KU LEUVEN

FACULTY OF PSYCHOLOGY AND
EDUCATIONAL SCIENCES

Assessment of early cognitive performance and social behaviour in the biAT/TPLH mouse models for Alzheimer's disease

Master's thesis submitted for the
degree of Master of Science in
Master of Psychology:
Theory and Research by
Celine Samaey

Supervisor: Prof. Detlef Balschun
Co-supervisor: Dr. Stijn Stroobants
In collaboration with: An Schreurs

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Summary

Alzheimer's disease (AD) is a neurocognitive disorder that affects over 40 million people worldwide and has become one of the main causes of death in developed countries. Its major neurobiological hallmarks are the extracellular Aβ plaques and the aggregation of hyperphosphorylated tau protein into intracellular neurofibrillary tangles (NFTs). The disease is characterized by a gradual onset and progressive decline of cognitive functions such as episodic memory, executive functions and language. Moreover, AD patients present behavioural and psychological signs and symptoms of dementia (BPSD), such as social withdrawal, apathy, depressive mood, aggression and diurnal rhythm disturbances. Currently, no cure exists for the disease. However, many authors have proposed that earlier intervention could improve AD prognosis and might even halt AD progression. Therefore, it is crucial to improve preclinical detection and diagnosis of AD based on biomarkers and behavioural markers.

In order to identify robust preclinical cognitive and non-cognitive changes in AD, we assessed cognitive functions and social behaviour in 3-month-old wild-type and transgenic mice over a period of three months. All mice had a C57BL/6J background. Two transgenic mouse models were used: 1) the biAT mouse model (APP.V717I x Tau.P301L) expressing both Aβ plaques and NFTs and 2) the TPLH mouse model (Tau.P301L) which only expresses tau pathology. All mice performed a variety of behavioural tasks to evaluate cognitive and non-cognitive impairments. More specifically, the test battery included 12 tasks (Morris water maze (MWM), context- and cue-dependent fear conditioning (CFR), spontaneous activity, rotarod, marble burying, elevated plus maze, open field, SPSN, tail suspension, tail withdrawal, nesting, and nesting 2.0), designed to assess hippocampus-dependent memory, exploratory, anxiety-related, social, depressive and nociceptive behaviour, and activities of daily living (ADLs).

We hypothesized that compared to age-matched controls, transgenic mice would show specific impairments in both cognitive and non-cognitive tasks. We expected significant differences in hippocampus-dependent memory function and anxiety-related, social and depressive behaviour, which are affected first in human patients. Furthermore, we expected little to no differences in exploratory behaviour, nociception and ADLs, since these functions are generally affected in later stages of the disease. Furthermore, we expected more severe impairments in the biAT than in the TPLH mouse model. In line with our expectations, transgenic mice showed decreased cognitive flexibility in the MWM. Moreover, transgenic mice displayed increased anxiety-related behaviour. However, transgenic mice also displayed decreased exploratory behaviour and reduced functioning in ADLs. Finally, there were no major differences in performance between the biAT and TPLH mouse models.

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Clarification of student's approach and contribution

The design of this study was formulated by my promotor and daily supervisor, who also obtained the approval of the Ethical Committee. The literature review got a kick-start thanks to the literature suggestions provided by my promotor and daily supervisor. Next, I individually gathered more relevant and in-depth literature to complete the literature review.

Before the start of the behavioural experiments, I frequently assisted my daily supervisor in the caretaking of the animals. I also assisted during the genotyping of the mice. My co-promotor explained and demonstrated all behavioural protocols used in this study and let me observe another student in the Morris water maze. The research work in this thesis was carried out by myself, under close supervision of my co-promotor and daily supervisor, between February and May 2017. During this three-month period, I was also responsible for the basic care and colour coding of the animals.

For the data-analyses, my co-promotor kindly provided some general guidelines, after which I individually analysed the data obtained in this study.

Finally, my promotor, co-promotor and daily supervisor provided feedback to this article-based manuscript.

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Introduction

Alzheimer's disease (AD), accounting for over 70% of all dementia cases, is the most common form of dementia (Jaworski et al., 2010), and is thought to affect over 40 million individuals worldwide (Selkoe & Hardy, 2016). According to the Alzheimer Association (2016), lifetime risk for AD at age 65 is 17% for women and 9% for men. At age 85, this percentage increases to 20% for women and 12% for men, illustrative of the fact that age is the strongest risk factor for AD. Yet, other risk factors like traumatic brain injury, low educational level and genetic susceptibility play a role in AD as well (American Psychiatric Association, 2013; Alzheimer Association, 2016).

AD is a complex, heterogeneous disease with a variable age of onset, rate of progression and development of pathology. Moreover, it has both genetic (70%) and environmental (30%) causes (Dorszewska, Prendecki, Oczkowska, Dezor, & Kozubski, 2016). An important distinction has to be made between familial (early onset, <65 years old) Alzheimer's disease (FAD), which comprises <5% of all AD cases, and the much more common sporadic Alzheimer's disease (SAD) or late onset AD (>65 years old), which accounts for over 95% of AD cases (Drummond & Wisniewski, 2017; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014).

FAD is inherited and symptoms usually present before the age of 65 years (Duthey, 2013). Most genetic mutations found in FAD are autosomal dominant mutations in presenilin 1 (PSEN1), presenilin 2 (PSEN2) or the amyloid precursor protein (APP), which alter the production of Aβ_{42}. However, these mutations only account for 5 to 10% of all FAD cases, thus the majority of FAD cases remains unexplained (Duthey, 2013; Drummond & Wisniewski, 2017).

SAD on the other hand, does not display autosomal-dominant inheritance. Nonetheless, genome wide association studies have identified over 30 loci that are involved in the development of AD (Bertram, Lill, Tanzi, 2010). The alipoprotein ϵ4 (APOE4) gene on chromosome 19 is especially important for the sporadic form of AD, although its specific mode of action is unknown (Goedert, & Spillantini, 2006). A review by Bertram and colleagues (2010) highlighted that APOE4 is involved in Aβ-aggregation and -clearance, as well as in inflammation and cerebrovascular events. The microtubule-associated protein tau (MAPT), and specific mutations in its gene, have been implicated in the hyperphosphorylation of tau in AD and constitute a risk factor for other neurodegenerative diseases

as well (Götz & Ittner, 2008).

Despite the distinction between familiar and sporadic, both types of AD have the same clinical features (Lehtovirta et al., 1996; American Psychiatric Association, 2013) and similar disease duration (Karran, Mercken, & De Strooper, 2011). AD progresses gradually through severe dementia and eventually death, with a mean survival of 10 years after diagnosis (American Psychiatric Association, 2013). The disease is defined by early memory deficits, especially in episodic memory, and gradual deterioration of other cognitive functions, including problems with working memory, executive functioning and language, object use and/or recognition, confusion in time and place and verbal fluency problems (Götz, & Ittner, 2008; Van der Jeugd et al., 2013; Webster et al., 2014). In addition to these cognitive deficits, AD is also characterized by several behavioural and psychological signs and symptoms of dementia (BPSD), which place a high burden on caregivers and the patient's family. Examples of these BPSD are changes in personality, deterioration of social skills, social withdrawal, emotional dulling, depression, aggression, behavioural disinhibition and even psychosis (Lyketsos et al., 2011; Van der Jeugd et al., 2013; Jaworski et al. 2010). Furthermore, patients with AD also display disturbances in diurnal rhythm and altered sleep-wake patterns (Van der Jeugd et al., 2013). Volicer, Harper, Manning, Goldstein and Satlin (2001) found that AD patients have less diurnal motor activity, but a higher nocturnal activity than healthy controls. Moreover, they displayed sundowning, the phenomenon in which AD symptoms and restlessness occur in the late afternoon and evening.

AD is often preceded by a period of mild cognitive impairment (MCI), a clinical condition in which cognitive decline is greater than expected given a person's age and educational level, but does not interfere with activities of daily living (ADLs) (Gauthier et al., 2006). During MCI, several cognitive deficits arise: impairments in executive functioning, attention and visuospatial memory, followed by impairments in verbal recall and finally impairments in general cognition (Webster et al., 2014). The disorder can be subdivided into amnestic MCI, which is characterized by isolated memory impairments, and non-amnestic or multidomain MCI with multiple cognitive deficits. Next to these cognitive impairments, MCI is also associated with increased neuropsychiatric symptoms, especially depression and anxiety (Palmer et al., 2007; Lyketsos et al., 2011). In general, people with MCI remain stable or even improve over time, but one-third to half of all

people with MCI progress to AD within five years (Gauthier et al., 2006; Palmer et al., 2007; Fischer et al., 2007). Neuropsychiatric symptoms in MCI, especially depression, apathy and anxiety, are a predictor of progression to AD (Palmer et al., 2007; Teng, Lu, & Cummings, 2007). Especially people with the amnestic subtype are at high risk of progression to AD, thus it is often regarded a prodromal phase of the disease (Gauthier et al., 2006).

