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Assessing Cognition In A Mouse Model Of Alzheimer's Disease

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Abstract

A major challenge in behavioural testing of Alzheimer's disease (AD) mouse models is a low degree of standardization and translatability of tasks to humans. Bussey-Saksida touchscreen systems employ numerous tasks allowing for assessment of advanced cognitive function in mice using paradigms similar to those used in humans. This system can be used to assess cognitive deficits in dementia.

Our study focused on testing visual discrimination, cognitive flexibility and attention of the APP/PS1 familial AD mouse model longitudinally to assess behavioural changes related to cognitive decline. Surprisingly, APP/PS1 mice did not demonstrate impairments in the 5-Choice Serial Reaction Time Task, which assesses attention and did not show robust deficits in the Pairwise Visual Discrimination task, which assesses visual discrimination and cognitive flexibility. Together, our findings suggest that APP/PS1 may not be a good model to evaluate these cognitive domains in AD, however other domains, namely visuospatial integration, may require closer attention.

Keywords

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

Alzheimer's Disease

Amyloid-beta ($A\beta$) plaques

Amyloid Precursor Protein (APP)

APP/PS1 Mouse Model

Attention

Cognitive Flexibility

Familial Alzheimer's Disease (FAD)

Pairwise Visual Discrimination (PVD)

Presenilin-1 (PS1)

Reversal Learning

Touchscreen

Co-Authorship Statement

Justin Mels performed all the touchscreen, locomotion, gait experiments and analyses in this thesis.

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List of Abbreviations

Abbreviation	Full Name
5-CSRTT	5-Choice Serial Reaction Time Task
A β	Amyloid Beta
Ach	Acetylcholine
AD	Alzheimer's Disease
AICD	APP Intracellular Domain
ANOVA	Analysis of Variance
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
APP/PS1	APP _(swe) /PS1 _(dE9)
BACE	Beta APP Cleaving Enzyme
CANTAB	Cambridge Neuropsychological Test Automated Battery
CTF	C-Terminal Fragment
hAPP	Human APP
ITI	Inter-Trial Interval
mAPP	Mouse APP
NMDA	N-Methyl-D-Aspartate
PS1	Presenilin-1
PVD	Pairwise Visual Discrimination
ROUT	Regression Outlier Removal
WT	Wild-type

1 Introduction

1.1 Alzheimer's Disease Overview

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by general brain atrophy and progressive cognitive impairment (Tawaneh and Holtzman, 2012). Globally, approximately 1 in 10 people over the age of 65 have AD (Alzheimer's Association, <https://www.alz.org/facts/#quickFacts>). AD is the most common form of dementia, with 60-80 % cases of dementia resulting from AD (Tawaneh and Holtzman, 2012). In 2015, it was estimated that 556,000 Canadians are affected with the disease and this number is expected to rise to 886,000 by 2030 according to a recent report from the Alzheimer' Society of Canada (Chambers et al., 2016). Women represent 65% of this number. The current annual total economic and societal burden of AD around the world exceeds 1 trillion Canadian dollars (Prince et al., 2016). Thus, developing effective therapeutics to combat the disease is of paramount importance (Prince et al., 2016).

The disease was first described following the study of Auguste Deter by Alois Alzheimer in 1901 (Hippius and Neundörfer, 2003). Auguste Deter showed a rapid progression of memory disturbances, sleep disruption, abnormal behaviours and confusion. Following her death in 1906, Dr. Alzheimer examined her brain and spotted anomalies in sections of the brain, consisting of senile plaques and neurofibrillary tangles (NFTs), which he deduced were the cause of her illness (Möller and Graeber, 1998).

Prior to death from AD, individuals often spend years suffering from poor health and disability. Quality of life becomes significantly diminished for the patients themselves, not to mention family members providing unpaid care (Kahle-Wroblewski et al., 2016). Studies assessing AD mortality rate indicate that 61% of 70-year-old individuals with AD are expected to die prior to age 80, compared to 30% of unaffected people expected to die in the same timeframe (Arrighi et al., 2010).

Most often AD is diagnosed following the exclusion of other causes of dementia, with official verification post-mortem (Prince et al., 2016). There are a multitude of symptoms characteristic of the disease. Among the most recognized are memory loss,

problem solving difficulties, confusion with time or place, difficulty completing familiar tasks, misplacing items, social withdrawal, mood and personality changes and speaking or writing problems. Prominent deficits also manifest in visuo-spatial relationship problems and decreased judgment in the form of impulsive and or compulsive behavior (Albert et al., 2001; Bidzan et al., 2012).

1.2 AD Neuropathology

Cognitive decline in AD is suspected to be a consequence of lesions resulting from toxic amyloid oligomers, extracellular amyloid β plaques and intracellular NFTs, which are the neuropathological hallmarks of the disease (Chen & Fernandez, 2001). However, an inherent difficulty with identifying AD is that not all individuals that present AD-like pathology display cognitive deficits (Nelson et al., 2009). As such it is important to understand the molecular processes that lead to the development of AD pathogenesis.

The production of amyloid β plaques arises from the abnormal processing of amyloid precursor protein (APP) (Figure 1). APP is a transmembrane protein that can be cleaved by 3 types of enzymatic activities, namely α , β and γ -secretases. Under normal conditions, the APP pathway is mediated by the cleavage of APP by α -secretase within the A β sequence, releasing soluble APP α (sAPP α) and an α C-terminal fragment (α CTF) (Zhang et al., 2011b). α CTF is then further broken down into the intracellular domain of APP (AICD) and a rapidly degraded fragment called P3 (Zhang et al., 2011b). Although the exact physiological functions of APP are not well defined, it has been suggested to be involved in a vast array of important processes such as synaptogenesis, neurite outgrowth, neuromuscular junction formation, calcium metabolism, cell adhesion and trafficking of neuronal protein along the axon (Stanga et al., 2016; Zhang et al., 2011b).

Under the amyloidogenic processing of APP, the APP holoprotein is first cleaved by the β -APP cleaving enzyme (BACE) generating soluble-APP β (sAPP β) and β C-terminal fragment (β CTF). Cleavage by γ -secretase activity of the β CTF produces AICD and A β (Zhang et al., 2011b). This β CTF cleavage mediated by γ -secretase activity can produce both A β 42 or A β 40, with the former being generated to a lesser extent but with greater amyloidogenic characteristics (Zhang et al., 2011b). A β 42 is more hydrophobic

and thus more prone to form fibrils (Zhang et al., 2011b). Numerous studies investigating Familial Alzheimer's Disease (FAD) mutations have found an increase in A β 42/A β 40 and thus have implicated higher A β 42 levels as the central tenet responsible for the formation of β -amyloid plaques (Borchelt et al., 1996; Iwatsubo et al., 1994; Scheuner et al., 1996; Selkoe and Hardy, 2016).

The accumulation of NFTs on the other hand results from the hyperphosphorylation and subsequent misfolding of a protein called tau. Tau is a microtubule associated protein (MAP) that is heavily phosphorylated (Iqbal et al., 2010). Tau is found abundantly in neurons of the central nervous system (CNS) and is responsible for stabilizing the structure of microtubules (Iqbal et al., 2010). Microtubules serve as the transport system of the neuron, providing a platform for intracellular transport. Under normal conditions, tau binds to tubulin, promoting the assembly of microtubules and maintaining their structure. However, when tau becomes hyperphosphorylated it is unable to bind to tubulin and does not promote assembly into microtubules (Iqbal et al., 2010). This pathological state of tau causes it to self-aggregate into insoluble paired helical filament conformations and forms NFTs inside neurons (Ackmann et al., 2000).

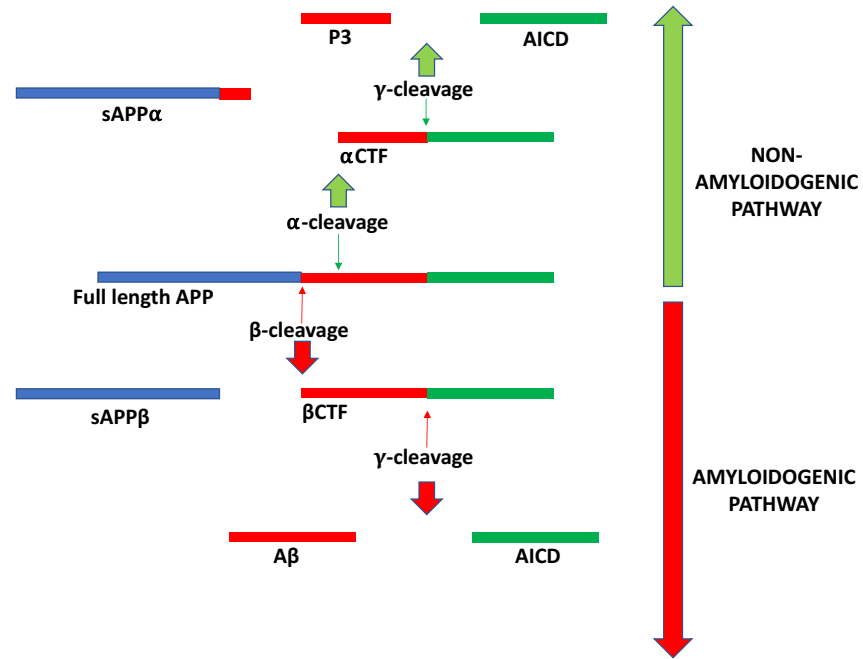


Figure 1. APP processing diagram.

Adapted from Zhang et al., 2011b.

1.3 Genetics of AD

There are two main classifications of AD cases, namely sporadic and familial. Approximately 95% of AD cases are classified as sporadic, with familial cases comprising roughly 5% (Alz. Association, 2016). Sporadic cases of AD have late-onset. Familial cases are mainly early-onset and are associated with mutations in APP, presenilin-1 (PS1) or presenilin-2 (PS2) genes (Selkoe and Hardy, 2016). Both types of AD cases lead to the oligomerization and gradual accumulation and deposition of these oligomers of A β in the brain in form of plaques and ultimately large-scale synaptic and neuronal dysfunction (Selkoe and Hardy, 2016).

Clearance failures of A β can be attributed to several causal factors, including apoE4 inheritance and faulty A β degradation (Bu, 2009). ApoE is a protein responsible for the transport of lipid and cholesterol molecules in the CNS through binding to ApoE cell surface receptors (Bu, 2009). There are three isoforms of the ApoE protein (ApoE2, 3 and 4), which differ from each other by a single amino acid (Mahley, 1988). Of these three isoforms, ApoE4 has been implicated as a genetic risk factor for AD (Bu, 2009). A β clearance in the brain is dependent upon these ApoE receptors. A β peptides show different binding affinity for each ApoE isoform, with the ApoE4 isoform binding A β with the lowest efficiency (Bu 2009; DeMattos et al., 2004). Consequently, the age at which amyloid deposition first appears is much earlier in people with ApoE4 genotype (Morris et al., 2010; Vemuri et al., 2010). Neurons possessing the ApoE4 genotype have also shown age-dependent decreases in spine density and reduced dendritic complexity in cortical neurons of mice (Dumanis et al., 2009). The brain of both humans and transgenic mice expressing ApoE4 present more plaque deposits than those expressing ApoE3 alone (DeMattos et al., 2004; Holtzman et al., 1999; Liu et al., 2013). Additionally, postmortem studies have identified carriers of the ApoE4 allele show a greater amyloid plaque load in both sporadic and familial AD instances (Bu, 2009).

Familial AD (FAD) can be caused by several gene mutations, however, most FAD cases can be attributed to missense mutations in at least 1 of 3 genes; APP, PS1 or PS2 (Scheuner et al., 1996; Selkoe and Hardy, 2016; Waring and Rosenberg, 2008). Eighteen exons are responsible for coding the APP gene, with exons 16 and 17 encoding

the β -amyloid sequence (Mullan et al., 1992). Mutations on the A β domain within APP that result in greater production of A β peptides lead to early-onset AD (Selkoe and Hardy, 2016). One of the most frequently studied of the 52 APP mutations identified is the APP Swedish (APP_{swe}) double mutation. This mutation results in two amino acids, lysine and methionine, to be substituted to asparagine and leucine respectively (Mullan et al., 1992). This mutation expressed *in vitro* has been shown to increase total A β levels, with increased production of both A β 40 and A β 42 (Cai et al., 1993; Johnston et al., 1994; Scheuner et al., 1996).

PS1 and PS2, found on chromosome 14 and chromosome 1 respectively, are relatively large transmembrane protein components of the γ -secretase complex (De Strooper et al., 2012). As previously discussed γ -secretase is responsible for cleaving the CTFs, either the α CTF following scission by α -secretase in the non-amyloidogenic pathway or the β CTF after cleavage by β -secretase in the amyloidogenic pathway. The most common cause of FAD results from a missense mutation within one of these two catalytic subunits which can alter the cleavage site of CTFs and result in increased production of A β 42 (Selkoe and Hardy, 2016).

FAD causing mutations occurs in both presenilin genes, however, they are far more prevalent in PS1, with over 150 different mutations being identified, whereas only about 10 have been found in the PS2 gene (De Strooper, 2007). In contrast to PS1 mutations with a high penetrance, PS2 mutations show a low penetrance and the associated dysfunction is highly variable in mutations of PS2 (Cai et al., 2015).

Presenilin mutations genetically appear to cause a gain of toxic function through an increase in the ratio of A β 42 to A β 40, which is more prone to aggregate (De Strooper 2007; De Strooper et al., 2012; Wolfe, 2007). A second view of presenilin mutations suggests a biochemical loss of function as they result in a partial loss of function of the γ -secretase complex leading to the incomplete digestion of A β and consequently affecting a number of downstream signaling pathways (DeStrooper, 2007). A third area of investigation concerning presenilin mutations and their contribution to AD pathogenesis involves their role in the regulation of neuronal calcium stores, underlying the calcium

hypothesis of AD (Tu et al., 2006). It is suggested that normally presenilins form calcium leak channels and serve a signaling function independent of γ -secretase activity, however, FAD mutations disrupt this function (Tu et al., 2006).

1.4 The Amyloid Hypothesis of AD

The predominant hypothesis in AD pathogenesis is concentrated around amyloid. The essence of the amyloid hypothesis is that an early initiating factor in AD is the imbalance of the increased production and decreased clearance of A β which leads to the accumulation of hydrophobic A β 42 peptides (Selkoe and Hardy, 2016). Soluble A β oligomers are suggested to contribute to the synaptic dysfunction characteristic in AD (Sakono and Zako, 2010). In addition to synaptic dysfunction, the aggregation of these peptides leads to the formation of insoluble plaques that trigger a further cascade of events leading to neuronal degeneration also characteristic in AD (Selkoe and Hardy, 2016). Data from autosomal dominant forms of AD support this hypothesis, indicating A β as a potential promising target for therapeutics (Selkoe and Hardy, 2016; Yamin et al., 2008). That is, all mutations that cause FAD, namely, mutations in either APP or presenilin, known to be part of the catalytic site of γ -secretase, lead to increased A β accumulation, supporting this hypothesis (Wolfe et al., 1999). Also, the amyloid hypothesis is strengthened by accumulation of A β in Down's syndrome patients (Iwatsubo et al., 1994; Selkoe, 1989). As the gene for APP is located on chromosome 21 and individuals with Down's syndrome possess three copies of this chromosome, this supports the notion that overexpression of APP leads to AD through the accumulation, aggregation and deposition of A β (Selkoe and Hardy, 2016). The amyloid hypothesis is further supported by the predisposition to AD provided by ApoE4 that has demonstrated impaired brain clearance of A β (Bu, 2009) and the induction of tau hyperphosphorylation by A β 42 oligomers (Jin et al., 2011).

Despite the predominance of the amyloid hypothesis as the framework for all AD research, the clarity of this hypothesis in sporadic AD cases is not as straightforward (Musiek and Holtzman, 2015). One of the hindrances of the amyloid hypothesis is the poor correlation between amyloid plaque deposition, neuronal death and clinical AD

symptoms, both anatomically and temporally (Musiek and Holtzman, 2015). Synaptic loss is one of the key features of AD related pathology. One of the drawbacks of the amyloid hypothesis is that A β plaque deposition can occur and result in no synapse loss (Musiek and Holtzman, 2015; Selkoe and Hardy, 2016). Conversely, synapse loss can also occur without the deposition of A β (Selkoe and Hardy, 2016). Thus, amyloid has been suggested to be a key initiating factor leading to a cascade of events resulting in AD pathology, however, it does not exclusively encompass the etiology of AD (Musiek and Holtzman, 2015).

1.5 The Cholinergic Hypothesis of AD

The core thesis of the cholinergic hypothesis of AD is the degeneration of cholinergic neurons in the basal forebrain leads to a loss of cholinergic neurotransmission (Craig et al., 2011). The premise of this hypothesis is that disruption of the cholinergic system precedes other pathological features observed in AD, such as A β plaques, NFTs, reactive microglia, inflammation and synaptic dysfunction (Auld et al., 2002; Bartus, 2000). Numerous studies in both rodents and non-human primates have found that normal cognitive function, particularly attention and memory encoding, is reliant on forebrain cholinergic activity (Bartus, 2000; Decker and McGaugh, 1991; Deutsch, 1971; Perry et al., 1999). Further support for this hypothesis is provided by pharmacological studies that systemically administered cholinergic receptor antagonists to monkeys and demonstrated impaired learning and performance on a wide range of memory tasks (Aigner and Mishkin, 1993; Miller and Desimone, 1993). Despite cholinergic mimetics having shown some therapeutic value in treating some symptoms of AD they have failed to show any benefits in decreasing A β production in AD patients (Francis et al., 1999).

Challenges of the cholinergic hypothesis of AD have been presented where acetylcholinesterase activity or choline acetyltransferase have been unaffected in the brains of patients with early-stage AD (Terry and Buccafusco, 2003). Furthermore, cholinergic system abnormalities alone fail to account for all the cognitive deficits, not to mention other behavioural peculiarities associated with AD (Terry and Buccafusco, 2003). Despite these limitations, targeting the cholinergic system for treatment and

therapeutics remains an area of focus in drug development in AD, but is just one of the multitude of systems impacted in the disease (McGleenon et al., 1999).

1.6 Treatments of AD

Despite the lack of a cure for AD, several drug treatments and interventions are available that may help to alleviate or stabilize some of the symptoms and cognitive deficits and improve the quality of life of patients (Bianchetti et al., 2006). Of the four current drugs available to Canadians, there are two main classes that these compounds fall under: cholinesterase inhibitors (ChEIs) and NMDA receptor antagonists (Massoud and Léger, 2011).

ChEIs fail to alter the progression of AD however, they do provide some cognitive and behavioural relief and demonstrate dose-dependent improvements in symptoms (McGleenon et al., 1999). One mechanism by which ChEIs exert their action is by inhibiting the enzyme acetylcholinesterase, which is responsible for degrading ACh in the synaptic cleft, thereby increasing cholinergic transmission (Massoud and Léger, 2011). The three ChEIs prescribed in Canada are Donepezil, Galantamine and Rivastigmine. Typically, patients with mild-to-moderate AD are prescribed a ChEI, whereas those with moderate-to-severe AD are prescribed memantine (Massoud and Léger, 2011).

Memantine falls under the second class of drugs available to Canadians for the treatment of AD and is often prescribed to those who cannot tolerate the side effects associated with ChEIs. It is an N-methyl-D-aspartate (NMDA) receptor antagonist that exerts its action on the glutamatergic system by blocking excessive NMDA receptor activity (Massoud and Léger, 2011). Dizziness, confusion, headache, nausea, vomiting and fatigue are just a few of numerous side effects that can be experienced when receiving a pharmacological treatment for AD (Bianchetti et al., 2006; Massoud and Léger, 2011). Further development and discovery of novel therapeutic agents to stabilize progression or delay disease onset remains vital to combatting AD.

1.7 Murine models of AD

Key pathological features in AD have been reproduced using molecular genetics to generate mouse models of AD (Spires and Hyman, 2005). With over 90% of a genetic overlap with humans, mice are an excellent species to use for genetic modification to investigate FAD (Chinwalla et al., 2002). Although the incidence of FAD is low in comparison to most AD cases that are sporadic, research has focused on FAD mouse models. A number of different AD mouse models have been generated to replicate behavioural features of the disease with a high degree of similarity (Elder et al., 2010, Selkoe, 2012). Furthermore, mice and humans share, to a large degree, the neural circuits that underlie episodic memory, namely those between the entorhinal cortex and hippocampus, which are susceptible to destruction in AD (Hall and Roberson, 2012).

Many transgenic mouse models of AD have been developed that express human APP transgenes, containing one to several FAD mutations (McGowan et al., 2006). APP deficient (APP^{-/-}) mice have been shown to display several abnormalities including decreased locomotion, weaker grip strength, decreased brain and body weights (Müller et al., 1994; Zheng et al., 1995). Additionally, these mice have demonstrated deficits in synaptic function and dysfunction of neuromuscular junction formation (Caldwell et al., 2013), suggesting that APP plays critical roles in normal development.

AD transgenic mice overexpressing a variant of APP with the Swedish FAD mutation (APP_{swe}) do not display deficits in cognitive function up to 24 months-old (Savonenko et al., 2003). Furthermore, these mice do not develop noticeable amyloid pathology until after 2 years of age. Given the importance of rapid testing, investigating models of AD with such delayed pathological and cognitive phenotypes provides little value to therapeutic development.

Another transgenic model overexpressing APP that has been well established in the literature is the TgCRND8 mouse line (Adalbert et al., 2009; Chishti et al., 2001; Romberg et al., 2013). This model expresses two APP mutations, namely the APP_{swe} and APP_{Ind} (Chishti et al., 2001). This mouse model has been previously investigated using an automated touchscreen cognitive test battery and impairments in memory, response

inhibition and attention were demonstrated (Romberg et al., 2013). Furthermore, these impairments coincided with substantial amyloid plaque pathology in both the cortex and hippocampus observed as early as 4-months of age in these mice (Adalbert et al., 2009; Chishti et al., 2001).

Like APP transgenic mice, presenilin transgenic mouse models have also been investigated in AD (De Strooper, 2007; Elder et al., 2010; Hernandez et al., 2001). Presenilins are known to be involved in a variety of molecular pathways, including the cleavage of APP by gamma-secretase (Elder et al., 2010). Interestingly, PS1 knockout mice die shortly after birth, with substantial skeletal and cerebral deformities (Tao et al., 2010). Single transgenic PS1 or PS2 mice fail to develop plaque pathology or cognitive deficits, despite increased levels of A β 42 (Elder et al., 2010; Hall and Roberson, 2012). However, when these single transgenic presenilin models are crossed with APP transgenic models, these mice display plaque deposition earlier and in greater abundance than APP transgenic mice alone (Elder et al., 2010; Jankowsky et al., 2004). Additional abnormalities range from age-dependent neuronal loss and impaired neurogenesis in the hippocampus to age-related impaired synaptic plasticity and spine morphology (Chui et al., 1999, Mohammad et al., 2004, Wen et al., 2004). Crossing presenilin mutant mice with hAPP transgenic mice allows for both a pathological and cognitive phenotype to be observed relatively quickly, in contrast to mAPP models (Elder et al., 2010; Hall and Roberson, 2012). As such, many current mouse models of AD are utilizing the co-injection of hAPP and presenilin transgenes resulting in a single transgene being bred (Hall and Roberson, 2011).

One aggressive mouse model of AD that exhibits pathology at a very early age is called 5xFAD. These transgenic mice contain five FAD mutations, three of which are on the APP transgene and two on presenilin-1 (Oakley et al., 2006). 5xFAD mice show amyloidosis as early as 2-months of age and the pathology progresses rapidly to fill the entire cortex and hippocampus by 6-months of age (Oakley et al., 2006). Although 5xFAD mice show rapid amyloid pathology accumulation, they fail to demonstrate any tangle or tauopathy characteristic of other APP transgenic models (Oakley et al., 2006). The progressive cognitive deficits observed in 5xFAD mice coincide with increasing

amyloid pathology as they age (Oakley et al., 2006). 5xFAD mice generate A β 42 almost exclusively and show a progressive degeneration of synapses beginning at 4-months of age (Oakley et al., 2006). Previous work has revealed cognitive deficits in associative learning, fear conditioning, hippocampal-dependent memory and spatial working memory in 5xFAD mice (Oakley et al., 2006). Our group has also observed attentional deficits in both male and female 5xFAD at 10-months of age using touchscreen systems (Masood, 2015).

Another transgenic AD mouse model of interest is called the triple transgenic (3xTG) model. This model, like 5xFAD, contains a Swedish FAD mutation of APP (APP_{swe}), and a mutation on presenilin-1. However, it differs from 5xFAD and TgCRND8 as it contains a tau mutation that contributes to the tangle pathology observed in 3xTG mice (Oddo et al., 2003). Pathological progression of the disease in 3xTG is similar to that in humans such that plaque deposition precedes the development of tangles (Oddo et al., 2003). In 3xTG, A β pathology appears in the neocortex at around four months of age and plaques and tangles increase in severity as the mice age (Oddo et al., 2006). A variety of cognitive deficits are prevalent in 3xTG mice, with deficits in associative learning, recognition and reference memory presenting around 4, 10 and 12 months respectively (Clinton et al., 2007; Nelson et al., 2007). 3xTG mice have been assessed using touchscreen tasks and multiple laboratories reporting deficits in sustained attention as early as ten months of age in male mice (Masood, 2015; Romberg et al., 2011). It has been suggested that deficits in sustained attention could be a consequence of an APP mutation as 5-Choice Serial Reaction Time Task (5-CSRTT) accuracy impairments have been found in both TgCRND8 and 3xTG mice, as both contain the APP_{swe} transgene mutation (Romberg et al., 2013).

