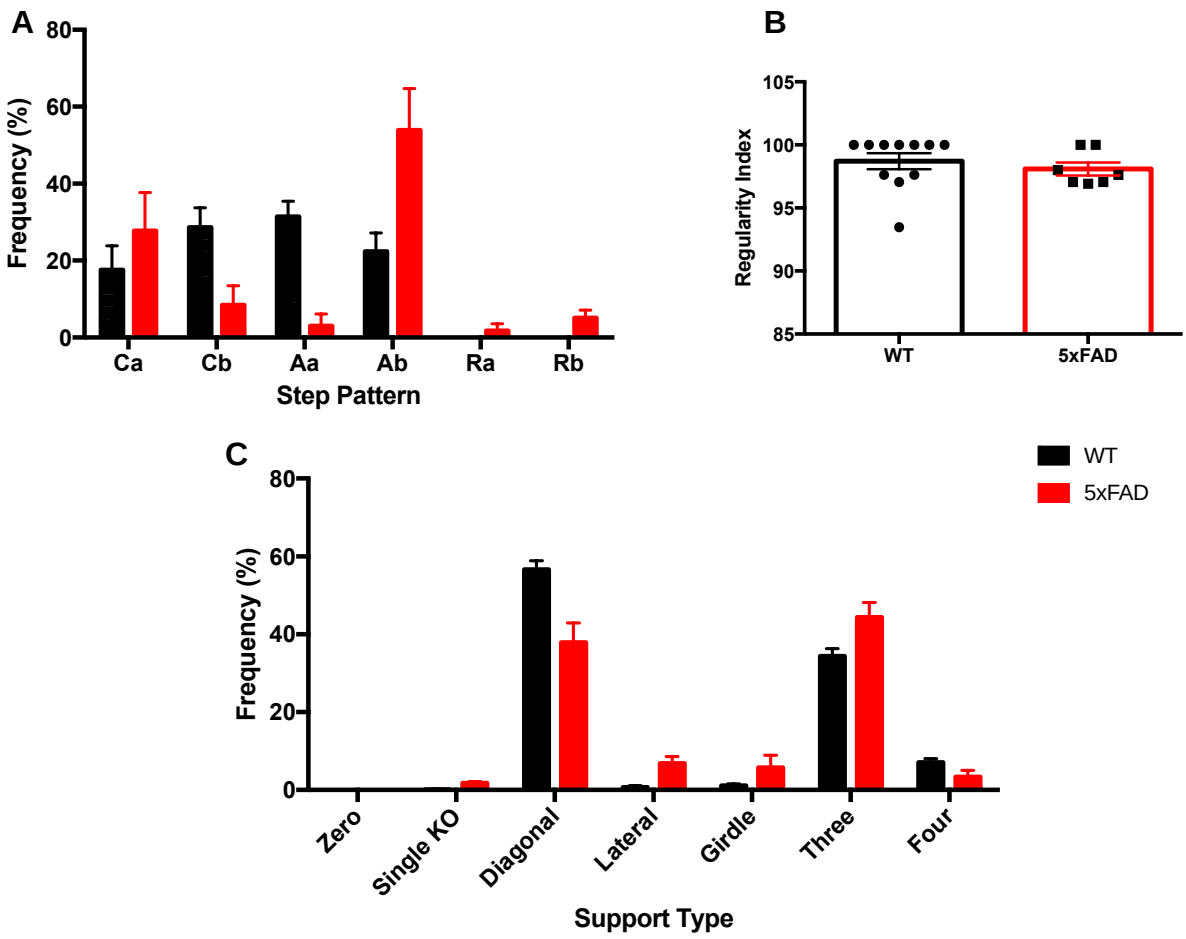


Figure 26. Step pattern and limb support measures in 15-month 5xFAD mice.

A) Mean ± SEM percentage occurrence of each of the step patterns, B) regularity index (%) and C) percentage occurrence of each of the possible limb support types in male 5xFAD mice (n=7) and their WT controls (n=11) following one month of free food (two-way ANOVA (A, C) or unpaired two-tailed t-test (B)).

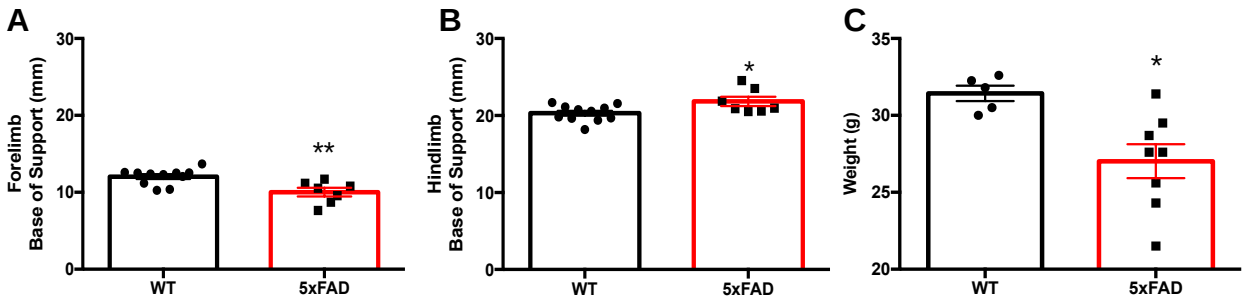


Figure 27. Base of support and weight in 15-month 5xFAD mice.

Mean ± SEM A) forelimb base of support (mm) and B) hindlimb base of support (mm) of male 5xFAD mice (n=7) and their WT controls (n=11) following one month of free food. Weights of WT (n=5) and 5xFAD mice (n=7) prior to transcardial perfusion (unpaired two-tailed t-test; *p<0.05, **p<0.005).

3.6 Neuromuscular Strength is not Affected in 5xFAD Mice at 14-Months of Age

It is possible that the gait impairments observed in 5xFAD mice are a result of perturbations in neuromuscular strength. And so, following gait assessments, neuromuscular strength was assessed using a grip-strength meter. Forelimb grip force was not significantly different between the 14-month-old male 5xFAD and WT mice ($t_{(20)}$ = 1.208, p=0.2413; Figure 28).

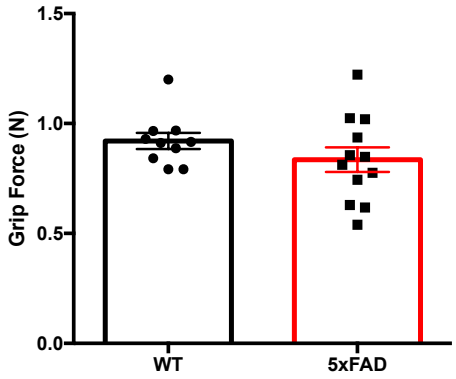


Figure 28. Forelimb grip force in 14-month old 5xFAD mice.

Mean ± SEM forelimb grip force of male 5xFAD mice (n=12) and their WT controls (n=10) at 14-months of age (unpaired two-tailed t-test).

3.7 Mild Food-Restriction Does Not Affect Amyloid Pathology in Male and Female 5xFAD Mice

In order to encourage mice to perform in the touchscreen tasks, the mice are mildly food restricted. Some studies have shown that caloric restriction (defined as a reduction of average calorie intake to 50-70% of the calories consumed *ad libitum*) has the ability to significantly reduce cognitive deficits in AD mouse models and humans (Halagappa et al., 2007; Schroeder et al., 2010; Witte et al., 2009; Wu et al., 2008). Various mouse models of AD also show a reduction in amyloid pathology following caloric restriction (Halagappa et al., 2007; Patel et al., 2005; Schroeder et al., 2010; Wang et al., 2005). Thus, we want to determine whether our food-restriction protocol would have any significant effect on amyloid pathology in these mice.

Three months of mild food-restriction in 5xFAD mice to 85% of their adult weight did not have a significant influence on amyloid pathology. Amyloid-beta immunoreactivity in the hippocampi and corticies of food-restricted 5xFAD mice did not significantly differ from that of free-feeding 5xFAD mice on a regular *ad libitum* diet. This was the case for both male (hippocampus ($t_{(6)}$ = 0.7622, p=0.4748, cortex ($t_{(6)}$ = 0.9598, p=0.3742); Figure 29) and female 5xFAD mice (hippocampus ($t_{(6)}$ = 0.9946, p=0.3583, cortex ($t_{(6)}$ = 1.237, p=0.2624); Figure 30). In addition, Thioflavin-S staining did not differ significantly in the hippocampi ($t_{(6)}$ = 0.2291, p=0.8264; Figure 31A, B) and cortex ($t_{(6)}$ = 1.168, p=0.2870; Figure 31C, D) of food-restricted female 5xFAD mice when compared to free-feeding female 5xFAD mice.

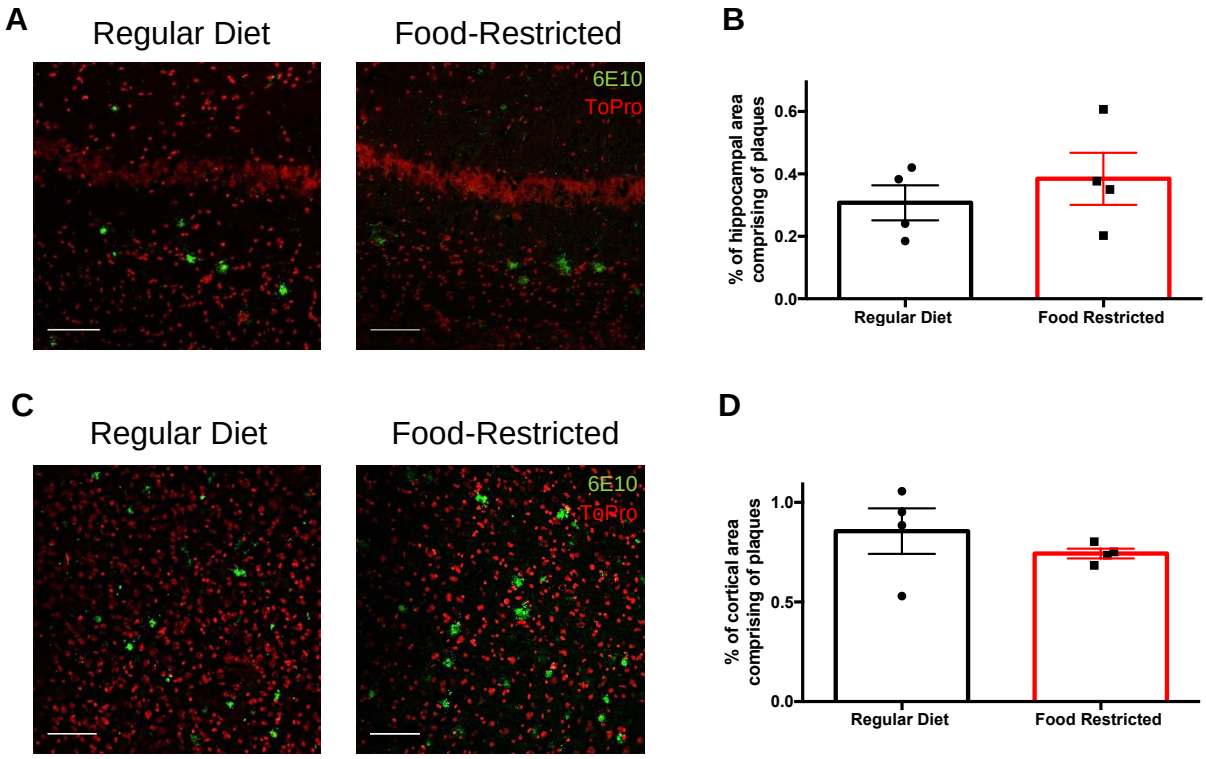


Figure 29. The effect of mild caloric restriction on 6E10 amyloid pathology in male 5xFAD mice at 6 months of age.

A) Representative images of amyloid-beta 6E10 antibody and ToPro-3 (20x magnification; scale bar = 100µm) and B) quantification (mean ± SEM) of 6E10 immunoreactivity in the hippocampi (CA1b) of mildly food-restricted (n=4; 3-4 slices/mouse) and free food male 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. C) Representative images of amyloid-beta 6E10 antibody and ToPro-3 (20x magnification; scale bar = 100µm) and D) quantification (mean ± SEM) of 6E10 immunoreactivity in the cortices of mildly food-restricted (n=4; 3-4 slices/mouse) and free food male 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. Unpaired two-tailed t-test.

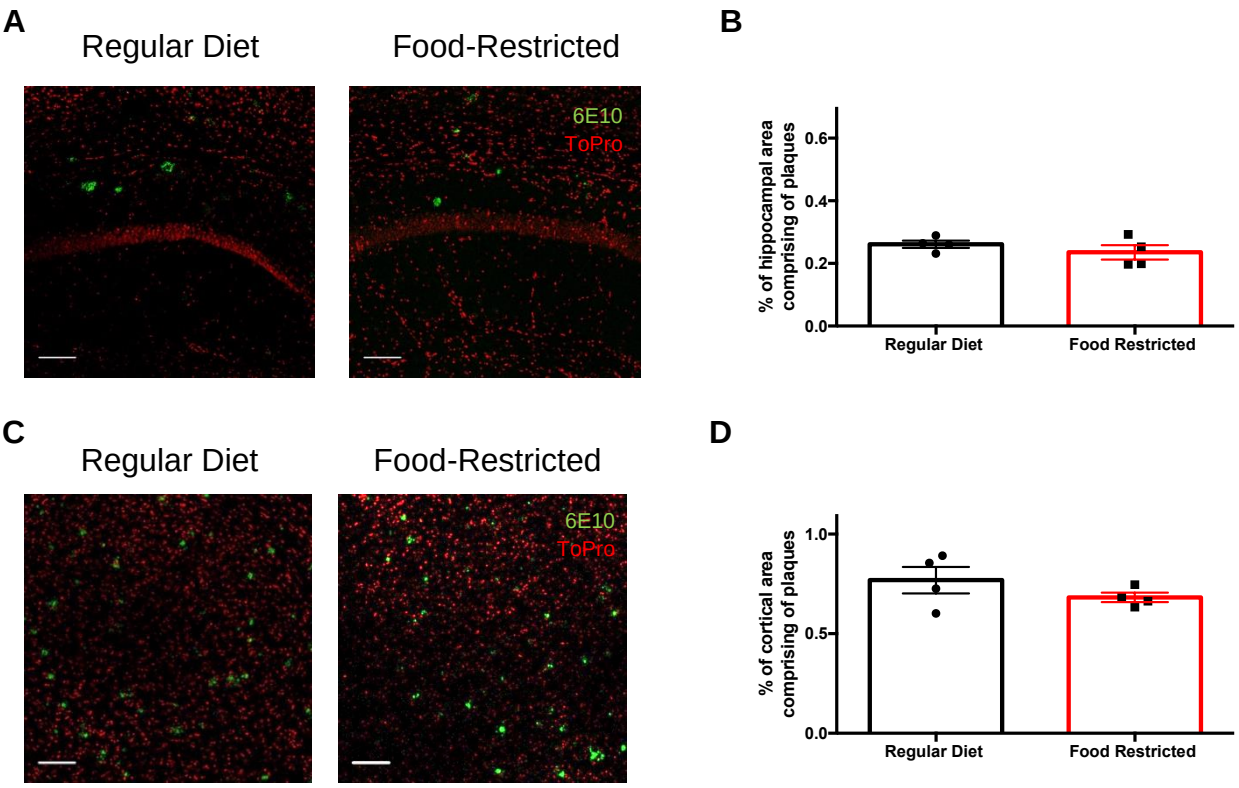


Figure 30. The effect of mild caloric restriction on 6E10 amyloid pathology in female 5xFAD mice at 6 months of age.