However, small changes in cognition and behaviour occur even before this MCI phase, and are labelled as preclinical changes. For example, impairments in episodic and semantic memory already arise near the end of the preclinical phase (Webster et al., 2014), with longitudinal studies describing significant impairments in episodic memory 10 to 12 years before symptom onset in patients with FAD (Bateman et al., 2012) and SAD (Amieva et al., 2008). Moreover, Hassenstab and colleagues (2016) found that in a sample of cognitively normal older adults, people with positive AD biomarkers, more specifically biomarkers in the cerebrospinal fluid (CSF) and neuroimaging biomarkers, performed worse on all cognitive measures than people without AD biomarkers. A meta-analysis by Bäckman and colleagues (2005) showed significant preclinical deficits in episodic memory as well as in global cognitive ability, perceptual speed and executive functioning. Furthermore, there were small differences in verbal ability, visuospatial skills and attention. Finally, according to Balsis, Carpenter and Storandt (2005) and Storandt (2008), initial personality changes and difficulties in attentional and inhibitory control are also prominent before clinical diagnosis of AD.

A definitive AD diagnosis can generally only be made after post-mortem examination of the neuropathological brain changes (Webster et al., 2014). However, patients can be diagnosed with "possible AD" or "probable AD" using both cognitive neuropsychological assessments and neurological examinations. AD is categorised as a subtype of the DSM-V Neurocognitive Disorder (NCD) and has four key criteria: 1) the criteria are met for major or mild NCD, 2) there is insidious onset and gradual progression of impairment in one or more cognitive domains, 3) criteria are met for either probable or possible AD as follows: probable AD is diagnosed if there is evidence of a causative AD genetic mutation from family history or genetic testing and if all three of the following are present: clear evidence of decline in memory and learning and at least one other cognitive domain, steadily progressive, gradual decline in cognition without ex-

tended plateaus and no evidence of mixed etiology. If these aren't present, possible AD should be diagnosed. 4) the disturbance is not better explained by cerebrovascular disease, another neurodegenerative disease, the effects of a substance, or another mental, neurological or systemic disorder (American Psychiatric Association, 2013). Important differential diagnoses of AD are other neurocognitive disorders (for example Lewy Body disease and frontotemporal dementia), other active neurological or systemic illnesses (for example thyroid disorders or vitamin-B12 deficiency) or late-life major depressive disorder. Given the specific neuropathology underlying AD, which will be explained in the next paragraph, imaging techniques are often applied to diagnose possible and probable AD. Structural MRI can be used to assess hippocampal volume, while fluorodeoxyglucose (FDG) PET and Pittsburgh compound B (PIB) PET are used to study glucose metabolism and amyloid deposition in relevant brain areas. More recently, F-AV-1451 PET scans are run to look at distribution of pathological tau in the brain (Hoenig et al., 2018).

AD is characterized by two major biological hallmarks in the brain: extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) from hyperphosphorylated tau (Spires-Jones & Hyman, 2014). Amyloid plaques are mainly composed of Aβ_{40} and Aβ_{42} amino acid polypeptides that are derived from the amyloid precursor protein (APP) by proteolytic cleavage. APP is cleaved at three cleavage sites, the β-, α-, and γ-secretase site, and different lengths of Aβ are created. Especially the Aβ_{42} protein is neurotoxic, forms oligomeric aggregates and eventually deposits as plaques. Transgenic mice with Aβ_{42} develop amyloid plaques, whereas Aβ_{40}-mice do not (Götz & Ittner, 2008). Moreover, Aβ_{40} seems to have a protective function, as it prevents Aβ_{42} from aggregating and forming plaques (Götz & Ittner, 2008). In general, Aβ levels rise because of both increased production and impaired elimination of Aβ_{42} (Jaworski et al., 2010).

NFTs, the second hallmark of AD, are formed by aggregated tau, a microtubule binding protein which is mainly found in axons, where it stabilizes microtubuli and likely plays a role in cellular transport processes (Spires-Jones & Hyman, 2014). Tau is a phosphoprotein and its activity is regulated by the degree of phosphorylation and its alternative splicing (Iqbal, Liu, Gong, & Grundke-Iqbal, 2010). In AD and other tauopathologies, tau is hyperphosphorylated and dissociates from the microtubuli, consequently forming NFTs (Götz & Ittner, 2008).

The amyloid cascade hypothesis, currently the dominant model for AD pathogenesis, states that amyloid pathology initiates the pathological cascade of AD leading to NFTs, synaptic dysfunction, neuronal cell death and dementia (Selkoe & Hardy, 2016; Mufson et al., 2016). This hypothesis is able to explain the pathology and the genetic risk factors that underly FAD and SAD, but does not consider the interaction of Aβ and tau (Karran et al., 2011). Several studies have demonstrated that Aβ plays a critical role in the development of AD, for example, increases in the Aβ_{42}/Aβ_{40} ratio or increases in the Aβ_{42} levels in the brain predispose individuals to developing AD (Karran et al., 2011). Furthermore, there is evidence that tau pathology arises downstream of amyloid pathology. Terwel and colleagues (2008) revealed that amyloid pathology precedes and actually induces tau pathology through the activation of GSK-3 isozymes. Moreover, Götz and colleagues (2004) report that β-amyloid induced an increase in the number of NFTs in mice, whereas the reverse was not observed, providing further evidence for the hypothesis that amyloid pathology precedes NFTs. Although the amyloid cascade hypothesis is mainly related to FAD, several reports indicate its occurrence in SAD as well (Dorszewska et al., 2016).

Neuropathological change in AD in biopsy or post-mortem tissue is typically ranked on three parameters to obtain an ABC score: a) histopathological assessment of Aβ-containing amyloid plaques according to Thal, Rüb, Orantes and Braak (2002), b) Braak staging of NFTs, and c) scoring of the neuritic amyloid plaques using a CERAD score (Webster et al., 2014; Mirra et al., 1991).

Thal and colleagues (2002) distinguish five distinct phases of Aβ deposition in the brain that describe the distribution pattern of pathology in AD. During the first phase, Aβ deposits are exclusively present in the neocortex. The second phase is characterized by Aβ depositions in the allocortex as well. In phase three, amyloid pathology has spread to the diencephalic nuclei, striatum and cholinergic nuclei of the basal forebrain. Brainstem nuclei become involved in the fourth phase, and in the last phase, Aβ-deposits infiltrate the cerebellum. People with probable AD exhibited Aβ phases three to five, whereas people without AD had limited pathology corresponding to the first three phases (Thal et al, 2002).

Braak and Braak (1991) examined the post-mortem distribution of NFTs in demented and non-demented participants and described the characteristic distribution pattern in six

stages. NFTs were numerous in people diagnosed with dementia, but not in non-demented controls. Moreover these NFTs displayed a robust, characteristic pattern of distribution with little inter-individual variation. NFTs first appear in the transentorhinal cortex in stage I and II. At the end of stage II, mild changes in the hippocampus appear. Next, in the limbic stages III and IV, the entorhinal cortex becomes affected. Finally, in the isocortical stages V and VI, large amounts of NFTs spread to all isocortical association areas.

It is clear that Aβ and tau pathology show a clear discrepancy in their distribution patterns. Deposition of amyloid plaques occurs early in AD and proceeds slowly. Aβ-pathology spreads from the neocortex through the allocortex, diencephalon, striatum and basal forebrain. Finally, it also spreads to the brainstem and cerebellum in the final stages of the disease (Spires-Jones & Hyman, 2014; Mufson et al., 2016). NFTs, on the other hand, first appear in the entorhinal cortex, hippocampal formation and association cortices. In later stages of the disease, NFTs invade the primary sensory areas (Spires-Jones & Hyman, 2014). This trajectory of tau pathology is consistent with early deficits in hippocampus-dependent functions, especially episodic memory (Webster et al., 2014). Several studies in AD patients indicate that NFTs correlate with the severity of cognitive symptoms, while the accumulation of amyloid plaques is uncorrelated with cognitive impairments in patients (Webster et al., 2014; Spires-Jones & Hyman, 2014; Iqbal et al., 2010). However, AD also involves other brain changes such as synapse loss, dysfunction of synapses and changes in synaptic plasticity, such as long-term potentiation (LTP) and -depression (LTD). These brain changes are directly or indirectly connected to Aβ and tau and correlate strongly with cognitive impairment in dementia as well (Spires-Jones & Hyman, 2014).