1.7.1 APP/PS1 Mouse Model of AD

Another mouse model of AD that utilizes the co-injection of hAPP and presenilin transgenes is the APP_{swe}/PSEN1dE9 (APP/PS1) mouse line (from here on referred to as APP/PS1 for simplicity). These mice were produced by co-injecting two expression plasmids, one containing humanized APP (hAPP) with the APP_{swe} mutation and the other containing human PS1 with exon 9 deletion, each controlled independently by mouse

prion promoter sequences. Co-injection of these two constructs into (C57BL/6 x C3H) F2 pronuclei resulted into insertion of the transgenes at a single locus (Jankowsky et al., 2004).

In APP/PS1 mice, A β deposits have been observed as early as 6 months of age, with an age-dependent increase in plaque deposition up to 9 months of age in the hippocampus and cortex and a progressive increase observed up to 12 months (Garcia-Alloza et al., 2006; Jankowsky et al., 2004; Ordóñez-Gutiérrez et al., 2013). Age-dependent increases in plaque number correlates with increases in levels of A β 42, which is more prone to aggregate than A β 40 (Garcia-Alloza et al., 2006; Jankowsky et al., 2004). Also, co-expression of PS1-d9 with mutated APP_{swe} accelerates the levels of A β 42 when compared to single APP_{swe} transgenic mice (Jankowsky et al., 2004). Coinciding with the timeline of plaque deposition, astrogliosis has been shown to develop in parallel, with the most severe gliosis occurring near plaques around 6 months of age (Kamphius et al., 2012). Additionally, decreased expression of the synaptic marker synaptophysin has been observed in the hippocampus of 20–24-month-old APP/PS1 mice (Ostapchenko et al., 2015). 20-24-month-old APP/PS1 mice have also demonstrated increased microglial activation in the hippocampus (Ostapchenko et al., 2015).

Electrophysiological studies of this mouse model have revealed that fibrillary A β induces hyperexcitability in pyramidal cells resulting in epileptic activity (Minkeviciene et al., 2009). Fibrillary A β was found to be the culprit in causing significant depolarization of pyramidal cells in 3 and 4.5 month-old APP/PS1 mice, resulting in seizure incidence of approximately 65% by 4.5 months of age (Minkeviciene et al., 2009). This provides the basis for the inherent difficulty of conducting a longitudinal study with this mouse model, as up to 50% of mice die by 12 months of age (Minkeviciene et al., 2009).

Context-dependent memory as assessed by fear-conditioning testing has been shown to be impaired as early as 4 to 6 months of age in APP/PS1 mice (Bonardi et al., 2011; Kilgore et al., 2010). 6 to 9-month-old APP/PS1 mice have shown deficits in discrimination learning (Filali et al., 2009). Spatial learning tested using the Barnes maze

task was shown to be intact in 7 month-old APP/PS1 mice (Reiserer et al., 2007). Similar findings have been revealed in this mouse model in Morris water maze (MWM) assessments, where transgenic mice showed no impairments at 12 months of age in the visible platform test but display deficits acquiring the hidden platform subtask and probe trial (Lalonde et al., 2005). Impairments in the MWM at 12 months of age in APP/PS1 mice have been replicated across a number of laboratories (O’Leary and Brown, 2009; Ostapchenko et al., 2015; Toledo and Inestrosa, 2010; Volianskis et al., 2010). Moreover, APP/PS1 mice have been previously investigated by our group longitudinally on a number of behavioural domains and have demonstrated age-dependent impairments on spatial memory tasks using both MWM and Barnes maze (Ostapchenko et al., 2015). These deficits in spatial working memory have also been observed at 15 months of age (O’Leary and Brown, 2009). Additionally, deficits in episodic memory using the repeated reversal and radial water maze tasks have also been described in 18 month-old APP/PS1 mice (Savonenko et al., 2005).

Motor coordination assessments through rotarod and grip strength tests have failed to reveal any deficits in motor function at 7 months of age (Lalonde et al., 2004). No differences in locomotor activity were apparent until 12 months of age where APP/PS1 demonstrated hypoactivity, that persisted at 15 months (Ostapchenko et al., 2015). These mice failed to demonstrate any anxiety-like behaviour as assessed by the elevated plus maze at 15 months (Ostapchenko et al., 2015). Other behavioural domains that have been previously examined in these mice include nest building and burrowing, which have been suggested to be an index of estimating general well-being in mice (Jirkof, 2014). APP/PS1 mice have shown declines in both nest building and burrowing activity at 18 months of age (Filali and Lalonde, 2009; Janus et al., 2015).

1.8 Touchscreen Systems

One critical issue that has been identified in cognitive research is the differences between tests of cognition in mice in pre-clinical research and those used in humans with AD (Schneider et al., 2014). One recent way to assess cognition has been by using an automated computerized test battery called the Cambridge Neuropsychological Test Automated Battery (CANTAB). CANTAB allows for the quick assessment of human

neuropsychiatric and neurodegenerative disorders. This test battery can identify early cognitive deficits in AD patients (Junkkila et al., 2012). Based on the CANTAB system, researchers developed a system for rodents that is fully automated and essentially mirrors human CANTAB testing, thereby increasing the translational potential for research from bench to bedside (Bussey et al., 2008).

The use of touchscreens to assess the cognitive abilities in rodents solves a number of problems typically encountered in conventional animal behaviour testing. Some of these common issues include lack of standardization, minimal automation, stressful animal testing environments and a lack of translatability across species (Bussey et al., 2008).

The first problem touchscreen testing aims to alleviate is the lack of standardization common in rodent behavioural testing across laboratories. Conventional behavioural testing is subject to a myriad of challenges including multiple experimenters handling animals and varying experiment timing throughout the light/dark cycle due to facility and equipment availability constraints. However, using touchscreen systems, operating procedures between laboratories has the potential to be identical. A multitude of tasks assessing different cognitive domains uses the same apparatus and only requires the correct mask to be inserted for the designated task. Second, touchscreen testing is fully automated where all response measures, latencies and omissions are recorded with millisecond accuracy and precision by a computer (Bussey et al., 2001). Automation removes human error common to conventional behavioural test recording and reduces animal handling, thus reducing variability and increasing replicability within experiments. Automation also allows for cognitive testing to become high throughput, whereby dozens of mice can be simultaneously tested in parallel and increase experimental statistical power. Third, touchscreen cognitive testing utilizes stress-averse procedures. This use of reward based, intrinsic motivation to perform provides a more natural response than one reproduced through eliciting a stress response (Bussey et al., 2012). The final problem touchscreen cognitive testing alleviates is a translatable testing method, where rodent touchscreen tasks are nearly identical to those performed by

clinical AD populations (Bussey et al., 2008). With high face validity, touchscreens are bridging the gap between cognitive testing in rodents, primates and humans.

Touchscreen systems are versatile, such that they can run multiple paradigms on a single chamber, flexible, whereby spatial location on a trial by trial basis automatically changes, and powerful, as custom images and stimuli locations can be manipulated to adjust the paradigm. Tasks already available that can be utilized on these chambers include pairwise visual discrimination (PVD), 5-choice serial reaction time task (5-CSRTT), pairwise associate learning (PAL), visuomotor conditional learning, location discrimination, trial unique nonmatching to location, autoshaping, extinction, progressive ratio, effort related choice, and the rodent continuous performance test (Bussey et al., 2008; Heath et al., 2015; Talpos et al., 2010). Task performance measured through parameters such as accuracy, omissions, numbers of varying touch response measures and response latencies are all recorded on a trial by trial basis and can be compiled digitally to be analyzed in greater detail over a large number of sessions.

1.8.1 Pairwise Visual Discrimination and Reversal Learning Task

Impairments in visual discrimination have been previously identified in AD patients (Cronin-Golomb et al., 1991; Nguyen et al., 2003; Pal et al., 2016; Quental et al., 2013). Cognitive flexibility, which is the capacity to change behaviour based on changing sets of rules or contingencies, is an important part of executive function and ultimately decision making (Dajani and Uddin, 2015). AD patients show impairments in decision-making processes and increases in risk taking behaviour (Ha et al., 2012). However, little research has investigated aspects of cognitive flexibility, as it is thought to remain largely intact until later stages of AD progression (Sahakian et al., 1993).

Using the pairwise visual discrimination (PVD) task on touchscreen systems provides a way to assess both visual discrimination and cognitive flexibility (Bussey et al., 2008) in rodents. Visual discrimination learning involves two processes. The first being able to learn to discriminate two stimuli, whereas the second is to associate one of the stimuli with a reward (Horner et al., 2013). The PVD task requires the mouse to learn that one of two stimuli presented is associated with a reward (S+) and the other is not (S-

). This task assesses visual perception and associative learning which is dependent on the components of the visual system and striatum (Horner et al., 2013). Following task acquisition, the stimulus-reward contingency is reversed, and the mouse must then learn to inhibit the previously learned response and form a new reward association with the former unrewarded stimulus. This is referred to as reversal learning and has been suggested to provide an evaluation of cognitive flexibility in rodents (Brigman et al., 2008; Izquierdo et al., 2006).

The neurochemical basis of cognitive flexibility is not fully understood, however the dopaminergic, glutamatergic and cholinergic systems have all been implicated as playing important roles within this domain of executive function (Berry et al., 2016; Brigman et al., 2008; Kolisnyk et al., 2013). Dopamine has been shown to play an important role in cognitive flexibility in both animals and humans (Berry et al., 2016; Klanker et al., 2013; Berry et al., 2016; Izquierdo et al., 2006). Intact functioning of the prefrontal cortex (PFC) and striatal-cortical loops have also been previously shown to be critical for task performance on the PVD touchscreen task (Graybeal et al., 2011; Horner et al., 2013; Bussey et al., 2008).

The glutamatergic system has also been shown to be involved in visual discrimination and cognitive flexibility. AD patients demonstrate reductions of NMDA receptors in the brain, which have also been correlated with decreased cognitive performance (Sze et al., 2001). In addition, NMDA receptor 2A knockout mice have shown impairments on the visual discrimination and reversal learning on the PVD task (Brigman et al., 2008). It has been suggested that these receptors act as a key molecular switch, enabling task acquisition (Barkus et al., 2012; Brigman et al., 2008).

Cholinergic transmission has been shown to be important in the modulation of cognitive flexibility (Prado et al., 2017; Ragozzino, 2003). The striatum and the prefrontal cortex are part of the cortico-basal-ganglia-thalamic circuit and cholinergic transmission is important to this network (Ragozzino, 2003). Cholinergic interneurons of the dorsomedial striatum have been suggested to be of critical importance in facilitating the selection of a new strategy (Prado et al., 2017). This effect has been shown to be

highly dependent on the activation of muscarinic receptors (Prado et al., 2017). However, the exact role of specific cholinergic circuits in modulating cognitive flexibility have been difficult to delineate as acetylcholine is present across widespread regions of the brain (Prado et al., 2017). Acetylcholine can signal both ionically and through G-protein coupled receptors both pre-synaptically and post-synaptically, that can influence a number of neuronal systems (Prado et al., 2017). Additionally, many neurons that release acetylcholine also release glutamate and GABA making it difficult to determine the specific cholinergic circuits involved in cognitive flexibility (Prado et al., 2017).

1.8.2 5-Choice Serial Reaction Time Task

One critical cognitive domain that has been suggested to deteriorate prior to language and visuospatial function early in AD individuals is attention (Baddeley et al., 2001; Bartko et al., 2011; Perry and Hodges, 1999; Romberg et al., 2013). Attention has been suggested to be divided into 3 main subtypes, namely sustained, divided and selective (Perry and Hodges, 1999). Sustained attention is the ability to maintain focused or remain vigilant over a period of time (Huntley et al., 2017). Divided attention refers to the ability to simultaneously perform two distinct tasks, whereas selective attention describes the ability to focus and switch attention to a target stimulus while ignoring irrelevant or distracting stimuli (Baddeley et al., 2001). All three of these divisions of attention have been identified as being affected in AD patients, however, divided and selective attention have been found to be compromised early in the disease, whereas sustained attention has been most commonly shown to remain unaffected until later in the disease progression (Perry and Hodges, 1999). However, the literature on deficits in sustained attention in AD patients has been far from conclusive, with multiple studies suggesting that it has been found to be impaired in mild AD and could be an early indicator of a transition from mild cognitive impairment (MCI) to dementia (Rizzo et al., 2000; Saunders and Summers, 2011).

Conflicting reports on attentional deficits are rooted in the inherent difficulty of assessing attention in a systematic way, particularly sustained attention (Huntley et al., 2017). There are a variety of behavioural tasks that have been developed to model attention in mice that attempt to provide comparable evaluations to those used in AD

patients. One of the most predominantly used is the 5-choice serial reaction time task (5-CSRTT) (Bartko et al., 2011; Bussey et al., 2012; Kolisnyk et al., 2013; Robbins, 1997; Romberg et al., 2013). This task was originally developed to assess arousal and attentional processes and identify the neurochemical pathways and brain regions associated within this aspect of cognition (Robbins, 1997). The touchscreen version of the 5-CSRTT requires responding to a light stimulus presented at random in one of five response windows on a digital screen. The duration that the light stimulus appears can be manipulated to shorter lengths, thereby increasing the attentional demand of the task. Deficits in sustained attention on the 5-CSRTT can be expressed as decreased accuracy, increased omissions and increased latencies to respond. Additionally, this task allows for the analysis of abnormal responding measures such as compulsivity and impulsivity, which are recorded as perseverative responses and premature responses respectively (Bartko et al., 2011). The 5-CSRTT not only provides a standardized, automated, stress-averse way to investigate attention, it also provides a method that is highly translatable across species (Romberg et al., 2011).

Impairments in sustained attention have been consistently reported in AD patients (Baddeley et al., 2001; Perry and Hodges, 1999). Furthermore, research using a touchscreen version of the 5-CSRTT from the CANTAB revealed deficits in response accuracy of patients with MCI (Sahakian and Coull, 1993). Similarly, multiple mouse models of AD have shown deficits in sustained attention using the 5-CSRTT (Driscoll et al., 2004; Romberg et al., 2011; Romberg et al., 2013). 5xFAD, TgCRND8 and 3xTg mice lines have all demonstrated deficits in sustained attention (Masood, 2015; Romberg et al., 2011). Each of these models have also demonstrated some degree of disruption of PFC activity (Crowe & Ellis-Davies, 2014; Romberg et al., 2011). The mPFC in particular, has been implicated in encoding information regarding action related outcomes, which has been demonstrated through lesion studies producing significant deficits on the 5-CSRTT (Benchenane et al., 2011; Fletcher et al., 2007).

Attention has been widely implicated as being modulated by the cholinergic system (Robbins, 1997; Passetti, 2002). However, other neurotransmitter systems including the serotonergic, dopaminergic and glutamatergic systems have also been

implicated in different aspects of behavioural inhibition and activation related to attention (Brigman et al., 2013; Robbins, 1997). Cholinergic neurons of the basal forebrain have been implicated as being of critical importance in the attentional function of humans, primates, rats and mice (Muir et al., 1995; Robbins, 1997; Romberg et al., 2011). The basal forebrain has cholinergic projections that extend to the PFC, which is a brain region highly involved in cognitive processing and executive function (Kolisnyk et al., 2013). Cholinergic dysfunction has been widely documented to decrease performance in the 5-CSRTT in both rats and mice (Bartko et al., 2011; Kolisnyk et al., 2013; Muir et al., 1995; Robbins, 1997). Rats with reductions in acetylcholinesterase activity demonstrate impairments detecting the stimulus location in the 5-CSRTT and these deficits are reversed by administration of cholinesterase inhibitors (Muir et al., 1995). Abnormalities in the cholinergic system are also one of the neurochemical hallmarks of AD (Perry et al., 1977). Deficits on the 5-CSRTT have been revealed following damage to the PFC through pharmacological induced lesioning (Robbins, 2002). Furthermore, 3xTg mice develop early plaque in the PFC that coincides with deficits in performance on the 5-CSRTT (Romberg et al., 2011). 5xFAD mice also demonstrate disrupted PFC activity (Crowe & Ellis-Davies, 2014).

Administration of cholinesterase inhibitors have shown to ameliorate deficits on the 5-CSRTT in mouse models of AD that exhibit both cholinergic dysfunction and A β pathology. Administration of donepezil in the 3xTg AD mouse model improved choice accuracy and vigilance on the 5-CSRTT (Romberg et al., 2011).

1.9 Non-cognitive dysfunction in AD

In addition to cognitive deficits, non-cognitive domains are also affected in AD (Bianchetti et al., 2006). Gait disturbances have been demonstrated in early-stage AD patients and gait problems appear more frequently in those who experience cognitive decline (Amboni et al., 2013). Gait dysfunction has been linked to an increased risk of developing AD and is a major contributor to increased risks of falls and immobility in AD patients (Amboni et al., 2013). Moreover, walking difficulties in AD patients has been suggested to originate from gait apraxia (Della Sala et al., 2004). Decreased mobility has been linked to additional health problems and increased risk of

comorbidities (McCarron et al., 2005). Hypoactivity has also been frequently observed both in AD patients and transgenic mouse models of AD (Ferguson et al., 2013; Iqbal et al., 2013). Until recently, gait has been generally viewed as a largely automated motor task, requiring minimal higher-level cognitive output however, increasing evidence has linked alterations in executive function and attention to gait disturbances (Amboni et al., 2013; Quental et al., 2013). These disturbances have been linked to the prevailing view that normal visuospatial functioning is linked to attention as the two appear to deteriorate in concert as the disease progresses (Quental et al., 2013).

One way to study the influence of executive function in gait is using a dual-task paradigm, in which individuals are instructed to perform a motor task, such as walking, while simultaneously performing another cognitive task, like a verbal fluency test (Amboni et al., 2013). Gait impairments are found to be more pronounced in subjects with AD on this task and the observed gait disturbances increase as the dual-task complexity increases or if the cognitive impairment is more severe (Amboni et al., 2013). This task requires the brain to unconsciously prioritize cognitive resources between both the motor task and the cognitive task as the demand for attentional resources increases (Amboni et al., 2013). Increased gait dysfunction has also been observed when AD patients are subject to distractions in a dual-task paradigm which manifest as a reduced stride length (Sheridan and Hausdorff, 2007).

However, gait in mouse models of AD has been largely overlooked and has yet to be studied in detail. Whether gait abnormalities in mouse models correlate with deficits in attention is unknown. Additionally, it is important to identify gait impairments in mouse models of AD as this could affect the performance of these mice on an array of behavioural tasks that require adequate motor function to complete tasks proficiently. Gait has been found to be affected in mouse models of AD exhibiting amyloid pathology (Schroer et al., 2010; Wirths et al., 2008; Yuan et al., 2017). In the 5xFAD line, mice displayed a shorter stride length (O'Leary et al., 2013). 5xFAD mice showed gait impairments as early as 12 months of age and showed impairments on rotarod and balance beam testing (Jawhar et al., 2012; O'Leary et al., 2013).

Changes in overall activity levels are also observed in AD patients, which could be indicative of motor control problems (Khachiyants et al., 2011). One brain region of importance that is affected and involved in the facilitation of movement is the pedunculopontine nucleus (PPN) (Mena-Segovia et al., 2004). The PPN is interconnected with the basal ganglia and increases in inhibitory neural activity to this region have been implicated in hypoactivity, whereas decreases in inhibition have been thought to contribute to hyperactivity (Mena-Segovia et al., 2004).

A large number of commonly used AD mouse models demonstrate increased locomotor activity (Arendash et al., 2001; Dumont et al., 2004; Hyde et al., 2005; Mori et al., 2013; Pietropaolo et al., 2008). Increased locomotor activity has been previously associated with cognitive problems in some mouse models of AD, with Tg2576 mice and 5xFAD mice exhibiting hyperactivity as high as twice that of their wild-type controls at 9-months of age (Gil-Bea et al., 2007; Yang et al., 2014). Conversely, other mouse models of AD with cognitive deficits, such as 3xTg mice, have demonstrated hypoactivity in open field tests (Filali et al., 2012; Sterniczuk et al., 2010). Furthermore, 5xFAD mice have also demonstrated reduced locomotor activity in other studies, showing that phenotypic variability exists even within the same AD mouse line (Bhattacharya et al., 2014). Granted that locomotor disturbances vary widely based on testing time of day and the specific mouse line in question.

The literature examining locomotor activity in APP/PS1 mice is also controversial. Some research has revealed APP/PS1 mice to exhibit hyperlocomotion at 7, 9 and 10-months of age (Cheng et al., 2013; Lok et al., 2013; Jansen et al., 2013). Whereas other studies have suggested that APP/PS1 mice display decreased locomotor activity (Ferguson et al., 2013; Lok et al., 2013). APP/PS1 mice studied in our group showed no differences in locomotor activity of APP/PS1 at 3, 6 and 9 months of age, but observed reduced locomotor activity at 15-months of age (Ostapchenko et al., 2015). Hypoactivity has been previously linked with increased anxiety levels in 3xTg mice (Blázquez et al., 2014; Nelson et al., 2007). However, work investigating anxiety in APP/PS1 mice displaying hypolocomotion found no evidence of anxious behaviour at 15

months of age (Ostapchenko et al., 2015). The reasons for these discrepancies are not clear and need to be investigated further.

With such a variable phenotype in mouse models of AD with respect to locomotor activity, gait and motor coordination, it is important to determine how these non-cognitive domains in APP/PS1 mice are affected in relation to attentional control and AD progression.

1.10 Study Objectives

Rationale:

A number of cognitive domains are impacted early in AD, with deficits in memory being the consensus deficiency identified (Elias et al., 2000). However, other non-mnemonic areas affected in AD, namely executive control, could provide some unique insights into the underlying disease processes that precede memory impairment. Despite this, routine assessment of executive function in animal models of AD is lacking. Importantly, a comprehensive cognitive assessment may allow for a better understanding of disease progression and the introduction of therapeutics earlier in the disease.

To minimize errors and biases, we used standardized, automated touchscreen tests to evaluate cognitive deficits. Utilizing touchscreens allows for a precise level of experimental control and task comparability as the setting for each task, as well as stimuli type, response and feedback all remain the same for each test conducted.

Of the most commonly used mouse models in AD research, many exhibit both amyloidosis and tauopathy. As a consequence, pathological progression of the disease in these models is often extremely rapid and widespread, making hypotheses relating to amyloid pathology or tauopathy independently difficult to delineate. The APP/PS1 model was selected as it solely exhibits amyloidosis and also demonstrates a slower disease progression than other commonly used mouse models. This allows for amyloid-induced behavioural dysfunction to be further defined and clarification as to when behavioural deficits begin in these mice.

Hypothesis: We hypothesize that the APP/PS1 mouse model of AD will show age-dependent deficits in cognitive flexibility and attention.

Objectives:

One objective of this project was to investigate sex differences in the APP/PS1 mouse model. Little research has investigated behavioural differences between sexes in AD mouse models, as females are often excluded from research due to the behavioural variability associated with the estrous cycle (Mezaine et al., 2007). This is important to investigate not only because there is a higher prevalence of AD in human females, but also due to a suggested greater rate of cognitive decline than age-matched males (Li and Singh, 2014). Additionally, females have been suggested to have a higher amyloid burden and greater plaque pathology than male counterparts (Devi et al., 2010). Numerous mouse models of AD have consistently demonstrated earlier-onset pathology in female mice and the accelerated pathology is thought to be caused by increased β -secretase processing of APP (Jankowsky and Zheng, 2017). This project encompassed both male and female mice, to test whether APP/PS1 females demonstrate poorer cognitive performance on the PVD and 5-CSRTT than males.

Another objective of this project was to provide a longitudinal characterization of behaviour in the APP/PS1 mouse model. Although several studies have looked at pathological changes in transgenic mouse models (Ghosal et al., 2009; Lalonde et al., 2013; O'Leary & Brown, 2009), longitudinal changes in cognition have yet to be studied in great detail. As such, we investigated APP/PS1 mice over their lifespan and expected to see increased deficits as mice grew older, as AD patients normally show a progression in cognitive deficits in concert with pathological disease progression.

A third objective was to investigate motor dysfunction in APP/PS1 mice. AD patients frequently develop symptoms affecting motor function in the latter stages of the disease, particularly gait abnormalities (Sheridan et al., 2003; Sheridan and Hausdorf, 2007). Similarly, several AD mouse models have demonstrated motor dysfunction and motor coordination deficits (Lalonde et al., 2013; O'Leary et al., 2013; Yuan et al., 2017;

Zheng et al., 1995). Given this, we expected to observe decreased locomotor activity as mice grew older and an increase in gait abnormalities with age.

In this study, my specific aims are:

1. Conduct a longitudinal assessment of visual discrimination and reversal learning in male APP/PS1 mice at 4, 7, and 10 months of age.
2. Conduct a longitudinal assessment of sustained attention in male and female APP/PS1 mice at 4, 7, and 10 months of age.
3. Evaluate non-cognitive measures of locomotion and gait in male and female APP/PS1 mice.

2 Materials and Methods

2.1 APP/PS1 Mice

The APP/PS1 mice (B6.Cg-Tg(APP^{swe},PSEN1^{dE9})85Dbo)Mmjax MMRCC Stock number 034832-JAX and wild-type age-matched control mice (C57BL/6J) (MMRCC Stock number 000664) used in this study were bred at Jackson Laboratory in Bar Harbor, Maine and shipped to Canada for behavioural testing. The APP/PS1 mice were generated by co-injecting two transgenes: a chimeric human/murine amyloid precursor protein (APP)_{swe}, which contains the Swedish mutations (K595N and M596L) and a human mutant presenilin 1 (PS1-dE9, exon 9 deleted variant). Both mutations are associated with early-onset Alzheimer's disease (Jankowsky et al., 2004). These two co-injected elements are driven by endogenous mouse prion protein promoter. The two vectors integrated at the same chromosomal locus (Rantamäki, et al., 2013; Jankowsky et al., 2004; Lalonde et al., 2005).