A) Representative images of amyloid-beta 6E10 antibody and ToPro-3 (20x magnification; scale bar = 100µm) and B) quantification (mean ± SEM) of 6E10 immunoreactivity in the hippocampi (CA1) of mildly food-restricted (n=4; 3-4 slices/mouse) and free food female 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. C) Representative images of amyloid-beta 6E10 antibody and ToPro-3 (20x magnification; scale bar = 100µm) and D) quantification (mean ± SEM) of 6E10 immunoreactivity in the cortices of mildly food-restricted (n=4; 3-4 slices/mouse) and free food male 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. Unpaired two-tailed t-test.

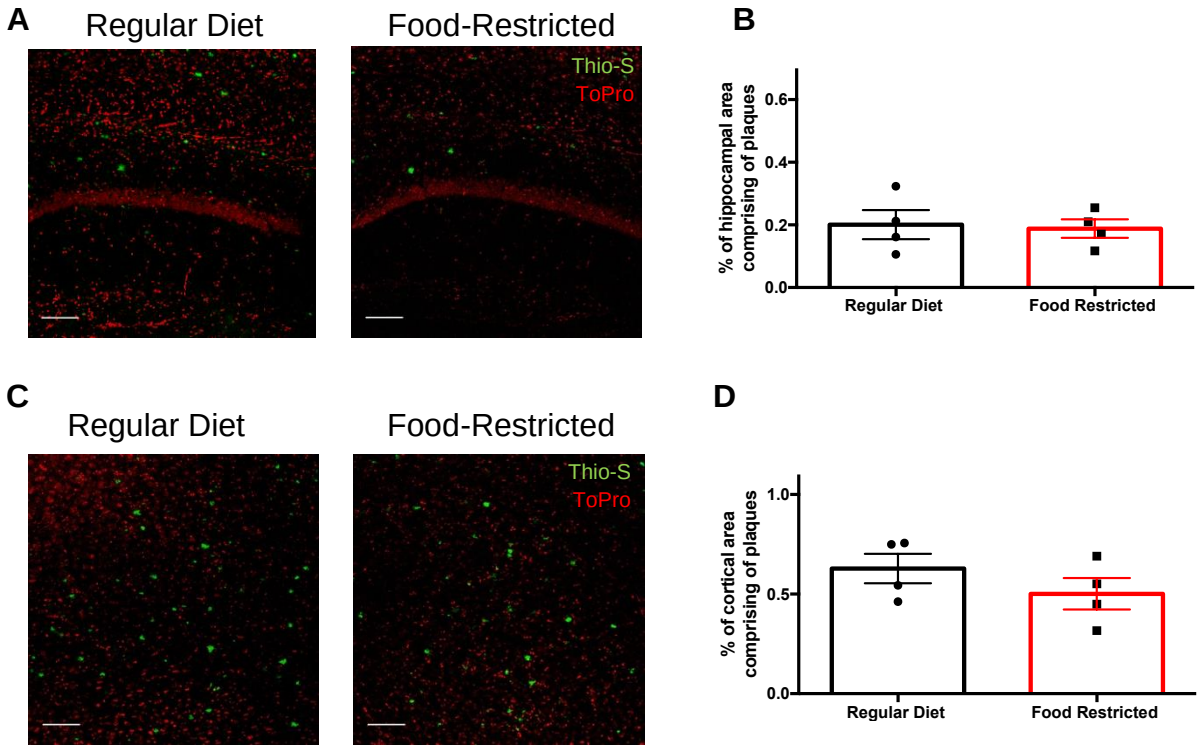


Figure 31. The effect of mild caloric restriction on Thioflavin-S amyloid pathology in female 5xFAD mice at 6 months of age.

A) Representative images of Thioflavin-S (Thio-S) and and ToPro-3 (20x magnification; scale bar = 100µm) and B) quantification (mean ± SEM) of Thio-S in the hippocampi (CA1b) of mildly food-restricted (n=4; 3-4 slices/mouse) and free food male 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. C) Representative images of Thioflavin-S and ToPro-3 (20x magnification; scale bar = 100µm) and D) quantification (mean ± SEM) of Thio-S in the cortices of mildly food-restricted (n=4; 3-4 slices/mouse) and free food male 5xFAD mice (n=4; 3-4 slices/mouse) at 6 months of age. Unpaired two-tailed t-test.

Chapter 4 - Discussion

4.1 5xFAD Mice Do Not Show Retinal Degeneration Due to the *Pdeb*^{rd1} allele

Although 5xFAD mice may carry the *Pdeb*^{rd1} allele for retinal degeneration, the presence of one *Pdeb*^{rd1} allele does not affect vision (Giménez and Montoliu, 2001) or, as we have observed, performance in the PVD task. In addition, young 5xFAD mice have been able to perform on a variety of other vision-requiring tasks without impairment. This includes the 5-CSRTT (Masood, 2015), the Y-maze (Oakley et al., 2006), and the Morris Water Maze (Ohno, 2006; Schneider et al., 2014), where the mouse is required to use spatial cues on the wall of a room to orient themselves in a water pool and find a hidden platform. Thus, *Pdeb*^{rd1} induced changes in retinal function does not affect the ability of 5xFAD mice to perform touchscreen tasks.

4.2 Visual Discrimination and Cognitive Flexibility is not Impaired in 5xFAD Mice

5xFAD mice did not show significant alterations in visual discrimination acquisition and reversal when compared to control mice. The lack of impairments in visual discrimination has also been observed in 21-month-old TASTPM (Harper et al., 2013), 18-month PDAPP (Harper et al., 2013) and APPSwDl/Nos2$^{-/-}$ (CVN) mice (Piiponniemi et al., 2017). Harper et al., used an apparatus similar to the Bussey-Saksida touchscreens, although their visual discrimination task used different images and did not consist of a reversal stage. TASTPM mice express two of the five mutations carried by 5xFAD mice: the Swedish APP K670N/M671L mutation, and a mutation in the gene for

presenilin 1 (M146V), resulting in cerebral Aβ deposition beginning at 3-months (Howlett et al., 2004). Meanwhile, PDAPP mice express the Indiana APP V717F mutation, leading to some plaque deposition by 10-12 months of age (Games et al., 1995; Hartman et al., 2005). CVN mice express a few mutations in human APP (Swedish K670N/M671L, Dutch E693Q, and Iowa D694N) along with a homozygous deletion of the gene for nitric oxide synthase from $Nos2^{-/-}$ (B6 129P2Nos2 tau1Lau/J) animals (Piiponniemi et al., 2017). These mice present dense Aβ deposits and extensive tau pathology by 52 weeks of age (Wilcock et al., 2008). Meanwhile, deficits in visual discrimination learning have been observed in the PVD task with 11-month old rTg4510 mice (Harper et al., 2013). These mice have tetracycline regulated expression of human P301L mutant Microtubule Associated Protein Tau, resulting in age related tau pathology (Harper et al., 2013). The rTg4510 mice performed with significantly less accuracy during the acquisition/learning phase of the PVD task (Harper et al., 2013). Meanwhile, 4-5 month old TgCRND8 mice were found to reverse the previously acquired association faster than WT mice (Romberg et al., 2013). TgCRND8 mice express the Swedish K670N/M671L and the Indiana V717F APP mutation, leading to plaque formation by 3 months of age (Chishti et al., 2001).

Interestingly, at the 10-month time-point, 5xFAD mice take significantly longer than WT mice to make a correct response and collect the reward in PVD. Significant delays in reward collection and task response were also observed in 10-13.5 month-old 5xFAD mice subject to the 5-CSRTT (Masood, 2015). Delays in reward collection have also been observed in CVN mice, but without delays in task response, leading Piiponniemi et al. (2017) to conclude that this was not likely due to gross motor impairments

(Piiponniemi et al., 2017). However, in our case, it is possible that this deficit results from a motor/gait impairment and/or a delay in cognitive processing – both of which have been observed in patients with AD (Dudgeon, 2010; Li et al., 2014b; O'keeffe et al., 1996; Sala et al., 2004).

Although we observed no significant differences in accuracy between the genotypes, mice performed better at 7-months than at 4- or 10-months of age. Treviño et al. (2013) demonstrated that the difficulty of visual discrimination depends on the degree of structural similarity between the CS+ and CS- images, affecting learning rate and maximum performance (Treviño et al., 2013). The three image sets used in our longitudinal study were subsequently assessed for their difficulty in three separate groups of WT mice at 4 months of age (Beraldo et al., unpublished). Image set 2 was significantly easier to acquire than image set 1 (a reduction in the number of sessions to criteria), likely leading to a significant increase in accuracy (% correct) compared to image set 3 during the 10 sessions of reversal (Beraldo et al., unpublished). Therefore, image sets could be manipulated to alter the difficulty of the PVD task, potentially allowing for magnification of deficits in visual discrimination and/or cognitive flexibility. Image sets could also be counterbalanced across time-points to avoid this issue.

4.3 The Sustained Attention of 5xFAD Mice is Affected by the Injection of Donepezil

Attention is affected by damage to the prefrontal cortex and/or striatum, and impairments in attention have been previously observed patients with AD (Perry and Hodges, 1999). The accuracy of mice in the 5-CSRTT task is significantly reduced by damage to the prefrontal cortex and/or striatum as well (Muir et al., 2012; Passetti et al.,

2003; Rogers et al., 2001). Various mouse models of cholinergic dysfunction also demonstrate deficits in attention, including M_1 muscarinic receptor deficient mice (Bartko, 2011), ChAT-ChR2-EYFP mice (Kolisnyk et al., 2013b) and $VAChT^{Six3\text{-}Cre\text{-}flox/flox}$ mice (Kolisnyk et al., 2013a), suggesting that cholinergic transmission is vital for attentional processes. Studies have also shown that the cholinergic system can be leveraged in order to improve attention. Galantamine was able to improve the attention of wild-type mice in the most demanding 0.6s stimulus length of the 5-CSRTT (Kolisnyk et al., 2013a). Meanwhile, donepezil has been shown to improve executive function and involuntary attention in human AD patients (Bohnen et al., 2005; Rokem et al., 2010) and in the 3xTG mouse model (Romberg et al., 2011). 3xTG mice were assessed in the 5-CSRTT at 9-months of age and were found to demonstrate significant deficits in response accuracy when compared to WT controls (Romberg et al., 2011). These attentional deficits were ameliorated following daily intraperitoneal injection of donepezil prior to the task (Romberg et al., 2011). Donepezil also improved the vigilance – the ability to maintain a constant level of attention across time – of these mice (Romberg et al., 2011). At high doses (4 mg/kg), the long-term administration of donepezil has also been shown to reduce the levels of soluble Aβ40 and Aβ42, reduce plaque deposition and prevent synapse loss in the Tg2576 mouse model of AD (Dong et al., 2009). This mouse model carries the Swedish K670N/M671L APP mutation (Hsiao et al., 1996). However, the administration of donepezil to 5xFAD mice – through implanted subcutaneous osmotic mini-pumps for the purpose of maintaining drug concentrations and increasing the efficacy of donepezil (Fara and Urquhart, 1984) – had little effect on attention as measured by the 5-CSRTT. However, when donepezil was administered

via intraperitoneal injection to replicate the study performed by Romberg et al. (2011), there was a significant reduction in the omission percentage of 5xFAD mice at the 0.6s stimulus length. This suggests that the injection of donepezil improved the ability of 5xFAD mice to attend to the stimulus display area throughout the duration of the task (Romberg et al., 2011). Deficits in vigilance have also been observed in patients with AD (Perry and Hodges, 1999).

Donepezil is normally recommended as a treatment for patients with mild to moderate AD that can tolerate the adverse side effects of the drug (Bishara et al., 2015). Continued treatment with donepezil in combination with memantine has also been associated with cognitive benefits in more advanced cases of AD, suggesting that it can be used for the treatment of severe AD as well (Howard et al., 2012). The administration of donepezil and other cholinesterase inhibitors should begin early and remain uninterrupted, as their benefits are rapidly lost and may not be fully regained upon re-administration (Bishara et al., 2015; Doody et al., 2001). In our experiment, the infusion of donepezil only lasted 28 days, after which the pump was removed and the drug was washed out. There is a possibility that the previous – although not statistically significant – benefits of donepezil on attention were lost during this time, and that the effects of donepezil following re-administration were insufficient to improve the performance of 5xFAD mice in all aspects of the task. It is also possible that the administration of donepezil began when cholinergic neuron death and dysfunction was already too pronounced. The number of ChAT+ neurons in these 5xFAD mice would already have been halved by 6 months of age (Devi and Ohno, 2010). As a result, enhancing cholinergic transmission past this age may be insufficient to entirely compensate for the

loss of acetylcholine-releasing neurons. So, if these experiments were to be repeated, donepezil treatment should commence prior to significant ChAT+ neuron death and dysfunction – or perhaps even earlier – as amyloid pathology begins to develop in 5xFAD mice at approximately 2 months of age (Oakley et al., 2006). In addition, treatment should continue until all assessments involving donepezil are completed.