The knowledge of neurobiological factors and genes underlying AD was largely non-existent up until 25 years ago. Since then, animal models, especially transgenic mouse models, have been developed (Khachaturian, 2005). These models have been instrumental in the increasing knowledge on AD mechanisms and the development of therapeutics (LaFerla, & Green, 2012). However, no animal model can fully replicate human AD pathology nor cognitive deficits. As a consequence, different animal models have been introduced for different research questions (Webster et al., 2014). The AD mouse models explained below constitute a non-exhaustive overview for illustrative purposes only, since

a full overview of AD mouse models would be beyond the scope of this thesis.

Transgenic models based on APP are the first and biggest subcategory of AD mouse models. The PDAPP mouse, created in 1995, was the first transgenic APP mouse model to successfully display many pathological features and cognitive impairments of AD (Götz et al., 2004; Webster et al., 2014). Later, following the discovery of the involvement of presenilins 1 and 2 in AD, PSEN1 and PSEN2 knock-out mice were created as well. Moreover, mouse models with pathogenic mutations in both APP and presenilins, for example APP/PS1 (Jankowski et al., 2001), were created, even though these mutations do not coexist in human AD. These mice carry higher Aβ_{42} concentrations in the brain and have an accelarated rate of Aβ deposition (Götz et al., 2004).

AD mouse models of the second category express tau pathology. The first transgenic tau models, established in 1995 as well, did not express NFT pathology, yet, they did model pre-tangle formation and hyperphosphorylation (Götz & Ittner, 2008). The identification of several pathogenic mutations in the MAPT gene in frontotemporal dementia (FTDP-17), led to the creation of new mouse models. These transgenic tau models, among which P301L, successfully displayed NFTs in neurons and glial cells (Götz et al., 2004; Götz & Ittner, 2008).

A third subcategory of AD mouse models, the multigenic mouse models, assesses the relationship of Aβ and NFTs. These multigenic mouse models more closely resemble human AD pathology and allow for precise monitoring of the interaction between Aβ and tau pathology. Common multigenic mouse models are 3xTg-AD (APP.SW x Tau.P301L), biGT (Tau.P301L x GSK3β-S9A) and biAT (APP.V717I x Tau.P301L). The latter displays a combined amyloid and tau pathology in the hippocampus and cortex that increases with age and mimics the pathology found in AD patients (Götz & Ittner, 2008; Jaworski et al., 2010; Drummond & Wisniewski, 2017, Terwel et al., 2008).

Next to the development of transgenic mouse models, several behavioural tasks have been designed to assess cognitive domains homologous to those in humans. Consequently, impairments in rodent cognition can be compared to deficits in human AD (Webster et al., 2014). Nonetheless, there are some translational issues in the use of mouse models, as is evident from the very low success rate (less than 1%) of clinical trials with treatments that were reported to have been successful in mouse models (Drummond & Wisniewski, 2017). First of all, transgenic mouse models mimic FAD, while this familial variant

only constitutes a minor amount of all AD cases. As mentioned above, over 95% of AD cases is sporadic (Drummond & Wisniewski, 2017). However, there is a great variety in the reported prevalence of FAD cases, with some studies reporting up to 25% of FAD (Zhao, Lu, Chew, & Mu, 2014). Next, Drummond and Wisniewski (2017) report that the majority of genetic mechanisms behind FAD are still unkown. Consequently, the genetic background used for transgenic mouse models is even less representative to the human AD etiology and pathology. Moreover, currently no animal model in itself is able to capture and express the entirety of human AD (Webster et al., 2014). According to Götz and colleagues (2004), mouse models are particularly inadequate for modelling neuronal loss and the spatiotemporal distribution of NFTs and amyloid plaques. Finally, the variability in behaviour displayed by mice is narrower than the behaviour displayed by humans and not all cognitive domains that are affected in human AD, for example language, can be modelled in mice. Yet, despite these limitations, behavioural testing in rodents remains relevant for human AD (Webster et al, 2014) and future models, based on improved understanding of the genetics of SAD, hold great promise for AD research (Onos, Sukoff Rizzo, Howell, & Sasner, 2016).

Several potential disease-modifying drugs have been proposed for AD, but currently there is no cure for the disease. However, many authors have attributed this lack of effect to the severity of the disease at the time of the start of treatment (Counts, Ikonomovic, Mercado, Vega, & Mufson, 2017; Emery, 2011). Consequently, early detection and diagnosis of AD is crucial for the development of treatments that might delay or even prevent AD. Several biomarkers, including early amyloid imaging, early tau imaging, levels of Aβ and tau in the cerebrospinal fluid (CSF), and hippocampal volume, have been proposed to detect AD in its early stages (Counts et al., 2017; Hoenig et al., 2018, Hassenstab et al., 2016; Selkoe & Hardy, 2016; Sperling & Johnson, 2013). Moreover, in their longitudinal study, Bateman and colleagues (2012) found that several pathophysiological and cognitive changes precede the diagnosis of AD with years and even decades. According to Fuentes (2012), there are even subtle changes in instrumental activities of daily living (IADLS) 10 years before the official diagnosis. Late-life psychiatric symptoms, especially late-life depression and anxiety, have been found to be a risk factor as well, often preceding MCI and AD (Donovan et al., 2018, Steenland et al., 2012). According to Jost and Grossberg (1996), 72 out of 100 AD patients experienced depression, changes

in mood, social withdrawal and suicidal thoughts more than two years before diagnosis. Assessing these cognitive and neuropsychiatric symptoms in an at-risk population is more straightforward than testing for the known biomarkers and, therefore, holds great promise. Neuropsychological and functional measures even outperformed CSF and MRI measures in predicting future AD in MCI patients (Cui et al., 2011) .

Therefore, this thesis aims to identify early behavioural and cognitive deficits in two transgenic mouse models: Tau.P301L (the TPLH mouse model) and APP.V717I x Tau.P301L (the biAT mouse model). These mouse models typically display severe tauopathy from 14 months onwards (Tau.P301L) and diffuse amyloid plaques at 10 months (APP.V717I x Tau.P301L) (Terwel et al., 2008). The selection of these models allows us to compare mice with tau pathology alone and mice with both Aβ and tau pathology, which is more representative of human AD. The behavioural read-outs from these models could indicate robust changes that arise early (already after three months) and could be used as behavioural markers to improve early detection of preclinical AD in humans. In combination with the biomarkers mentioned above, behavioural markers should make it possible to identify people in the earliest stages of AD, when drug modification might still be effective (Counts et al., 2016). Furthermore, identification of early functional parameters would allow more sensitive evaluation of therapeutic efficacy in preclinical trials with these mouse models.

Several cognitive and behavioural domains that are typically impaired in human AD, were assessed in this study. Reference memory, closely related to semantic memory in humans, refers to learned knowledge for a specific task that remains constant for that task and was operationalised in the Morris water maze (MWM) and the contextual- and cued fear conditioning task (CFR). Moreover, the MWM was also used to assess spatial working memory as well as executive functions, more specifically cognitive flexibility. Locomotor activity, circadian rhythm and sundowning were measured in the 23 hours spontaneous activity task. Locomotor activity and motor coordination were also judged in the open field and on the rotarod. Social behaviour was assessed using the sociability and preference for social novelty task (SPSN). Activities of daily living were operationalised in nesting tasks, while nociceptive behaviour was measured in the tail withdrawal task. Finally, neuropsychiatric symptoms such as anxiety and depressive symptoms were evaluated with the elevated plus maze, marble burying task and tail sus-

pension box (Webster et al., 2014). These paradigms allowed us to identify early changes in cognitive performance and social behaviour of the TPLH and biAT mouse models.

Methods

Animals

Wild-type and transgenic mice were bred and housed in the animalium of the Laboratory of Biological Psychology. Founder transgenic AD mice were kindly provided by reMYND nv (Heverlee, Belgium). All animals were group-housed under standard conditions (constant temperature and humidity; normal 12hr light/dark cycle starting at 8am), with *ad libitum* access to food and water.

Our sample consisted of 38 C57BL/6J males with an average age of 2.48 months (SD = 0.51) at the start of our experiments, of which 13 wild-type (mean age = 2.48, SD = 0.51), 13 TPLH (Tau.P301L) (mean age = 2.49, SD = 0.53) and 12 biAT (APP.V717I x Tau.P301L) (mean age = 2.46, SD = 0.54) animals. Throughout all behavioural tests, the experimenter was blind to the genotype of the animals. All animal experiments were approved by the KU Leuven Ethical Committee and in accordance with the European Directive 2010/63/EU.

Genotyping

Transgenic mouse DNA was extracted using the AccuStart II Mouse Genotyping Kit (Quantabio). 2 mm tail snips were submerged in 75 μl Extraction Reagent and heated to 95°C for 30 minutes. Next, the samples were cooled to room temperature and an equal amount of Stabilisation Buffer was added. Finally, 1 μl of extract was used in a 25 μl PCR reaction to amplify APP-V717I gene products. Samples were further processed by agarose gel electrophoresis and visualised by GelRed dye and UV illumination (Figure S1).