The first cohort of APP/PS1 male mice and wild-type littermate controls consisted of twenty-four pairs. Twelve male pairs were originally used as cohort 1 for the PVD task, with an additional three pairs from a second cohort being added later due to the high mortality rate. The second half of cohort 1, consisting of twelve male pairs of APP/PS1 mice and wild-type littermate controls, were used for the 5-CSRTT.

The first cohort of APP/PS1 female mice and wild-type littermate controls consisted of twelve pairs used for the 5-CSRTT. Due to extremely high rates of mortality, a second cohort of three pairs and a third cohort of three pairs were additionally added, for a total of eighteen pairs to be used for the 5-CSRTT.

To note, like AD patients, mouse models of AD have been reported to exhibit higher incidences of spontaneous seizures (Jackson et al., 2015; Minkeviciene et al., 2009). Although the literature surrounding APP/PS1 spontaneous seizure incidence is not exhaustive, one study using video-EEG recordings in these mice detected at least one spontaneous seizure occurring between 3 and 4.5 months of age with the prevalence of seizures increasing as they grew older (Minkeviciene et al., 2009). In this project, by 12

months of age, only 52% of males and 22% of females from the beginning of the study were alive (Figure 2).

An additional cohort of fourteen free-feeding male APP/PS1 pairs and wild-type littermate controls were used to investigate non-cognitive deficits in locomotion and gait at 14 and 18-months of age. Several mice required treatment for dermatitis with two wild-type males requiring euthanasia prior to reaching 14 months old. Additionally, four APP/PS1 males died, leaving the total number of free-feeding APP/PS1 males and WT littermate controls at 10 and 12 mice respectively at the 14-month testing age. An additional two free-feeding APP/PS1 and three wild-type mice died or were euthanized prior to behavioural experiments at 18 months of age, leaving a total of eight free-feeding APP/PS1 and nine free-feeding wild-type mice.

All animals were tattooed on the tail with a unique animal identification number prior to the beginning of any behavioural assessment. Behavioural testing began at 4 months of age for all cohorts of mice subject to touchscreen testing as for this mouse line amyloid plaque deposits in the hippocampus and cortex are observed around 4-5 months of age (Minkeviciene, et al., 2009; Shemer et al., 2006).

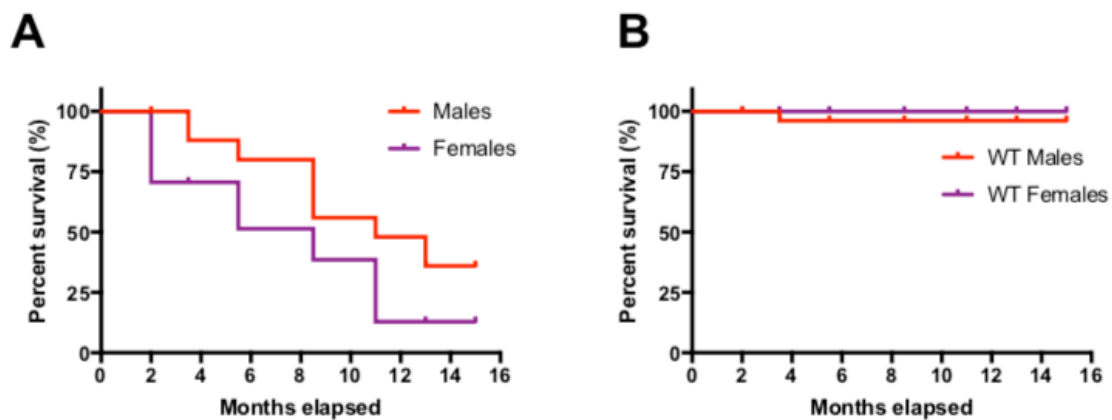


Figure 2. Percent survival (%) of male and female mice used in touchscreen testing.

A) APP/PS1 male and female mice (APP Males n=24, APP Females n=15) **B)** Wild-type male and female mice (WT Males n=28, WT Females n=18)

2.2 Housing and Food Restriction

Mice were individually housed in Robarts behavioural facility in a room with a 12-hour light/dark cycle (lights turn on at 7:00 A.M.) to facilitate control of food intake. Social isolation increases the risk of developing dementia and it is consistently found in elderly at risk of AD, therefore our protocol models this risk factor in mice. Temperature and humidity of the room were maintained between 22 and 25 degrees Celsius and between 40-60% humidity. All behavioural testing were performed during the light cycle. Mice had *ad libitum* water access. Mice subject to touchscreen testing were food restricted prior to behavioural testing, beginning at 3.5 months of age and animal weights were measured three times weekly to ensure maintenance of 85% free-feeding weight throughout the experiment. Mice were fed (Harlan Tekland Chow) daily, averaging 2.5g for males and 2.0g for females. Mice were fed following their behavioural testing session each day according to their weight. Cages were changed and fresh water was supplied bi-weekly. Free-feeding mice were provided *ad-libitum* access to food in addition to water. The animals were handled in compliance with the Canadian Council of Animal Care at the University of Western Ontario with an approved protocol (Protocol # 2016-103 and 2016#104). The detailed food restriction standard operating procedure can be found in Appendix 1.

2.3 Touchscreen System

Mice were trained in Bussey-Saksida automated Mouse Touchscreen Systems (Campden Instruments) for each of the touchscreen tasks. The apparatus consists of a sound and light attenuated ventilated box, which houses a testing chamber (Figure 3A and Figure 3B). Each chamber is comprised of a tone generator, a house light, a reward tray and a monitor connected to infrared touchscreen sensors controlled by ABET II Touch software v.2.20.3 (Lafayette Instruments). Mice view stimuli through a black mask, which is selected based on the task being assessed (Figure 3C and Figure 3D).

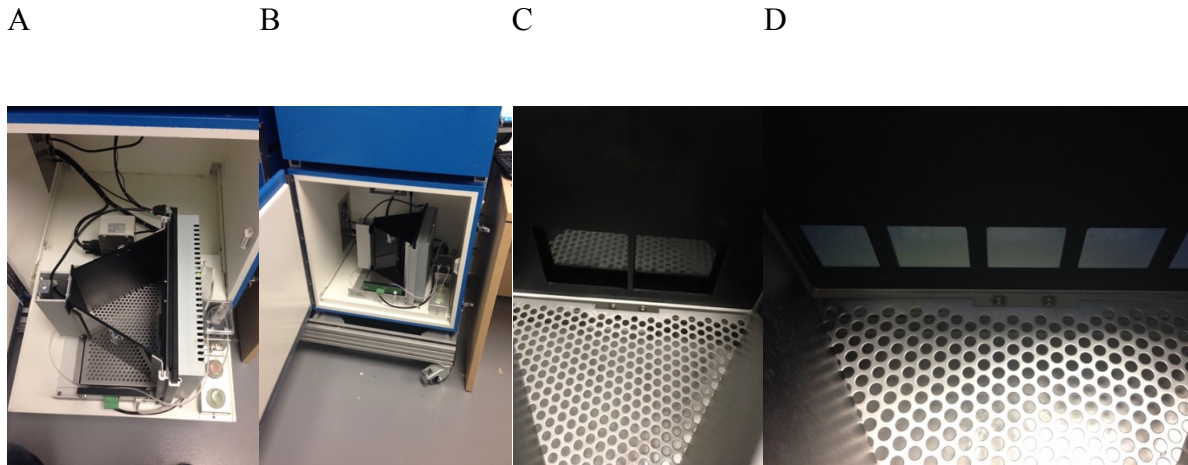


Figure 3. Images of the touchscreen apparatus and masks for each of the tasks.

A) B) The left side of chamber houses a reward tray that dispenses strawberry milkshake. Each chamber is housed in a sound and light attenuated box. The right side of the chamber consists of a touchscreen covered by a black Perspex mask with **C)** two 7.5x7.5cm windows for PVD and **D)** five 2x2 cm windows for the 5-CSRTT.

2.3.1 Touchscreen Pre-training

Throughout behavioral testing, each mouse was subject to one session per day. For both PVD and the 5-CSRTT, each mouse was subject to the same pre-training regimen. The only difference between the pre-training procedures on the two tasks was that for PVD the stimulus was a random image displayed in one of two response windows (Figure 3C), and for the 5-CSRTT the stimulus was a white square displayed in one of five response windows (Figure 3D).

Habituation was completed in 4 sessions with the first session involving the mouse being placed in the chamber for 10 minutes with all lights turned off and no stimulus or reward presented. The second and third sessions are 20 minutes with the reward tray light initially turned on. The reward tray is primed with 150ul strawberry milkshake reward (Neilson – Saputo Dairy) and an audio tone is played. Once the mouse enters and subsequently leaves the reward tray, the reward tray light is turned off. After a 10 second delay, the tray light is turned on again, accompanied by a tone and a 7ul delivery of reward. The fourth session is 40 minutes in length and reward presentation follows the same protocol observed in sessions 2 and 3 (Figure 4 and 6).

After habituation sessions, mice are submitted to a series of training sessions. The first being initial touch, in which the stimulus (random images for PVD and a white square for 5-CSRTT) is paired with the reward. During initial touch, the stimulus is pseudo randomly displayed in one of the windows for 30 seconds. After 30 seconds the image is removed, food is delivered (7ul) coinciding with the illumination of the tray light and audio tone. Entry to collect the reward extinguishes the tray light and the inter trial interval (ITI) begins. The ITI is the period of time between trials. Following 20 seconds ITI, another stimulus is displayed. If the mouse touches the screen while the stimulus is displayed and in the correct location, the image disappears, a tone is played and 3x reward is delivered. Mice are required to complete 30 trials in 60 minutes to achieve criterion. The initial touch schedule is repeated until criterion is reached.

Once criterion is achieved on initial touch, the mouse is subjected to the must touch session (phase III). Session conditions remain the same as for initial touch except

that the mouse must touch the stimulus to elicit the tone and reward. The mouse is again required to complete 30 trials in 60 minutes to achieve criterion (Figure 4 and 6).

The next stage of training is the must initiate session. Session conditions remain the same as for must touch with the addition of an initial reward delivery being made and the tray light being turned on to commence the session. The mouse is required to nose poke and exit the reward tray prior to a stimulus being displayed. The criterion to proceed through this session is the completion of 30 trials in 60 minutes. Sessions are repeated until criterion is attained (Figure 4).

The final training stage is the punish incorrect session. This schedule trains the mouse to initiate following an ITI and not to touch an incorrect window. Conditions remain the same as for the must initiate schedule, except that if a mouse touches the incorrect location (where the stimulus is not displayed) the house light is turned on for 5 seconds and no reward is dispensed. Following this time out period, the house light is turned off and the ITI of 20 seconds begins. No time limit is imposed on the duration of time the stimulus is displayed, so no omission score is calculated and no correction trials are required. To successfully pass this schedule and advance to the training phase requires the completion of 24/30 trials correctly or better within 60 minutes for 2 consecutive sessions (Figure 4 and 6). Mice unable to reach criterion after 30 sessions are removed from the study.

2.3.2 Pairwise Visual Discrimination (PVD) and Reversal

Task acquisition begins with a delivery of 7ul reward and the first trial begins following the mouse exiting the reward magazine. A S+ and S- image are presented in one of the two windows (Figure 5). The left/right order of these images is pseudo random and no order repeats more than 3 times. A touch at the location where the S+ stimulus is presented results in a reinforcement (7ul of reward) into the reward magazine. Like training, this delivery coincides with the illumination of the tray light and a tone. Once a mouse collects the reward and exits the reward tray, the ITI of 20 seconds begins. Following the ITI, the reward tray light is turned on again and requires the mouse to enter to initiate the next trial. An incorrect response (touching the S- image) will cause a 5

second “time out” with the house light turned on. After the “time out”, the house light is turned off and the ITI begins. After the ITI, the reward tray light is turned on and the mouse must nose poke the reward tray to start the correction trial. During a correction trial, the left/right order of the S+ and S- images is repeated from the last trial and this cycle continues until the correct choice is made. Correction trial results do not contribute toward criteria for session completion. Criterion is attained or the mouse is deemed to have acquired the task when it correctly performs at least 24/30 trials within 60 minutes, for two consecutive days.

After reaching criterion, each mouse is subject to additional 2 baseline sessions that follow the same guidelines as those in the acquisition sessions. However, there is no criterion score required to pass. Each baseline session ends following the completion of 30 trials or after 60 minutes has elapsed.

After completion of the 2 baseline sessions, each mouse is subject to 10 consecutive reversal learning sessions. Reversal sessions are subject to the same initiation, pseudo random presentation, time out and ITI procedures as previously described in acquisition and baseline sessions. However, a correct response is now defined as touching the location where the S- stimulus was presented and will elicit the reward to be dispensed (7ul reward) into the reward tray (Figure 5). As was the case with baseline sessions, reversal sessions require no score to pass and each session ends after the completion of 30 trials or 60 minutes has elapsed.

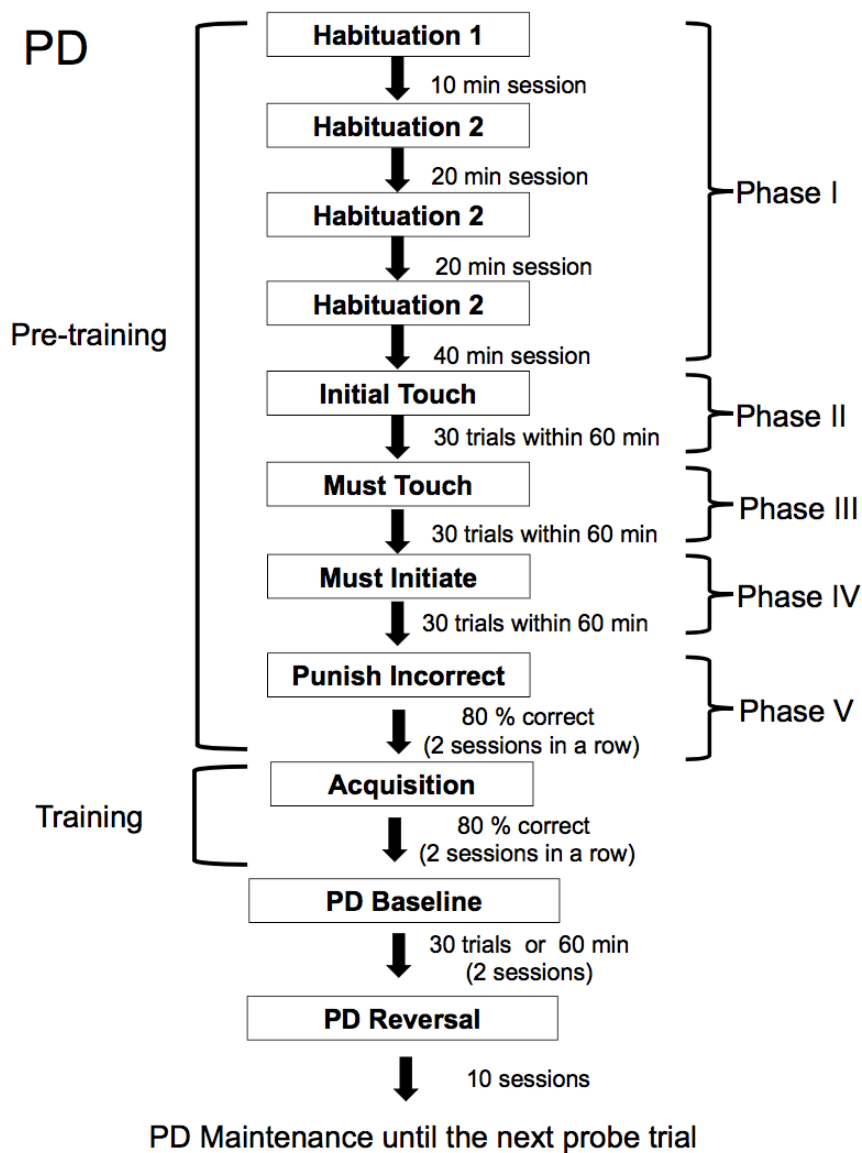


Figure 4. Flow chart of the pairwise visual discrimination schedules.

Pre-training schedules are shown at the top with each phase identified on the right and progression criteria located below each schedule. Adapted from Romberg et al., 2011.

Two weeks after each mouse has completed their final (10th) reversal session, the mouse is subject to 2 retention reversal sessions. Procedures and criterion for retention reversal are the same as reversal sessions.

Following the completion of the retention reversal sessions, maintenance sessions are run once weekly until each mouse is old enough to begin the next time point acquisition sessions. This schedule is identical to the punish incorrect schedule previously described. No criterion needs to be achieved and each session ends after 30 trials have been completed or after 60 minutes have elapsed. The detailed standard operating procedure for the PVD task can be found in Appendix 2.

2nd, 3rd and 4th time points are performed identically to the first-time point with different image sets for each time point. The second, third and fourth time points of testing begins at seven, ten and fourteen months of age respectively (Figure 5).

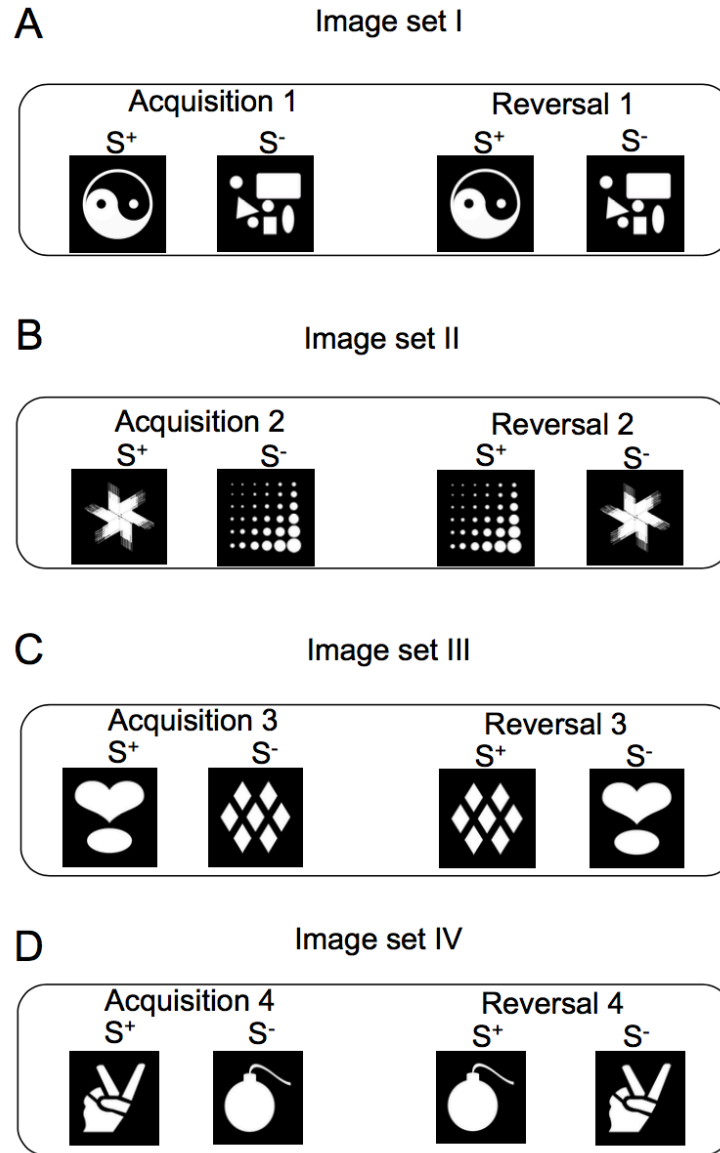


Figure 5. Image sets for the pairwise visual discrimination task during acquisition and reversal.

S⁺ indicating the stimulus paired with reward and S⁻ representing an unrewarded stimulus. **A)** First image set for 4 month-old time point **B)** Second image set for 7 month-old time point **C)** Third image set for 10 month-old time point **D)** Fourth image set for 14 month-old time point.

ABET II software collects a variety of parameters during the training, acquisition and reversal trials on the PVD task to assess performance. These include:

Trials Completed: the number of trials completed within the maximum time of 60 minutes.

Accuracy: the number of trials resulting in the correct image being selected. Calculated by number of correct trials divided by the total number of trials X 100. $(\# \text{ correct} / (\# \text{ correct} + \# \text{ incorrect}) \times 100$.

Correction Errors: the number of trials completed following an incorrect response.

Correct Touch Latency: the duration of time it takes for the mouse touch the display following stimulus onset.

Reward Collection Latency: the duration of time following a nose poke to the correct stimulus that it takes for mouse to enter the reward tray to collect the reward.

2.3.3 5-Choice Serial Reaction Time Task (5-CSRTT)

Following the successful progression through the five pre-training phases of the 5-CSRTT (Figure 6) as previously described, each mouse was trained to respond to a 4 second flash of light in one of the five response windows. Upon reaching criterion of greater than 80% correct responses and fewer than 20% of trials omitted for 3 consecutive days, each mouse was then required to reach the same criterion with the presentation of a 2-second stimulus duration. This period is collectively called the training phase.

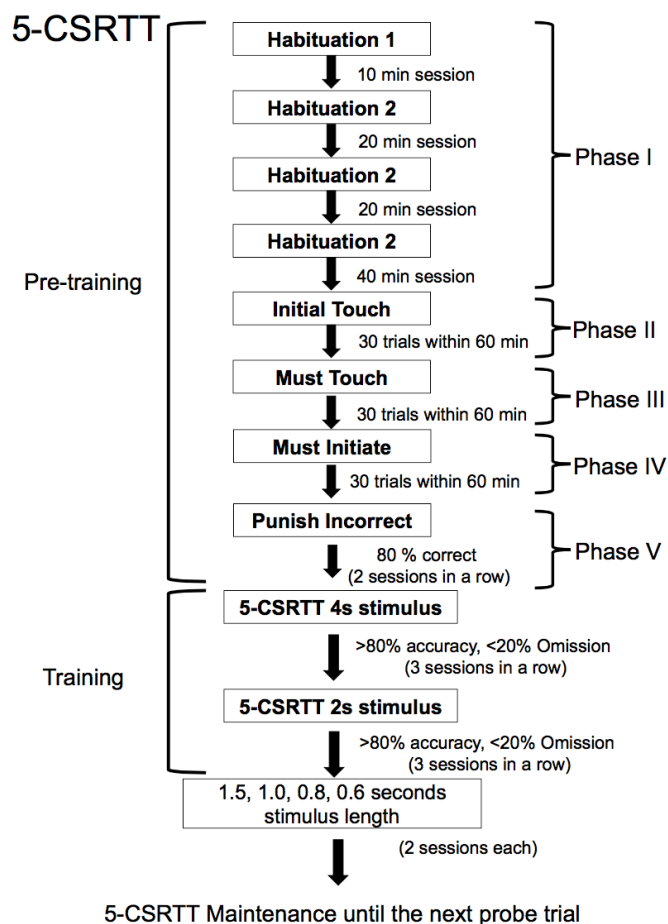


Figure 6. Flow chart of the 5-CSRTT schedules.

Pre-training schedules are shown at the top with each phase identified on the right and schedule progression criteria located below each schedule. Adapted from Masood, 2015.

After criterion was reached for 3 consecutive sessions on the 2-second stimulus duration, each mouse was subject to a series of probe sessions. Each mouse was randomly pre-assigned to one of four counterbalanced subgroups, which determined the sequence of presentation for probe stimulus durations. Furthermore, the order of probe schedule presentation was counterbalanced across each of the four time points. These probe stimulus durations included 1.5s, 1.0s, 0.8s and 0.6s for 4-month, 7-month and 10-month time points. At the 14-month time point, an additional stimulus duration of 0.4s was added to increase the attentional demand of the task. Each mouse was subject to completing each probe schedule duration for 2 consecutive sessions. Following each probe stimulus duration session set, each mouse returned to complete two sessions of the 2-second stimulus duration prior to beginning the next probe session set (referred to as inter-probe sessions). Following the completion of probe trials, mice were subject to a weekly maintenance session until the next time point was reached. At subsequent time points, mice completed five sessions of a 2-second stimulus duration (referred to as re-baseline sessions) prior to beginning the sequence of probe trials. The detailed standard operating procedure for the 5-CSRTT can be found in Appendix 3.

For probe trials, the performance measures were analyzed as an average between each set of two probe trials for each stimulus duration. In addition to trials completed, accuracy, correct touch latency and reward collection latency being recorded in PVD, omissions, premature responses as well as perseverative responses are recorded.

Omission: percentage of trials where no response is made; $(\text{omitted trials} / \text{total trials}) \times 100$

Premature Response: responses made prior to the onset of stimulus during the 5 second ITI $(\text{premature trials} / \text{total trials})$

Perseverative Correct: number of repeated nose pokes into the reward tray during presentation of the light stimulus or during the ITI made following the collection of reward to response windows, prior to the initiation of the next trial

2.4 Longitudinal Testing

Two cohorts of mice were used, one cohort in the PVD task (Figure 7) and the other in the 5-CSRTT (Figure 8). Both cohorts of mice were tested longitudinally on their respective task. Testing was conducted at 4, 7, 10 and 14 months of age for both tasks. Between time points, mice were subject to a weekly maintenance schedule to ensure that they did not forget how to use the touchscreen system. This schedule was implemented to ensure retraining on the apparatus was not required at subsequent time points. Upon reaching the subsequent testing time point, a 5 session 2-second re-baseline period was performed prior to the beginning of the next set of probe trials for the 5-CSRTT. For PVD, mice were subjected to a new image set.