4.4 Open-Field Locomotion in 5xFAD mice

Literature regarding locomotion on an open-field in male 5xFAD mice has been limited and inconsistent. One study showed that male 5xFAD mice have a significant reduction in locomotion and rearing activity at 9 months of age compared to WT littermates (Schneider et al., 2014). These mice also appear to be less anxious, spending significantly more time in the center of the maze (Schneider et al., 2014). Another study found that male 5xFAD mice only travel significantly less than their WT controls at 12 and 15 months of age and reared significantly less at 9, 12 and 15 months (O'Leary, 2013). However, they recorded no significant differences in center time (O'Leary, 2013). The mice in these studies were not food-restricted (O'Leary, 2013; Schneider et al., 2014). Another study claims that 5xFAD mice are hyperactive at 9 and 12 months of age (Yang et al., 2014). We only observe significant changes at 14 months of age (with no significant differences in locomotion), where food-restricted male 5xFAD mice spend significantly less time rearing than their WT controls. Both O'Leary (2013) and Schneider et al. (2014) suggest that a reduction in rearing activity with age could be due to impairments in the motor coordination required by a mouse to stand on their hindlimbs. Decreases in rearing have also been reported in other non-AD mouse models that walk with an irregular gait and thus lack the ability to maintain balance upon

rearing (Baik et al., 1995; Nelson and Young, 1998). The fourteen-month-old male 5xFAD mice in our study demonstrated changes in weight-bearing capacity, and balance (Gensel et al., 2006). This also has been observed by O'Leary et al. in 10, 13 and 16 month-old male and female 5xFAD mice as well (O'Leary, 2013; O'Leary et al., 2013). In addition, our 14-month-old male 5xFAD mice also had a significantly lower regularity index, suggesting that these mice have an impairment in interlimb coordination (Parvathy and Masocha, 2013). Overall, this suggests that the reduction in rearing activity observed in our mice was likely was a result of gait impairments.

4.4.1 *Donepezil reduces anxiety in 5xFAD mice*

The anxiolytic effects of donepezil have been previously observed in patients with AD. When compared to AD patients given the placebo, those given donepezil (5mg/day for 28 days) showed a significant reduction in anxiety (relative to baseline) as measured by the 12-item Neuropsychiatric Inventory (Feldman et al., 2001; Gauthier et al., 2002). Similar effects have also been observed in three mouse models of anxiety (Nakamura and Kurasawa, 2001) and a valproic acid-induced model of autism (Kim et al., 2014). There are not yet any studies evaluating the effects of donepezil on the anxiety-like behavior of AD mouse models.

In addition to locomotor and rearing activity, open-field locomotion can also be used to measure anxiety-like behavior. Small rodents tend to prefer to be in corners rather than out in the open to avoid potential predators, and the time spent in the edges of an open-field can be used as a measure of anxiety-like behaviour. Less anxious animals will spend significantly more time in the center of open-field and less time closer to the walls

of the box (Carola, 2002; Kulesskaya and Voikar, 2014). Like Kim et al. (2014), we also observe a significant increase in center time following treatment with donepezil, suggesting that donepezil also had anxiolytic effects on 10-month male 5xFAD mice. This also implies that the donepezil did indeed inhibit acetylcholinesterase in these mice. An acetylcholinesterase activity assay could always be run in tissue, blood or urine samples taken from mice given donepezil to confirm this.

4.4.2 *The effect of food-restriction on locomotion and gait*

In older mice, long-term caloric restriction has been shown to lead to hyperlocomotion in an open-field (Halagappa et al., 2007), while short-term caloric-restriction in mice appears to cause hypoactivity (Kuhla et al., 2013). In one study, male and female 3-month-old 3xTG mice that were food-restricted for 7 or 14 months prior both demonstrated a significant increase in open-field locomotion (Halagappa et al., 2007). Meanwhile the 3xTG mice that were given food *ad libitum* exhibited reduced locomotion and exploratory behaviour compared to WT controls (Halagappa et al., 2007). Kuhla et al. found that after 4 weeks of food restriction, C57BL/6 mice travelled significantly less distances in an open-field compared to controls. However, this was not observed after 20 or 74 weeks of food-restriction. In another study, caloric restriction in female C3B10RF[1] mice (a long-lived F1 hybrid strain) 11-15 or 31-35 months of age led to an increase in locomotor activity in a runwheel cage but not in an open field (Ingram et al., 1987). After being given food *ad libitum* for one month, both WT and 5xFAD mice travelled less distances in the open-field than they did when they were still on food-restriction one month prior, suggesting that the long-term food-restriction led to

hyperlocomotion in these mice. However, because we used the same cohort of mice for both of these experiments, it is also possible that these mice have already habituated to the open-field boxes and their surroundings. Since this cohort of mice aged between the two locomotor assessments, we also cannot rule out the gait impairments that come with age as a contributor to the reduction in locomotion (O'Leary, 2013; O'Leary et al., 2013). For these reasons, we also evaluated gait in 5xFAD before and after one month of free food. Because the weights of the 5xFAD mice and their WT controls were significantly different, we cannot compare the static gait parameters. However, the dynamic gait parameters of 15-month-old 5xFAD mice after 1 month of free-food did not significantly differ from what is described below, thus changes in gait are likely not responsible for the reductions in locomotion.

4.5 Gait Impairments in 5xFAD Mice

Patients with AD also demonstrate impairments in gait – although the dominating type of gait disorder depends on the severity of the disease (O'keeffe et al., 1996; Sala et al., 2004). These gait impairments contribute to the increase in fall risk and immobility in AD patients compared to age matched controls (Amboni et al., 2013; Muir et al., 2012; Nutt, 2013). Gait is also affected in various mouse models of AD. Six month old APP/PS1 knock-in mice also exhibit an abnormal gait, with a reduction in average stride length but no apparent neurogenic muscular fiber atrophy (Wirths et al., 2008). These mice express the Swedish K670N/M671L and London V717I APP mutation, in addition to two presenilin 1 knock-in mutations PS1 M233T and PS1 L235P (Wirths et al., 2008). Alterations in gait have also been observed in the TG2576 mouse model of AD at 6

months – prior to expected plaque accumulation (Schroer et al., 2010). At 12 months of age, female 5xFAD mice demonstrate impairments in gait, motor coordination and balance – with no differences in motor strength (O'Leary et al., 2013). Similar to what has been observed in other mouse models of AD, we also observed gait impairments in male 5xFAD mice at 14 months of age in the absence of differences in neuromuscular function and weight. Male 5xFAD mice demonstrate a significant increase in the hindlimb base of support, accompanied by a decrease in the forelimb base of support. The further apart the paws are placed during locomotion, the less likely the animal is to fall – thus a large base of support can compensate for an instable gait (Liu et al., 2013b). Changes in base of support have also been observed in patients with AD, where they widen their base to increase stability (Nutt, 2013). However, the reduction in forelimb base of support suggests that there may have been a shift in how the weight was carried in the 5xFAD mice – which could also result in an increase in the hindlimb base of support (Gensel et al., 2006). This is supported by the decrease in the paw print area and paw intensity of forepaws, which is often affected in response to a reduction in forelimb weight bearing (Gensel et al., 2006; Neumann et al., 2009). There is also a slight decrease in the paw print area of the hindpaws, but the forepaws play a more important role in supporting the body weight during walking (Neumann et al., 2009). The decrease in maximum paw area during the stance phase is mostly attributed to the significant decrease in print width. There were no significant differences between the weights of the WT and 5xFAD mice, so the changes in these gait parameters cannot be attributed to differences in weight. Forelimb and hindlimb paw angle was also significantly increased in 5xFAD mice. This larger paw placement is an indication of

dysfunctional ataxia (Hannigan and Riley, 1988). Lastly, 5xFAD mice have significantly shorter stride lengths and slower swing speeds than their WT controls. Therefore, 5xFAD mice would take longer than their WT controls to travel the same distance.

The gait impairments we observed in 14-month-old 5xFAD mice could explain the delays we observe on the touchscreens at the 10-month time-point (when mice were between 10-13.5 months of age) and the reduction in rearing activity observed during open-field locomotion. Remarkably, at least for the PVD task, the 5xFAD mice performed as well as their controls, suggesting that touchscreen tasks can be used effectively even in mice with changes in gait. However, the gait of 5xFAD mice must be taken into consideration when subjecting them to other behavioural assessments.

Further studies need to be done to investigate the reasons behind the extensive gait impairments observed in 5xFAD mice and other AD mouse models. Cholinergic neuron death and dysfunction in brain structures associated with gait – such as the pedunculopontine nucleus (Mesulam, 2013) – could be the cause of what we have observed, as these areas are also perturbed in patients with AD (Mesulam, 2013). Problems with gait and balance have been associated with impairments in cholinergic function, and cholinergic augmentation via donepezil has been shown to improve gait and balance while reducing fall frequency in patients with mild AD (Mancini et al., 2015; Montero-Odasso et al., 2015; Segev-Jacubovski et al., 2011). Some improvements in gait were sustained and continued to improve over a four month period (Segev-Jacubovski et al., 2011). The deletion of VAChT in the peduncolopontine and laterodorsal tegmental nuclei cholinergic neurons in $VAChT^{En1\text{-}Cre\text{-}flox/flox}$ mice impairs motor learning/coordination, which continues to deteriorate with age (Janickova et al.,

2017). In addition, at 2-5 months of age, these mice also demonstrate a significant reduction in stride length and swing speed on the Catwalk (Janickova et al., 2017). Deficits in balance on the Catwalk become more pronounced at 13-16 months of age (Janickova et al., 2017). We also observed these impairments in 14-month male 5xFAD mice, suggesting that the death of cholinergic neurons in theses mice could have led to perturbations in gait as well. After all, 5xFAD mice do demonstrate a significant reduction of ChAT, a marker of cholinergic neurons (Devi and Ohno, 2010; Francis et al., 1999). At 6-months, 5xFAD mice have 50% less ChAT+ positive neurons than their WT controls (Devi and Ohno, 2010). However, neither the infusion or intraperitoneal injection of donepezil had significant effects on gait. There is a possibility that the administration of donepezil began when cholinergic neuron death and dysfunction was already too severe – such that inhibiting acetylcholinesterase would not be capable of enitrely compensating for the loss and dysfunction of cholinergic neurons in these mice. As previously discussed, it is also possible that, because donepezil administration did not begin early in life and remain uninterrupted, the benefits of donepezil were rapidly lost and not fully regained upon re-administration (Bishara et al., 2015; Doody et al., 2001). This should be taken into consideration if these series of experiments were to be repeated, ensuring that treatment begins early and continues until all donepezil-related evaluations are complete. There are currently no published studies assessing the effects of donepezil on gait in AD mouse models.

4.6 Food-restriction had no effect on amyloid pathology in 5xFAD mice

Caloric restriction has been shown to reduce cognitive impairments in both humans and mouse models of AD (Halagappa et al., 2007; Schroeder et al., 2010; Witte et al., 2009; Wu et al., 2008). In mice, food-restriction is associated with a reduction in pathology, and could confound our behavioural experiments (Halagappa et al., 2007; Patel et al., 2005). Male and female 3xTG mice that have been food-restricted for 14 months showed significantly lower levels of Aβ40, Aβ42 and hyperphosphorylated tau than their controls (Halagappa et al., 2007). A significant reduction in Aβ-plaque accumulation and astrocytic activation was also observed following 15 weeks of caloric-restriction in male APP/PS1 mice (Patel et al., 2005). These mice carry the Swedish K670N/M671L mutation of APP and the M146L mutation of presinilin 1 (Patel et al., 2005). Both Patel et al. (2005) and Halagappa et al. (2007) defined caloric-restriction as 60% of the *ad libitum* diet. Our food-restriction protocol is more mild, reducing food consumption to maintain 85% of adult weight beginnning at 12 weeks of age. We did not obseve signficant differences in amyloid pathology in male and female 5xFAD mice following 2-3 months of food resctriction. There are strong associations between pathology and cognitive function, suggesting that cognition was likely unaffected by our food-restriction protocol. However, there remains a possibility that food-restriction can affect cognition through mechanisms that may not be related to Aβ or tau pathologies – like insulin sensitivity (Halagappa et al., 2007), but this will need to be explored further. This experiment could be repeated with the same food-restriction protocol but beginning at 3

months of age (when we begin to food restrict our mice) and with a longer duration to evaluate the effects of long-term caloric-restriction on 5xFAD mice, as this would be more respresntative of the 5xFAD mice used in our study.

4.7 Conclusions, Significance and Future Directions

In summary, male 5xFAD mice do not demonstrate deficits in visual discrimination and cognitive flexibility in the PVD task at 4, 7 and 10-month time-points. The lack of impairments in this task suggests that male 5xFAD mice are not a good model of visual discrimination or cognitive flexibility in AD. It is also possible that this touchscreen task is not sensitive enough to detect impairments in these particular cognitive domains in 5xFAD mice. However, the variety of parameters recoded by the touchscreen apparatus still allowed us to detect latencies in task response and reward collection when these mice were 10-13.5 months of age (at the 10-month time-point). This could have been the result of delays in cognitive processing and/or locomotor impairments. Locomotor activity assessments demonstrated that these mice were not significantly more or less active than their WT controls. Meanwhile, gait analyses revealed that 14-month-old male 5xFAD mice exhibit severe impairments in gait. Most notably, the stride lengths are significantly shorter and the swing speeds are significantly slower in male 5xFAD mice when compared to their controls. This suggests that it would take male 5xFAD mice significantly longer than their WT controls to travel the same distances. Further studies need to be done to evaluate the reasons behind these gait impairments in 5xFAD mice and pinpoint the brain regions involved, as there were no significant differences in weight and neuromuscular function between male 5xFAD mice and their

WT controls. Recent studies demonstrating the effects of cholinergic dysregulation on gait suggests that the death and dysfunction of cholinergic neurons in 5xFAD mice may play a role (Janickova et al., 2017). Gait and locomotor activity in male 5xFAD mice was also not significantly affected by food-restriction. In conclusion, impairments in gait could explain the delays observed in the PVD and other touchscreen tasks, although it did not affect their accuracy on either of the tasks. This suggests that gait does not significantly influence or confound the performance of mice in the PVD task. However, gait must be taken into consideration when subjecting old 5xFAD mice to other behavioural assessments. These findings also suggest that the 5xFAD mouse model can be used as an animal model of non-cognitive function in AD. Drugs aimed at improving motor function in AD patients could use 5xFAD mice as their pre-clinical model. Gait is an important non-cognitive domain to treat in AD patients, as it contributes to the increase in fall risk and immobility in AD patients compared to age matched controls (Amboni et al., 2013; Muir et al., 2012; Nutt, 2013). Immobility is associated with changes in social behaviour, personality and deteriorations in mental health, along with poorer outcomes and more co-morbidities (McCarron et al., 2005).