Behavioural measures

Spontaneous activity

To gain insight in general exploration, diurnal pattern of locomotor activity, motor function and general arousal, spontaneous activity was monitored every 30 minutes during 23 hours. Mice were individually placed in transparent home cages filled with 400 ml

bedding and with modified cage tops that prevented them from climbing. Activity was recorded using three infrared beams and the Mouse4Win program.

Nesting

Making nests is natural murine behaviour, as nests are important for thermoregulation and are associated with reproduction and shelter. Moreover, research has shown that hippocampal lesions are associated with decreased nesting behaviour, which can be interpreted as deterioration in the ability to perform activities of daily living, an early symptom of AD (Deacon, 2012). The animals were placed in individual cages filled with 0.5 cm of normal bedding and a piece of paper. After 23 hours, the quality of the paper nests was assessed on a 5-point scale (Deacon, 2012). A largely untouched nest with over 90% of material still intact, was given a score of 1. 2 points were given for a partially torn-up nest, with 50 to 90% intact. When less than 50% of the material was intact without identifiable nest site, a score of 3 was given. A 4-point nest has a clearly identifiable but flat nest site, whereas a 5-point nest is a nearly perfect crater with walls higher than the animal's body height on 50% of the circumference. Exemplar pictures of the different scores are presented in Figure S2.

Nesting 2.0

The nesting was repeated with cylindrical 2 cm nesting material (Cocoon), which is more comparable to the Nestlet nesting material used by Deacon (2012), instead of paper, exactly 50 days after the first nesting task. The same scoring system as before was applied (Deacon, 2012). Exemplar pictures of the different scores obtained in this thesis are presented in Figure S3.

Rotarod

The accelerating rotarod is used to evaluate motor coordination. During the training phase, mice were placed on the rotarod for two minutes, followed by three minutes of resting. The testing phase consisted of four trials, each maximally lasting five minutes on the accelerating rod, during which the rotation speed progressively increased from 4 to 40 rpm. In between test trials, mice were given at least a five minute break, since the inter-trial interval was 10 minutes.

Elevated plus maze

Anxiety-related behaviour was assessed with the elevated plus maze. This plus-shaped maze has two arms enclosed by walls and two open arms without walls. Entries in both the closed and the open arms, as well as percentage of time spent in the open arms, were recorded by infrared beams. Each mouse was placed in the left closed arm of the maze and following one minute of habituation, exploratory activity was recorded for 10 minutes.

Open field

In order to assess general exploration of a novel open arena and anxiety-like behaviour, mice were placed in a brightly illuminated transparent plexiglas arena (50 x 50 x 30 cm) inside an enclosed cupboard (Figure S4). After one minute of habituation, open field exploration was monitored during 10 minutes with ANY-maze tracking software (Stoelting Co., IL, USA). For our analyses, parameters of interest were total path length and time spent in both the periphery and the center. Moreover, we also reviewed the number of corner and center entries as well as the average distance to center.

Sociability/preference for social novelty (SPSN) test

The three-step SPSN protocol, consisting of a 5-minute acclimation phase and two 10-minute testing phases, was used to assess sociability and social memory (Naert, Callaerts-Vegh, & D'Hooge, 2011). A transparent plexiglas box (94 x 28 x 30 cm) with three chambers separated by division walls was placed in an enclosed cupboard with dim lighting (Figure S5). During the acclimation phase, the animals could move freely in the central chamber (29 x 28 x 30 cm). Empty cylindrical wire cages were present in the left and right chambers (36 x 28 x 30 cm each). In the sociability trial, the second phase of this test, one stranger mouse was placed in the wire cage in either the left or right chamber while the other wire cage remained empty. The chamber in which the first stranger mouse was presented, was alternated between test mice. The test animal was placed in the central chamber and was allowed to explore all chambers freely. Finally, in the preference for social novelty trial, a second stranger mouse was placed in the empty cage, while the first stranger animal remained in the same location as in the previous trial. The test mouse was placed in the central chamber and could explore all chambers freely.

Explorative behaviour was tracked using ANY-maze video tracking. Moreover, time spent sniffing an animal cage was scored manually during the trials. The stranger mice were group-housed C57BL/6J males that had already served as stranger mice in other SPSN experiments. Every stranger mouse was used only once per day.

Tail suspension

The tail suspension test is one of the most widely used tests to assess depression-like behaviour and effectiveness of antidepressants in mice (Cryan, Mombereau, & Vassout, 2005). The mice were suspended by their tails with tape in a suspension box for six minutes. Their escape-oriented behaviours were video-recorded and scored (Can et al., 2012). Immobility, the dependent variable, was measured in several ways: the average distance from the center, the latency to immobility, immobility time and number of immobile episodes.

Marble burying

To assess digging activity as well as OCD and anxiety-like behaviour, mice were placed individually in a large cage containing a five centimetre-thick layer of standard bedding and 22 glass marbles distributed equally along the cage walls at approximately 2 cm distance from each other. The animals were left undisturbed in a quiet room for 30 minutes, after which the number of marbles that were buried with bedding for at least two thirds, were counted.

Morris water maze

The Morris water maze was used to assess hippocampus-dependent spatial learning and memory. The round pool was filled with opacified water at 25 $\pm$ 1 °C and contained a transparent escape platform. The Ethovision system (Noldus, Wageningen) was used to track the mouse while in the pool. During the acquisition phase, each mouse was placed inside the pool four times a day at a different starting position with an intertrial interval between 15 and 30 minutes. Two acquisition blocks were performed, each from Monday to Friday (five days), followed by a pause during the weekend and a probe trial thereafter (on day 6 and 11). During these probe trials, the escape platform was removed from the pool. The mice were placed inside the pool opposite of the platform position and tested

for 100 seconds. Finally, during the third week, the escape platform was moved opposite to the original platform position. Each mouse performed four reversal trials a day from different starting positions a day for five days. After the weekend, on day six, retention of spatial information was again tested with a probe trial.

Performance in the acquisition trials was assessed by analysing average pathlength, mean velocity and escape latency. Key parameters of interest for the probe trials were time spent in each quadrant and number of entries in each quadrant. For the reversal trials, average pathlength, mean velocity and escape latency were measured. Finally, inflexibility during the reversal trials was operationalised by the time spent at the previous platform position.

Context- and cue-dependent fear conditioning

Fear conditioning and retention was studied using a four-day protocol. On the first day, animals were placed inside the test chamber (context A: dark room, grid floor, unscented) and were allowed to adjust for five minutes. Their mobility was recorded using a force transducer with a sampling rate of 50 Hz (MED Associates Inc., St. Albans, Vermont, USA). 24 hours later, animals were again placed in the same test chamber (context A) for fear conditioning. After two minutes of acclimatisation, two auditory cues (4 kHz, 80 dB) were administered for 30 seconds with a one-minute inter-stimulus interval. The auditory cue co-terminated with a two second 0.2 mA foot shock administered through the grid floor of the test chamber. During these three minutes, shock-induced freezing was monitored. Another 24 hours later, during the contextual fear phase, the animals returned to the test chamber (context A) for five minutes of exploration. At least 90 minutes later, the animals were again placed in the test chamber, however, in a different context (context B: brightly illuminated, a white plastic sheet covering the grid, scented with peppermint oil) for the cued fear phase. After three minutes without stimulus presentation, the auditory cue was presented alone for another three minutes. Animal movements were recorded by the force transducer. Finally, exactly 21 days after the testing phase, the animals were again tested in both context A (contextual fear phase) and context B (cued fear phase) to study fear retention.

Tail withdrawal

The tail withdrawal test evaluates the ability of an animal to detect nociceptive stimuli, more specifically heat. In order to reduce handling restraint and variability in handling by the experimenter, as well as to reduce stress, the mice were slightly restricted by entering a plastic cylinder voluntarily. The distal half of the tail was placed in warm water twice at four increasing temperatures (47°C, 49°C, 51°C and 53°C respectively) as previously described by Leo, Straetemans, D'Hooge and Meert (2008). Latency to respond to this heat stimulus by flexing the tail strenuously was measured manually using a stopwatch. Animals were removed from the water immediately after responding or after a 25 second cut-off time, to avoid tissue damage. Between same-temperature trials, an interval of at least 15 minutes was adopted. The between-temperatures interval was at least 30 minutes.

Statistical analysis

For the data analysis of the open field and tail suspension test, we used the analysis tools provided by ANY-maze (Stoelting Co., IL, USA). RStudio version 1.1.383 (RStudio Team, USA) was used for data analysis of all other behavioural measures as well as for the creation of the figures. One-way ANOVA was performed either using the raw data or transformed data when the assumptions of normality, homoscedasticity or independence were violated. In case of robust violations of these assumptions, a non-parametric Kruskal-Wallis test, or one-way ANOVA on ranks, was performed. Datasets with multiple datapoints for each animal were analysed using linear mixed effects models. Data are presented as mean + two times the standard error of mean (2 x SEM).