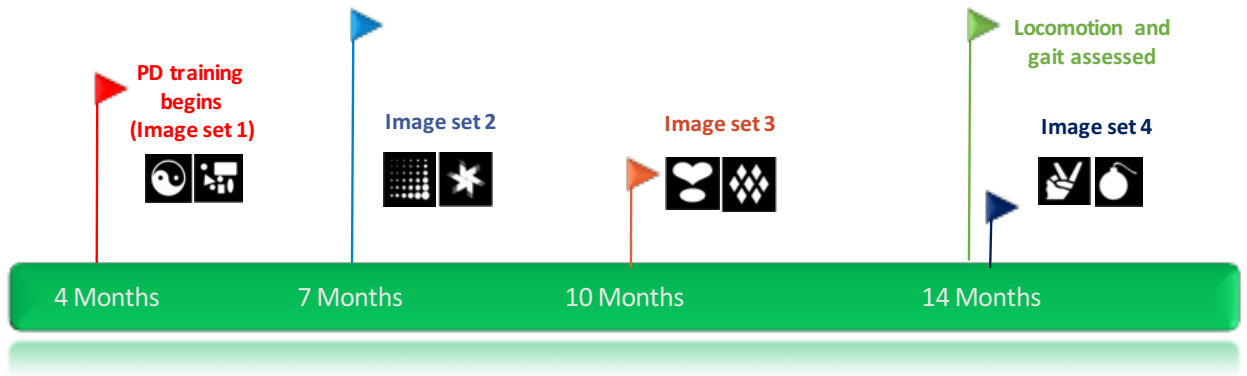


Figure 7. PVD experiment timeline.

Illustrations of image sets used at each time-point and the respective mouse age.

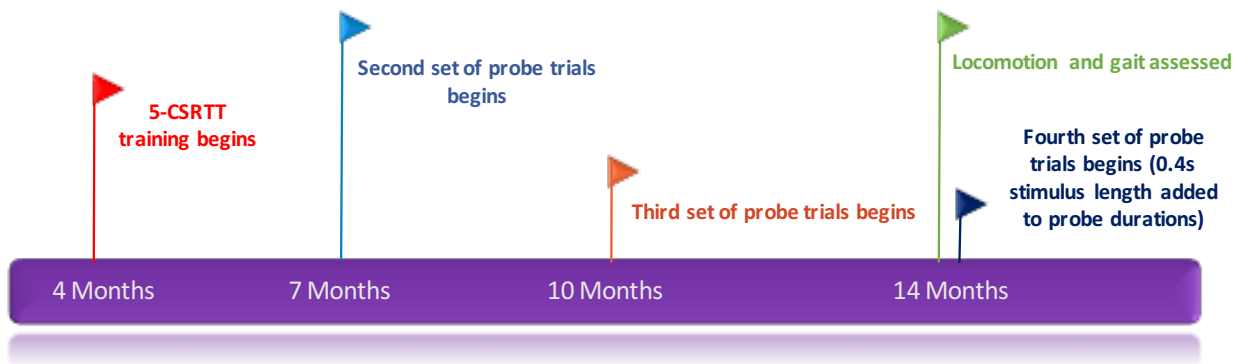


Figure 8. 5-CSRTT experiment timeline.

Illustration of operating procedure sequence at each time-point and respective mouse age.

2.5 Gait Analysis

Mouse gait parameters were measured using the Catwalk gait analysis system (Noldus Information Technology, Wageningen, Netherlands). In brief, the Catwalk is an automated, video based gait analysis system that is comprised of a high speed colour video camera, an enclosed walkway with a glass bottom, a fluorescent lamp that emits light inside the glass bottom and a recording analysis software to examine gait of mice. Mice were subject to an acclimation period once entering the room: 5 minutes with the lights dimmed and door open, 5 minutes with the lights off and door open and 5 minutes in complete darkness. Mice were individually placed in the walkway and permitted to walk from one end of the glass plate to the other. The recordings took place in complete darkness. Footprints were captured using the Catwalk 7.1 software.

2.6 Open-Field Locomotion

Mice were placed in the center of open field locomotor boxes (30x30x20cm) following a 30-minute acclimation period to the room. Locomotor activity was recorded in blocks of 5 minutes intervals for the duration of the 2-hour session (Omnitech Electronics, Columbus, USA). Boxes were cleaned with 70% ethanol prior to and following sessions to ensure that odor traces were eliminated.

2.7 Statistical Analysis

GraphPad Prism 7 was used to perform statistical analyses. Data are presented as $\pm$ SEM. Student's t-test was used when comparing two experimental groups, and two-way repeated measures (RM) analysis of variance (ANOVA) or a two-way ANOVA was used when multiple experimental groups were assessed. Sidak's multiple comparisons post-hoc comparison was used if results of ANOVA were found to be significant. The regression outlier removal (ROUT) method was utilized with a false discovery rate maximum (Q) of 1% to remove any outliers.

3 Results

3.1 Pairwise Visual Discrimination and Reversal Learning in APP/PS1 Male Mice

Individuals afflicted with AD have been shown to exhibit deficits in many aspects of nonmnemonic domains of cognition, namely executive control and attention (Romberg et al., 2013). Deficiencies in visuospatial function have been shown to be significant factors in the functional outcomes of AD patients (Quental et al., 2013). One way to assess visuospatial function is using the PVD task (Bussey et al., 2008). A number of brain structures are involved in visuospatial function, including the PFC and the perirhinal cortex (Romberg et al., 2013). Using the PVD task will allow us to identify if these cognitive deficits are replicated in APP/PS1 mice and further evaluate the longitudinal cognitive profile of this mouse model of AD.

Performance of APP/PS1 male mice compared to control mice did not differ throughout the pre-training phases on the touchscreen PVD task (**Figure 9**; RM-ANOVA, no effect of genotype, $F_{(1,27)} = 0.5565$, $p = 0.4621$; main effect of training phase $F_{(3,33)} = 41.8$, $p < 0.0001$; and no interaction effect, $F_{(3,33)} = 2.224$, $p = 0.0917$).

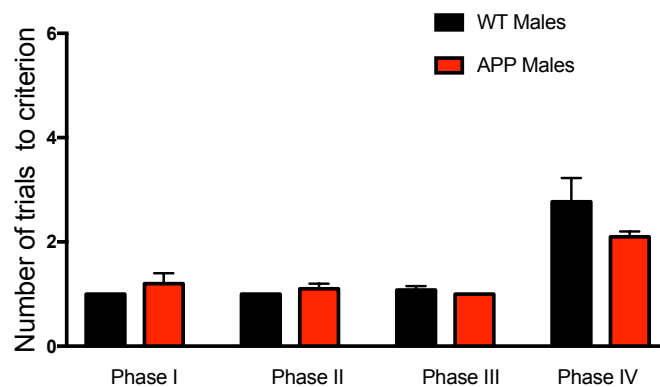


Figure 9. Pre-training learning phases of pairwise visual discrimination.

Number of sessions required for APP/PS1 male mice to advance through each phase of pre-training. (WT males $n=15$, APP males $n=14$, data are Mean $\pm$ SEM). Repeated-measures two-way ANOVA.

At 4 months, APP/PS1 mice showed no difference in the learning phase of the pairwise discrimination task compared to controls, as indicated by the number of sessions to criterion (**Figure 10A**; $t_{(27)} = 1.195$, $p = 0.2424$). During the reversal phase, there was no difference between genotypes in terms of trials completed (**Figure 10B** $F_{(1, 27)} = 0.1812$, $p = 0.6737$), percentage of correct responses (**Figure 10C**; RM-ANOVA, no effect of genotype $F_{(1,27)} = 3.179$, $p = 0.0859$; main effect of session $F_{(9,243)} = 92.01$, $p < 0.0001$; and no interaction effect $F_{(9,243)} = 0.8863$, $p = 0.5381$), number of correction errors (**Figure 10D**; RM-ANOVA, no effect of genotype $F_{(1,27)} = 2.07$, $p = 0.1617$; main effect of session $F_{(9,243)} = 116.1$, $p < 0.0001$), response latency (**Figure 10E**; RM-ANOVA, no effect of genotype $F_{(1,27)} = 3.495$, $p = 0.0724$; main effect of session $F_{(9,243)} = 21.52$, $p < 0.0001$; and no interaction effect $F_{(9,243)} = 0.8847$, $p = 0.5395$) and reward collection latency (**Figure 10F**; RM-ANOVA, no effect of genotype $F_{(1,27)} = 0.3924$, $p = 0.53638$; main effect of session $F_{(9,243)} = 7.572$, $p < 0.0001$; and no interaction effect $F_{(9,243)} = 0.9729$, $p = 0.4630$).

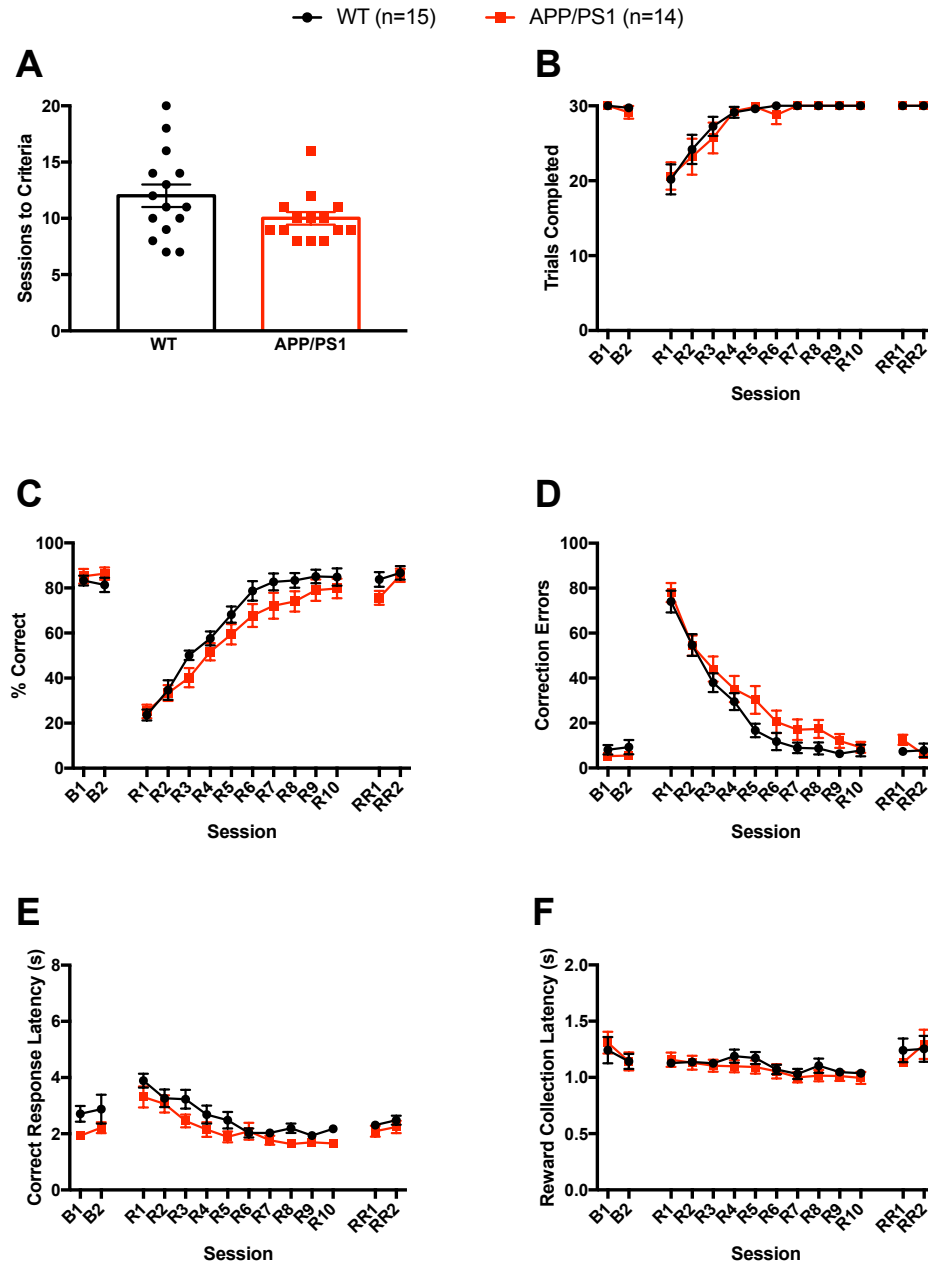


Figure 10. Learning and reversal learning performance of males on PVD task at 4-months of age.

A) Pairwise discrimination learning – Sessions to criteria. **B)** Reversal learning –Number of trials completed per session. **C)** Reversal learning –Mean accuracy. **D)** Reversal learning – Number of correction trials completed. **E)** Reversal learning – Mean correct response latency. **F)** Reversal learning – Mean reward collection latency (WT males n=15, APP males n=14, data are mean $\pm$ SEM). * $p < 0.05$. Repeated-measures two-way ANOVA.

At 7 months, the number of sessions APP/PS1 male mice took to learn the task (pairwise discrimination) did not differ from that of controls (**Figure 11A**; $t_{(25)} = 0.7037$, $p = 0.4881$). At this age, mice did not show deficits in reversal learning. We did not observe any difference between genotypes in regards to number of trials completed (**Figure 11B**; no effect of genotype, $F_{(1,25)} = 1.464$, $p = 0.2376$; main effect of session $F_{(9,225)} = 12.24$, $p < 0.0001$; no interaction effect, $F_{(9,225)} = 0.9922$, $p = 0.4473$), percentage of correct responses (**Figure 11C**; no effect of genotype, $F_{(1,25)} = 1.467$, $p = 0.2376$; main effect of session, $F_{(9,225)} = 12.24$, $p < 0.0001$; significant interaction effect, $F_{(9,225)} = 0.9922$, $p = 0.0473$), number of correction errors (**Figure 11D**; no effect of genotype, $F_{(1,25)} = 0.9129$, $p = 0.3485$; main effect of session, $F_{(9,225)} = 114.6$, $p < 0.0001$; no interaction effect, $F_{(9,225)} = 0.5449$, $p = 0.8407$), response latency (**Figure 11E**; no effect of genotype, $F_{(1,25)} = 0.05292$, $p = 0.8199$; main effect of session, $F_{(9, 225)} = 15.61$, $p < 0.0001$; no interaction effect, $F_{(9,225)} = 1.364$, $p = 0.2056$), or reward collection latency (**Figure 11F**; no effect of genotype, $F_{(1,25)} = 0.01689$, $p = 0.8976$; main effect of session, $F_{(9,225)} = 3.958$, $p = 0.0001$; no interaction effect, $F_{(9,225)} = 0.7458$, $p = 0.6666$).

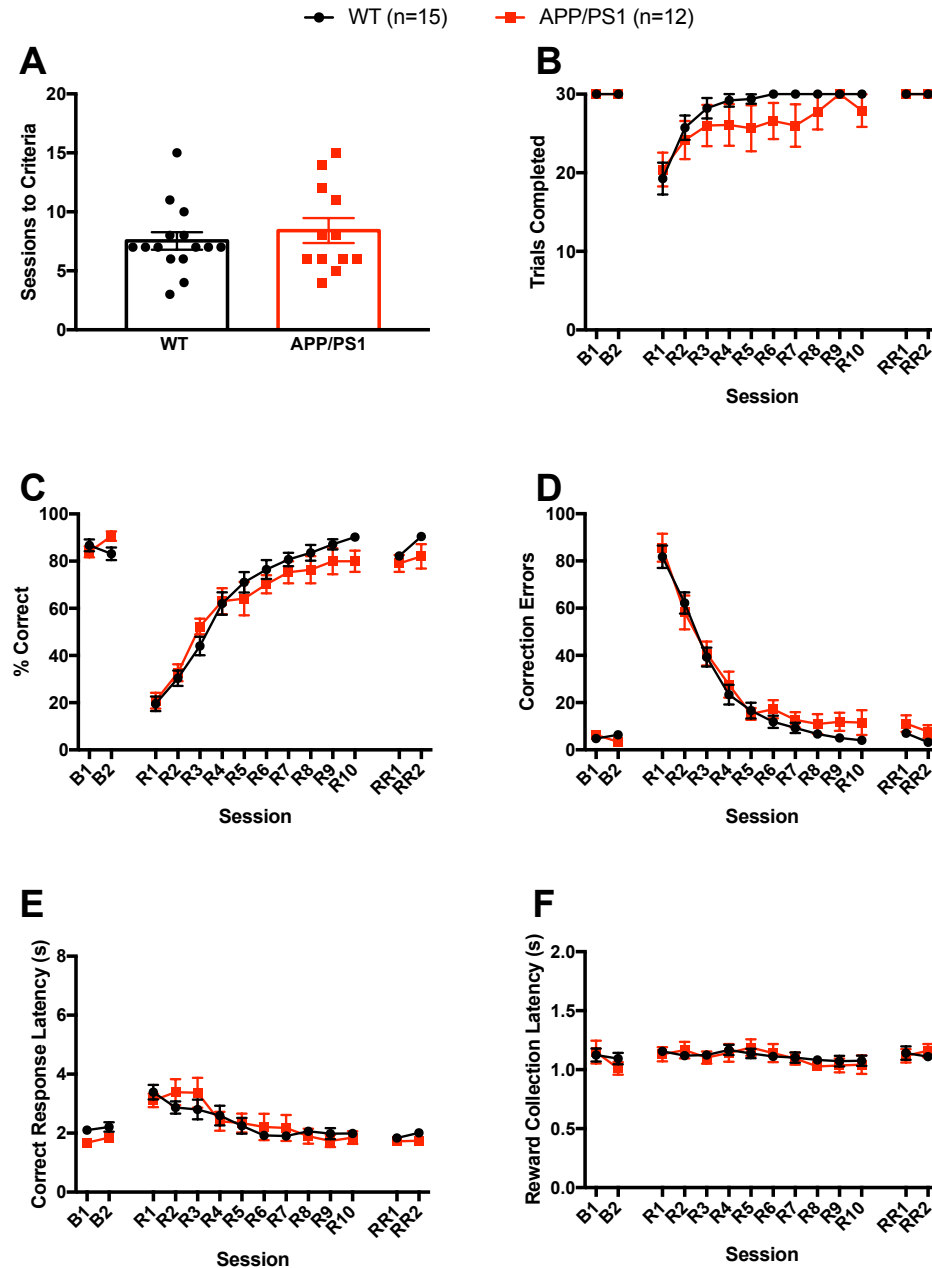


Figure 11. Learning and reversal learning performance of males on PVD task at 7-months of age.

A) Pairwise discrimination learning – Sessions to criteria. **B)** Reversal learning – Number of trials completed per session. **C)** Reversal learning – Mean accuracy. **D)** Reversal learning – Number of correction trials completed. **E)** Reversal learning – Mean correct response latency. **F)** Reversal learning – Mean reward collection latency (WT males n=15, APP males n=12, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

At 10 months, no differences were observed between genotypes to learn the pairwise visual discrimination task (**Figure 12A**; $t_{(21)} = 1.703$, $p = 0.1041$). Additionally, mutant mice did not show deficits in the reversal learning as they do not differ from controls in regards to number of trials completed (**Figure 12B**; no effect of genotype, $F_{(1,21)} = 0.01426$, $p = 0.9061$; main effect of session, $F_{(9,189)} = 6.266$, $p < 0.0001$; no interaction effect, $F_{(9,189)} = 0.3467$, $p = 0.9580$), percentage of correct responses (**Figure 12C**; no effect of genotype, $F_{(1,21)} = 0.1263$, $p = 0.7258$; main effect of session, $F_{(9,189)} = 25.96$, $p < 0.0001$; no interaction effect $F_{(9,189)} = 0.9589$, $p = 0.4756$), number of correction errors (**Figure 12D**; no effect of genotype, $F_{(1,21)} = 0.00041$, $p = 0.9839$; main effect of session, $F_{(9,189)} = 57.7$, $p < 0.0001$; no interaction effect, $F_{(9,189)} = 1.348$, $p = 0.2149$). Likewise, response latency (**Figure 12E**; no effect of genotype, $F_{(1,21)} = 0.07602$, $p = 0.7855$; main effect of session, $F_{(9,189)} = 9.398$, $p < 0.0001$; no interaction effect, $F_{(9,189)} = 0.5718$, $p = 0.8192$), or reward collection latency (**Figure 12F**; no effect of genotype, $F_{(1,21)} = 0.2199$, $p = 0.6439$; no effect of session, $F_{(9,189)} = 0.4379$, $p = 0.9133$; no interaction effect, $F_{(9,189)} = 0.4379$, $p = 0.9133$) did not differ between 10 month-old APP/PS1 and WT control mice.

In summary, male APP/PS1 mice demonstrated no deficits in visual discrimination learning or on reversal learning when tested longitudinally at 4-months, 7-months and 10-months of age compared to their WT controls.

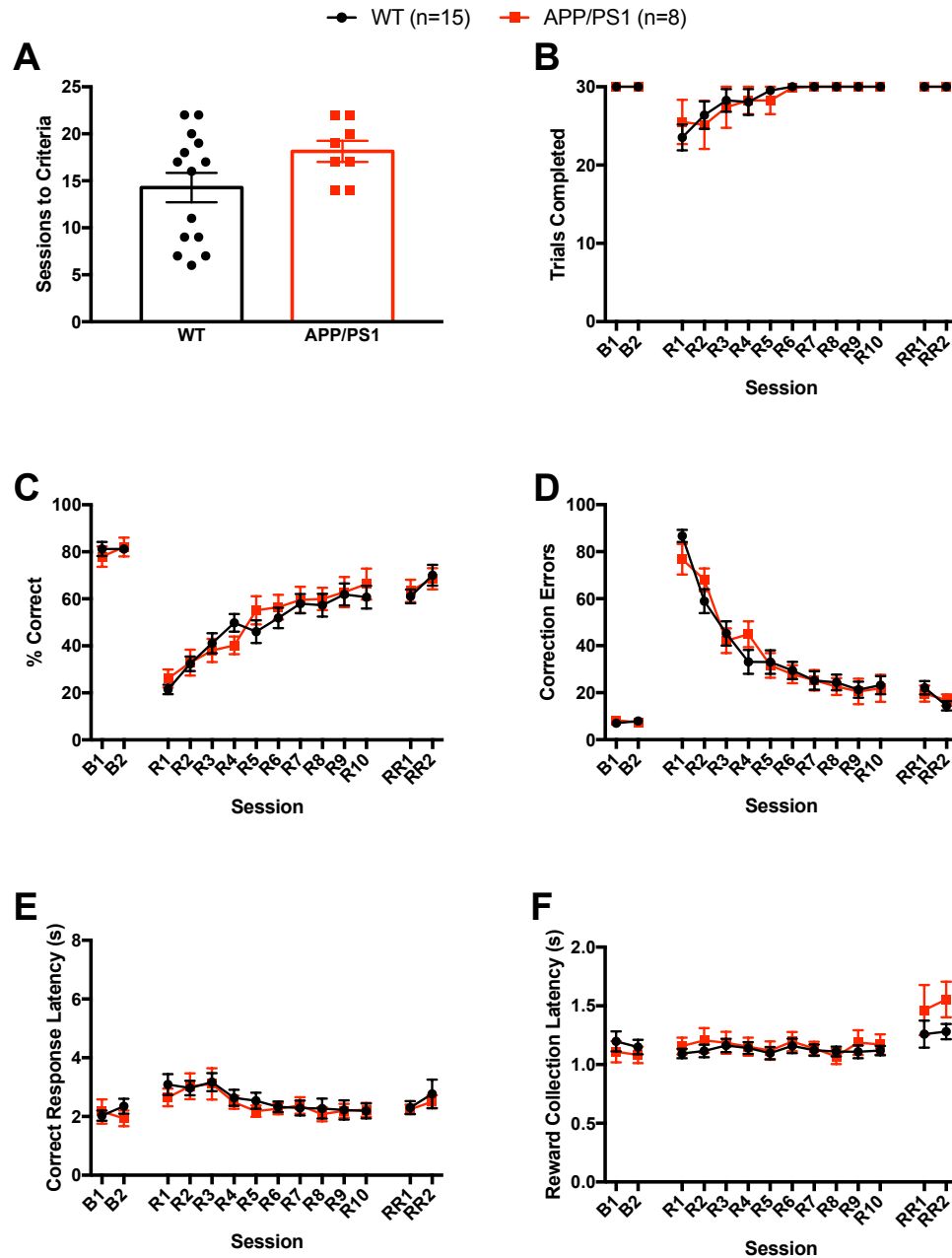


Figure 12. Learning and reversal learning performance of males on PVD task at 10-months of age.

A) Pairwise discrimination learning – Sessions to criteria. **B)** Reversal learning – Number of trials completed per session. **C)** Reversal learning – Mean accuracy. **D)** Reversal learning – Number of correction trials completed. **E)** Reversal learning – Mean correct response latency. **F)** Reversal learning – Mean reward collection latency (WT males $n=15$, APP males $n=8$, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

3.2 5-Choice Serial Reaction Time Task

Sustained attention has been found to be impaired consistently in AD patients (Baddeley et al., 2001; Perry and Hodges, 1999). Attention in humans and mice is reliant upon the cholinergic system. For adequate cognitive processing and executive function the cholinergic neurons of the basal forebrain that project to the PFC are of critical importance (Kolisnyk et al., 2013). One of the most comprehensive and widely tasks used to evaluate attention is the 5-CSRTT. This task allows for the identification of deficits in sustained attention and response control.

3.2.1 Learning Performance in APP/PS1 Male and Female Mice

The performance of male APP/PS1 mice on the 4 seconds training phase of the 5-CSRTT did not differ from controls (**Figure 13A**; no effect of genotype, $F_{(1,20)} = 4.236$, $p = 0.0528$; no effect of stimulus duration, $F_{(1,20)} = 0.1408$, $p = 0.7115$; no interaction effect, $F_{(1,20)} = 1.84$, $p = 0.1900$). However, *post-hoc* analysis revealed that males took a significantly shorter number of sessions to reach criterion at the 2-second stimulus duration ($t_{(20)} = 2.436$, $p = 0.0243$), suggesting that APP/PS1 males could learn the task faster than WT controls.