Infusion of donepezil had minor effects on the performance of male 5xFAD mice in the 5-CSRTT when compared to the 5xFAD mice that received saline + 10% DMSO. Meanwhile, the injection of donepezil reduced the omission percentage and therefore improved the vigilance of in 5xFAD mice. Treatment with donepezil also had no significant effect on gait in these mice. Donepezil had anxiolytic effects on the 5xFAD mice, significantly increasing the amount of time spent in the center of the open-field. This effect has also been observed in 7-month old 5xFAD mice that have been treated

with galantamine, another cholinesterase inhibitor (Bhattacharya et al., 2014), AD patients (Gauthier, 2012) and mouse models of anxiety (Nakamura and Kurasawa, 2001). This suggests that donepezil did indeed inhibit acetylcholinesterase as intended. However, acetylcholinesterase activity assays can be performed to confirm this. For reasons described previously, if this entire experiment were to be repeated, donepezil should be administered early and remain uninterrupted for the entire duration of donepezil related experiments to achieve maximum effects.

Lastly, none of the male 5xFAD mice were homozygous for *Pdeb*^{rd1}, and *Pdeb*^{rd1} genotype did not affect performance in the PVD task. In addition, 2-3 months of mild food-restriction in 5xFAD mice did not significantly affect amyloid pathology, suggesting that our food-restriction protocol also did not alter amyloid pathology in 5xFAD mice and that touchscreen food restriction protocols are compatible with the study in AD mice.

References

Akasofu, S., Kimura, M., Kosasa, T., Sawada, K., and Ogura, H. (2008). Study of neuroprotection of donepezil, a therapy for Alzheimer's disease. Chem. Biol. Interact. *175*, 222–226.

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., et al. (2000). Inflammation and Alzheimer's disease. Neurobiol. Aging *21*, 383–421.

Albert, M.S. (1996). Cognitive and neurobiologic markers of early Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. *93*, 13547–13551.

de-Almada, B.V.P., de-Almeida, L.D., Camporez, D., de-Moraes, M.V.D., Morelato, R.L., Perrone, A.M.S., Belcavello, L., Louro, I.D., and de-Paula, F. (2011). Protective effect of the APOE-e3 allele in Alzheimer's disease. Braz. J. Med. Biol. Res. *45*, 8–12.

Amboni, M., Barone, P., and Hausdorff, J.M. (2013). Cognitive Contributions to Gait and Falls: Evidence and Implications. Mov. Disord. Off. J. Mov. Disord. Soc. *28*, 1520–1533.

Arnold, H.M., Burk, J.A., Hodgson, E.M., Sarter, M., and Bruno, J.P. (2002). Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. Neuroscience *114*, 451–460.

Baik, J.H., Picetti, R., Saiardi, A., Thiriet, G., Dierich, A., Depaulis, A., Le Meur, M., and Borrelli, E. (1995). Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature *377*, 424–428.

Bari, A., Dalley, J.W., and Robbins, T.W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat. Protoc. *3*, 759–767.

Bartko, S.J. (2011). Intact attentional processing but abnormal responding in M1 muscarinic receptor-deficient mice using an automated touchscreen method. Neuropharmacology *61*, 1366–1378.

Bartus, R.T., Dean, R.L., Beer, B., and Lippa, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. Science *217*, 408–414.

Beach, T.G., Monsell, S.E., Phillips, L.E., and Kukull, W. (2012). Accuracy of the Clinical Diagnosis of Alzheimer Disease at National Institute on Aging Alzheimer's Disease Centers, 2005–2010. J. Neuropathol. Exp. Neurol. *71*, 266–273.

Bekris, L.M., Yu, C.-E., Bird, T.D., and Tsuang, D.W. (2010). Genetics of Alzheimer Disease. J. Geriatr. Psychiatry Neurol. *23*, 213–227.

Bhattacharya, S., Haertel, C., Maelicke, A., and Montag, D. (2014). Galantamine Slows Down Plaque Formation and Behavioral Decline in the 5XFAD Mouse Model of Alzheimer's Disease. PLoS ONE *9*.

Birks, J.S. (2006). Cholinesterase inhibitors for Alzheimer's disease. In Cochrane Database of Systematic Reviews, (John Wiley & Sons, Ltd),.

Bishara, D., Sauer, J., and Taylor, D. (2015). The pharmacological management of Alzheimer's disease. Prog. Neurol. Psychiatry *19*, 9–16.

Bohnen, N., Kaufer, D., Hendrickson, R., Ivanco, L., Lopresti, B., Koeppe, R., Meltzer, C., Constantine, G., Davis, J., Mathis, C., et al. (2005). Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry *76*, 315–319.

Boncristiano, S., Calhoun, M.E., Kelly, P.H., Pfeifer, M., Bondolfi, L., Stalder, M., Phinney, A.L., Abramowski, D., Sturchler-Pierrat, C., Enz, A., et al. (2002). Cholinergic changes in the APP23 transgenic mouse model of cerebral amyloidosis. J. Neurosci. Off. J. Soc. Neurosci. *22*, 3234–3243.

Braak, H., and Braak, E. (1991). Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. Brain Pathol. Zurich Switz. *1*, 213–216.

Brigman, J.L., and Rothblat, L.A. (2008). Stimulus specific deficit on visual reversal learning after lesions of medial prefrontal cortex in the mouse. Behav. Brain Res. *187*, 405–410.

Brigman, J.L., Bussey, T.J., Saksida, L.M., and Rothblat, L.A. (2005). Discrimination of multidimensional visual stimuli by mice: intra- and extradimensional shifts. Behav. Neurosci. *119*, 839–842.

Brigman, J.L., Feyder, M., Saksida, L.M., Bussey, T.J., Mishina, M., and Holmes, A. (2008). Impaired discrimination learning in mice lacking the NMDA receptor NR2A subunit. Learn. Mem. Cold Spring Harb. N *15*, 50–54.

Brigman, J.L., Daut, R.A., Wright, T., Gunduz-Cinar, O., Graybeal, C., Davis, M.I., Jiang, Z., Saksida, L.M., Jinde, S., Pease, M., et al. (2013). GluN2B in corticostriatal circuits governs choice learning and choice shifting. Nat. Neurosci. *16*, 1101–1110.

Brookmeyer, R., Johnson, E., Ziegler-Graham, K., and Arrighi, H.M. (2007). Forecasting the global burden of Alzheimer's disease. Alzheimers Dement. *3*, 186–191.

Buée, L., Bussière, T., Buée-Scherrer, V., Delacourte, A., and Hof, P.R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Rev. *33*, 95–130.

Burdick, D., Soreghan, B., Kwon, M., Kosmoski, J., Knauer, M., Henschen, A., Yates, J., Cotman, C., and Glabe, C. (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. J. Biol. Chem. *267*, 546–554.

Burns, A., Gauthier, S., and Perdomo, C. (2007). Efficacy and safety of donepezil over 3 years: an open-label, multicentre study in patients with Alzheimer's disease. Int. J. Geriatr. Psychiatry *22*, 806–812.

Bussey, T.J., Muir, J.L., Everitt, B.J., and Robbins, T.W. (1997). Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. Behav. Neurosci. *111*, 920–936.

Bussey, T.J., Saksida, L.M., and Rothblat, L.A. (2001). Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. Behav. Neurosci. *115*, 957–960.

Bussey, T.J., Saksida, L.M., and Murray, E.A. (2003). Impairments in visual discrimination after perirhinal cortex lesions: testing "declarative" vs. "perceptual-mnemonic" views of perirhinal cortex function. Eur. J. Neurosci. *17*, 649–660.

Bussey, T.J., Padain, T.L., Skillings, E.A., Winters, B.D., Morton, A.J., and Saksida, L.M. (2008). The touchscreen cognitive testing method for rodents: how to get the best out of your rat. Learn. Mem. Cold Spring Harb. N *15*, 516–523.

Bussey, T.J., Holmes, A., Lyon, L., Mar, A.C., McAllister, K. a. L., Nithianantharajah, J., Oomen, C.A., and Saksida, L.M. (2012). New translational assays for preclinical modelling of cognition in schizophrenia: the touchscreen testing method for mice and rats. Neuropharmacology *62*, 1191–1203.

Carli, M., Robbins, T.W., Evenden, J.L., and Everitt, B.J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav. Brain Res. *9*, 361–380.

Carola, V. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav. Brain Res. *134*, 49–57.

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

Chudasama, Y., and Robbins, T.W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J. Neurosci. Off. J. Soc. Neurosci. *23*, 8771–8780.

Clapcote, S.J., Lazar, N.L., Bechard, A.R., Wood, G.A., and Roder, J.C. (2005). NIH Swiss and Black Swiss Mice Have Retinal Degeneration and Performance Deficits in Cognitive Tests. Comp. Med. *55*, 310–316.

Conejero-Goldberg, C., Gomar, J.J., Bobes-Bascaran, T., Hyde, T.M., Kleinman, J.E., Herman, M.M., Chen, S., Davies, P., and Goldberg, T.E. (2014). APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. Mol. Psychiatry *19*, 1243–1250.

Contestabile, A. (2011). The history of the cholinergic hypothesis. Behav. Brain Res. *221*, 334–340.

Cummings, J.L., Morstorf, T., and Zhong, K. (2014). Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res. Ther. *6*, 37.

Cutuli, D., De Bartolo, P., Caporali, P., Tartaglione, A.M., Oddi, D., D'Amato, F.R., Nobili, A., D'Amelio, M., and Petrosini, L. (2013). Neuroprotective effects of donepezil against cholinergic depletion. Alzheimers Res. Ther. *5*, 50.

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature *391*, 387–390.

Devi, L., and Ohno, M. (2010). Phospho-eIF2α Level Is Important for Determining Abilities of BACE1 Reduction to Rescue Cholinergic Neurodegeneration and Memory Defects in 5XFAD Mice. PLoS ONE *5*.

Dong, H., Yuede, C.M., Coughlan, C.A., Murphy, K.M., and Csernansky, J.G. (2009). Effects of donepezil on amyloid-beta and synapse density in the Tg2576 mouse model of Alzheimer's disease. Brain Res. *1303*, 169–178.

Doody, R.S., Geldmacher, D.S., Gordon, B., Perdomo, C.A., Pratt, R.D., and Donepezil Study Group (2001). Open-label, multicenter, phase 3 extension study of the safety and efficacy of donepezil in patients with Alzheimer disease. Arch. Neurol. *58*, 427–433.

Downes, J.J., Roberts, A.C., Sahakian, B.J., Evenden, J.L., Morris, R.G., and Robbins, T.W. (1989). Impaired extra-dimensional shift performance in medicated and unmedicated Parkinson's disease: evidence for a specific attentional dysfunction. Neuropsychologia *27*, 1329–1343.

Dudgeon, S. (2010). Rising tide: the impact of dementia on Canadian society : a study (Alzheimer Society of Canada).

Efange, S.M.N., Garland, E.M., Staley, J.K., Khare, A.B., and Mash, D.C. (1997). Vesicular Acetylcholine Transporter Density and Alzheimer's Disease. Neurobiol. Aging *18*, 407–413.

Fara, J., and Urquhart, J. (1984). The value of infusion and injection regimens in assessing efficacy and toxicity of drugs. Trends Pharmacol. Sci. *5*, 21–25.

Feldman, H., Gauthier, S., Hecker, J., Vellas, B., Subbiah, P., Whalen, E., and Donepezil MSAD Study Investigators Group (2001). A 24-week, randomized, double-blind study of donepezil in moderate to severe Alzheimer's disease. Neurology *57*, 613–620.

Francis, P.T., Palmer, A.M., Snape, M., and Wilcock, G.K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. J. Neurol. Neurosurg. Psychiatry *66*, 137–147.

Fukui, T., and Lee, E. (2009). Visuospatial Function is a Significant Contributor to Functional Status in Patients With Alzheimer's Disease. Am. J. Alzheimers Dis. Dementias® *24*, 313–321.

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., and Gillespie, F. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. Nature *373*, 523–527.

Gauthier, S. (2012). Pharmacological treatment of Alzheimer's disease. Alzheimers Dement. J. Alzheimers Assoc. *8*, P2.

Gauthier, S., Feldman, H., Hecker, J., Vellas, B., Ames, D., Subbiah, P., Whalen, E., Emir, B., and Donepezil MSAD Study Investigators Group (2002). Efficacy of donepezil on behavioral symptoms in patients with moderate to severe Alzheimer's disease. Int. Psychogeriatr. *14*, 389–404.

Gensel, J.C., Tovar, C.A., Hamers, F.P.T., Deibert, R.J., Beattie, M.S., and Bresnahan, J.C. (2006). Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. J. Neurotrauma *23*, 36–54.

German, D.C., Yazdani, U., Speciale, S.G., Pasbakhsh, P., Games, D., and Liang, C.-L. (2003). Cholinergic neuropathology in a mouse model of Alzheimer's disease. J. Comp. Neurol. *462*, 371–381.

Gilmor, M.L., Erickson, J.D., Varoqui, H., Hersh, L.B., Bennett, D.A., Cochran, E.J., Mufson, E.J., and Levey, A.I. (1999). Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. J. Comp. Neurol. *411*, 693–704.

Giménez, E., and Montoliu, L. (2001). A simple polymerase chain reaction assay for genotyping the retinal degeneration mutation (Pdeb(rd1)) in FVB/N-derived transgenic mice. Lab. Anim. *35*, 153–156.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., and James, L. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature *349*, 704–706.