Results

Spontaneous activity

A linear mixed effects model indicated a main effect of time on the activity level of the animals ($F(45, 1581.1) = 19.0941$, $p < 0.000$), but no effect of genotype ($F(2, 35) = 1.0957$, $p = 0.3455$). There was however a significant interaction effect between time and genotype ($F(90, 1581.1) = 2.358$, $p < 0.000$) (Figure 1). Overall, wild-type mice were more active during the habituation phase and at the first nocturnal peak, yet this difference disappears during the second and third nocturnal peak and the light phase. Dunnett's test was used for comparing transgenic and wild-type mice. There was a significant difference between APPxTau and wild-type mice with APPxTAU animals being less active ($p = 0.0066$), but not between Tau and wild-type animals ($p = 0.3923$).

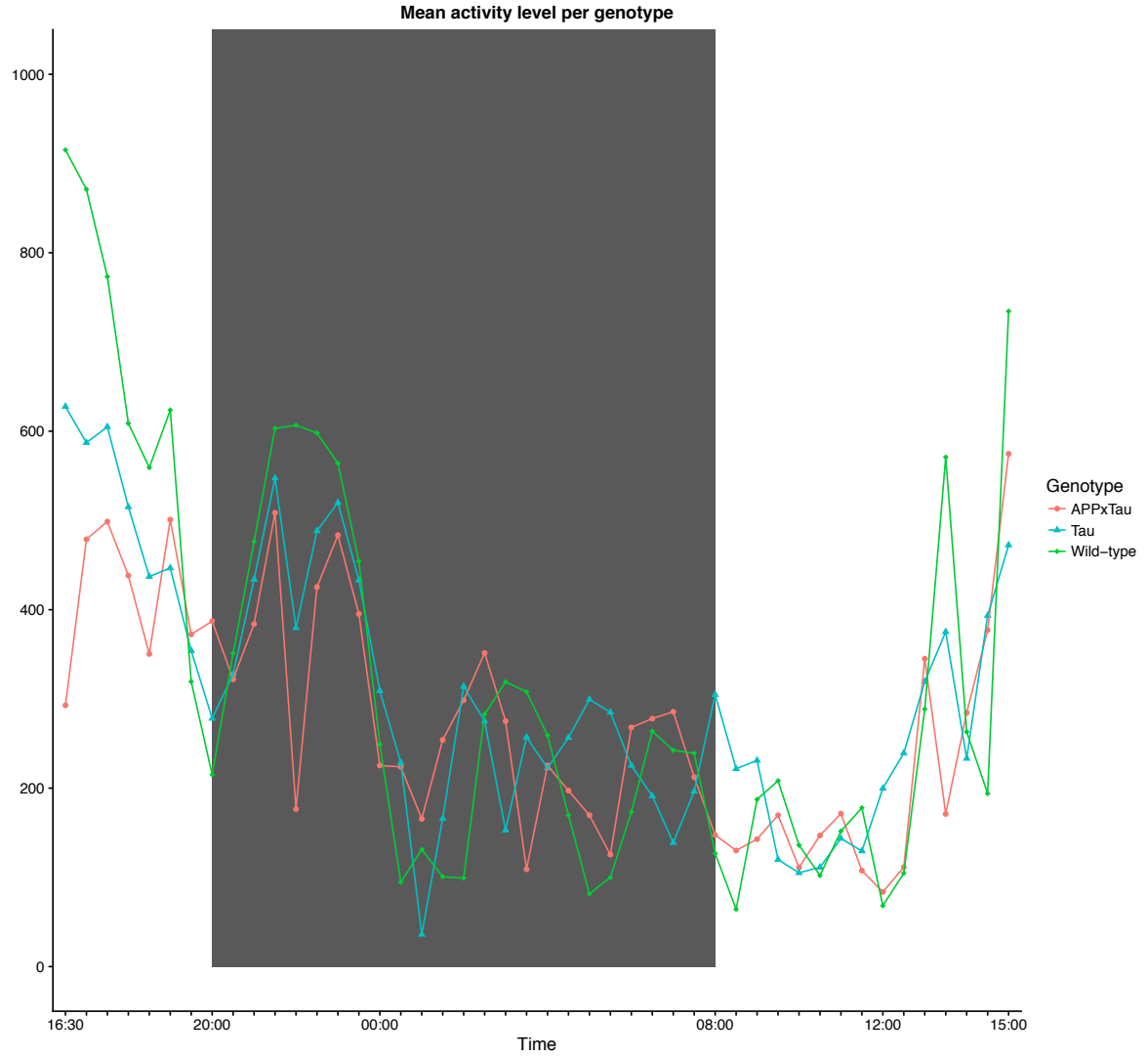

Figure 1. Mean activity pattern for all genotypes. Time of the day had a significant main effect on the activity level (p <0.000) and there was a significant interaction effect between time and genotype (p <0.000). Wild-type animals were more active during the habituation phase and the first nocturnal peak, but these differences disappeared in later nocturnal peaks and the day phase. The dark background indicates the period when the lights were off.

Nesting

Visual inspection of the data suggests an unequal distribution of the scores over the different genotypes, with higher scores for wild-type and Tau mice, and on average lower scores for APPxTau (Figure 2). Mean nesting scores per group are 4.0385 (SD = 0.6279),

3.8462 ($SD = 0.8006$) and 3.0000 ($SD = 1.1677$) for wild-type, Tau and APPxTau mice respectively. A non-parametric Kruskal-Wallis ANOVA revealed that this difference is significant ($\chi^2 = 7.7956$, $p = 0.0203$). Post-hoc Dunn testing showed a significant difference between wild-type and APPxTau ($p = 0.0253$), but no significant difference between wild-type and Tau, nor between Tau and APPxTau.

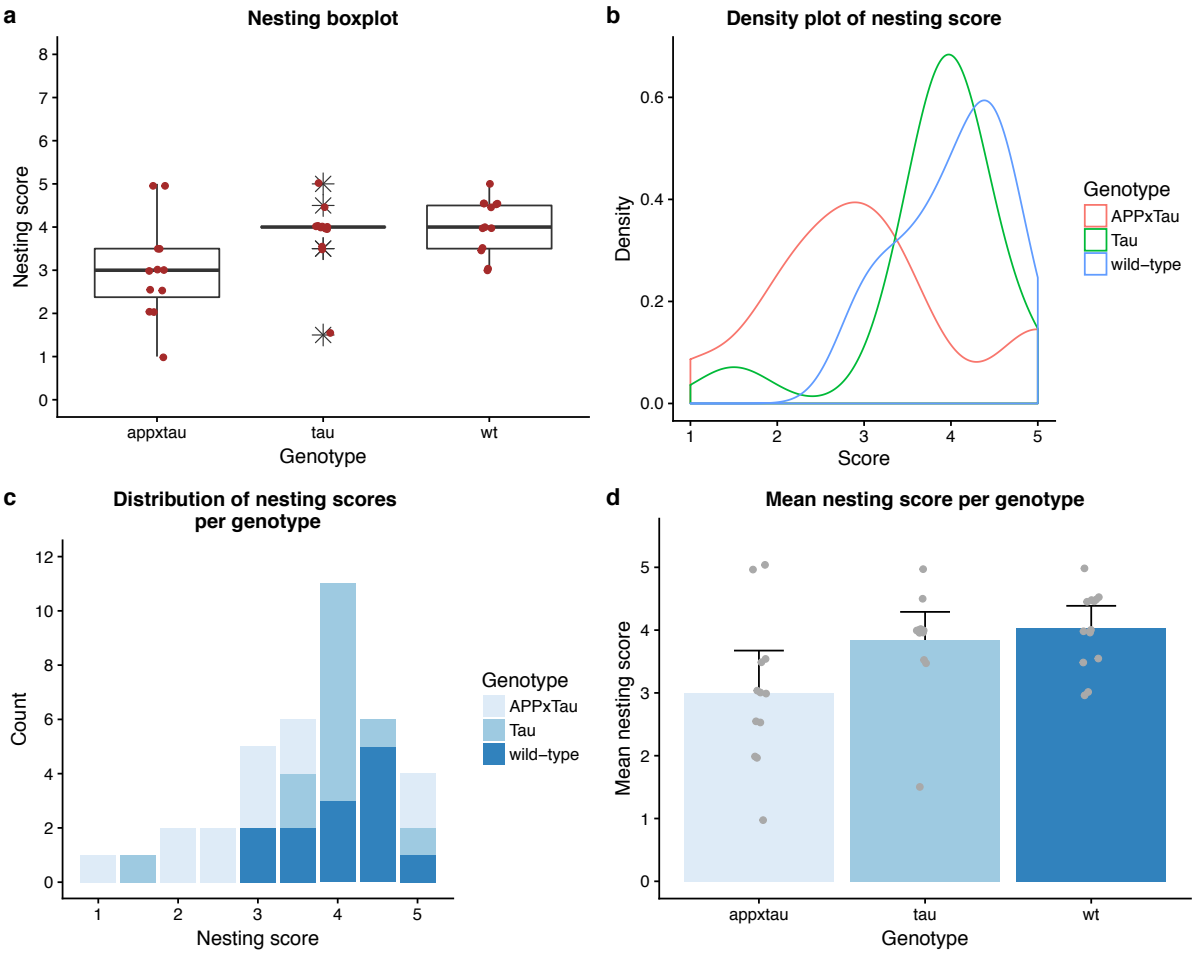

Figure 2. (a) Nesting boxplot. Individual datapoints are presented on the plot. * indicates outliers. (b) Density plot of nesting scores per genotype. The distribution mode largely overlaps for Tau and wild-type animals, whereas the mode for APPxTau is markedly lower. (c) Distribution of nesting scores per genotype. (d) Mean nesting score per genotype. Dunn test showed a significant difference between wild-type and APPxTau ($p = 0.0253$). Individual datapoints are presented on the plot.