Female APP/PS1 mice required significantly fewer sessions to reach criterion on the training phase of the 5-CSRTT (**Figure 13B**; main effect of genotype, $F_{(1,24)} = 12.2$, $p = 0.0019$; main effect of stimulus duration, $F_{(1,24)} = 5.922$, $p = 0.0228$; no interaction effect $F_{(1,24)} = 0.9865$, $p = 0.3305$). *Post-hoc* analysis showed that APP/PS1 females also took a significantly shorter number of sessions to reach criterion at the 2-second stimulus duration ($t_{(24)} = 2.559$, $p = 0.0172$). These data suggest that, similar to males, APP/PS1 females seem to learn the task faster than WT controls.

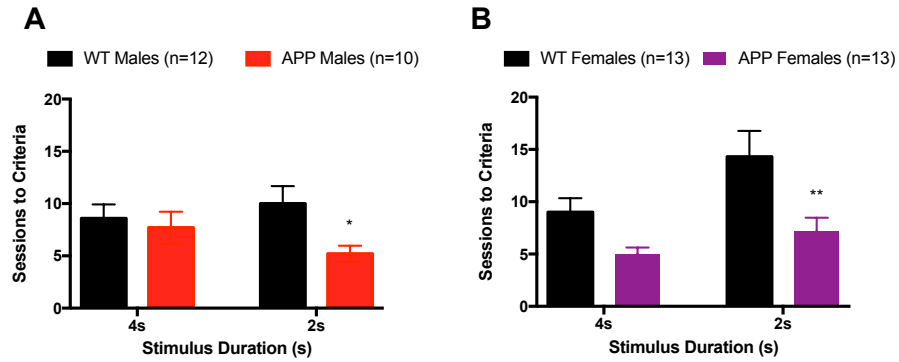


Figure 13. Performance of males and females on learning phases of 5-CSRTT at 4-months of age.

Number of session to attain >80% accuracy and <20% omission on trials for three consecutive days. **A)** Male sessions to criteria for 4 second and 2 second stimulus durations (WT males n=12, APP males n=10). **B)** Female sessions to criteria for 4 second and 2 second stimulus durations (WT females n=13, APP females n=13). (Data are mean $\pm$ SEM, * p <0.05, ** p <0.005).

3.2.2 Attentional Performance of Male APP/PS1 in the 5-CSRTT

4 month-old male APP/PS1 mice performance on the 5-CSRTT probe trials did not differ between genotypes across stimulus durations in terms of percentage of correct responses (**Figure 14A**; no effect of genotype, $F_{(1,20)} = 0.7281$, $p = 0.4036$; main effect of stimulus duration, $F_{(3,60)} = 39.41$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 2.125$, $p = 0.1065$), as well as percentage of trials omitted (**Figure 14B**; no effect of genotype, $F_{(1,20)} = 2.342$, $p = 0.1416$; main effect of stimulus duration, $F_{(3,60)} = 42.87$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 1.316$, $p = 0.2775$). Likewise, number of premature responses, (**Figure 14C**; no effect of genotype, $F_{(1,20)} = 1.19$, $p = 0.2882$; main effect of stimulus duration, $F_{(3,60)} = 3.523$, $p = 0.0202$; no interaction effect, $F_{(3,60)} = 1.629$, $p = 0.1920$), number of perseverative responses (**Figure 14D**; no effect of genotype, $F_{(1,20)} = 0.06901$, $p = 0.7955$; no effect of stimulus duration, $F_{(3,60)} = 1.238$, $p = 0.3037$; no interaction effect, $F_{(3,60)} = 0.07771$, $p = 0.9718$), correct response latency (**Figure 14E**; no effect of genotype, $F_{(1,20)} = 0.00091$, $p = 0.9762$; main effect of stimulus duration, $F_{(3,60)} = 7.845$, $p = 0.0002$; no interaction effect, $F_{(3,60)} = 0.3221$, $p = 0.8093$) or reward collection latency (**Figure 14F**; no effect of genotype, $F_{(1,20)} = 0.1604$, $p = 0.6930$; no effect of stimulus duration, $F_{(3,60)} = 0.9968$, $p = 0.4006$; no interaction effect, $F_{(3,60)} = 0.5395$, $p = 0.6570$) did not differ between 4 month-old APP/PS1 males. In summary, APP/PS1 male mice do not display attentional impairments in the 5-CSRTT at 4-months of age.

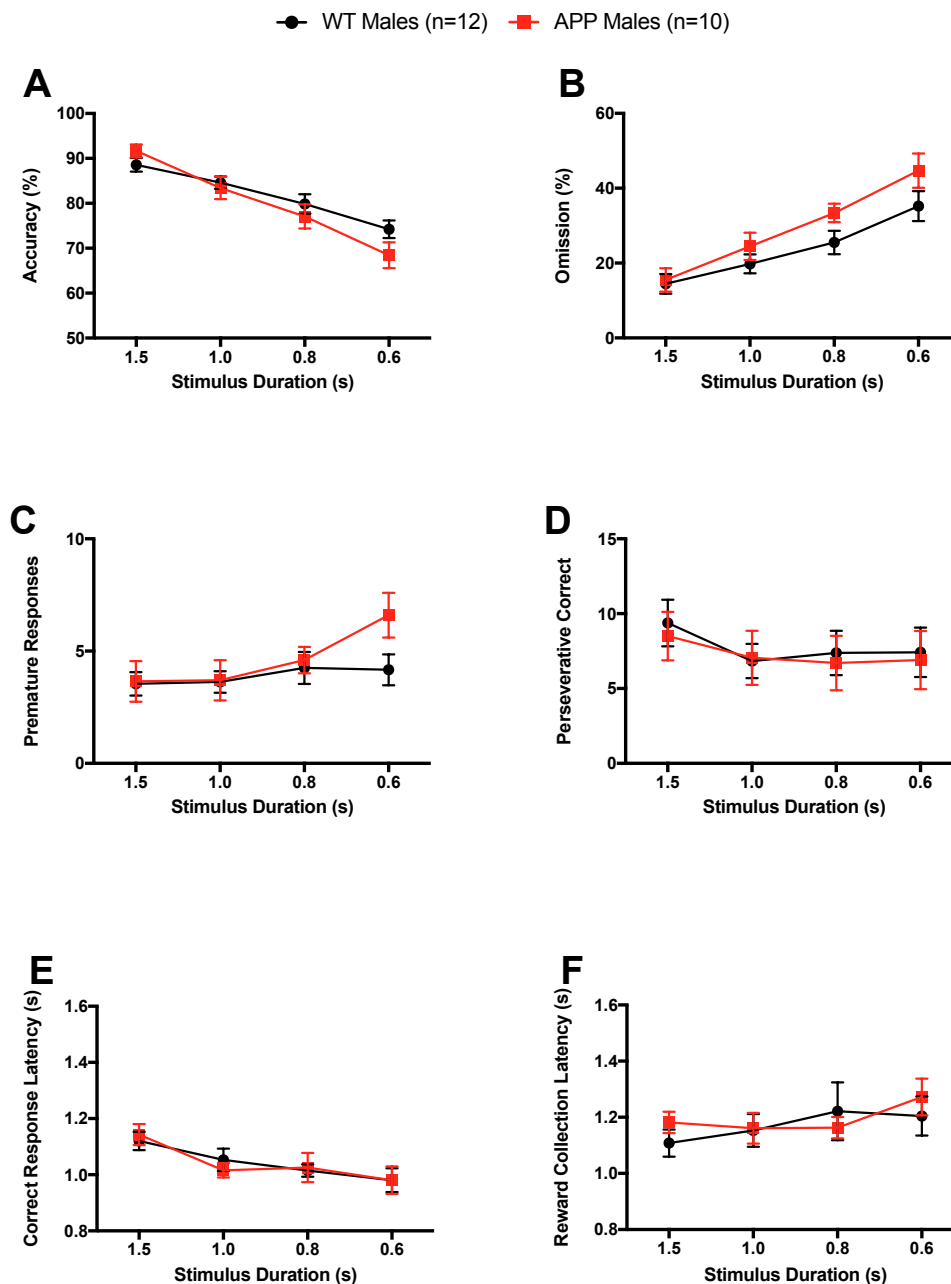


Figure 14. Response and performance measures of males during 5-CSRTT probe trials at 4-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. **A)** Mean accuracy **B)** Mean omission **C)** Mean number of premature responses **D)** Mean number of perseverative responses **E)** Mean correct response latency **F)** Mean reward collection latency. (WT males n=12, APP males n=10, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

7 month-old male APP/PS1 mice performance on the 5-CSRTT probe trials did not show any attentional deficit when compared to WT controls as measured by percentage of correct responses (**Figure 15A**; no effect of genotype, $F_{(1,20)} = 0.03262$, $p = 0.8585$; main effect of stimulus duration, $F_{(3,60)} = 26.81$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 0.89$, $p = 0.4516$), percentage of trials omitted (**Figure 15B**; no effect of genotype, $F_{(1,20)} = 7.5e^{-7}$, $p = 0.9993$; main effect of stimulus duration, $F_{(3,60)} = 25.56$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 0.3918$, $p = 0.7593$), number of premature responses, (**Figure 15C**; no effect of genotype, $F_{(1,20)} = 0.003129$, $p = 0.9559$; no effect of stimulus duration, $F_{(3,60)} = 2.458$, $p = 0.0715$; no interaction effect, $F_{(3,60)} = 1.565$, $p = 0.2073$), number of perseverative responses (**Figure 15D**; no effect of genotype, $F_{(1,20)} = 0.2168$, $p = 0.6466$; no effect of stimulus duration, $F_{(3,60)} = 4.458$, $p = 0.0068$; no interaction effect, $F_{(3,60)} = 0.4192$, $p = 0.7399$), correct response latency (**Figure 15E**; no effect of genotype, $F_{(1,20)} = 0.7108$, $p = 0.4091$; main effect of stimulus duration, $F_{(3,60)} = 12.14$, $p < 0.0001$; no interaction effect, $F_{(3,60)} = 0.2146$, $p = 0.8859$) or reward collection latency (**Figure 15F**; no effect of genotype, $F_{(1,20)} = 4.261$, $p = 0.0522$; no effect of stimulus duration, $F_{(3,60)} = 1.02$, $p = 0.3900$; no interaction effect, $F_{(3,60)} = 0.3599$, $p = 0.7822$). In summary, APP/PS1 male mice do not display impairments in the 5-CSRTT at 7-months of age.

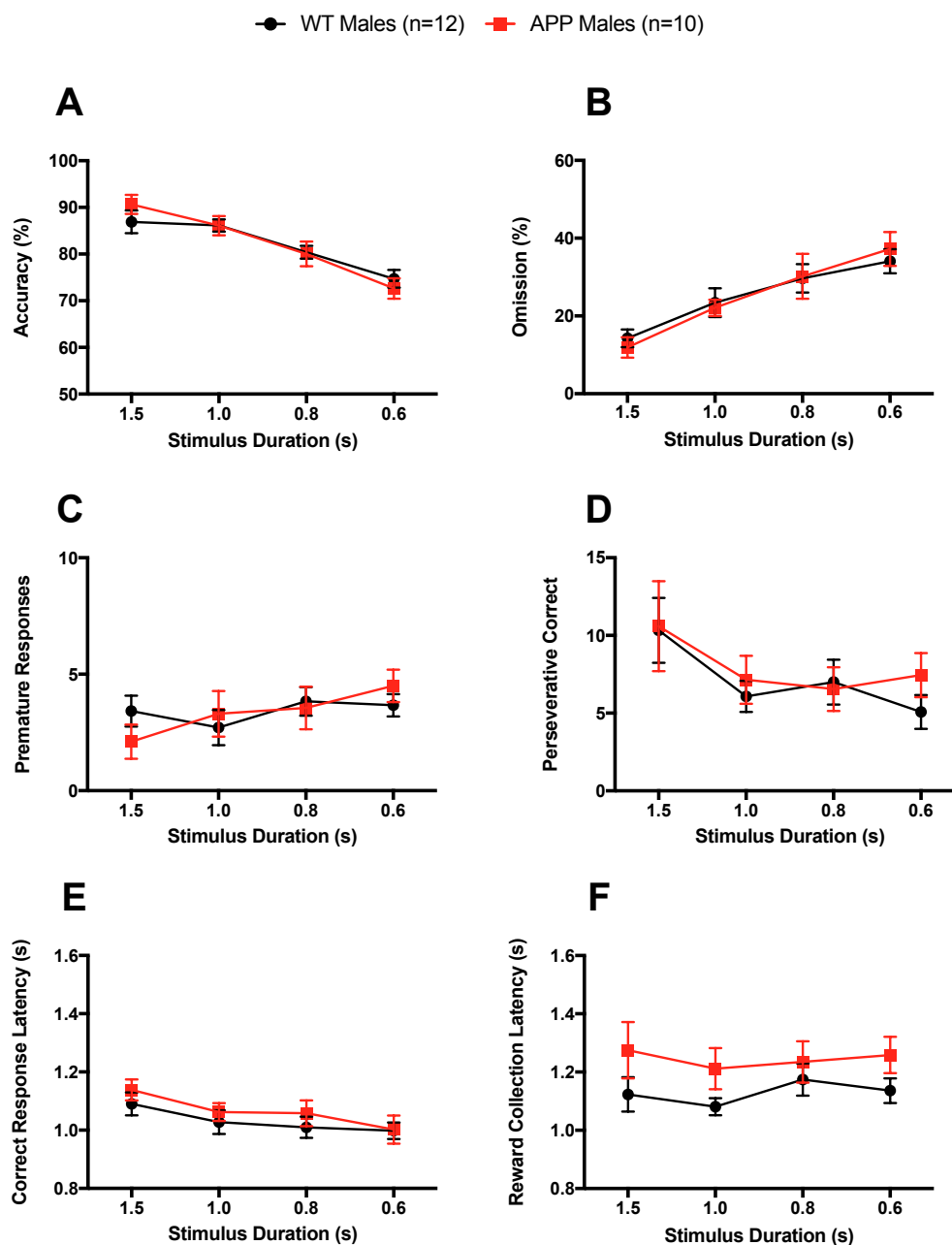


Figure 15. Response and performance measures of males during 5-CSRTT probe trials at 7-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. **A)** Mean accuracy **B)** Mean omission **C)** Mean number of premature responses **D)** Mean number of perseverative responses **E)** Mean correct response latency **F)** Mean reward collection latency. (WT males n=12, APP males n=10, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

10 month-old APP/PS1 male mice performance on the 5-CSRTT probe trials did not differ from controls across stimulus durations in terms of percentage of correct responses (**Figure 16A**; no effect of genotype, $F_{(1,18)} = 0.2426$, $p = 0.6283$; main effect of stimulus duration, $F_{(3,54)} = 10.43$, $p < 0.0001$; no interaction effect, $F_{(3,54)} = 2.619$, $p = 0.0601$), percentage of trials omitted (**Figure 16B**; no effect of genotype, $F_{(1,18)} = 1.144$, $p = 0.2990$; main effect of stimulus duration, $F_{(3,54)} = 31.05$, $p < 0.0001$; no interaction effect, $F_{(3,54)} = 1.362$, $p = 0.2643$), number of premature responses, (**Figure 16C**; no effect of genotype, $F_{(1,18)} = 0.9399$, $p = 0.3452$; no effect of stimulus duration, $F_{(3,54)} = 1.348$, $p = 0.2685$; no interaction effect, $F_{(3,54)} = 0.9771$, $p = 0.4104$), number of perseverative responses (**Figure 16D**; no effect of genotype, $F_{(1,18)} = 0$, $p > 0.9999$; no effect of stimulus duration, $F_{(3,54)} = 0.2617$, $p = 0.8526$; no interaction effect, $F_{(3,54)} = 1.576$, $p = 0.2058$), correct response latency (**Figure 16E**; no effect of genotype, $F_{(1,18)} = 0.2297$, $p = 0.6375$; main effect of stimulus duration, $F_{(3,54)} = 30.92$, $p < 0.0001$; no interaction effect, $F_{(3,54)} = 1.198$, $p = 0.3193$) or reward collection latency (**Figure 16F**; no effect of genotype, $F_{(1,18)} = 0.9749$, $p = 0.3365$; no effect of stimulus duration, $F_{(3,54)} = 0.5888$, $p = 0.6250$; no interaction effect, $F_{(3,54)} = 0.1456$, $p = 0.9320$). In summary, APP/PS1 male mice do not display impairments in the 5-CSRTT at 10-months of age.

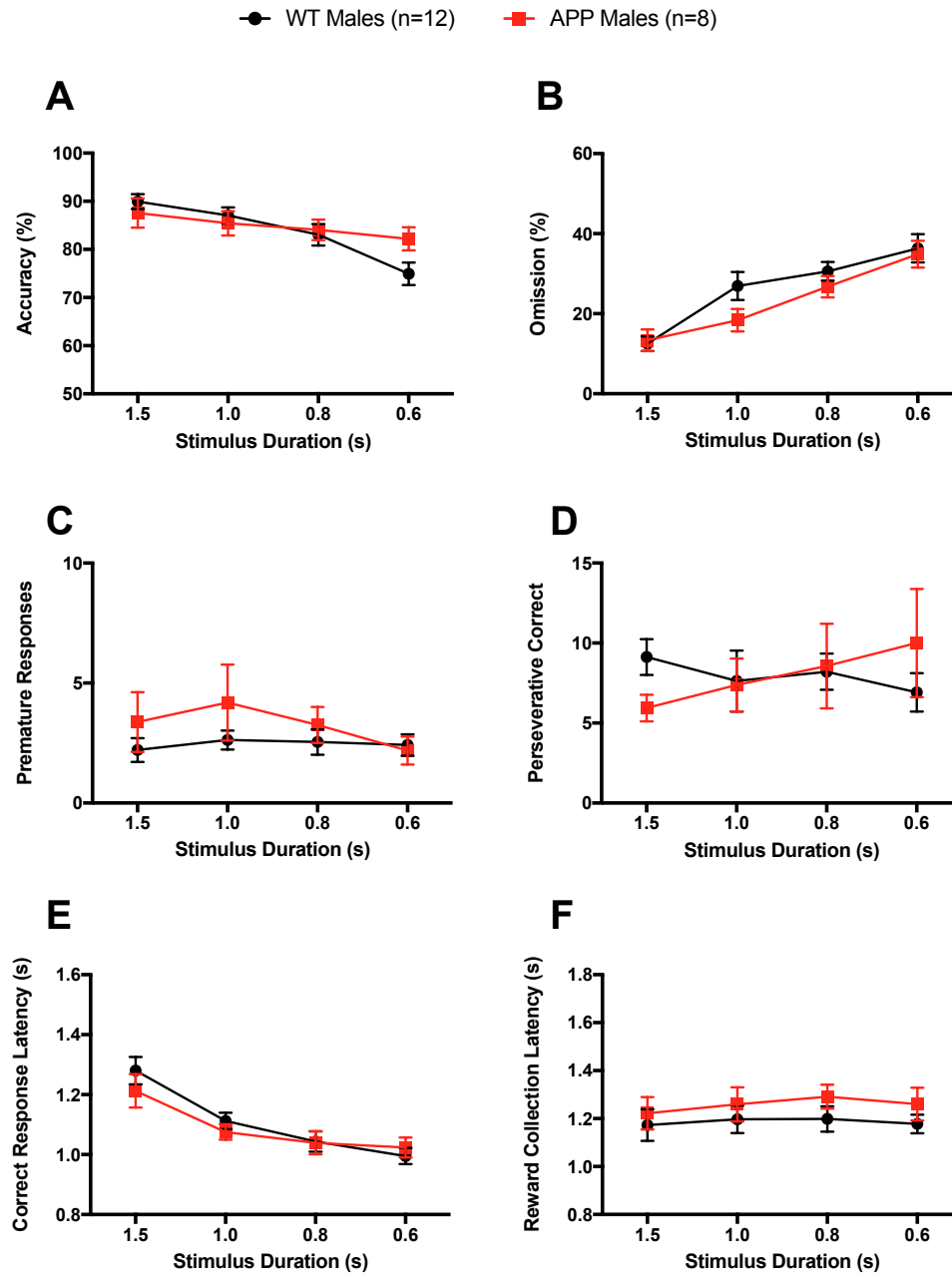


Figure 16. Response and performance measures of males during 5-CSRTT probe trials at 10-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. A) Mean accuracy B) Mean omission C) Mean number of premature responses D) Mean number of perseverative responses E) Mean correct response latency F) Mean reward collection latency. (WT males n=10, APP males n=8, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

3.2.3 Attentional Performance of Female APP/PS1 in the 5-CSRTT

4 month-old female APP/PS1 mice performance on the 5-CSRTT probe trials did not differ between genotypes across stimulus durations in terms of percentage of correct responses (**Figure 17A**; no effect of genotype, $F_{(1,24)} = 2.432$, $p = 0.1320$; main effect of stimulus duration, $F_{(3,72)} = 36.77$, $p < 0.0001$; no interaction effect, $F_{(3,72)} = 0.4644$, $p = 0.7080$), percentage of omitted trials (**Figure 17B**; no effect of genotype, $F_{(1,24)} = 2.825$, $p = 0.1058$; main effect of stimulus duration, $F_{(3,72)} = 49.66$, $p < 0.0001$; main interaction effect, $F_{(3,72)} = 3.039$, $p = 0.0345$), number of premature responses (**Figure 17C**; no effect of genotype, $F_{(1,24)} = 0.2561$, $p = 0.6174$; no effect of stimulus duration, $F_{(3,72)} = 1.626$, $p = 0.1909$; no interaction effect, $F_{(3,72)} = 1.705$, $p = 0.1736$) and number of perseverative responses (**Figure 17D**; no effect of genotype, $F_{(1,24)} = 2.699$, $p = 0.1135$; no effect of stimulus duration, $F_{(3,72)} = 2.361$, $p = 0.0785$; no interaction effect, $F_{(3,72)} = 0.7217$, $p = 0.5423$). However, 4 month-old APP/PS1 female mice were faster to touch the screen after the stimulus than controls, that is, they showed shorter response latency than wild-type control females (**Figure 17E**; main effect of genotype, $F_{(1,24)} = 7.07$, $p = 0.0137$; main effect of stimulus duration, $F_{(3,72)} = 18.94$, $p < 0.0001$; no interaction effect, $F_{(3,72)} = 0.01953$, $p = 0.9963$). APP/PS1 females also showed shorter reward collection latencies than WT control mice (**Figure 17F**; no effect of genotype, $F_{(1,24)} = 6.135$, $p = 0.0207$; main effect of stimulus duration, $F_{(3,72)} = 2.771$, $p = 0.0477$; no interaction effect, $F_{(3,72)} = 1.035$, $p = 0.3822$). In summary, APP/PS1 female mice do not display impairments in the 5-CSRTT at 4-months of age.

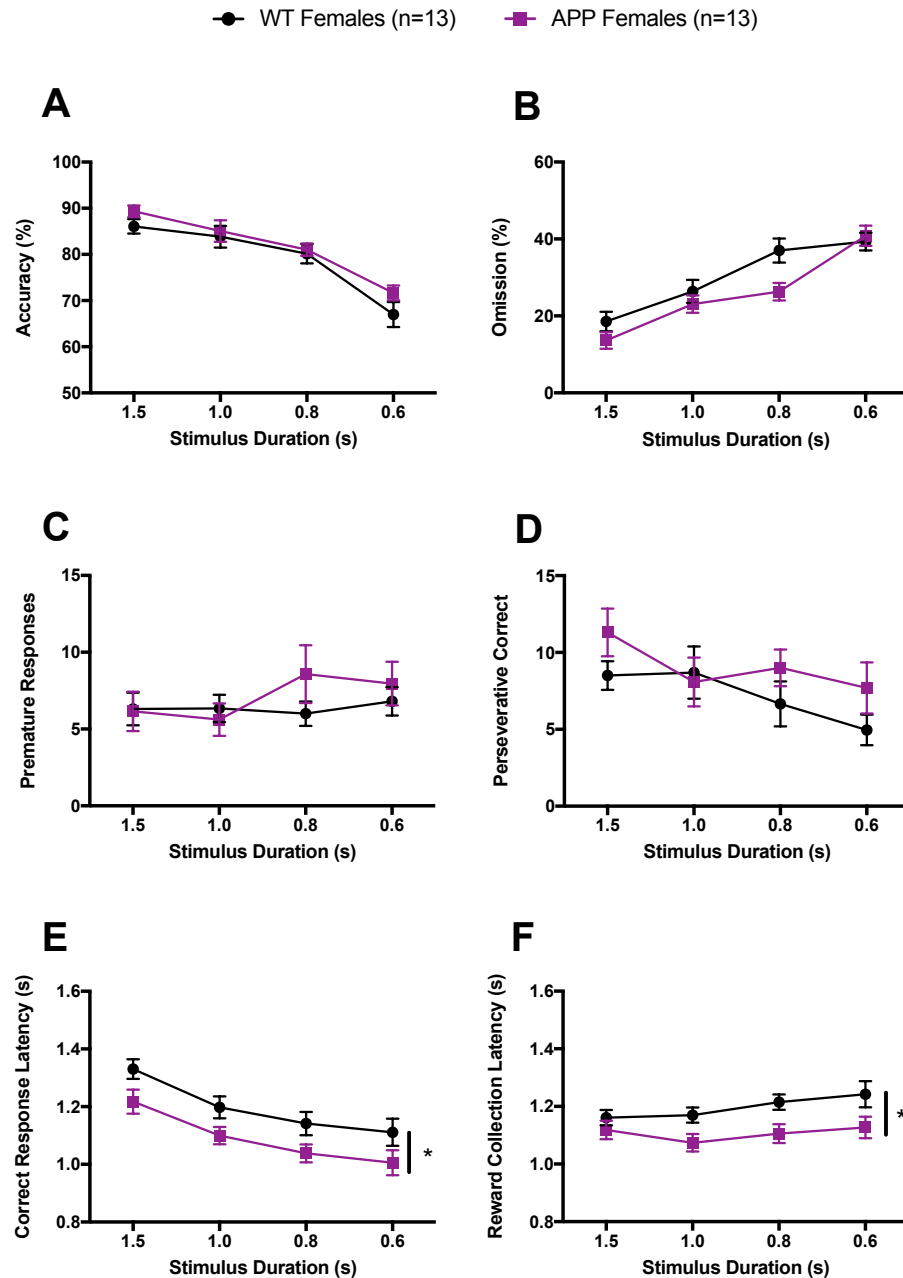


Figure 17. Response and performance measures of females during 5-CSRTT probe trials at 4-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. **A)** Mean accuracy **B)** Mean omission **C)** Mean number of premature responses **D)** Mean number of perseverative responses **E)** Mean correct response latency **F)** Mean reward collection latency. (WT females n=13, APP females n=13, data are mean $\pm$ SEM). * $p < 0.05$, ** $p < 0.005$. Repeated-measures two-way ANOVA.