Halagappa, V.K.M., Guo, Z., Pearson, M., Matsuoka, Y., Cutler, R.G., LaFerla, F.M., and Mattson, M.P. (2007). Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. *26*, 212–220.

Hannigan, J.H., and Riley, E.P. (1988). Prenatal ethanol alters gait in rats. Alcohol *5*, 451–454.

Harper, A.J., Billa, S., Potts, S., Richardson, J.C., Murray, T.K., O'Neill, M.J., Hutton, M., and Dix, S.L. (2013). Characterisation of three lines of transgenic mice expressing pathology relevant to Alzheimer's disease using touchscreens: Performance of PDAPP, TASTPM and rTg4510 mice on the acquisition of spatial and visual discriminations. *618.05/G5*.

Hartman, R.E., Izumi, Y., Bales, K.R., Paul, S.M., Wozniak, D.F., and Holtzman, D.M. (2005). Treatment with an amyloid-beta antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease. J. Neurosci. Off. J. Soc. Neurosci. *25*, 6213–6220.

Howard, R., McShane, R., Lindesay, J., Ritchie, C., Baldwin, A., Barber, R., Burns, A., Dening, T., Findlay, D., Holmes, C., et al. (2012). Donepezil and Memantine for Moderate-to-Severe Alzheimer's Disease. N. Engl. J. Med. *366*, 893–903.

Howlett, D.R., Richardson, J.C., Austin, A., Parsons, A.A., Bate, S.T., Davies, D.C., and Gonzalez, M.I. (2004). Cognitive correlates of Abeta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. Brain Res. *1017*, 130–136.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science *274*, 99–102.

Ingram, D.K., Weindruch, R., Spangler, E.L., Freeman, J.R., and Walford, R.L. (1987). Dietary Restriction Benefits Learning and Motor Performance of Aged Mice. J. Gerontol. *42*, 78–81.

Janickova, H., Rosborough, K., Al-Onaizi, M., Kljakic, O., Guzman, M.S., Gros, R., Prado, M.A.M., and Prado, V.F. (2017). Deletion of the vesicular acetylcholine transporter from pedunculopontine/laterodorsal tegmental neurons modifies gait. J. Neurochem. *140*, 787–798.

Juottonen, K., Laakso, M.P., Insausti, R., Lehtovirta, M., Pitkänen, A., Partanen, K., and Soininen, H. (1998). Volumes of the entorhinal and perirhinal cortices in Alzheimer's disease. Neurobiol. Aging *19*, 15–22.

Karran, E., Mercken, M., and Strooper, B.D. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat. Rev. Drug Discov. *10*, 698–712.

Kawas, C.H. (2003). Early Alzheimer's Disease. N. Engl. J. Med. *349*, 1056–1063.

Kaye, J.A. (1998). Diagnostic challenges in dementia. Neurology *51*, S45–S52; discussion S65–S67.

Kim, J.-W., Seung, H., Kwon, K.J., Ko, M.J., Lee, E.J., Oh, H.A., Choi, C.S., Kim, K.C., Gonzales, E.L., You, J.S., et al. (2014). Subchronic Treatment of Donepezil Rescues Impaired Social, Hyperactive, and Stereotypic Behavior in Valproic Acid-Induced Animal Model of Autism. PLoS ONE *9*.

Klinkenberg, I., Sambeth, A., and Blokland, A. (2011). Acetylcholine and attention. Behav. Brain Res. *221*, 430–442.

Kolisnyk, B., Al-Onaizi, M.A., Hirata, P.H.F., Guzman, M.S., Nikolova, S., Barbash, S., Soreq, H., Bartha, R., Prado, M.A.M., and Prado, V.F. (2013a). Forebrain Deletion of the Vesicular Acetylcholine Transporter Results in Deficits in Executive Function, Metabolic, and RNA Splicing Abnormalities in the Prefrontal Cortex. J. Neurosci. *33*, 14908–14920.

Kolisnyk, B., Guzman, M.S., Raulic, S., Fan, J., Magalhães, A.C., Feng, G., Gros, R., Prado, V.F., and Prado, M.A.M. (2013b). ChAT–ChR2–EYFP Mice Have Enhanced Motor Endurance But Show Deficits in Attention and Several Additional Cognitive Domains. J. Neurosci. *33*, 10427–10438.

Kryger, G., Silman, I., and Sussman, J.L. (1999). Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. Structure *7*, 297–307.

Kuhla, A., Lange, S., Holzmann, C., Maass, F., Petersen, J., Vollmar, B., and Wree, A. (2013). Lifelong Caloric Restriction Increases Working Memory in Mice. PLOS ONE *8*, e68778.

Kulesskaya, N., and Voikar, V. (2014). Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: role of equipment and procedure. Physiol. Behav. *133*, 30–38.

Leonard, J.A. (1959). Five choice serial reaction apparatus. Med. Res. Counc. Appl. Psychol. Unit Rep. *326*, 59.

Li, X., Rastogi, P., Gibbons, J.A., and Chaudhury, S. (2014a). Visuo-cognitive skill deficits in Alzheimer's disease and Lewy body disease: A comparative analysis. Ann. Indian Acad. Neurol. *17*, 12.

Li, X.-L., Hu, N., Tan, M.-S., Yu, J.-T., and Tan, L. (2014b). Behavioral and Psychological Symptoms in Alzheimer's Disease. BioMed Res. Int. *2014*, e927804.

Lindenberger, U., Marsiske, M., and Baltes, P.B. (2000). Memorizing while walking: increase in dual-task costs from young adulthood to old age. Psychol. Aging *15*, 417–436.

Liu, C.-C., Kanekiyo, T., Xu, H., and Bu, G. (2013a). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat. Rev. Neurol. *9*, 106–118.

Liu, Y., Ao, L.J., Lu, G., Leong, E., Liu, Q., Wang, X.H., Zhu, X.L., Sun, T.F.D., Fei, Z., Jiu, T., et al. (2013b). Quantitative gait analysis of long-term locomotion deficits in classical unilateral striatal intracerebral hemorrhage rat model. Behav. Brain Res. *257*, 166–177.

Maarouf, C.L., Kokjohn, T.A., Whiteside, C.M., Macias, M.P., Kalback, W.M., Sabbagh, M.N., Beach, T.G., Vassar, R., and Roher, A.E. (2013). Molecular Differences and Similarities Between Alzheimer's Disease and the 5XFAD Transgenic Mouse Model of Amyloidosis. Biochem. Insights *6*, 1–10.

Macdonald, I., DeBay, D., O'Leary, T., Reid, A., Cash, M., Jollymore, C., Mawko, G., Burrell, S., Martin, E., Bowen, C., et al. (2013). Cerebral glucose metabolism, pathology and behaviour in the 5XFAD mouse model of Alzheimer's disease. Alzheimers Dement. J. Alzheimers Assoc. *9*, P246–P246.

Mancini, M., Fling, B.W., Gendreau, A., Lapidus, J., Horak, F.B., Chung, K., and Nutt, J.G. (2015). Effect of augmenting cholinergic function on gait and balance. BMC Neurol. *15*.

Maragos, W.F., Chu, D.C., Young, A.B., D'Amato, C.J., and Penney, J.B. (1987). Loss of hippocampal [3H]TCP binding in Alzheimer's disease. Neurosci. Lett. *74*, 371–376.

Masood, T. (2015). Determining Attention Deficits In Mouse Models Of Alzheimer's Disease Using Touchscreen Systems. Electron. Thesis Diss. Repos.

McCarron, M., Gill, M., McCallion, P., and Begley, C. (2005). Health co-morbidities in ageing persons with Down syndrome and Alzheimer's dementia. J. Intellect. Disabil. Res. *49*, 560–566.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Jr., C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., et al. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. *7*, 263–269.

Mesulam, M.-M. (2013). Cholinergic Circuitry of the Human Nucleus Basalis and Its Fate in Alzheimer's Disease. J. Comp. Neurol. *521*, 4124–4144.

Montero-Odasso, M., Muir-Hunter, S.W., Oteng-Amoako, A., Gopaul, K., Islam, A., Borrie, M., Wells, J., and Speechley, M. (2015). Donepezil improves gait performance in older adults with mild Alzheimer's disease: a phase II clinical trial. J. Alzheimers Dis. JAD *43*, 193–199.

Motulsky, H.J., and Brown, R.E. (2006). Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinformatics *7*, 123.

Muir, J.L., Everitt, B.J., and Robbins, T.W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. Cereb. Cortex N. Y. N 1991 *6*, 470–481.

Muir, S.W., Speechley, M., Wells, J., Borrie, M., Gopaul, K., and Montero-Odasso, M. (2012). Gait assessment in mild cognitive impairment and Alzheimer's disease: The effect of dual-task challenges across the cognitive spectrum. Gait Posture *35*, 96–100.

Mullan, M. (1992). Familial Alzheimer's disease: second gene locus located. BMJ *305*, 1108–1109.

Nakamura, K., and Kurasawa, M. (2001). Anxiolytic effects of aniracetam in three different mouse models of anxiety and the underlying mechanism. Eur. J. Pharmacol. *420*, 33–43.

Nelson, R.J., and Young, K.A. (1998). Behavior in mice with targeted disruption of single genes. Neurosci. Biobehav. Rev. *22*, 453–462.

Neumann, M., Wang, Y., Kim, S., Hong, S.M., Bilgen, M., and Liu, J. (2009). Assessing gait impairment following experimental traumatic brain injury in mice. J. Neurosci. Methods *176*, 34–44.

Newman, M., Musgrave, F.I., and Lardelli, M. (2007). Alzheimer disease: Amyloidogenesis, the presenilins and animal models. Biochim. Biophys. Acta BBA - Mol. Basis Dis. *1772*, 285–297.

Nikolaev, A., McLaughlin, T., O'Leary, D.D.M., and Tessier-Lavigne, M. (2009). APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature *457*, 981–989.

Nutt, J.G. (2013). Higher-level gait disorders: An open frontier. Mov. Disord. *28*, 1560–1565.

Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., et al. (2006). Intraneuronal beta-amyloid

aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J. Neurosci. Off. J. Soc. Neurosci. *26*, 10129–10140.

Ohno, M. (2006). Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1. Eur. J. Neurosci. *23*, 251–260.

O'keeffe, S.T., Kazeem, H., Philpott, R.M., Playfer, J.R., Gosney, M., and Lye, M. (1996). Gait Disturbance in Alzheimer's Disease: A Clinical Study. Age Ageing *25*, 313–316.

O'Leary, T. (2013). CHARACTERIZATION OF AGE-RELATED CHANGES IN MOTOR ABILITY AND LEARNING AND MEMORY IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE.

O'Leary, T., Robertson, A., Chipman, P., Rafuse, V., and Brown, R. (2013). Motor dysfunction in the 12-month-old 5xFAD mouse model of Alzheimer's disease. Alzheimers Dement. J. Alzheimers Assoc. *9*, P497.

Pal, A., Biswas, A., Pandit, A., Roy, A., Guin, D., Gangopadhyay, G., and Senapati, A.K. (2016). Study of visuospatial skill in patients with dementia. Ann. Indian Acad. Neurol. *19*, 83–88.

Parvathy, S.S., and Masocha, W. (2013). Gait analysis of C57BL/6 mice with complete Freund's adjuvant-induced arthritis using the CatWalk system. BMC Musculoskelet. Disord. *14*, 14.

Passetti, F., Levita, L., and Robbins, T.W. (2003). Sulpiride alleviates the attentional impairments of rats with medial prefrontal cortex lesions. Behav. Brain Res. *138*, 59–69.

Patel, N.V., Gordon, M.N., Connor, K.E., Good, R.A., Engelman, R.W., Mason, J., Morgan, D.G., Morgan, T.E., and Finch, C.E. (2005). Caloric restriction attenuates Aβ-deposition in Alzheimer transgenic models. Neurobiol. Aging *26*, 995–1000.

Perry, R.J., and Hodges, J.R. (1999). Attention and executive deficits in Alzheimer's disease. Brain *122*, 383–404.

Piiponniemi, T.O., Bragge, T., Vauhkonen, E.E., Vartiainen, P., Puoliväli, J.T., Sweeney, P.J., and Kopanitsa, M.V. (2017). Acquisition and reversal of visual discrimination learning in APPSwDI/Nos2−/− (CVN) mice. Neurosci. Lett. *650*, 126–133.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C.P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. J. Alzheimers Assoc. *9*, 63–75.e2.

Proulx, É., Fraser, P., McLaurin, J., and Lambe, E.K. (2015). Impaired Cholinergic Excitation of Prefrontal Attention Circuitry in the TgCRND8 Model of Alzheimer's Disease. J. Neurosci. Off. J. Soc. Neurosci. *35*, 12779–12791.

Quental, N.B.M., Brucki, S.M.D., and Bueno, O.F.A. (2013). Visuospatial Function in Early Alzheimer's Disease—The Use of the Visual Object and Space Perception (VOSP) Battery. PLOS ONE *8*, e68398.

Robbins, T.W., James, M., Owen, A.M., Sahakian, B.J., McInnes, L., and Rabbitt, P. (1994). Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dement. Basel Switz. *5*, 266–281.

Rogers, R.D., Baunez, C., Everitt, B.J., and Robbins, T.W. (2001). Lesions of the medial and lateral striatum in the rat produce differential deficits in attentional performance. Behav. Neurosci. *115*, 799–811.

Rokem, A., Landau, A.N., Garg, D., Prinzmetal, W., and Silver, M.A. (2010). Cholinergic Enhancement Increases the Effects of Voluntary Attention but Does Not Affect Involuntary Attention. Neuropsychopharmacology *35*, 2538–2544.

Romberg, C., Mattson, M.P., Mughal, M.R., Bussey, T.J., and Saksida, L.M. (2011). Impaired Attention in the 3xTgAD Mouse Model of Alzheimer's Disease: Rescue by Donepezil (Aricept). J. Neurosci. *31*, 3500–3507.

Romberg, C., Horner, A.E., Bussey, T.J., and Saksida, L.M. (2013). A touch screen-automated cognitive test battery reveals impaired attention, memory abnormalities, and increased response inhibition in the TgCRND8 mouse model of Alzheimer's disease. *34*, 731–744.