Nesting 2.0

In contrast to the first nesting task, the scores per group were more equally distributed in this second nesting task (wild-type: $M = 4.1154$, $SD = 1.2442$; Tau: $M = 4.4615$, $SD = 0.6602$ and APPxTau: $M = 4.0000$, $SD = 1.1282$) (Figure 3, panel b and c). The Kruskal-Wallis test was non-significant, indicating no difference in nesting score between groups ($\chi^2 = 1.3588$, $p = 0.5069$) (Figure 3, panel a and d).

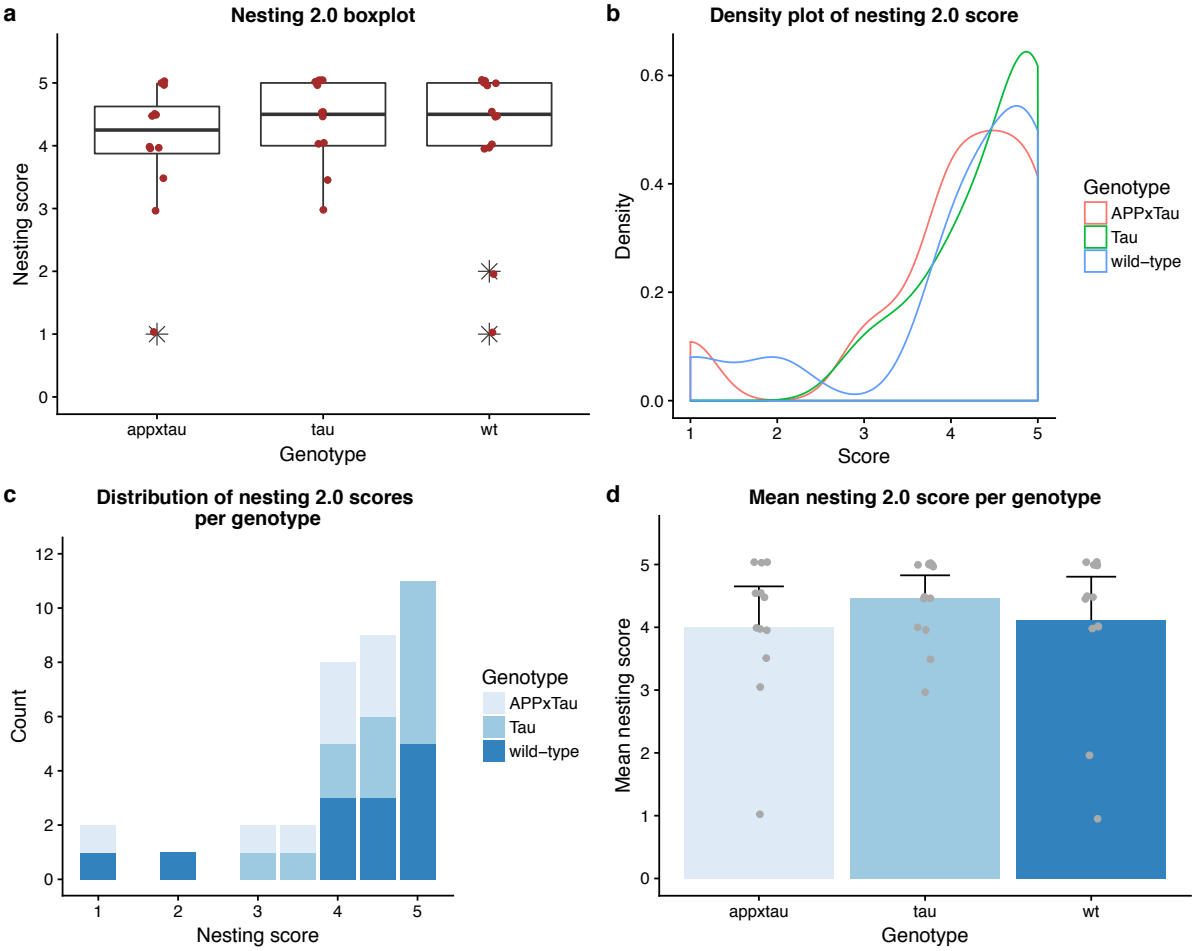

Figure 3. (a) Nesting 2.0 boxplot. Individual datapoints are presented on the plot. * indicates outliers. (b) Density plot of nesting 2.0 scores per genotype. Unlike in the first nesting test, distributions for all genotypes show considerable overlap. (c) Distribution of nesting 2.0 scores per genotype. (d) Mean nesting 2.0 score per genotype. The Kruskal-Wallis ANOVA was non-significant, indicating no difference in nesting score between groups ($p = 0.5069$). Individual datapoints are presented on the plot.

Rotarod

The mean amount of falls during the two-minute training session is shown in Figure 4, panel a (wild-type: $M = 0.9231$, $SD = 0.9541$; Tau: $M = 0.8462$, $SD = 0.9871$ and APPxTau: $M = 0.2500$, $SD = 0.6216$). There was no significant difference between groups in number of falls during this training session ($\chi^2 = 4.9947$, $p = 0.0823$).

Figure 4 (panels b - f) also depicts the mean latency to fall per genotype for all test trials. The linear mixed-effects model showed a significant effect of trial number on the latency to fall ($F(3,104.97) = 3.6993$, $p = 0.0141$): motor coordination improved over time with significant differences between trial 1 and trials 2, 3 and 4 ($p = 0.0272$, $p = 0.0101$, $p = 0.0069$, respectively). However, the effect of genotype was marginally non-significant ($F(2,35) = 3.1000$, $p = 0.0576$). There was no interaction effect between trial number and genotype ($F(3,104.97) = 0.6836$, $p= 0.6632$).