7 month-old female APP/PS1 mice performance on the 5-CSRTT probe trials did not differ between genotypes across stimulus durations in terms of percentage of correct responses (**Figure 18A**; no effect of genotype, $F_{(1,21)} = 1.267$, $p = 0.2731$; main effect of stimulus duration, $F_{(3,63)} = 25.96$, $p < 0.0001$; no interaction effect, $F_{(3,63)} = 0.896$, $p = 0.4483$), percentage of trials omitted (**Figure 18B**; no effect of genotype, $F_{(1,21)} = 3.537$, $p = 0.0740$; main effect of stimulus duration, $F_{(3,63)} = 42.12$, $p < 0.0001$; no interaction effect, $F_{(3,63)} = 2.353$, $p = 0.0805$), number of premature responses (**Figure 18C**; no effect of genotype, $F_{(1,21)} = 1.406$, $p = 0.2489$; no effect of stimulus duration, $F_{(3,63)} = 0.06379$, $p = 0.9788$; no interaction effect, $F_{(3,63)} = 0.2174$, $p = 0.8840$), number of perseverative responses (**Figure 18D**; no effect of genotype, $F_{(1,21)} = 0.005387$, $p = 0.9422$; no effect of stimulus duration, $F_{(3,63)} = 0.4145$, $p = 0.7432$; no interaction effect, $F_{(3,63)} = 0.1426$, $p = 0.9341$). Furthermore, 7 month-old APP/PS1 female mice did not differ between genotypes in correct response latency compared to wild-type control females (**Figure 18E**; no effect of genotype, $F_{(1,21)} = 2.578$, $p = 0.1233$; main effect of stimulus duration, $F_{(3,63)} = 11.75$, $p < 0.0001$; no interaction effect, $F_{(3,63)} = 0.3145$, $p = 0.8148$) and reward collection latency (**Figure 18F**; no effect of genotype, $F_{(1,21)} = 1.327$, $p = 0.2623$; no effect of stimulus duration, $F_{(3,63)} = 2.15$, $p = 0.1027$; no interaction effect, $F_{(3,63)} = 0.4002$, $p = 0.7533$). In summary, APP/PS1 female mice do not display impairments in the 5-CSRTT at 7-months of age.

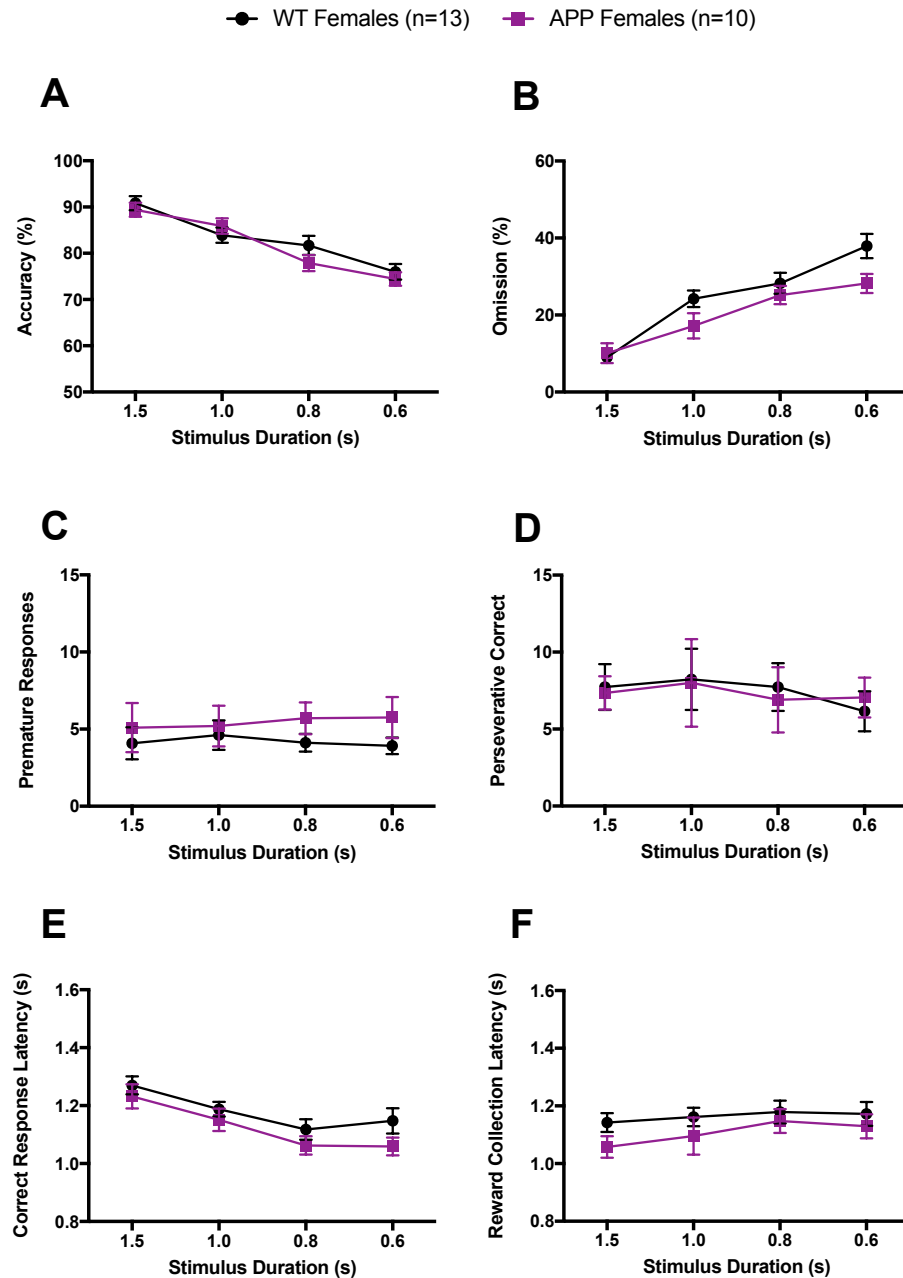


Figure 18. Response and performance measures of females during 5-CSRTT probe trials at 7-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. **A)** Mean accuracy **B)** Mean omission **C)** Mean number of premature responses **D)** Mean number of perseverative responses **E)** Mean correct response latency **F)** Mean reward collection latency. (WT females n=13, APP females n=10, data are mean $\pm$ SEM). * $p < 0.05$. Repeated-measures two-way ANOVA.

10 month-old APP/PS1 female mice performance on the 5-CSRTT probe trials did not differ from controls across stimulus durations in terms of percentage of correct responses (**Figure 19A**; no effect of genotype, $F_{(1,19)} = 0.1851$, $p = 0.6719$; main effect of stimulus duration, $F_{(3,57)} = 15.32$, $p < 0.0001$; no interaction effect, $F_{(3,57)} = 0.5234$, $p = 0.6679$), percentage of trials omitted (**Figure 19B**; no effect of genotype, $F_{(1,19)} = 2.366$, $p = 0.1405$; main effect of stimulus duration, $F_{(3,57)} = 34.73$, $p < 0.0001$; no interaction effect, $F_{(3,57)} = 0.3144$, $p = 0.8149$), number of premature responses, (**Figure 19C**; no effect of genotype, $F_{(1,19)} = 1.318$, $p = 0.2651$; no effect of stimulus duration, $F_{(3,57)} = 1.427$, $p = 0.2443$; no interaction effect, $F_{(3,57)} = 0.4799$, $p = 0.6975$), number of perseverative responses (**Figure 19D**; no effect of genotype, $F_{(1,19)} = 0.5427$, $p = 0.4703$; no effect of stimulus duration, $F_{(3,57)} = 1.82$, $p = 0.1537$; no interaction effect, $F_{(3,57)} = 0.1454$, $p = 0.9322$), correct response latency (**Figure 19E**; no effect of genotype, $F_{(1,19)} = 0.02513$, $p = 0.8757$; main effect of stimulus duration, $F_{(3,57)} = 16.69$, $p < 0.0001$; no interaction effect, $F_{(3,57)} = 1.085$, $p = 0.3630$) or reward collection latency (**Figure 19F**; no effect of genotype, $F_{(1,19)} = 0.01581$, $p = 0.9012$; no effect of stimulus duration, $F_{(3,57)} = 1.191$, $p = 0.3215$; no interaction effect, $F_{(3,57)} = 0.0387$, $p = 0.9897$).

In summary, APP/PS1 female mice did not demonstrate deficits in performance when compared to their WT controls in the 5-CSRTT at all time-points. Data analysis for 14 month-old female mice performance on the 5-CSRTT probe trials was not performed as several females died prior to reaching 14-months of age and thus, the number of surviving animals was insufficient to obtain statistical power.

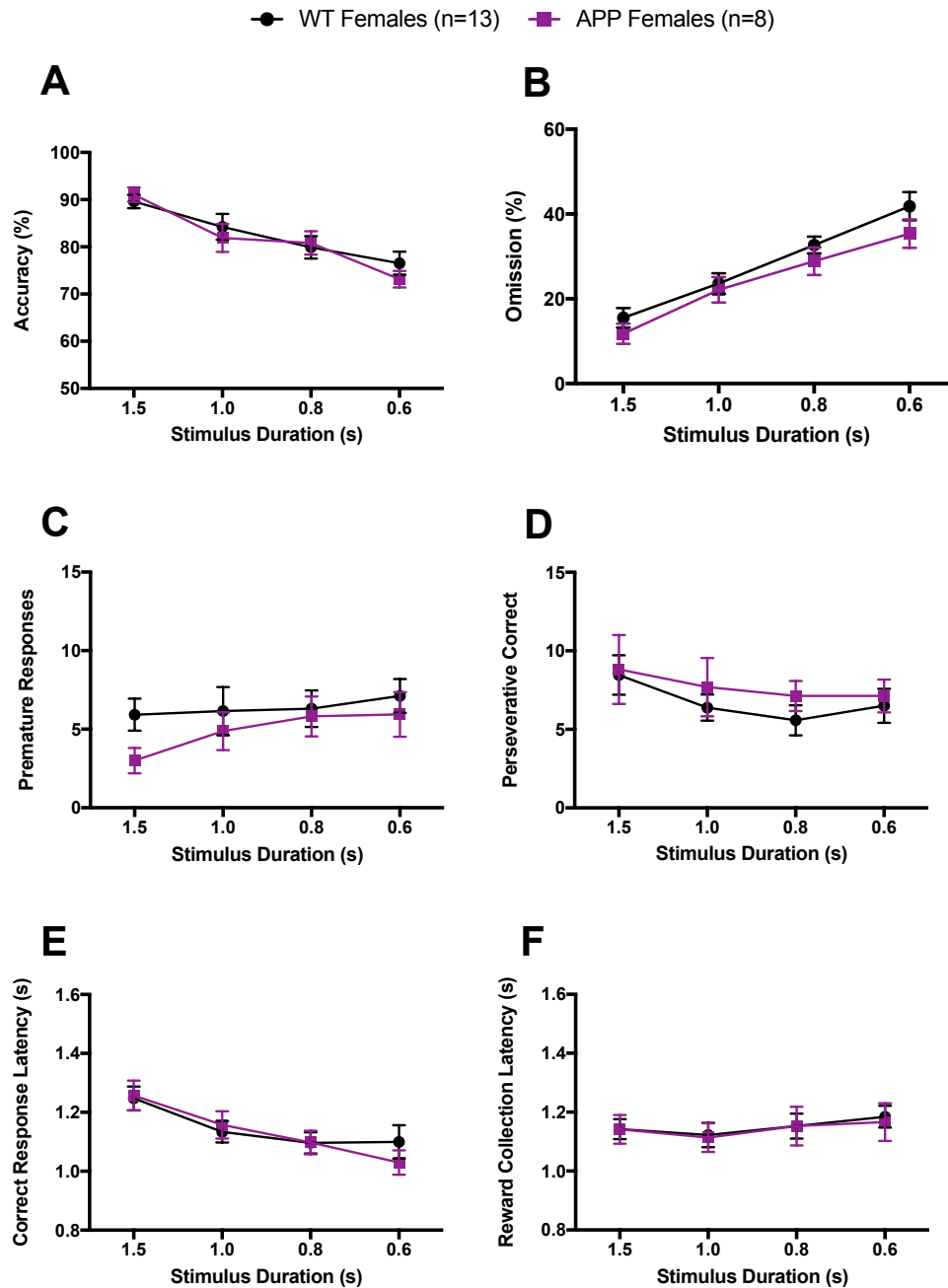


Figure 19. Response and performance measures of females during 5-CSRTT probe trials at 10-months of age.

Mice performed a sequence of probe trial schedules and the mean values of the sessions are illustrated. **A)** Mean accuracy **B)** Mean omission **C)** Mean number of premature responses **D)** Mean number of perseverative responses **E)** Mean correct response latency **F)** Mean reward collection latency. (WT females n=13, APP females n=8, data are mean $\pm$ SEM). Repeated-measures two-way ANOVA.

3.2.4 Sustained attention analysis on male APP/PS1 mice at 0.6s stimulus duration in the 5-CSRTT

APP/PS1 mouse performance at the most challenging 0.6s stimulus length was also analyzed by dividing sessions into bins of 10 trials to assess sustained attentional performance on the 5-CSRTT. The accuracy of APP/PS1 mice did not differ between genotypes at 4 months (**Figure 20A**; no effect of genotype, $F_{(1,20)} = 1.549$, $p = 0.2277$; no effect of bin, $F_{(4,80)} = 1.238$, $p = 0.3014$; no interaction effect, $F_{(4,80)} = 0.8679$, $p = 0.4870$), 7 months (**Figure 20B**; no effect of genotype, $F_{(1,20)} = 0.8444$, $p = 0.3691$; no effect of bin, $F_{(4,80)} = 0.4907$, $p = 0.7425$; no interaction effect, $F_{(4,80)} = 2.124$, $p = 0.0855$), 10 months (**Figure 20C**; no effect of genotype, $F_{(1,18)} = 3.933$, $p = 0.0628$; no effect of bin, $F_{(4,72)} = 0.4896$, $p = 0.7433$; no interaction effect, $F_{(4,72)} = 0.9697$, $p = 0.4295$). Similarly, the percentage of omissions did not differ between genotypes at 4 months (**Figure 20D**; no effect of genotype, $F_{(1,20)} = 2.715$, $p = 0.1150$; no effect of bin, $F_{(4,80)} = 1.778$, $p = 0.1415$; no interaction effect, $F_{(1,80)} = 0.1204$, $p = 0.9748$), 7 months (**Figure 20E**; no effect of genotype, $F_{(1,20)} = 0.3184$, $p = 0.5789$; main effect of bin $F_{(4,80)} = 3.925$, $p < 0.0058$; significant interaction effect, $F_{(4,80)} = 2.997$, $p = 0.0233$), 10 months (**Figure 20F**; no effect of genotype, $F_{(1,18)} = 0.08125$, $p = 0.7789$, no effect of bin $F_{(4,72)} = 1.119$, $p = 0.3543$; no interaction effect, $F_{(4,72)} = 0.1821$, $p = 0.9470$). In summary, male APP/PS1 mice do not display sustained attention deficits at the 0.6s stimulus duration.

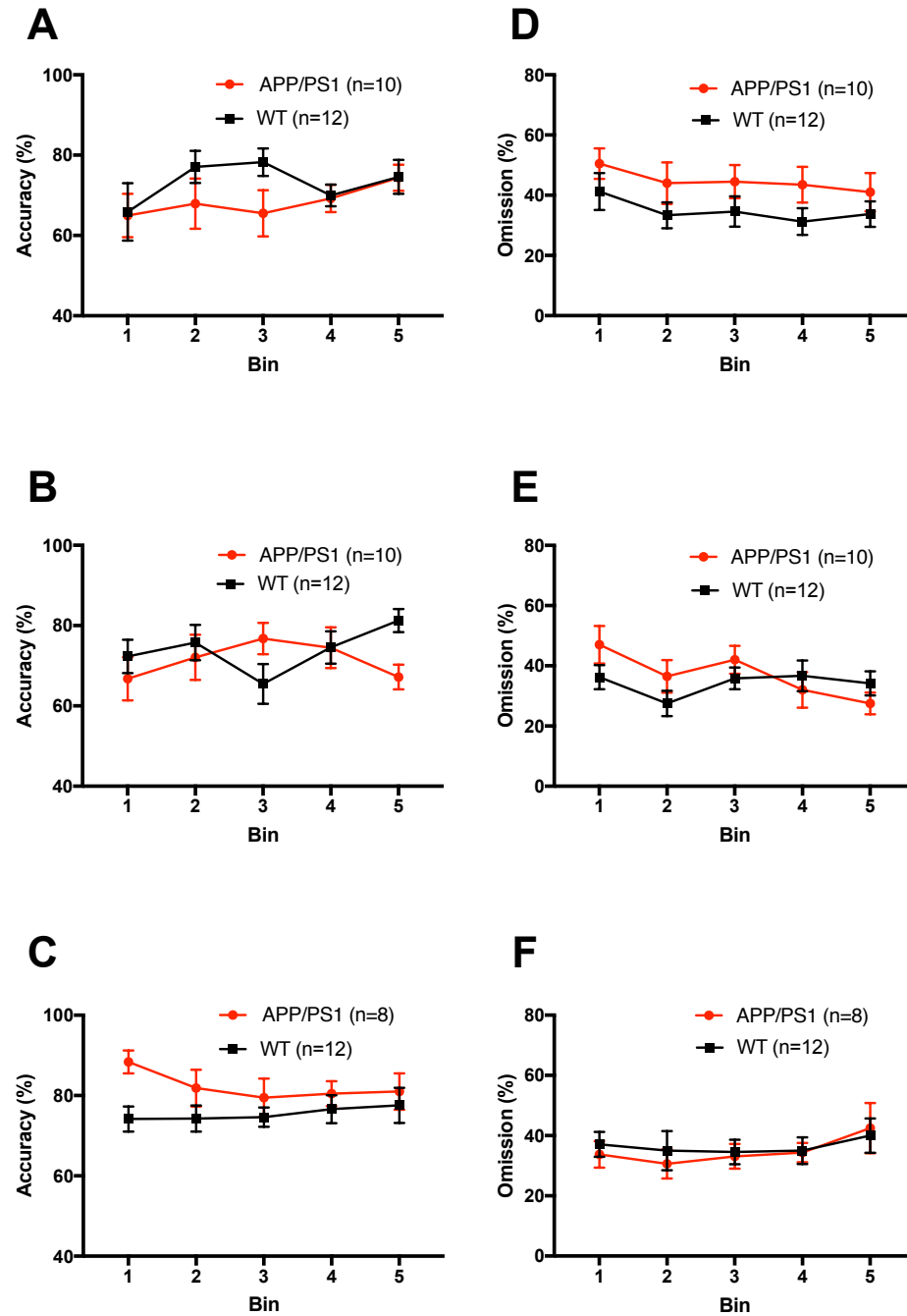


Figure 20. Male APP/PS1 mice vigilance during the 0.6s stimulus duration of the 5-CSRTT.

Mean accuracy $\pm$ SEM (%) and omission (%) at **A**) and **B**) 4 months, **C**) and **D**) 7 months, and **E**) and **F**) 10 months. Trials are divided into bins of 10 trials each. Two-way ANOVA.

3.2.5 Sustained attention analysis on female APP/PS1 mice at 0.6s stimulus duration in the 5-CSRTT

Similarly, female APP/PS1 performance was analyzed dividing sessions into bins of 10 trials each at the most challenging 0.6s stimulus length to assess sustained attention in the 5-CSRTT. The accuracy of APP/PS1 females did not differ across bins and there were no difference between genotypes at 4 months (**Figure 21A**; no effect of genotype, $F_{(1,24)} = 2.379$, $p = 0.1361$; no effect of bin, $F_{(4,96)} = 1.563$, $p = 0.1903$; no interaction effect, $F_{(4,96)} = 0.962$, $p = 0.4320$), 7 months (**Figure 21B**; no effect of genotype, $F_{(1,21)} = 1.23$, $p = 0.2799$; no effect of bin, $F_{(4,84)} = 0.2014$, $p = 0.9370$; no interaction effect, $F_{(4,84)} = 0.8116$, $p = 0.5212$) and 10 months (**Figure 21C**; no effect of genotype, $F_{(1,19)} = 1.573$, $p = 0.2249$; no effect of bin, $F_{(4,76)} = 1.829$, $p = 0.1319$; no interaction effect, $F_{(4,76)} = 0.2248$, $p = 0.9238$). The percentage of omissions did not differ between genotypes at 4 months (**Figure 21D**; no effect of genotype, $F_{(1,24)} = 0.1949$, $p = 0.6628$; no effect of bin, $F_{(4,96)} = 1.922$, $p = 0.1130$; no interaction effect, $F_{(4,96)} = 0.5069$, $p = 0.7308$), 7 months (**Figure 21E**; no effect of genotype, $F_{(1,21)} = 4.3$, $p = 0.0506$; no effect of bin, $F_{(4,84)} = 0.3957$, $p = 0.8112$; no interaction effect, $F_{(4,84)} = 0.5444$, $p = 0.7036$) and 10 months (**Figure 21F**; no effect of genotype, $F_{(1,19)} = 1.686$, $p = 0.2097$; no effect of bin, $F_{(4,76)} = 1.204$, $p = 0.3159$; no interaction effect, $F_{(4,76)} = 0.123$, $p = 0.9738$). In summary, female APP/PS1 mice do not demonstrate any notable deficits in sustained attention at the 0.6s stimulus duration in the 5-CSRTT compared to WT controls.

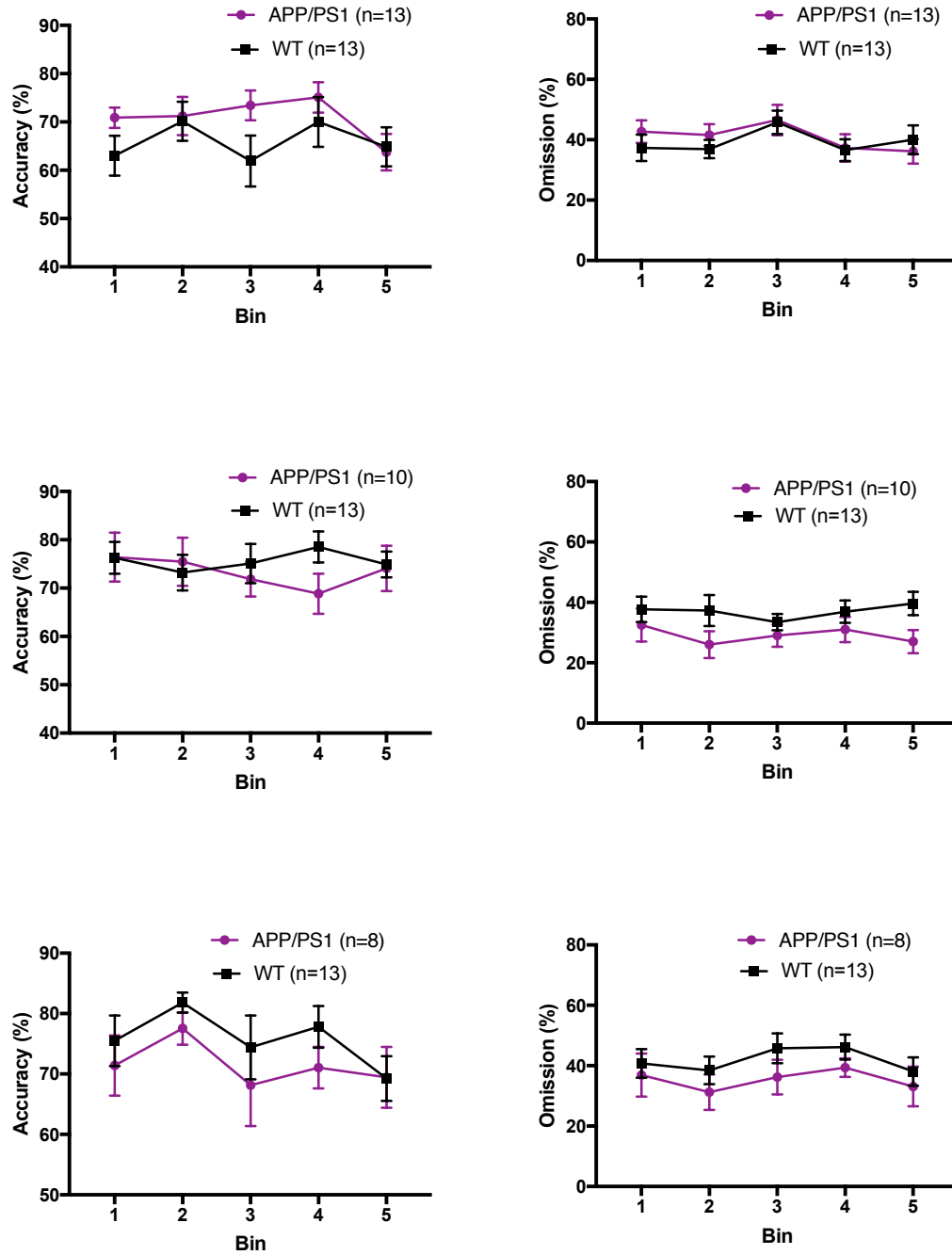


Figure 21. Female APP/PS1 mice vigilance during the 0.6s stimulus duration of the 5-CSRTT.