Sahakian, B.J., and Coull, J.T. (1993). Tetrahydroaminoacridine (THA) in Alzheimer's disease: an assessment of attentional and mnemonic function using CANTAB. Acta Neurol. Scand. Suppl. *149*, 29–35.

Sahakian, B.J., Owen, A.M., Morant, N.J., Eagger, S.A., Boddington, S., Crayton, L., Crockford, H.A., Crooks, M., Hill, K., and Levy, R. (1993). Further analysis of the cognitive effects of tetrahydroaminoacridine (THA) in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. Psychopharmacology (Berl.) *110*, 395–401.

Sala, S.D., Spinnler, H., and Venneri, A. (2004). Walking difficulties in patients with Alzheimer's disease might originate from gait apraxia. J. Neurol. Neurosurg. Psychiatry *75*, 196–201.

Sarter, M., Hasselmo, M.E., Bruno, J.P., and Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res. Brain Res. Rev. *48*, 98–111.

Schneider, F., Baldauf, K., Wetzel, W., and Reymann, K.G. (2014). Behavioral and EEG changes in male 5xFAD mice. Physiol. Behav. *135*, 25–33.

Schroeder, J.E., Richardson, J.C., and Virley, D.J. (2010). Dietary manipulation and caloric restriction in the development of mouse models relevant to neurological diseases. Biochim. Biophys. Acta BBA - Mol. Basis Dis. *1802*, 840–846.

Schroer, W., Wahl, K., Ehrenstrom, R., Butano, V., James, K.J., Timson, B.F., and Zimmerman, S.D. (2010). Early gait alterations in the TG2576 mouse model of Alzheimer's disease: A preliminary analysis. Alzheimers Dement. J. Alzheimers Assoc. *6*, S215–S216.

Segev-Jacubovski, O., Herman, T., Yogev-Seligmann, G., Mirelman, A., Giladi, N., and Hausdorff, J.M. (2011). The interplay between gait, falls and cognition: can cognitive therapy reduce fall risk? Expert Rev. Neurother. *11*, 1057–1075.

Selkoe, D.J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol. Med. *8*, 595.

Spires, T.L., and Hyman, B.T. (2005). Transgenic Models of Alzheimer's Disease: Learning from Animals. NeuroRX *2*, 423–437.

Sun, A., Nguyen, X.V., and Bing, G. (2002). Comparative analysis of an improved thioflavin-s stain, Gallyas silver stain, and immunohistochemistry for neurofibrillary tangle demonstration on the same sections. J. Histochem. Cytochem. Off. J. Histochem. Soc. *50*, 463–472.

Sze, C., Bi, H., Kleinschmidt-DeMasters, B.K., Filley, C.M., and Martin, L.J. (2001). N-Methyl-D-aspartate receptor subunit proteins and their phosphorylation status are altered selectively in Alzheimer's disease. J. Neurol. Sci. *182*, 151–159.

Treviño, M., Oviedo, T., Jendritza, P., Li, S.-B., Köhr, G., and Marco, R.J.D. (2013). Controlled variations in stimulus similarity during learning determine visual discrimination capacity in freely moving mice. Sci. Rep. *3*, 1048.

Wang, J., Ho, L., Qin, W., Rocher, A.B., Seror, I., Humala, N., Maniar, K., Dolios, G., Wang, R., Hof, P.R., et al. (2005). Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. *19*, 659–661.

Wilcock, D.M., Lewis, M.R., Van Nostrand, W.E., Davis, J., Previti, M.L., Gharkholonarehe, N., Vitek, M.P., and Colton, C.A. (2008). Progression of amyloid pathology to Alzheimer's disease pathology in an amyloid precursor protein transgenic mouse model by removal of nitric oxide synthase 2. J. Neurosci. Off. J. Soc. Neurosci. *28*, 1537–1545.

Winters, B.D., Bartko, S.J., Saksida, L.M., and Bussey, T.J. (2010). Muscimol, AP5, or scopolamine infused into perirhinal cortex impairs two-choice visual discrimination learning in rats. Neurobiol. Learn. Mem. *93*, 221–228.

Wirths, O., Breyhan, H., Schafer, S., Roth, C., and Bayer, T.A. (2008). Deficits in working memory and motor performance in the APP/PS1ki mouse model for Alzheimer's disease. Neurobiol. Aging *29*, 891–901.

Witte, A.V., Fobker, M., Gellner, R., Knecht, S., and Flöel, A. (2009). Caloric restriction improves memory in elderly humans. Proc. Natl. Acad. Sci. *106*, 1255–1260.

Wu, L., and Zhao, L. (2016). ApoE2 and Alzheimer's disease: time to take a closer look. Neural Regen. Res. *11*, 412–413.

Wu, P., Shen, Q., Dong, S., Xu, Z., Tsien, J.Z., and Hu, Y. (2008). Calorie restriction ameliorates neurodegenerative phenotypes in forebrain-specific presenilin-1 and presenilin-2 double knockout mice. Neurobiol. Aging *29*, 1502–1511.

Yang, C., Feng, R., Yang, L., Jiang, S., Liu, N., Yan, L., Liu, Y., Yang, C., and Wang, K. (2014). Phenotyping age-sependent changes in the 5xFAD Alzheimer's Disease Model mice (LB556). FASEB J. *28*, LB556.

Yue, W., Li, Y., Zhang, T., Jiang, M., Qian, Y., Zhang, M., Sheng, N., Feng, S., Tang, K., Yu, X., et al. (2015). ESC-Derived Basal Forebrain Cholinergic Neurons Ameliorate the Cognitive Symptoms Associated with Alzheimer's Disease in Mouse Models. Stem Cell Rep. *5*, 776–790.

Zhang, Y., Thompson, R., Zhang, H., and Xu, H. (2011). APP processing in Alzheimer's disease. Mol. Brain *4*, 3.

Zheng, H., and Koo, E.H. (2006). The amyloid precursor protein: beyond amyloid. Mol. Neurodegener. *1*, 5.

Appendices

APPENDIX 1

TITLE: Food restriction for adult mice (12 weeks or older)

PREPARED BY: Matthew Cowan

1.0 PURPOSE

1.1 Standardized body weight at 85% of adult baseline weight is required to motivate mice to perform tasks designed to assess attention or cognitive ability, where successful completion results in the presentation of a food reward.

2.0 SCOPE

2.1 This SOP applies to all research personnel that are employing food restriction of mice for use in the Bussey-Saksida touch screen chambers.

3.0 RESPONSIBILITIES

3.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

4.0 NOTES

4.1 The validity of results obtained from behavioral phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare, and are familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

4.2 The majority of the mouse behavior studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

4.3 Environmental factors may contribute to the levels of mouse anxiety. Temperature, humidity, ventilation, noise intensity and light intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained.

4.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day (day time difference should not be more than 90 min) because physiological and biochemical parameters change throughout the day.

5.0 EQUIPMENT

5.1 Electronic balance accurate to 0.01g (Ex: Scout Pro, Ohaus)

5.2 Rodent fur dye (Ex: fine tip marker from Ketchum, tattoo machine)

6.0 PROCEDURE

6.1 **Acclimation and identification:**

6.1.1 Record the weight of each mouse when it arrives in the behavior facility. Mice should be allowed to acclimatize to the environment for 5 days (minimum) with ad libitum food and water. Do not conduct any experimental procedure during this acclimatization period.

6.1.2 During acclimatization a unique visual identification number should be applied to the back (or tail) of each mouse using for instance, a semi-permanent fur dye, or a tattoo machine. It is important to ensure that each animal can be reliably and easily identified.

6.2 **Determining individual food consumption:**

6.2.1 To determine the amount of food each mouse is consuming per day it is important to keep only 2 or 3 mice per cage (obs: Ideally 2 mice should be kept per cage. To avoid having single housed cages it might be necessary to keep 3 mice per cage).

6.2.2 Weigh out the food that is going to be provided to the mice making sure that an amount of food in excess of what could possibly be consumed in a day is going to be supplied (6g per mouse per day should be enough). Food should be provided in a Petri dish (or similar container) on the cage floor. The next day, weigh the food that remains and estimate the amount of food consumed per mouse Mice should not be found without food at this stage. Repeat the procedure the next two days. Use the average of the three days for individual food consumption.

6.3 **Food restriction to 85% of baseline adult (12 weeks or older) body weight:**

6.3.1 We have determined the body weight baseline for male and female mice in a C57BL/6 background from ages ranging from 4-24 weeks (see below Fig1: Male weight curve and Fig2: Female weight curve). We observed that the average weight of an adult male ranging from 13 to 22 weeks is around 30g (13 weeks: 28.5g±0.6; 22 weeks: 31.2g±0.9) while the average weight of an adult female ranging from 12-24 weeks is around 22g (12 weeks: 20.0g±0.51; 24 weeks: 23.6g±0.67). Thus, **to reach 85% of the average adult body weight adult male mice weight should be reduced to ±25.5g and adult female mouse weight should be reduced to ±18.7g**.

6.3.2 Weigh each mouse daily, 7 days a week. For males, provide 2.0 – 3.0 g of food per 25-35 g of mouse per day. For females, provide 1.5-2.0 g of food per 20-23g of mice. Proceed until body weight is reduced to 85% of baseline.

6.3.3 Cages of mice on food restriction must be flagged with orange labels so that animal care staff is aware of them. Mice should be monitored for changes in appearance or signs of aggression. The weight decrease is intended to be gradual and may take 7-15 days.

6.3.4 In the C57Bl/6 background it is common to find obese mice (males >30g, females>22g). Obese mice are less motivated to complete food rewarded tasks therefore, it is essential that all subjects within an experimental group weight approximately the same prior to the experiment **(adult male mice weight should be reduced to ±25.5g and adult female mouse**

weight should be reduced to ±18.7g). Thus, obese mice must be food restricted longer until they get to the appropriate weight.

6.4 **Maintenance at 85% of baseline adult body weight:**

6.4.1 When 85% of baseline is reached, male mice can be safely maintained through the testing period by providing 2.5g of food per mouse per day, as they are going to receive the strawberry shake during the tests. In case of females provide 2.0g of food per mouse per day**. Caution –2.5 g per male mouse or 2.0 g per female mouse is <u>an average</u> of food to keep mice at 85% of their weigh. This amount must be carefully monitored and properly adjusted**.

6.4.2 Each mouse must be weighed every second day. No individual's weight can be allowed to fall below 80% of its pre-established adult baseline. Mice between 80-85% of baseline weight should receive up to 3.0g of food per mouse per day on the cage floor until they are stable at 85% again.

6.4.3 Mice should be weighed and fed after completion of the day's experiment to ensure the experimental food reward is motivating and that rationed food is consumed in advance of the next days schedule.

6.4.4 Group housed mice may compete for rationed food. Break the food pellets into several pieces so that each mouse can manipulate and eat their own. Watch for changes in body condition and aggressive behavior immediately after the addition of food to the cage. If the weights of cage mates begin to diverge the heavier mouse should be separated.

7.0 HEALTH & SAFETY

7.1 General laboratory procedures should be followed, which include: no eating or drinking. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

8.0 REFERENCES / ASSOCIATED MATERIALS

JAX mice strain C57BL/6J mean weight by age and sex.

http://jaxmice.jax.org/support/weight/000664.html

http://www.nature.com/nprot/journal/v8/n10/full/nprot.2013.122.html

http://www.nature.com/nprot/journal/v8/n10/full/nprot.2013.123.html

http://www.nature.com/nprot/journal/v8/n10/full/nprot.2013.124.html

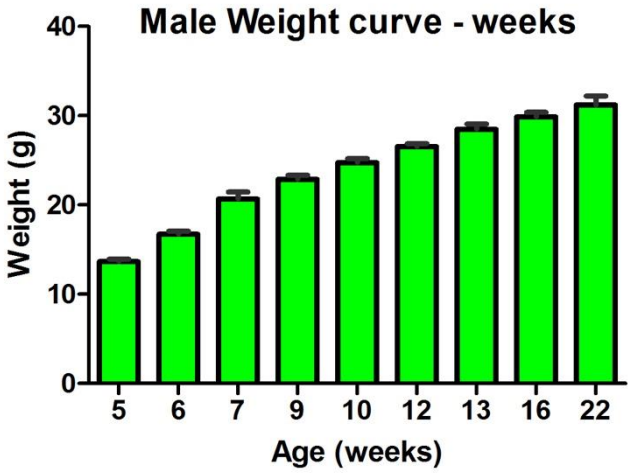


Figure 1: Male weight curve.

Figure 2: Female weight curve.

APPENDIX 2

TITLE: 2-Choice Pairwise Visual Discrimination Task
Standard Operating Procedure

PREPARED BY: Matthew Cowan

1.0 INTRODUCTION

The PD task has been designed to measure effects of drugs and other manipulations (ex: genetic) on attentional performance. The test is performed in a specially designed touchscreen-based automated chamber with 2 response locations (left and right windows) using food reinforcers to maintain performance. The PD task requires the subject to learn to associate a food reward with a nose-poke response to one image (S+ stimulus) when it appears in one of the windows and ignore a second visually distinct image (S- stimulus) appearing simultaneously in the other location. After the task is learned reversal learning is attempted where the food reward becomes linked to the former S- stimulus and responses to the former S+ stimulus go unrewarded. Reversal learning of the PD task is useful for measuring effects of different manipulations on the functioning of the prefrontal cortex.

2.0 EQUIPMENT

- Mouse Touch Screen Systems and ABET II
- 89540CAM Pairwise (Visual) Discrimination (PD) Task with Cambridge Amendment

3.0 PROCEDURE

3.1 Testing the hardware:

A quick test of the hardware should be done prior to every days training or testing. To do the hardware testing, follow procedures indicted below:

a. From the main menu the Execution Manager, select the boxes you wish to test.
b. Click the ‘Open/Load Schedule’ icon and select ‘2-Touch MouseTestLines’ and click ‘Open’.
c. Click the play icon. The boxes are now ready to test.

See Table 1 for Inputs to activate a response and output response expected.

Table 1: Action necessary and output response expected.