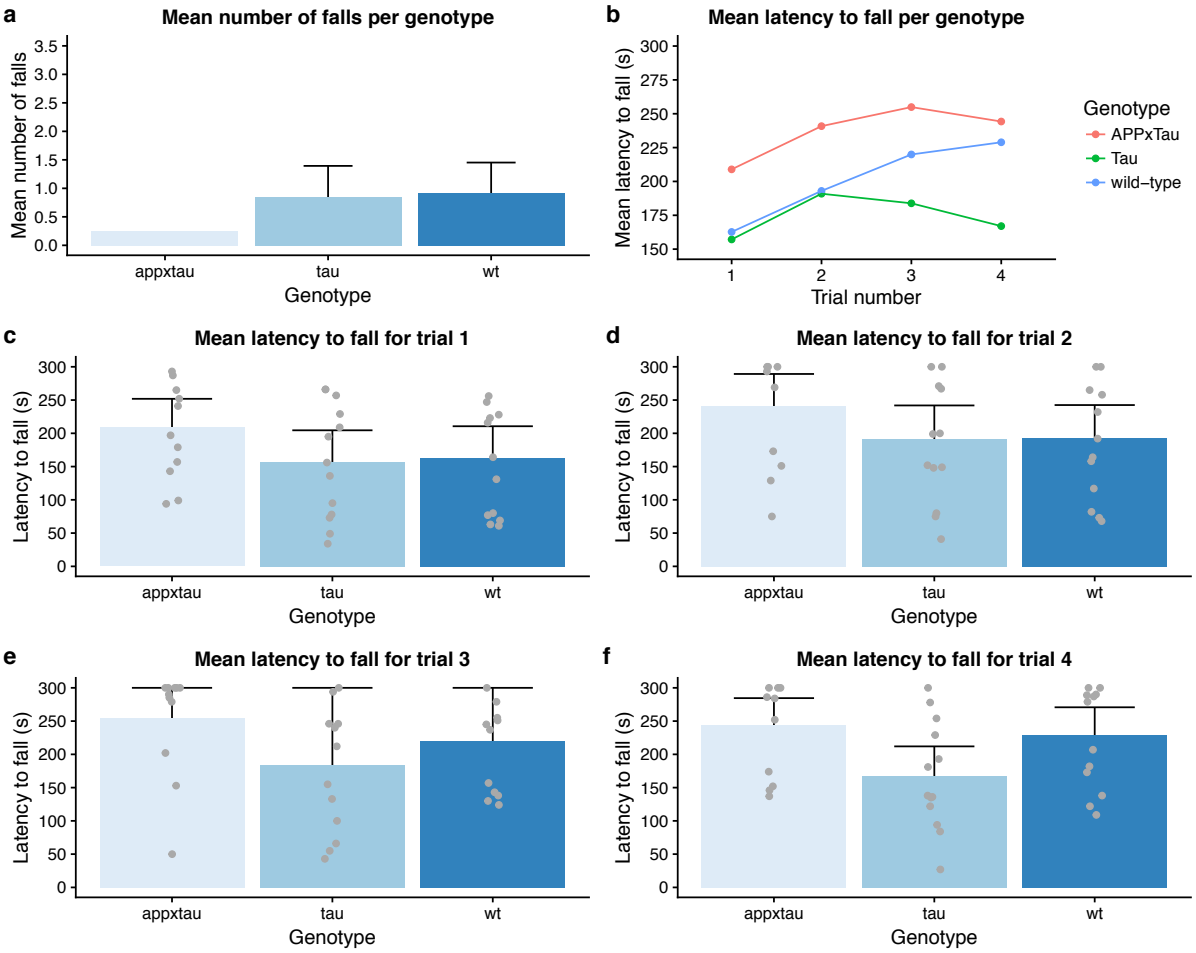

Figure 4. (a) Mean number of falls during the training phase. There was no significant difference between groups in number of falls during the training sessions ($p = 0.0823$). (b) Mean latency to fall per genotype for all test trials. Linear mixed-effects model showed a significant effect of trial number on the latency to fall ($p = 0.0141$). The effect genotype was marginally non-significant ($p = 0.0576$). (c) - (f) Mean latency to fall for each trial. Individual datapoints are presented on the plot.

Elevated plus maze

There was a significant effect of genotype on total number of crossings in all arms ($\chi^2 = 27.323$, $p < 0.000$). More specifically, a post-hoc Dunn test revealed a significant difference between wild-type and APPxTau ($p < 0.000$) and wild-type and Tau animals ($p < 0.000$): wild-type animals had a higher number of crossings (Figure 5, panel c). Moreover, the number of crossings in the closed arms differed significantly between genotypes ($\chi^2 =$

27.33, p <0.000), especially between wild-type and APPxTau (p <0.000) and wild-type and Tau (p <0.000), with wild-type animals crossing the infrared beams in the closed arms significantly more often (Figure 5, panel b). There was a marginally significant difference between genotypes on the number of crossings in the open arms ($\chi^2 = 6.1369$, $p = 0.0465$), with a significant post-hoc difference between wild-type and Tau animals ($p = 0.0402$): wild-type animals crossed the infrared beams more often than Tau mice (Figure 5, panel a). Finally, the percentage of time spent in the open arms was significantly influenced by genotype ($\chi^2 = 19.11$, p <0.000), with wild-type animals spending significantly more time in the open arms than APPxTau ($p = 0.0203$) and Tau animals (p <0.000) (Figure 5, panel d).

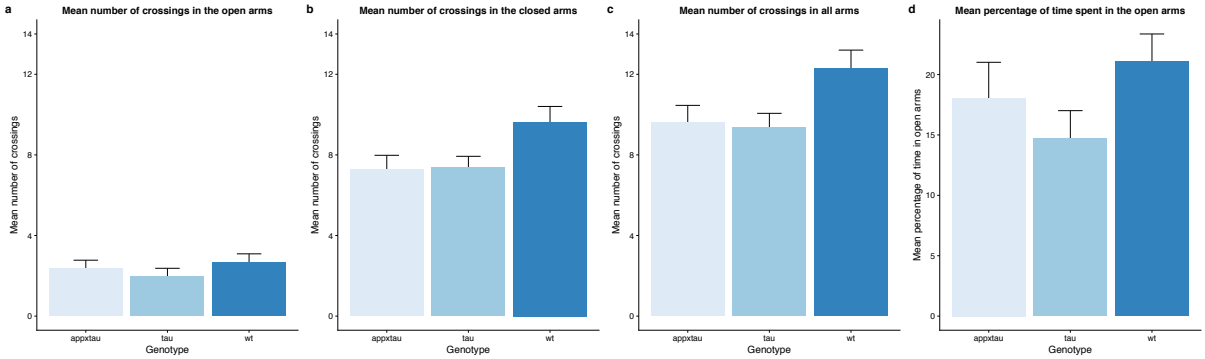

Figure 5. (a) - (c) Mean number of crossings in the open, closed and all arms of the elevated plus maze. Overall, wild-type mice had a higher number of crossings in both the open and the closed arms. (d) Percentage of time spent in the open arms per genotype, with wild-type mice spending significantly more time in the open arms than APPxTau and Tau mice ($p = 0.0203$ and p <0.000).

Open field

There were several parameters of interest for the open field task. First, there was a significant difference in the total distance travelled ($F(2,35) = 10.8438$, p <0.000). Tukey testing revealed a significant difference between wild-type and Tau and between wild-type and APPxTau, but no difference between Tau and APPxTau ($p = 0.048$, p <0.000 and p = 0.079 respectively): wild-type animals explored the arena significantly more than Tau and APPxTau mice (Figure 6).

Average distance from the center also differed significantly between the genotypes

($F(2,35) = 3.3056$, $p = 0.048$). Tukey testing indicated a significant difference between wild-type and Tau genotypes with wild-type animals being further away from the center on average than Tau mice ($p = 0.038$). There were no significant differences between wild-type and APPxTau and Tau and APPxTau mice ($p = 0.446$ and $p = 0.408$). Furthermore, there were no significant differences between genotypes in the number of entries to, time spent in and distance travelled in the center zone ($F(2,35) = 2.9436$, $p = 0.066$; $F(2,35) = 1.8842$, $p = 0.167$; $F(2,35) = 3.2462$, $p = 0.051$) (Figure 7, panel a).

Considering the corner zones, there was a significant effect of genotype on the number of entries ($F(2,35) = 10.1278$, $p < 0.000$). A Tukey test indicated a significant difference between wild-type and APPxTau ($p < 0.000$): wild-type mice entered the corner zones significantly more often. However, there was no significant difference between wild-type and Tau ($p = 0.059$) and Tau and APPxTau mice ($p = 0.090$). As expected from the overall difference in the total distance travelled, there was a significant effect of genotype on the distance travelled in the corner zones ($F(2,35) = 12.6136$, $p < 0.000$). The Tukey test again showed a significant difference between wild-type and Tau and wild-type and APPxTau, but not between Tau and APPxTau genotypes ($p = 0.008$, $p < 0.000$ and $p = 0.180$, respectively), with wild-type mice having travelled a bigger distance in the corner zones. The effect of genotype on time spent in the corner zones was non-significant ($F(2,35) = 1.3526$, $p = 0.272$) (Figure 7, panel b).

Finally, we evaluated their behaviour in the periphery (Figure 7, panel c). There was a significant effect of genotype on the number of entries to the periphery ($F(2,35) = 9.4205$, $p = 0.001$). According to the Tukey test, wild-type and Tau genotypes entered the periphery significantly more than APPxTau mice ($p = 0.002$ and $p = 0.001$, respectively). The difference between wild-type and Tau mice was non-significant ($p = 0.970$). Time spent in the periphery also differed significantly depending on the genotype ($F(2,35) = 4.5871$, $p = 0.017$): wild-type animals spent more time in the periphery than Tau mice ($p = 0.013$). There was no significant difference between wild-type and APPxTau and Tau and APPxTau animals ($p = 0.195$ and $p = 0.473$). As expected, the genotypes also differed significantly in their distance travelled in the periphery ($F(2,35) = 13.5622$, $p < 0.000$). Tukey testing indicated a significant difference between wild-type and Tau and wild-type and APPxTau mice ($p = 0.001$, $p < 0.000$), but not between Tau and APPxTau mice ($p = 0.519$): wild-type animals again travelled a longer distance in the periphery

than Tau and APPxTau mice.