Mean accuracy $\pm$ SEM (%) and omission (%) at **A)** and **B)** 4 months, **C)** and **D)** 7 months, and **E)** and **F)** 10 months. Trials are divided into bins of 10 trials each. Two-way ANOVA.

3.3 Locomotor Activity in Male APP/PS1 Mice

Mice were tested in open-field boxes to examine locomotor behaviour at 14 months of age. To assess whether food restriction impacted locomotion in mice we tested two different cohorts, one cohort that had previously been subjected to cognitive testing on touchscreens, and therefore was food restricted, and naïve mice of the same age that were kept on *ad libitum* food regimen. These experiments provided information of whether mice that undergo touchscreen testing can be used for conventional behavioral studies.

3.3.1 Locomotor activity of food restricted and free-feeding 14-month old male APP/PS1

Our analysis of locomotor activity of 14 month-old APP/PS1 food restricted male mice and their WT controls indicated that these mice did not show differences in distance travelled every 5 minutes (**Figure 22A**; RM-ANOVA, no effect of genotype, $F_{(1,28)} = 3.694$, $p = 0.0649$; main effect of time, $F_{(23,644)} = 12.07$, $p < 0.0001$; no interaction effect, $F_{(23,644)} = 0.791$, $p = 0.7448$), or total distance travelled over two hours (**Figure 22B**; $t_{(27)} = 1.872$, $p = 0.0721$). Interestingly, these food restricted mice failed to habituate to the environment (**Figure 22A**) as at the end of two hours in the chamber they were still as active as they were when they were introduced to the box.

Naïve, *ad libitum* fed APP/PS1 male mice walked significantly larger distances every five minutes than controls (**Figure 22C**; RM-ANOVA, main effect of genotype, $F_{(1,20)} = 15.02$, $p = 0.0009$; main effect of time, $F_{(23,460)} = 25.08$, $p < 0.0001$; main interaction effect, $F_{(23,460)} = 2.09$, $p = 0.0024$). *Post-hoc* analysis revealed APP/PS1 naïve males were significantly hyperactive when compared to controls (**Figure 22C**). Naïve APP/PS1 male mice also travelled a larger total distance over two hours (**Figure 22D**; $t_{(20)} = 3.876$, $p = 0.0009$). Also, locomotor activity of free-fed APP/PS1 and WT controls decreased with time. Mice from both genotypes were mainly quiet on the second hour of the test (**Figure 22C**) indicating 14 month-old free-fed APP/PS1 and WT controls show habituation to the environment.

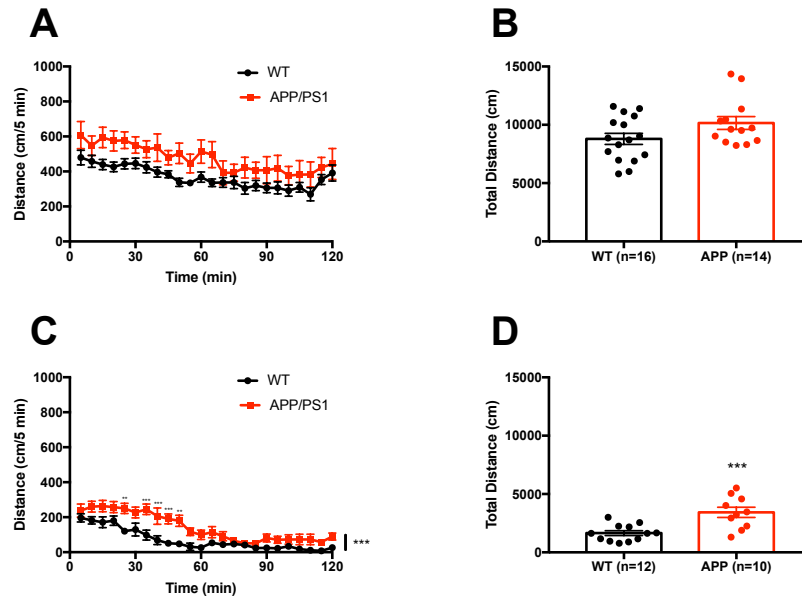


Figure 22. Spontaneous locomotor activity of food restricted APP/PS1 male mice and free-feeding APP/PS1 male mice and their wild-type littermate controls at 14 months of age.

A) Total locomotor activity of males subject to food restriction (APP n=14; WT n=16). **B)** Total distance travelled of males subject to food restriction **C)** Total locomotor activity of free-feeding males (APP n=10; WT n=12). **D)** Total distance travelled of free-feeding males. Two-way repeated measures ANOVA (**A,C**) or unpaired two-tailed t-test (**B,D**). * $p < 0.005$, *** $p < 0.001$ between genotypes.

To note, food restricted APP/PS1 males were significantly more active than *ad libitum* APP/PS1 males (**Figure 23A**; RM-ANOVA, main effect of genotype, $F_{(1,22)} = 23.52$, $p < 0.0001$; main effect of time, $F_{(23,506)} = 14.42$, $p < 0.0001$; no interaction effect, $F_{(23,506)} = 0.6498$, $p = 0.8935$; **Figure 23C**; $t_{(21)} = 9.087$, $p < 0.0001$). Likewise, food restricted wild-type males were hyperactive when compared to age matched *ad libitum* wild-type males (**Figure 23B**; RM-ANOVA, main effect of genotype, $F_{(1,26)} = 151.5$, $p < 0.0001$; main effect of time, $F_{(23,598)} = 13.61$, $p < 0.0001$; no interaction effect, $F_{(23,598)} = 0.9707$, $p = 0.5021$; **Figure 23C**; $t_{(26)} = 12.31$, $p < 0.0001$).

To assess whether touchscreen testing contributed for the differences observed in locomotor activity, after the conclusion of the final time point of touchscreen testing mice were maintained food restricted for one month in their individual homecages without any testing. After one month, locomotor activity was reassessed (mice were 18 month old by then). Similar to results seen at 14 months of age, food restricted APP/PS1 mice and their WT controls did not differ in distance travelled every 5 minutes (**Figure 24A**; RM-ANOVA, no effect of genotype, $F_{(1,23)} = 0.1071$, $p = 0.7464$; main effect of time, $F_{(23,529)} = 12.02$, $p < 0.0001$; no interaction effect, $F_{(23,529)} = 0.8163$, $p = 0.7120$), or total distance travelled (**Figure 24B**; $t_{(23)} = 0.3273$, $p = 0.7464$). As previously observed, these mice failed to habituate to the locomotor chamber, suggesting that food restriction, and not the touchscreen testing might be causing the hyperactivity in these mice.

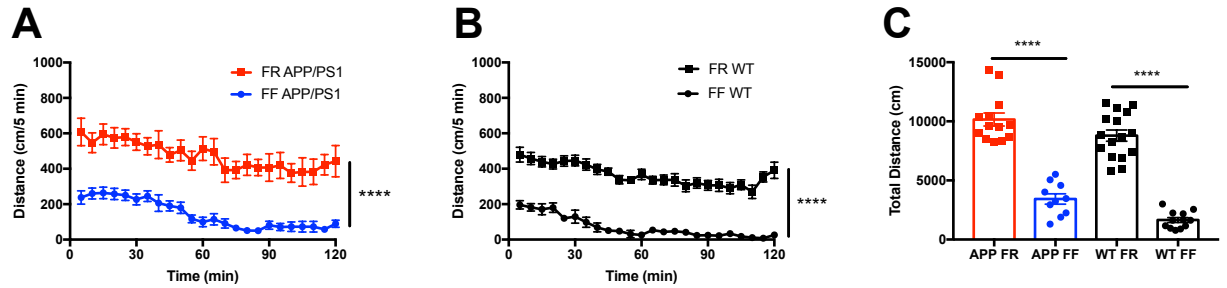


Figure 23. Spontaneous locomotor activity of food restricted and free-feeding APP/PS1 male mice and their wild-type littermate controls at 14 months of age.

A) Total locomotor activity of APP/PS1 (FR n=14; FF n=10). **B)** Total locomotor activity of wild-type controls (FR n=16; FF n=12). **C)** Total distance travelled of food restricted and free feeding APP/PS1 and WT mice. Two-way repeated measures ANOVA (**A,B**) or unpaired two-tailed t-test (**C**) ****p<0.0001 between genotypes.

Likewise, naïve *ad libitum* fed APP/PS1 male mice travelled significantly larger distances every five minutes and showed normal habituation to the novel environment over time (**Figure 24C**; RM-ANOVA, main effect of genotype, $F_{(1,15)} = 15.73$, $p = 0.0012$; main effect of time, $F_{(23,345)} = 19.3$, $p < 0.0001$; main interaction effect, $F_{(23,345)} = 5.057$, $p < 0.0001$). APP/PS1 male mice also moved a greater total distance over the two hours (**Figure 24D**; $t_{(15)} = 3.265$, $p = 0.0052$).

Food restricted APP/PS1 males were still hyperactive when compared to *ad libitum* APP/PS1 males at 18 months of age (**Figure 25A**; RM-ANOVA, main effect of genotype, $F_{(1,15)} = 18.74$, $p = 0.0006$; main effect of time, $F_{(23,345)} = 12.96$, $p < 0.0001$; no interaction effect, $F_{(23,345)} = 1.025$, $p = 0.4321$; **Figure 25C**; $t_{(16)} = 6.116$, $p < 0.0001$). Food restricted wild-type males locomotor activity was also significantly larger when compared to *ad libitum* wild-type males at 18 months of age (**Figure 25B**; RM-ANOVA, main effect of genotype, $F_{(1,23)} = 78.74$, $p < 0.0001$; main effect of time, $F_{(23,529)} = 9.623$, $p < 0.0001$; main interaction effect, $F_{(23,529)} = 2.862$, $p < 0.0001$; **Figure 25C**; $t_{(22)} = 8.286$, $p < 0.00$).

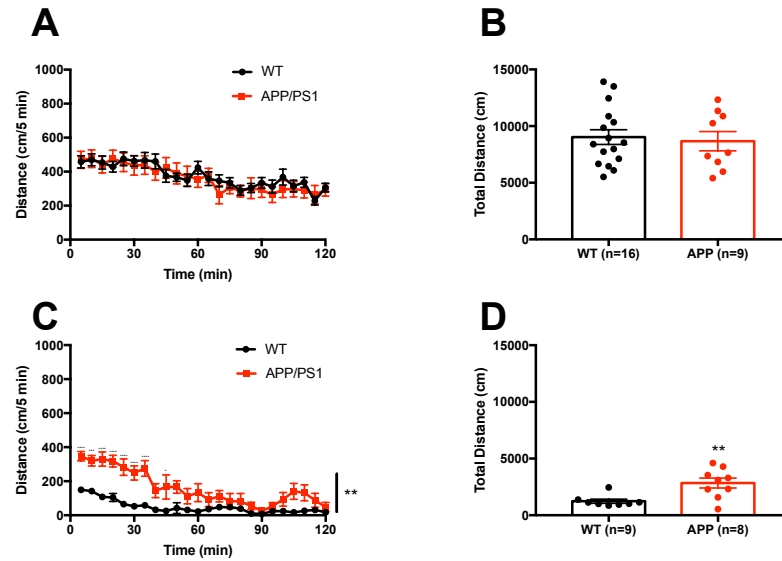


Figure 24. Spontaneous locomotor activity of food restricted APP/PS1 male mice and free-feeding APP/PS1 male mice and their wild-type littermate controls at 18 months of age.

A) Total locomotor activity of males subject to food restriction (APP n=9; WT n=16). **B)** Total distance travelled of males subject to food restriction **C)** Total locomotor activity of free-feeding males (APP n=8; WT n=9). **D)** Total distance travelled of free-feeding males. Two-way repeated measures ANOVA (**A,C**) or unpaired two-tailed t-test (**B,D**). ** $p < 0.005$, *** $p < 0.001$ between genotypes.

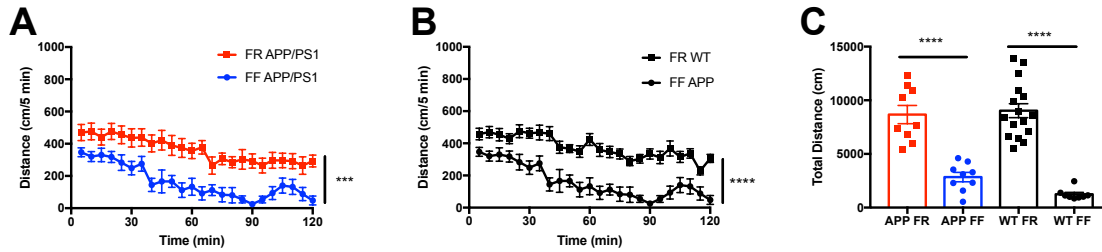


Figure 25. Spontaneous locomotor activity of food restricted and free-feeding APP/PS1 male mice and their wild-type littermate controls at 18 months of age.

A) Total locomotor activity of APP/PS1 (FR n=9; FF n=8). **B)** Total locomotor activity of wild-type controls (FR n=16; FF n=9). **C)** Total distance travelled of food restricted and free feeding APP/PS1 and WT mice. Two-way repeated measures ANOVA (**A,B**) or unpaired two-tailed t-test (**C**). *** $p < 0.001$, **** $p < 0.0001$ between genotypes.

3.4 Gait Analyses in Male APP/PS1 Mice

Intact motor function and the ability to walk is important for a variety of behavioural assessments. In particular, touchscreen tasks require mice to be able to move within the apparatus to perform the task proficiently. Proper gait involves a number of components beyond simple automated motor activity. It requires an ongoing balance of adequate visuospatial abilities and attention to the surrounding environment (Amboni et al., 2013). Impairments in gait develop early in AD patients and gait dysfunction has been linked to an increased risk of developing AD (Amboni et al., 2013; Kluger et al., 1997). Several mouse models of AD have demonstrated gait abnormalities, including 5xFAD mouse models (Lee, 2017). Furthermore, changes in executive function have been suggested to be related to motor symptoms and possibly precede these physical abnormalities (Sheridan et al., 2003). It has also been suggested that gait speed may also serve as a predictor of dementia (Buracchio et al., 2010; Inzitari et al., 2007; Waite et al., 2005). Thus, we used the automated CatWalk system to investigate whether 14-month-old APP/PS1 mice present deficits in gait. As the cohort of mice used in the touchscreen experiments were food restricted, we also tested a naïve cohort of male APP/PS1 and controls, with *ad libitum* access to food to evaluate if food restriction had any effect on gait in these mice.

3.4.1 Gait analysis in food-restricted 14-month old APP/PS1 male mice.

We did not observe differences between genotypes on any of the static gait parameters assessed (**Table 1**), nor on step patterns (**Figure 26A-B**; RM-ANOVA, no effect of genotype, $F_{(1,120)} = 1.34^{-10}$, $p > 0.9999$; main effect of step pattern, $F_{(5,120)} = 24.19$, $p < 0.0001$; no interaction effect $F_{(5,120)} = 2.211$, $p = 0.0575$) or regularity index (**Figure 26C**; $t_{(20)} = 0.9658$, $p = 0.3457$). Differences between genotypes appeared in the dynamic gait parameters with APP/PS1 displaying significantly shorter stand durations on both forelimbs (**Table 2**; $t_{(20)} = 3.19$, $p = 0.0046$) and hindlimbs (**Table 2**; $t_{(20)} = 3.9$, $p = 0.0009$). Additionally, APP males displayed a quicker hindlimb stand index (**Table 2**; $t_{(20)} = 4.13$, $p = 0.0005$) than wild-type males. Furthermore, APP/PS1 mice and controls differed in the type of support used (**Figure 26D**; RM-ANOVA, no effect of genotype,

$F_{(1,140)} = 9.9^{-10}$, $p > 0.9999$; main effect of type of support, $F_{(6,140)} = 581.3$, $p < 0.0001$; main interaction effect $F_{(6,140)} = 14.08$, $p < 0.0001$). *Post-hoc* analysis revealed APP/PS1 males spend a higher relative occurrence percentage in diagonal support and a lower relative occurrence percentage on three paws (**Figure 26D**). Weights of 14 month-old male food restricted mice did not differ between genotypes (**Figure 26E**; $t_{(20)} = 1.792$, $p = 0.0883$).

Table 1. Static gait parameters of 14-month old food restricted APP/PS1 male mice. Static gait parameters were measured at 14 months in male APP/PS1 and their WT littermates. Values are expressed as mean $\pm$ SEM for both forelimbs and hindlimbs (unpaired two-tailed t-test).

Parameter	Wild-Type (n=12)	APP/PS1(n=10)
Paw angle (degrees)		
Forelimb	10.57 $\pm$ 2.94	8.177 $\pm$ 2.05
Hindlimb	5.954 $\pm$ 1.31	7.917 $\pm$ 1.65
Paw print area (mm ²)		
Forelimb	18.8 $\pm$ 1.93	20.54 $\pm$ 1.47
Hindlimb	18.62 $\pm$ 2.36	17.88 $\pm$ 2.21
Print length (mm)		
Forelimb	5.909 $\pm$ 0.22	6.015 $\pm$ 0.14
Hindlimb	6.567 $\pm$ 0.25	6.356 $\pm$ 0.25
Print width (mm)		
Forelimb	6.66 $\pm$ 0.30	6.776 $\pm$ 0.22
Hindlimb	6.576 $\pm$ 0.43	6.195 $\pm$ 0.37
Max contact area (mm ²)		
Forelimb	14.05 $\pm$ 1.49	15.9 $\pm$ 1.21
Hindlimb	14.26 $\pm$ 2.06	14 $\pm$ 1.81
Paw pressure (arbitrary units)		
Forelimb	88.45 $\pm$ 1.75	90.83 $\pm$ 1.72
Hindlimb	96.92 $\pm$ 3.19	102.5 $\pm$ 3.35

Table 2. Dynamic gait parameters of 14-month old food restricted APP/PS1 male mice.

Dynamic gait parameters were measured at 14 months in food restricted male APP/PS1 and their WT littermates. Values are expressed as mean $\pm$ SEM for both forelimbs and hindlimbs (unpaired two-tailed t-test; **p<0.005, ***p<0.001 between genotypes).

Parameter	Wild-Type (n=12)	APP/PS1(n=10)
Base of support (mm)		
Forelimb	12.34 $\pm$ 0.39	12.54 $\pm$ 0.38
Hindlimb	23.99 $\pm$ 0.74	22.93 $\pm$ 0.82
Stand duration (s)		
**Forelimb	0.1696 $\pm$ 0.01	0.137 $\pm$ 0.01
***Hindlimb	0.1833 $\pm$ 0.01	0.141 $\pm$ 0.01
Swing duration (s)		
Forelimb	0.1271 $\pm$ 0.01	0.112 $\pm$ 0.01
Hindlimb	0.1088 $\pm$ 0.01	0.105 $\pm$ 0.01
Swing speed (m/s)		
Forelimb	0.4392 $\pm$ 0.03	0.529 $\pm$ 0.03
Hindlimb	0.535 $\pm$ 0.05	0.567 $\pm$ 0.04
Stride length (mm)		
Forelimb	51.62 $\pm$ 1.27	54.76 $\pm$ 1.14
Hindlimb	51.25 $\pm$ 1.52	54.96 $\pm$ 1.04
Stand index (m/s)		
Forelimb	-7.301 $\pm$ 0.61	-8.181 $\pm$ 0.53
***Hindlimb	-7.035 $\pm$ 0.47	-9.859 $\pm$ 0.50

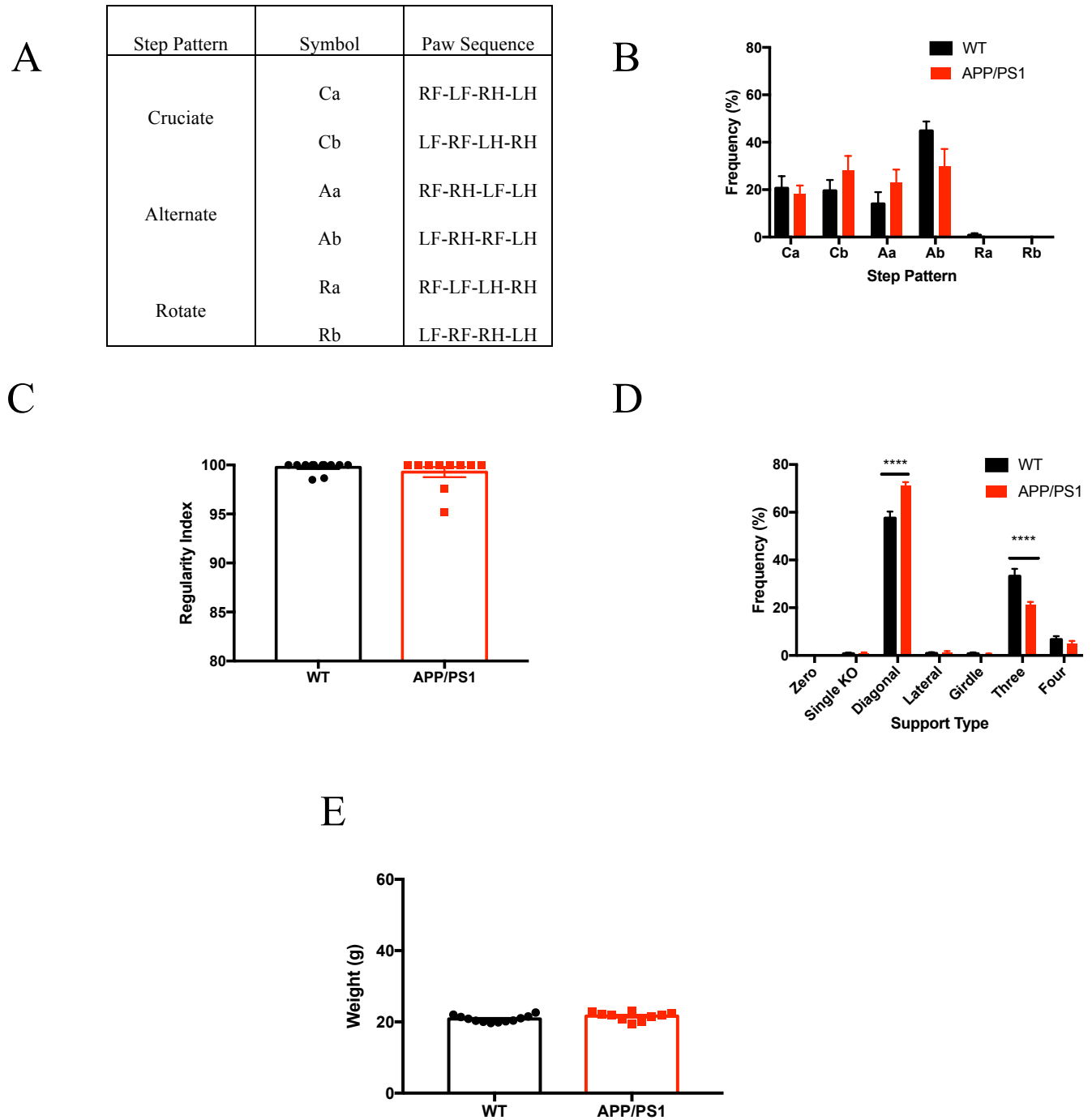


Figure 26. Step patterns and support type measures in 14-month old food restricted APP/PS1 male mice.

A) B) Individual step pattern, **C)** regularity index (%), **D)** possible support types in percentage of walking time and **E)** weights $\pm$ SEM of male APP/PS1 ($n=10$) and their WT controls ($n=12$). Repeated measures two-way ANOVA (**B and D**) or unpaired two-tailed t-test (**C and E**). **** $p<0.0001$ between genotypes.

3.4.2 Gait analysis in free-feeding APP/PS1 14-month old male mice

Differences between genotypes appeared in the static gait parameters of free feeding males at 14 months of age with APP/PS1 displaying a significantly smaller forelimb paw print area (**Table 3**; $t_{(18)} = 2.305$, $p = 0.0333$) despite their heavier weight (**Figure 27E**). On dynamic gait parameters, APP males showed a slower forelimb swing speed (**Table 4**; $t_{(18)} = 2.494$, $p = 0.0226$) than wild-type males. Differences in the step pattern used were revealed between genotypes of free feeding male mice at 14 months of age (**Figure 27B**; RM-ANOVA, no effect of genotype, $F_{(1,108)} = 0$, $p > 0.9999$; main effect of step pattern, $F_{(5,108)} = 15.94$, $p < 0.0001$; main interaction effect, $F_{(5,108)} = 4.282$, $p = 0.0014$). *Post-hoc* analysis revealed APP/PS1 males spend a higher relative occurrence percentage in the alternate a (Aa) step pattern than wild-type controls (**Figure 27B**). Additionally, APP/PS1 males displayed a lower regularity index than wild-type control mice (**Figure 27C**; $t_{(18)} = 2.267$, $p = 0.0359$). 14 month-old free feeding male APP/PS1 and wild-type mice did not differ on the type of support used (**Figure 27D**; RM-ANOVA, no effect of genotype, $F_{(1,126)} = 0$, $p > 0.9999$; main effect of support type, $F_{(6,126)} = 541.7$, $p < 0.0001$; main interaction effect, $F_{(6,126)} = 1.057$, $p = 0.3920$). 14 month-old free feeding male mice displayed a significant difference in weight between genotypes (**Figure 27E**; $t_{(18)} = 2.829$, $p = 0.0111$).