Schedule	Inputs to activate (use your fingers)	Output response
2-Touch Mouse Test Lines	Enter feeder tray	House light on during tray beam break, audio tone plays, pump activates ***Tray light stays on when finger removed.***
	Touch right window	Solid white square appears on screen
	Enter feeder tray	Square removed from screen, tray light goes off.
	Repeat first 3 actions, **this time touching left window**	See above output responses
	Block Front IR activity beams	Count goes up by 1 for each beam blockage. House light turns on, ***stays on until feeder tray is re-entered***
	Block Back IR activity beams	Count goes up by 1 for each beam blockage

3.2 Testing the feeder and mask

- A quick test of the feeder should be done prior to every days training or testing. Manually switch on the feeder pump and make sure the food is delivered.
- If clogged, the tubing can be cleared by using a 5ml syringe with 21 gauge needle to force water through.
- Build up does occur in the tubing and gradually reduce the flow rate even if it does not clog completely so replace the tubing in each chamber every 2 months.
- Make sure the PD Mask is inserted (2 windows).
- Reward provided is Neilson Strawberry milkshake (SM) (Saputo Inc. Montreal Quebec. H1P1X8). This milkshake can be found in most grocery stores (including Wall Mart and Superstore).

3.3 Pre-training

- Animals may require food restriction before task training and it will be required throughout the experiment (see relevant SOPs: “Food restriction in young mice” or “Food restriction in adult mice”).

- Divide each group of subjects (Ex: 5xFAD colony females or APP colony males, etc.) into 2 counter-balanced sub groups containing both wt and transgenic mice to control for the time of day the experiment is performed and the particular cabinet being used in case of an equipment failure.
- Pre-select a pair of images to be used in the discrimination/reversal task for each age point required. Preselecting 5 pairs allows for 5 potential data sets over the life of each cohort and prevents those images from being displayed during the training and maintenance phases. All training schedules should be checked for which images they will display.

3.4 Training Procedures

3.4.1 Basic training schedule

IMPORTANT: for both training and probe trials, each mouse is submitted to one session per day.

Stage 1: Habituation1: 1 session. Load the PD_habituation_1_v2 schedule from the PD v3 subdirectory in the ABETII software. The session duration is set to 600s (10 minutes), and the number of trials is left to unlimited. Mouse is left in the chamber for 10 min. All lights are turned off. No stimulus or reward is presented. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 2: Habituation2a: 2 sessions. Load the PD_habituation_2_v2 schedule from the PD v3 subdirectory in the ABETII software. The session duration is set to 1200s (20 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 20 min sessions. The tray light is going to be initially turned on. A tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivered for 6000ms (150µl). The program waits for the mouse to enter the food tray. When the mouse leaves the reward tray, the reward tray light is turned off. There is a 10s delay before the tray light is turned on, a tone is played and SM is then delivered for 280ms (7 µl). If the mouse is in the reward tray at the end of the 10s delay, an extra 1s is added to the delay. The procedure is repeated until the session ends. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 3: Habituation2b: 1 session. Load the habituation2 schedule from the PD v3 subdirectory in the ABETII software. The session duration is set to 2400s (40 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 40 min. Reward presentation is the same as described in stage 2. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 4: "Initial touch": (usually 1 session). Load the schedule 'PD_Initial_Touch_Training_v3' from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure

that “Image Time” is 30s; ‘Feed Pulse Time” is 280ms; “tone duration” is 1000 ms, and ITI period is 20s. The stimulus (any image not designated for use in discrimination/reversal trials) is displayed in either the left or right window. The other window is blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. After a delay (**Image Time – 30s**) the image is removed and food is delivered (**‘Feed Pulse Time –280ms**). Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency is 3 KHz. The tone duration is (1000 ms). Entry to collect the food turns off the tray light and starts the ITI. After the **ITI period (20s)** another image is displayed. If the mouse touches the screen while the image is displayed (where the image is displayed), the image is removed, a tone will be played and 3 x food is delivered immediately. Collection of this reward again starts the ITI and then progresses to the next image. Touch training is performed with the house light off.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 5: “Must touch”: Number of session varies for individual mouse. Run the schedule ‘PD_Must_Touch_Training_v3’ from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No Trials to 30. Make sure **tone duration is set to 1000 ms** (from the ‘Tone Duration’ variable) and **ITI period is set to 20s**.
The stimulus, an image selected pseudo randomly (no image shown twice in a row) from a list which **must not include** any of the images to be used in discrimination/reversal trials. The stimulus is presented in only one window at a time. The other window is blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (20s) another image is displayed.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 7 sessions a mouse does not reach criterion for “must touch”, take it back one step; that is, retrain the mouse on “PD_Initial_touch_RETRAIN_v3” until it reaches criterion and repeat the “PD_Must_touch_Training_v3” training. If after 7 sessions of the second attempt of “must touch” the mouse does not reach criterion, remove it from the study.

Stage 6: “Must initiate”: Number of session varies for individual mouse. Run the schedule ‘PD_Must_Initiate_Training_v3’ from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** (from the ‘Tone Duration’ variable) and **ITI period is set to 20s**.

A free delivery of food is made and the tray light is turned on. The mouse **must** nose poke and exit the reward tray before a stimulus is displayed randomly on the screen. The stimulus, an image selected pseudo randomly (no image shown twice in a row) from a list which **must not include** any of the images to be used in discrimination/reversal trials. The stimulus is presented in only one window at a time. The other window is blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period the tray light is again illuminated. The mouse **must** nose poke and exit the reward tray before the next image is displayed.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 5 sessions a mouse does not reach criterion for "must initiate", take it back one step; that is, retrain the mouse on "PD_must_touch_RETRAIN_v3" until it reaches criterion and repeat the "PD_Must_Initiate_Training_v3". If after 5 sessions of the second attempt of "must initiate" the mouse does not reach criterion, remove it from the study.

Stage 7: "Punish incorrect". Number of session varies for individual mouse. Run the schedule 'PD_Punish_Incorrect_Training_v3' from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** and **ITI period is set to 20s**.
This schedule trains the mouse to both initiate after an ITI and not to touch an incorrect location. As for previous training described above, except if a mouse touches an incorrect (blank) location the **house light will be turned ON for 5s** and **no reward is given**. Once the time out period finishes the house light is turned OFF again and the **ITI period begins (20s)**. There is no time limit on the display of the image (no omissions score) and no correction trials.
Criterion: Completion of 24/30 trials or better within 60 min for 2 consecutive sessions

IMPORTANT: If after 30 sessions (30 days) the mouse does not reach criterion for "Punish incorrect", remove it from study.

3.4.2 PD task acquisition, baseline and reversal learning , 1st time point

Stage 8: PD task acquisition, 1st time point. Number of session varies for individual mouse. Run the 'PD_Acquisition_1_v3' from the PD v3 subdirectory schedule. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure tone duration is set to 1000 ms, ITI period is set to 20s, Food/CM pulse time [280ms (7 µl SM), time out (TO, 5s, paired with overhead light).

The session begins with a priming delivery of reinforcer ***[280ms (7 µl SM)]*** and on exiting the food magazine the first trial begins. Following tray exit a S+ image and a S- image are presented in either of the 2 windows. The left/right ordering of the S+ and S- images is pseudo random with no ordering repeated more than 3 times. A correct response, touching at the location in which the S+ stimulus was presented, will trigger the presentation of reinforce [**280ms (7 µl SM)**] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is **(1000 ms tone)**. The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (20s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial. An incorrect response, i.e. touching the S- image will cause a time out (**TO, 5s**) and the house light to be turned ON. After the TO, the house light will be turned OFF and the "**ITI" will begin** (**20s)**. After the ITI the tray light will come on and the subject must enter and exit the food tray to start the correction trial. In a correction trial the left/right ordering of the S+/S- images is repeated from the previous trial and repeated each subsequent trial until a correct choice is made. The results of correction trials do not count toward criteria for completion of the session.

Criterion: 24/30 trials correct within 60 min, for 2 consecutive days.

Stage 9: PD baseline, 1st time point. Run for 2 sessions immediately after reaching PD acquisition criteria. Load the 'PD_Baseline_1_v3' schedule from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure tone duration is set to 1000 ms, ITI period is set to 20s, Food/CM pulse time [280ms (7 µl SM), time out (TO, 5s).
The session begins with a priming delivery of reinforce [**280ms (7 µl SM)]** and on exiting the food magazine the first trial begins. Following tray exit a S+ image and a S- image are presented in either of the 2 windows. The left/right ordering of the S+ and S- images is pseudo random with no ordering repeated more than 3 times. A correct response, touching at the location in which the S+ stimulus was presented, will trigger the presentation of reinforce [**280ms (7 µl SM)**] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is **(1000 ms tone)**. The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (20s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial. An incorrect response, i.e. touching the S- image will cause a time out (**TO, 5s**) and the house light to be turned ON. After the TO, the house light will be turned OFF and the "**ITI" will begin** (**20s)**. After the ITI the tray light will come on and the subject must enter and exit the food tray to start the correction trial. In a correction trial the left/right ordering of the S+/S- images is repeated from the previous trial and repeated each subsequent trial until a correct choice is made. The results of correction trials do not count toward criteria for completion of the session.

Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Stage 10: PD task reversal, 1st time point. Run for 10 sessions immediately after completing PD baseline criteria. Load the 'PD_Reversal_1_v3' schedule from the PD v3 subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure tone duration is set to 1000 ms, ITI period is set to 20s, Food/CM pulse time [280ms (7 µl SM), time out (TO, 5s). The session begins with a priming delivery of reinforce [**280ms (7 µl SM)]** and on exiting the food magazine the first trial begins. Following tray exit a S+ image and a S- image are presented in either of the 2 windows. The left/right ordering of the S+ and S- images is pseudo random with no ordering repeated more than 3 times. A correct response is **now defined as** touching at the location in which the S- stimulus was presented and will trigger the presentation of reward [**280ms (7 µl SM)**] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is **(1000 ms tone)**. The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (20s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial. An incorrect response, i.e. touching the S+ image will cause a time out (**TO, 5s**) and the house light to be turned ON. After the TO, the house light will be turned OFF and the **ITI will begin** (**20s)**. After the ITI the tray light will come on and the subject must enter and exit the food tray to start the correction trial. In a correction trial the left/right ordering of the S+/S- images is repeated from the previous trial and repeated each subsequent trial until a correct choice is made. The results of correction trials do not count toward criteria for completion of the session.

Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Stage 11: PD Maintenance. Run the schedule 'PD_Maintenance_1_v3' from the PD v3 subdirectory. This schedule is identical to 'PD_Punish_Incorrect_Training_v3'. Run this schedule once per week until the subjects are old enough to begin **PD task acquisition, 2nd time point.**

Criterion: There is no score required to pass, the session ends after 30 trials have been completed or 60 min has elapsed.

Subsequent time points: 2nd, 3rd, 4th, etc. time points are performed identically to the 1st time point using the appropriately named schedules which contain unique S+ and S- images. However as subjects age it is possible that acquiring the PD task will take longer or fail to occur. This may require adjusting subsequent time points or dropping subjects from the study according to previously stated criteria.

APPENDIX 3

TITLE: 5-Choice Serial Reaction Time Task (5-CSRTT) Standard Operating Procedure

PREPARED BY: Matthew Cowan

1.0 INTRODUCTION

The 5-CSRT task has been designed to measure effects of drugs and other manipulations (ex: genetic) on attentional performance (and stimulus control). The test is performed in a specially designed touchscreen-based automated chamber with multiple response locations ('five-screens") using food reinforcers to maintain performance. The 5CSRTT is useful for measuring effects of different manipulations on various aspects of attentional control, including sustained, selective and divided attention – and is relevant to the definition of neural systems of attention and has applications to human disorders such as attention deficit/hyperactivity disorder (ADHD) and Alzheimer's disease.

4.0 EQUIPMENT

- Mouse Touch Screen Systems and ABET II
- 89543CAM 5-Choice Serial Reaction Time Task with Cambridge Amendment
-

5.0 PROCEDURE

5.1 Testing the hardware:

A quick test of the hardware should be done prior to every days training or testing. To do the hardware testing, follow procedures indicted below:

a. From the main menu the Execution Manager, select the boxes you wish to test.
b. Click the 'Open/Load Schedule' icon and select 'Touch MouseTestLines' and click 'Open'.
c. Click the play icon. The boxes are now ready to test.

See Table 1 for Inputs to activate a response and output response expected.

Table 1: Action necessary and output response expected.

Schedule	**Inputs to activate (use your fingers)**	**Output response**
Touch Mouse Test Lines	Touch Grid 1	Image in all grid spaces, Grid 1 has 30% of full white, grids 2 to 4 have full white image.

		Activate the Tray to clear all images and proceed with test.
	Touch Grid 2	Image in all grid spaces, Grid 2 has 40% of full white, all other grids have full white image. ***Activate the Tray to clear all images and proceed with test.***
	Touch Grid 3	Image in all grid spaces, Grid 3 has 50% of full white, all other grids have full white image. ***Activate the Tray to clear all images and proceed with test.***
	Touch Grid 4	Image in all grid spaces, Grid 4 has 70% of full white, all other grids have full white image. ***Activate the Tray to clear all images and proceed with test.***
	Touch Grid 5	Full white (bright) image in all grid spaces. Pulses Sound_On 500ms ***Activate the Tray to clear all images and proceed with test.***
	Enter the feed-tray	House-light and tray-light illuminate and feeder - 800ms
	Exit the feed-tray	Houselight and tray light extinguish

	Block Front IR activity beams	House-light illuminates
	Block Back IR activity beams	Tray-light illuminates

5.2 Testing the feeder and mask

- A quick test of the feeder should be done prior to every days training or testing. That is, turn on manually the switch on the feeder pump and make sure the food is delivered.
- Make sure the 5CSRT Mask is inserted (5 windows).
- Reward provided is Neilson Strawberry milkshake (SM) (Saputo Inc. Montreal Quebec. H1P1X8). This milkshake can be found in most grocery stores (including Wall Mart and Superstore).

5.3 Pre-training

- Animals need to be food restricted before task training and throughout experiment (see relevant SOPs: “Food restriction in young mice” or Food restriction in adult mice”).
- Divide the subjects of each group to be tested (Ex: Group 1: 5xFAD females, Group 2: APP males) into 4 sub-groups (A, B, C, D). Groups must be counter-balanced for genotype (wt x mutant). Each subgroup is going to follow specific testing schedules during probe trial (see Table 2).

5.4 Training Procedures

5.4.1 Basic training schedule

IMPORTANT: for both training and probe trials, each mouse is submitted to one session per day.

Stage 1: Habituation1: 1 session. Load the habituation1 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 600s (10 minutes), and the number of trials is left to unlimited. Mouse is left in the chamber for 10 min. All lights are turned off. No stimulus or reward is presented. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 2: Habituation2a: 2 sessions. Load the habituation2 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 1200s (20 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 20 min sessions. The tray light is going to be initially turned on. A tone is played and the food-tray/magazine is primed with strawberry milkshake (SM) delivered for 6000ms (150µl). The program waits for the mouse to enter the food tray. When the mouse

leaves the reward tray, the reward tray light is turned off. There is a 10s delay before the tray light is turned on, a tone is played and SM is then delivered for 280ms (7 µl). If the mouse is in the reward tray at the end of the 10s delay, an extra 1s is added to the delay. The procedure is repeated until the session ends.

Stage 3: Habituation2b: 1 session. Load the habituation2 schedule from the CAM-5choice subdirectory in the ABETII software. The session duration is set to 2400s (40 minutes), and the number of trials is left to unlimited. The mouse is left in the chamber for 40 min. Reward presentation is the same as described in stage 2. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.

Stage 4: "Initial touch": (usually 1 session). Load the schedule '5-choice Mouse Initial Touch Training' from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure that "Image Time" is 30s; 'Feed Pulse Time" is 280ms; "tone duration" is 1000 ms, and ITI period is 5s. The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. After a delay (**Image Time – 30s**) the image is removed and food is delivered (**'Feed Pulse Time –280ms**). Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency is 3 KHz. The tone duration is (100**0 ms**). Entry to collect the food turns off the tray light and starts the ITI. After the **ITI period (5s)** another image is displayed. If the mouse touches the screen whilst the image is displayed (where the image is displayed), the image is removed, a tone will be played and 3 x food is delivered immediately. Collection of this reward again starts the ITI and then progresses to the next image. Touch training is performed with the house light off.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 5: "Must touch": Number of session varies for individual mouse. It can go from ~1-7 days (median: 2 days). Run the schedule '5-choice Mouse Must Touch Training' from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No Trials to 30. Make sure **tone duration is set to 1000 ms** (from the 'Tone Duration' variable) and **ITI period is set to 5s**.
The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row. The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches blank part of the screen. Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency default is 3 KHz. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period (5s) another image is displayed.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 7 sessions a mouse does not reach criterion for "must touch", take it back one step; that is, retrain the mouse on "Initial touch" until it reaches criterion and repeat the "Must touch" training. If after 7 sessions of the second attempt of "must touch" the mouse does not reach criterion, remove it from the study.

Stage 6: "Must initiate": Number of session varies for individual mouse. It usually takes ~1-2 sessions (i.e. 1 or days). Run the schedule '5-Choice Must Initiate Training' from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** (from the 'Tone Duration' variable) and **ITI period is set to 5s**.
A free delivery of food is made and the tray light is turned on. The mouse must nose poke and exit the reward tray before a stimulus is displayed randomly on the screen. The stimulus (a white square) is displayed randomly in one of the 5 windows. The stimulus is presented in only one window at a time. The other windows are left blank. The position is chosen pseudo randomly, such that the stimulus will not be displayed in the same position more than 3 times in a row.) The mouse must touch the stimulus to elicit tone/food response. There is no response if mouse touches the blank parts of the screen. Food delivery is accompanied by illumination of the tray light and a tone. Entry to collect the food turns off the tray light and starts the ITI. After the ITI period the tray light is again illuminated. The mouse must nose poke and exit the reward tray before the next image is displayed.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

IMPORTANT: If after 5 sessions a mouse does not reach criterion for "must initiate", take it back one step; that is, retrain the mouse on "must touch" until it reaches criterion and repeat the "Must Initiate" training. If after 5 sessions of the second attempt of "must initiate" the mouse does not reach criterion, remove it from the study.

Stage 7: "Punish incorrect". Number of session varies for individual mouse. It can go from ~2-30 days (median: 9 days). Run the schedule '5-Choice Mouse Punish Incorrect Training' from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 30. Make sure **tone duration is set to 1000 ms** and **ITI period is set to 5s**.
This schedule trains the mouse to both initiate after an ITI and not to touch an incorrect location. As for previous training described above, except if a mouse touches an incorrect (non-illuminated) location the **house light will be turned ON for 5s** and **no reward is given**. Once the time out period finishes the house light is turned OFF again and the **ITI period begins (5s)**. The mouse must then complete a correction trial: the image and position from the previous trial are kept the same and the mouse must repeat the same trial until a correct response to the image is made, at which point it will receive a tone and reward.
Criterion: Completion of 23/30 trials or better within 60 min for 2 consecutive sessions

IMPORTANT: If after 30 sessions (30 days) the mouse does not reach criterion for "Punish incorrect", remove it from study.

5.4.2 5-CSRT Training to baseline

Stage 8: 5-CSRT training to baseline- 4s stimulus. Number of session varies for individual mouse. It can go from ~4-30 days (median: 11 days). Run the '5CSRTT_2s_Var1' from the CAM-5choice subdirectory schedule. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50. Make sure tone duration is set to 1000 ms, ITI period is set to 5s, Food/CM pulse time [280ms (7 µl SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

*The session begins with a priming delivery of reinforce [**280ms (7 µl SM)]** and on exiting the* food magazine the first trial begins. Following tray exit, a "**Delay interval" (5s)** begins at the end of which a stimuli is presented in one of the 5 stimuli grid spaces on the LCD touch screen. The sequence of presentations of the stimuli is a pseudorandom schedule such that there are 4 presentations at each spatial location within a block of 20 trials. The subject must respond within a time period defined (**limited hold period 5s**). A correct response, touching at the location in which the stimulus was presented, will trigger the presentation of reinforce [**280ms (7 µl SM)**] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is **(1000 ms tone)**. The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (5s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial and start the Delay' interval. An incorrect response, i.e. touching a location other than where the stimulus was presented, or making no response at all (an omission) within the limited hold period, will cause a time out (**TO, 5s**) as identified house light turned ON. After the TO, the house light will be turned OFF and the "**ITI Incorr" will begin** (**5s)**. After the ITI incorr period the tray light will come on and the subject must enter and exit the food tray to start the next trial and start the Delay' interval. A premature response is recorded when a touch is made in one of the response grid areas during the Delay and also results in a TO.
> 80% accuracy = [number of Correct trials / Total number of trials responded to (correct and incorrect)]
< 20% omissions = [number of trials missed / number of trials presented]

Criterion: 80% accuracy or better, 20% omission or less, 3 consecutive days, minimum 30 trials completed per session.

Stage 9: 5-CSRT training to baseline- 2s stimulus. Number of session varies for individual mouse. Number of session varies for individual mouse. It can go from ~5-30 days (median: 12 days). Run the '5CSRTT_2s_Var1 schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50. . Make sure tone duration is set to 1000 ms, ITI period is set to 5s, Food/CM pulse time [280ms (7 µl SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

The session begins with a priming delivery of reinforce [**280ms (7 µl SM)]** and on exiting the food magazine the first trial begins. Following tray exit, a "**Delay interval" (5s)** begins at the end of which a stimuli is presented in one of the 5 stimuli grid spaces on the LCD touch screen. The sequence of presentations of the stimuli is a pseudorandom schedule such that there are 4 presentations at each spatial location within a block of 20 trials. The subject must respond within a time period defined (**limited hold period 5s**). A correct response, touching at the location in which the stimulus was presented, will trigger the presentation of reinforce [**280ms (7 µl SM)**] into the food magazine. Food delivery is accompanied by illumination of the tray light and a tone. The tone duration is **(1000 ms tone)**. The subject collects the food by making an entry at the food magazine. On exiting the food tray the **ITI (5s)** will begin. After the ITI period, the tray light comes on again and the mouse must enter and exit the food tray to start the next trial and start the Delay' interval. An incorrect response, i.e. touching a location other than where the stimulus was presented, or making no response at all (an omission) within the limited hold period, will cause a time out (**TO, 5s**) as identified house light turned ON. After the TO, the house light will be turned OFF and the '**ITI Incorr' will begin** (**5s)**. After the ITI incorr period the tray light will come on and the subject must enter and exit the food tray to start the next trial and start the Delay' interval. A premature response is recorded when a touch is made in one of the response grid areas during the Delay and also results in a TO.
Criterion: 80% accuracy or better, 20% omission or less, 3 consecutive days, 50 trials must be completed per session.

5.4.3 Testing schedules

Stage 10: First probe trial evaluation. Subjects will not progress through the training at exactly the same rate. The first set of probe trials for a group begins once the last mouse in that group has passed the 2s stimulus performance criteria (Stage 9). Subjects that have completed the Stage 9 before the slowest subject are maintained on food restriction and repeat Stage 9 before performing their probe trial. There is no minimum performance criterion for subjects to advance through the probe trials. The order of performance of probe trials for each counter-balanced group varies according to Table 2.

- For the 2s stimulus run the '5CSRTT_2s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 1.5s stimulus run the '5CSRTT_1.5s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 1s stimulus run the '5CSRTT_1s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.

- For the 0.8s stimulus run the '5CSRTT_0.8s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.
- For the 0.6s stimulus run the '5CSRTT_0.6s_Var1schedule from the CAM-5choice subdirectory. Set the Session: Max_Schedule_Time to 60 minutes, i.e. 60:00. Set the Max No. Trials to 50.

Table 2: Order of stimulus duration for individual groups (1st probe trial evaluation)

# of consecutive sessions	Stimulus duration throughout sessions for Sub-group A	Stimulus duration throughout sessions for Sub-group B	Stimulus duration throughout sessions for Sub-group C	Stimulus duration throughout sessions for Sub-group D
2	0.6s	0.8s	1.0s	1.5s
2	2.0s	2.0s	2.0s	2.0s
2	1.5s	0.6s	0.8s	1.0s
2	2.0s	2.0s	2.0s	2.0s
2	1.0s	1.5s	0.6s	0.8s
2	2.0s	2.0s	2.0s	2.0s
2	0.8s	1.0s	1.5s	0.6s

Table 3: Setting adjustment for additional variables in probe trials

Variable	Value
Session Length	60 min
Food/CM pulse time	280 ms
DELAY	5s
Time out	5s
Limited Hold Value	5s

Stage 11: Reusing same mouse cohort for a new probe trial

- All subjects are maintained on food restriction for 1 month.

- Subjects perform one 2s stimulus trial per week during the interval between probe trials.

Stage 12: Second probe trial (and all subsequent probe trials) evaluation

- Mice should be re-baselined at 2s for 5 consecutive days before beginning the next probe trial (Stage 9: >80% Accuracy, <20% omissions). Depending on how long it's been since the previous probe trial it might be necessary to rebaseline them at 4s first (Stage 8). If they are not re-baselined the second probe trial will not be accurate.
- A second probe trial should be performed according to the order shown in Table 4.

Table 4: Order of stimulus duration for individual groups (2nd probe trial evaluation)

# of consecutive sessions	Stimulus duration throughout sessions for Sub-group A	Stimulus duration throughout sessions for Sub-group B	Stimulus duration throughout sessions for Sub-group C	Stimulus duration throughout sessions for Sub-group D
2	1.5s	0.6s	0.8s	1.0s
2	2.0s	2.0s	2.0s	2.0s
2	1.0s	1.5s	0.6s	0.8s
2	2.0s	2.0s	2.0s	2.0s
2	0.8s	1.0s	1.5s	0.6s
2	2.0s	2.0s	2.0s	2.0s
2	0.6s	0.8s	1.0s	1.5s

Curriculum Vitae

Name: Wai-Jane Virginia Lee

Education:

The University of Western Ontario
London, Ontario, Canada
2011 - 2015 HSp.BMSc

The University of Western Ontario
London, Ontario, Canada
2015-2017 MSc.

Professional Affiliations:

Canadian Association for Neuroscience (CAN)

Southern Ontario Neuroscience Association (SONA)

Honours and Awards:

Southern Ontario Neuroscience Association Trainee Award
2016

Abstracts and Presentations:

June 2017. Poster Presentation - Robarts Research Retreat (London, CA)
Cognitive and Non-Cognitive Phenotypes in the 5xFAD mouse model of Alzheimer's Disease

June 2017. Poster Presentation - Canadian Association for Neuroscience Annual Meeting (Montreal, CA)
Cognitive and Non-Cognitive Phenotypes in the 5xFAD mouse model of Alzheimer's Disease

May 2017. Poster Presentation - Southern Ontario Neuroscience Association Annual Meeting (St.Catherines, CA)
Cognitive and Non-Cognitive Phenotypes in the 5xFAD mouse model of Alzheimer's Disease

March 2017. Poster Presentation - London Health Research Day (London, CA)
Cognitive and Non-Cognitive Phenotypes in the 5xFAD mouse model of Alzheimer's Disease

October 2016. Poster Presentation - Anatomy and Cell Biology Research Day (London, CA)
Hyperlocomotion in the 5xFAD and APP/PS1 mouse models of Alzhiemer's Disease following touchscreen evaluation

June 2016. Poster Presentation - Robarts Research Retreat (London, CA)
Gait Disturbances in the 5xFAD transgenic mouse model of Alzheimer's Disease

June 2016. Poster Presentation - Canadian Association for Neuroscience Annual Meeting (Toronto, CA)
Gait Disturbances in the 5xFAD transgenic mouse model of Alzheimer's Disease

May 2016. Poster Presentation - Southern Ontario Neuroscience Association Annual Meeting (Waterloo, CA)
Gait Disturbances in the 5xFAD transgenic mouse model of Alzheimer's Disease

May 2016. Poster Presentation - Neuroscience Research Day (London, CA)
Gait Disturbances in the 5xFAD transgenic mouse model of Alzheimer's Disease

March 2016. Poster Presentation - London Health Research Day (London, CA)
Gait Disturbances in the 5xFAD transgenic mouse model of Alzheimer's Disease

Related Work Experience:

Teaching Assistant
Neuroscience 4000E
The University of Western Ontario
2015-2016

Teaching Assistant
Neuroscience 9500b
The University of Western Ontario
2017