Table 3. Static gait parameters of 14-month old ad libitum feeding APP/PS1 male mice.

Values are expressed as mean $\pm$ SEM for both forelimbs and hindlimbs (unpaired two-tailed t-test; * $p < 0.05$ between genotypes).

Parameter	Normal Diet Wild-Type (n=10)	Normal Diet APP/PS1(n=10)
Paw angle (degrees)		
Forelimb	8.52 $\pm$ 2.76	9.628 $\pm$ 1.83
Hindlimb	9.347 $\pm$ 2.21	10.51 $\pm$ 2.21
Paw print area (mm ²)		
*Forelimb	33.55 $\pm$ 1.46	29.5 $\pm$ 0.98
Hindlimb	32.14 $\pm$ 1.60	28.68 $\pm$ 2.14
Print length (mm)		
Forelimb	7.477 $\pm$ 0.23	6.952 $\pm$ 0.12
Hindlimb	7.599 $\pm$ 0.28	7.331 $\pm$ 0.25
Print width (mm)		
Forelimb	7.892 $\pm$ 0.17	7.387 $\pm$ 0.19
Hindlimb	8.329 $\pm$ 0.23	7.908 $\pm$ 0.34
Max contact area (mm ²)		
Forelimb	26.86 $\pm$ 1.30	23.57 $\pm$ 0.99
Hindlimb	25.33 $\pm$ 1.38	21.73 $\pm$ 1.95
Paw pressure (arbitrary units)		
Forelimb	112 $\pm$ 2.86	104.8 $\pm$ 2.21
Hindlimb	108.8 $\pm$ 3.07	107.9 $\pm$ 1.79

Table 4. Dynamic gait parameters of 14-month old ad libitum feeding APP/PS1 male mice.

Values are expressed as mean $\pm$ SEM for both forelimbs and hindlimbs (unpaired two-tailed t-test; * $p < 0.05$, between genotypes).

Parameter	Normal Diet Wild-Type (n=10)	Normal Diet APP/PS1(n=10)
Base of support (mm)		
Forelimb	13.62 $\pm$ 0.49	13.24 $\pm$ 0.54
Hindlimb	28.15 $\pm$ 0.83	27.43 $\pm$ 0.73
Stand duration (s)		
Forelimb	0.201 $\pm$ 0.01	0.197 $\pm$ 0.01
Hindlimb	0.214 $\pm$ 0.01	0.2175 $\pm$ 0.01
Swing duration (s)		
Forelimb	0.128 $\pm$ 0.01	0.1395 $\pm$ 0.00
Hindlimb	0.104 $\pm$ 0.01	0.11 $\pm$ 0.01
Swing speed (m/s)		
*Forelimb	0.4765 $\pm$ 0.04	0.3785 $\pm$ 0.01
Hindlimb	0.5495 $\pm$ 0.04	0.4835 $\pm$ 0.03
Stride length (mm)		
Forelimb	54.53 $\pm$ 2.17	50.65 $\pm$ 1.27
Hindlimb	52.27 $\pm$ 2.17	49.12 $\pm$ 1.49
Stand index (m/s)		
Forelimb	-4.446 $\pm$ 0.21	-3.726 $\pm$ 0.28
Hindlimb	-5.911 $\pm$ 0.54	-6.25 $\pm$ 0.41

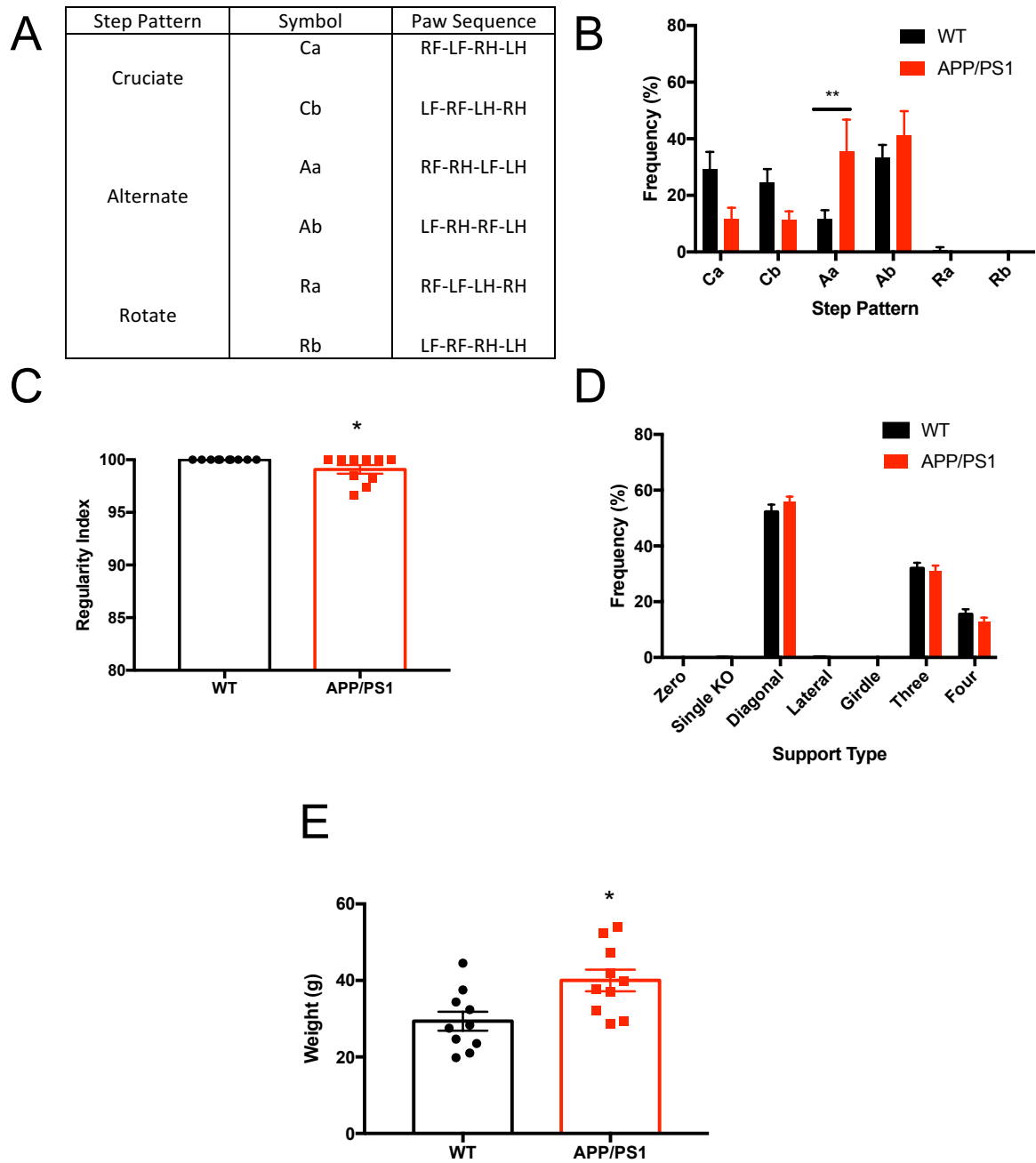


Figure 27. Step pattern and support type measures in 14-month old ad libitum feeding APP/PS1 male mice.

A) B) Individual step pattern, **C)** regularity index (%), **D)** possible support types in percentage of walking time and **E)** weights $\pm$ SEM of male APP/PS1 ($n=10$) and their WT controls ($n=10$). Repeated measures two-way ANOVA (**B** and **D**), **** $p<0.0001$, $p<0.05$, ** $p<0.01$ between genotypes or unpaired two-tailed t-test (**C** and **E**).

4 Discussion

4.1 APP/PS1 male mice do not display impairments in visual discrimination or cognitive flexibility

Although an array of nonmnemonic deficits present themselves early in AD progression, deficits in visual discrimination are not commonly found to be prominent in AD patients (Romberg et al., 2013; Sahakian et al., 1993). Visual discrimination is reliant on visual perception and basic associative learning processes involving the visual areas of the brain and the striatum (Romberg et al., 2013). On the other hand, reversal learning/cognitive flexibility is observed in AD patients (Traykov et al., 2014). Visual discrimination and reversal learning can be examined in mice using the PVD and reversal touchscreen task which measures the ability of mice to recognize and differentiate visual stimuli (Romberg et al., 2013). Male APP/PS1 mice do not display impairments in visual discrimination. Previous research in a number of similar AD mouse models have also revealed no impairments in visual discrimination. TgCRND8 mice, which express human APP_{swe} and develop typical AD amyloid pathology, have shown improvement on the touchscreen visual discrimination task compared to age matched WT controls (Romberg et al., 2013).

Furthermore, no impairments were found on visual discrimination in 5xFAD mice using the PVD touchscreen task (Lee, 2017). 5xFAD male performance showed no differences at 4, 7 and 10 months of age in accuracy on the PVD task (Lee, 2017). Additionally, 3xTg mice did not show differences on this task (Lee, 2017). From this we can gather that APP/PS1 mice are able to perceive and discriminate visually presented images and are able to associate these stimuli with a reward accurately.

Lesioning of the orbitofrontal cortex in mice has previously revealed reversal learning deficits and increased perseverative responding, further supporting the role of the PFC in cognitive flexibility (Chudasama and Robbins, 2003; Remmelink et al., 2016). Reversal learning not only allows for the assessment of being able to form novel stimulus-reward contingencies but also provides an insight into cognitive flexibility by measuring the ability to inhibit previously rewarded responses (Romberg et al., 2013). It

is thought to be mediated by the PFC, striatum and hippocampus (Izquierdo et al., 2006; Prado et al., 2017). However, much of the research in AD patients has focused little attention to cognitive flexibility (Sahakian et al., 1993). Collectively, transgenic mouse models of AD, including APP/PS1 mice show no significant deficits in reversal learning using the PVD touchscreen task (Lee, 2017; Piipponniemi et al., 2017). Given these findings, it is possible that cognitive flexibility remains relatively stable in mouse models of AD until very late stages of disease progression, similar to AD patients (Romberg et al., 2013; Sahakian et al., 1993).

Deficits in reversal learning have been shown in APP/PS1 male mice at 6-7 months of age using a paradigm without food restriction and individual housing (Remmelink et al., 2016). In this study, APP/PS1 male mice were placed in an automated home cage containing a wall with three holes whereby mice could navigate using both visual and tactile stimuli and were analyzed continuously for 4 days (Remmelink et al., 2016). This difference in experimental design could provide an explanation as to why deficits are observed in APP/PS1 mice in the Remmelink et al. study whereas, no deficits in reversal learning was observed in our analysis.

4.2 Attention is not impaired longitudinally in male and female APP/PS1 mice

Sustained attention is the ability to remain vigilant or alert over a period of time (Huntley et al., 2017). However, the exact relationship of sustained attention and AD progression is not particularly well understood. Deficits in sustained attention have been proposed to serve as an early transition indicator of MCI to dementia or present early in AD progression (Rizzo et al., 2000; Summers and Saunders, 2012). Moreover, deficits in sustained visual attention and problems with response control have been observed frequently in AD patients as well as individuals afflicted with mild cognitive impairment (MCI) (Baddeley et al., 2001; Sahakian and Coull, 1993; Traykov et al., 2007). Whereas other work has deemed deficits in sustained attention to not appear until later stages of AD (Perry and Hodges, 1999).

Previous work using touchscreen systems with two mouse models of AD harboring the APP_{swe} transgene have revealed deficits in sustained visual attention (Romberg et al., 2011; Romberg et al., 2013). Male 3xTg mice presented decreased accuracy performance on the 5-CSRTT. Furthermore, 5xFAD mice who carry the APP_{swe} mutation also display impairments on the 5-CSRTT in sustained attention (Masood, 2015). Similar deficits in task accuracy on the 5-CSRTT have also been revealed in mice with prefrontal cortex (PFC) damage (Muir et al. 2012). Moreover, assessment using the 5-CSRTT in rats has correlated accuracy performance with reductions in acetylcholine in the medial PFC (Dalley et al., 2004). Decreased performance on the task is proposed to manifest from insufficient or disrupted cholinergic activity (Bartko et al., 2011; Kolisnyk et al., 2013; Muir et al., 1995; Robbins, 1997). Acetylcholine in the PFC has been shown to be altered by A β oligomers (Chen et al., 2013). Deficits in attention in TgCRND8 mice have similarly been suggested to be linked to disruptions in cholinergic signaling in the medial PFC (Romberg et al., 2013). Our assessments did not reveal any impairments in attention or response control longitudinally in male APP/PS1 mice. Interestingly, while TgCRND8, 5xFAD and 3xTg mice have been shown to present disruption of cholinergic tone, APP/PS1 mice up to 12 month seem to have the basal forebrain cholinergic neurons intact (Hernandez et al., 2001; Jaffar et al., 2001; Perez et al., 2007). However, this issue is still controversial as APP/PS1 female mice have been shown to exhibit impairments in muscarinic transmission as well as cholinergic dysfunction alongside amyloid accumulation in an age-dependent manner (Machova et al., 2008, Machova et al., 2010). Although further studies are necessary to clearly determine whether cholinergic neurons are preserved in APP/PS1 mice, it is tempting to speculate that the lack of attention deficits in these mice could be attributed to no apparent disruption in cholinergic signaling.

Another explanation as to why APP/PS1 mice do not display deficits in attention could lie in the expression of APP. APP is suggested to be involved in a multitude of cellular and physiological functions (O'Brien and Wong, 2011). APP overexpression has predominantly demonstrated positive effects on cell growth and health (O'Brien and Wong, 2011). Additionally, a number of studies have indicated that the overexpression of APP may indeed be neuroprotective (Clarke et al., 2007; Goodman and Mattson, 1994;

Smith-Swintosky et al., 1994). Furthermore, overexpression of APP has been demonstrated to provide neuroprotection in rats subject to middle cerebral artery occlusion (Clarke et al., 2007). Given these findings, APP overexpression could provide an explanation as to why 4 month old males and females seemed to learn the attention task faster than WT controls. The beneficial effects provided to neurons by APP overexpression could be providing a resilience that may not be observed if APP expression was at normal baseline levels. As such, this resiliency would preserve function of a number of key neural pathways and brain structures involved in cognition that would otherwise deteriorate under baseline APP expression conditions.

4.3 Locomotor activity in APP/PS1 male mice

We also evaluated APP/PS1 mice using conventional behaviour methodologies to assess non-cognitive dysfunction. Previous work has revealed hypolocomotor activity at 15-months of age in APP/PS1 male mice compared to WT littermate controls (Ostapchenko et al., 2015). Similar findings have also been reported at 7 and 13-months of age in APP/PS1 male mice (Lalonde et al., 2004; Lee et al., 2004). However, in our study no differences in locomotor behaviour were found in APP/PS1 mice at 14-months of age. One common protocol difference in these experiments compared to those conducted in our study was the lack of a food restriction protocol, which could provide one explanation for the results. Our cohorts of APP/PS1 were food restricted from 3.5 months of age onward. Locomotor activity of mouse models of AD has been previously investigated, however findings have been far from consistent and a high degree of variability within strains has also been prevalent (Webster et al., 2014). These inconsistencies are amplified once food restriction protocols are taken into consideration. Food restriction in AD mouse models can have differential effects on locomotor behaviour based on the duration of the intervention. Long-term food restriction has led to increased locomotor activity in 10-month old 3xTg mice, whereas shorter term intervention has been shown to induce decreased locomotor activity in C57BL/6 wild-type mice (Halgappa et al., 2007; Kuhla et al., 2013). Food restricted mice have been reported to reduce both their exploration of a new environment and their overall locomotor activity (Tucci et al., 2006).

Food restricted APP/PS1 mice also failed to habituate to the open field. Typically, mice subject to an open-field readily explore the new environment and their activity levels decrease over time as they habituate to the novel environment (Konsolaki et al., 2016). However, the cohorts of mice we subjected to long-term food restriction and touchscreen testing did not habituate to the open-field chamber at 14 months of age. Moreover, this cohort of mice failed to habituate following a one-month period of no touchscreen testing, suggesting food restriction may have contributed to the observed persistent locomotion in this cohort of mice and not touchscreen testing. Rats subject to food restriction have previously exhibited increased locomotor activity prior to feeding times (Boulos and Logothetis, 1990).

4.4 APP/PS1 mice do not display robust gait impairments at 14-months of age.

A key relationship between cognitive dysfunction and gait impairments exists in AD patients, with gait problems increasing the risk of falls and immobility in these individuals (Amboni et al., 2013; Montero-Odasso et al., 2017; Sheridan et al., 2003; Raudino, 2013; Wennberg et al., 2017). Some studies have suggested that gait measures can help to predict the risk of future cognitive decline (Thomas et al., 2002; Verghese et al., 2007). Gait has also been found to be affected in mouse models of AD (Schroer et al., 2010; Wirths et al., 2008). 5xFAD mice have demonstrated gait impairments as early as 12 months of age (O’Leary et al., 2013). Male 5xFAD mice have demonstrated a shift in their base of support, similar to what has been previously observed in patients with AD which is proposed to compensate for gait instability (Lee et al., 2017; Liu et al., 2013).

Given the differences observed between food restricted and free feeding mice in locomotion, we also tested if there were any differences in gait between food restricted and free feeding APP/PS1 mice. However, no robust deficits in gait was observed in both cohorts of APP/PS1, suggesting that these mice did not have any motor function deficits that would have inhibited their ability to complete the touchscreen tasks. As gait has a neurochemical basis rooted in the cholinergic system, specifically the pedunculopontine nucleus (PPN) which has also been implicated as important in attention (Janickova et al.,

2017; Mena-Segovia et al., 2004; Pahapill and Lozano, 2000), the lack of robust gait deficits in APP/PS1 is not unexpected.

4.5 Conclusions, Limitations and Future Studies

The primary goal of this project was to determine if we could identify early cognitive deficits in the APP/PS1 mouse model of AD using touchscreen tasks. However, our findings failed to reveal any cognitive deficit using touchscreens. Additionally, conventional behaviour assessments used to characterize non-cognitive domains in this AD mouse model did not reveal any robust phenotype. Behavioural analyses in very old mice modelling neurodegenerative diseases is rare due to the increased laboratory costs and the heterogeneity associated with aged mice. Despite our best efforts, the extreme mortality incidence from epileptic seizures common to this mouse model provided challenges beyond what was anticipated.

In summary, the APP/PS1 mouse model does not appear to be a good model to investigate some of the fundamental cognitive and non-cognitive domains commonly affected in AD. No robust deficits in visual discrimination were demonstrated at 4, 7, 10 and 14-months of age in male APP/PS1 mice in the PVD task. Additionally, no impairments in divided attention were found in both male and female APP/PS1 mice longitudinally in the 5-CSRTT. Sustained attention also appears to remain intact in this mouse model of AD, as no impairments were revealed upon assessing the vigilance of these mice in the 5-CSRTT at the most cognitively demanding stimulus duration. Based on previous work investigating the cholinergic system in APP/PS1, it appears that the neural pathways responsible for attention in this mouse model remain functional. Furthermore, no significant impairments in locomotor activity or gait were found in APP/PS1 mice utilized in touchscreen testing.

Limitations of this study primarily revolve around the use of an appetitive operant paradigm, whereby the use of food for task motivation can have an effect on appetite (Horner et al., 2013). Moreover, this could cause an unintended interaction effect with the changes in physiology due to food restriction (Horner et al., 2013). Collectively, the lack of robust impairments in visual discrimination, reversal learning, divided attention and

sustained attention, suggests that APP/PS1 lacks face validity to assess these aspects of cognition in AD. Additionally, APP/PS1 mice do not appear to be a good model to assess the non-cognitive domains of gait and locomotor activity affected in AD. Future experiments should test whether other aspects of cognition are affected in this model using touchscreen tasks. To date, APP/PS1 mice have been shown to have significant impairments in hippocampal-dependent learning and memory on a variety of conventional behaviour assessments (Jankowsky et al., 2005). It would be interesting to test older APP/PS1 mice (18-24 month-old) on attention and visual discrimination to determine whether they would show deficits.

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5 Appendices

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

PREPARED BY: Matthew Cowan

PURPOSE

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.

SCOPE

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

RESPONSIBILITIES

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

NOTES

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.

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

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.

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.

EQUIPMENT

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

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

PROCEDURE

Acclimation and identification:

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.

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.

Determining individual food consumption:

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).

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.

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

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: $28.5\text{g} \pm 0.6$; 22 weeks: $31.2\text{g} \pm 0.9$) while the average weight of an adult female ranging from 12-24 weeks is around 22g (12 weeks: $20.0\text{g} \pm 0.51$; 24 weeks: $23.6\text{g} \pm 0.67$). Thus, **to reach 85% of the average adult body weight adult male mice weight should be reduced to $\pm 25.5\text{g}$ and adult female mouse weight should be reduced to $\pm 18.7\text{g}$.**

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.

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.

In the C57Bl/6 background it is common to find obese mice (males $>30\text{g}$, females $>22\text{g}$). 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 $\pm 25.5\text{g}$ and adult female mouse weight should be reduced to $\pm 18.7\text{g}$**). Thus, obese mice must be food restricted longer until they get to the appropriate weight.

Maintenance at 85% of baseline adult body weight:

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 an average of food to keep mice at 85% of their weigh. This amount must be carefully monitored and properly adjusted.**

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.

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.

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.

HEALTH & SAFETY

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.

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>

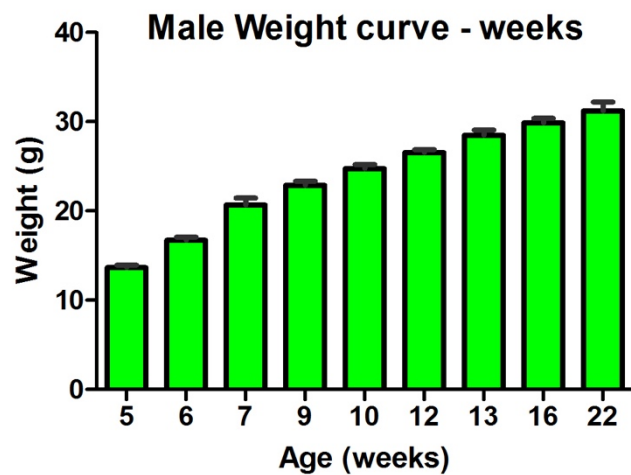


Figure 1: Male weight curve.

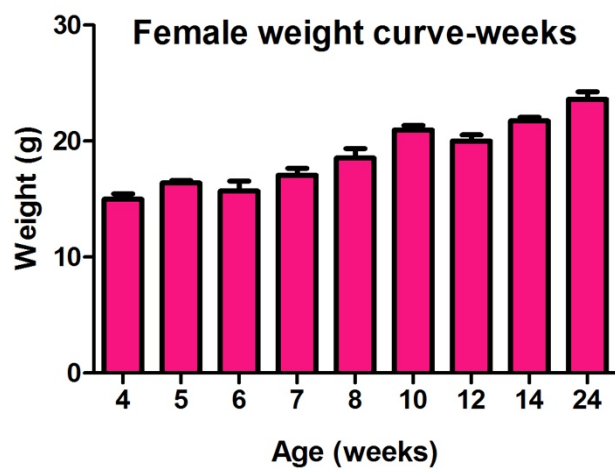


Figure 2: Female weight curve.

Appendix B: 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.

EQUIPMENT

Mouse Touch Screen Systems and ABET II

89540 CAM Pairwise (Visual) Discrimination (PD) Task with Cambridge Amendment

PROCEDURE

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 <i>Tray light stays on when finger removed.</i>
	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, <i>stays on until feeder tray is re-entered</i>
	Block Back IR activity beams	Count goes up by 1 for each beam blockage

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).

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.

Training Procedures

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 (150 μ l). 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 μ l). 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.

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 μ l SM), time out (TO, 5s, paired with overhead light).

The session begins with a priming delivery of reinforcer [280ms (7 μ l 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 μ l 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 μ l SM), time out (TO, 5s).

The session begins with a priming delivery of reinforce [**280ms (7 μ l 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 μ l 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 μ l SM), time out (TO, 5s). The session begins with a priming delivery of reinforce [280ms (7 μ l 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 μ l 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 C: 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.

EQUIPMENT

Mouse Touch Screen Systems and ABET II

89543CAM 5-Choice Serial Reaction Time Task with Cambridge Amendment

PROCEDURE

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. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 2	Image in all grid spaces, Grid 2 has 40% of full white, all other grids have full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 3	Image in all grid spaces, Grid 3 has 50% of full white, all other grids have full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 4	Image in all grid spaces, Grid 4 has 70% of full white, all other grids have full white image. <i>Activate the Tray to clear all images and proceed with test.</i>
	Touch Grid 5	Full white (bright) image in all grid

		spaces. Pulses Sound_On 500ms <i>Activate the Tray to clear all images and proceed with test.</i>
	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

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).

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).

Training Procedures

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 (1000 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-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 μ l SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

The session begins with a priming delivery of reinforce [280ms (7 μ l 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 μ l 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 incorrect 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 μ l SM), Delay interval (5s), time out (TO, 5s) and ITI Incorr (5s).

The session begins with a priming delivery of reinforce [280ms (7 μ l 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 μ l 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 incorrect 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.

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

6 Curriculum Vitae

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