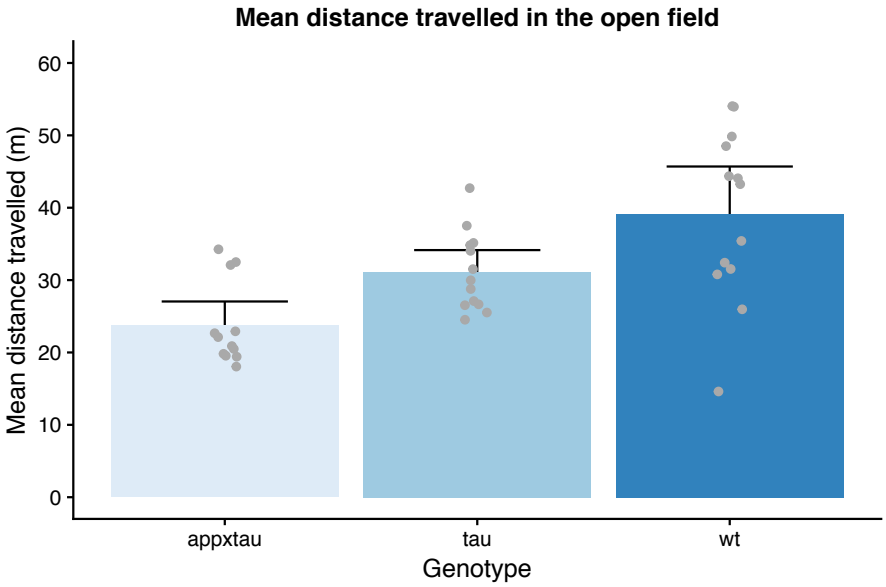

Figure 6. Total distance travelled differed significantly between genotypes (p <0.000): on average, wild-type animals explored the arena significantly more than Tau and APPxTau mice.

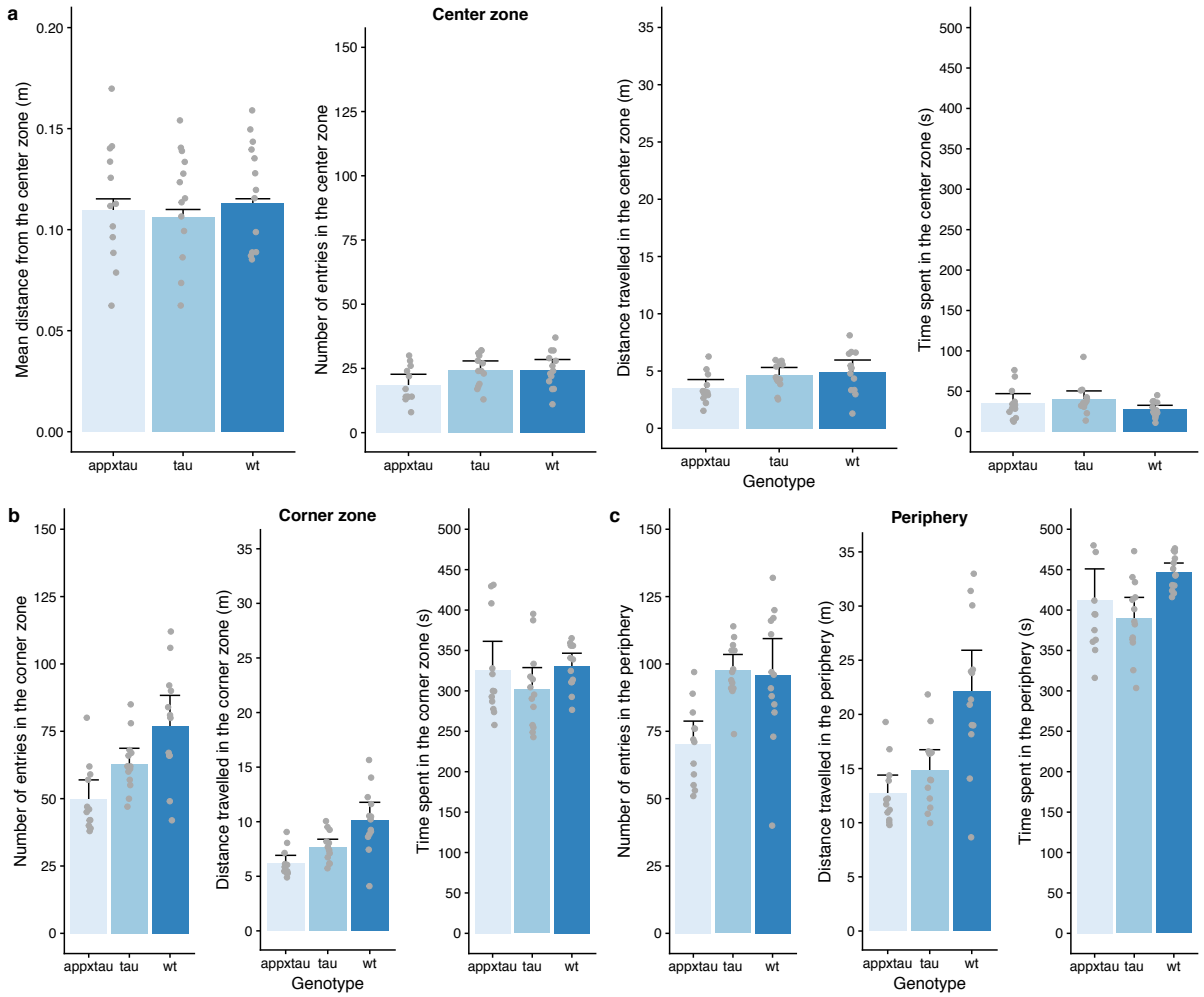

Figure 7. (a) Average distance from, number of entries to, distance travelled in and time spent in the center zone. On average, wild-type mice were further away from the center than Tau mice ($p = 0.038$). There was no effect of genotype on the number of entries to, distance travelled in and time spent in the center zone. (b) Number of entries to, distance travelled in and time spent in the corner zones. Wild-type animals entered the corner zones significantly more often than APPxTau mice (p <0.000). Wild-type animals also travelled more than Tau and APPxTau mice (p <0.000), but there was no effect of genotype on time spent in the corner zones. (c) Number of entries to, distance travelled in and time spent in the periphery. Wild-type and Tau mice entered the periphery significantly more than APPxTau mice ($p = 0.001$), wild-type animals spent more time in the periphery than Tau mice ($p = 0.017$) and they travelled further than Tau and APPxTau mice (p <0.000). Individual datapoints are presented on the plot.

Sociability/preference for social novelty (SPSN) test

A one-way ANOVA revealed a main effect of genotype on the total distance travelled in the arena during the 5-minute acclimation stage ($F(2,35) = 9.1428$, $p = 0.001$). The post-hoc Tukey test showed that wild-type mice travelled significantly further than APPxTau and Tau mice ($p = 0.001$ and $p = 0.010$, respectively). During the 10-minute sociability stage, there was again a main effect of genotype on the distance travelled ($F(2,35) = 7.9311$, $p = 0.001$): wild-type animals travelled further than APPxTau and Tau mice ($p = 0.002$ and $p = 0.015$, respectively). This main effect of genotype was also present in the social novelty stage ($F(2,35) = 8.7778$, $p = 0.001$). Wild-type animals again travelled a significantly longer distance than APPxTau and Tau animals ($p = 0.001$ and $p = 0.020$, respectively) (Figure 8).

Overall, a linear mixed-effects model revealed a main effect of stage ($F(1,102) = 4.4766$, $p = 0.0368$) and an interaction effect of stage and chamber ($F(1,102) = 7.6612$, $p = 0.0067$) on the time spent sniffing. There was no main effect of genotype or chamber on the time spent sniffing in the sociability stage. However, their interaction was significant ($F(2,34) = 4.4157$, $p = 0.0197$): Tau animals spent more time sniffing the empty cage than the stranger 1 cage, whereas wild-type and APPxTau mice spent more time sniffing the stranger 1 cage. In the social novelty stage, the chamber significantly influenced time spent sniffing ($F(1,34) = 4.9710$, $p = 0.0325$), with all animals sniffing the stranger 2 mouse significantly longer ($p = 0.0267$) (Figure 9).

Finally, there was a significant effect of stage on the number of entries in the stranger 1 chamber ($F(2,68) = 229.3698$, $p<0.000$), with more entries in the sociability than in the social novelty stage ($p <0.000$). Number of entries in the stranger 2 chamber was significantly affected by genotype ($F(2,34) = 7.2637$, $p = 0.002$), stage ($F(2,68) = 424.0932$, $p <0.000$) and their interaction ($F(4,68) = 6.9938$, $p <0.000$). More specifically, wild-type animals entered the stranger 2 chamber significantly more than Tau and APPxTau mice ($p = 0.006$ and $p = 0.003$, respectively). Furthermore, animals entered the stranger 2 chamber significantly more in the sociability stage than in the social novelty stage ($p <0.000$) (Figure 10). There was no significant effect of genotype or stage on time spent in either the stranger 1 or stranger 2 chamber.

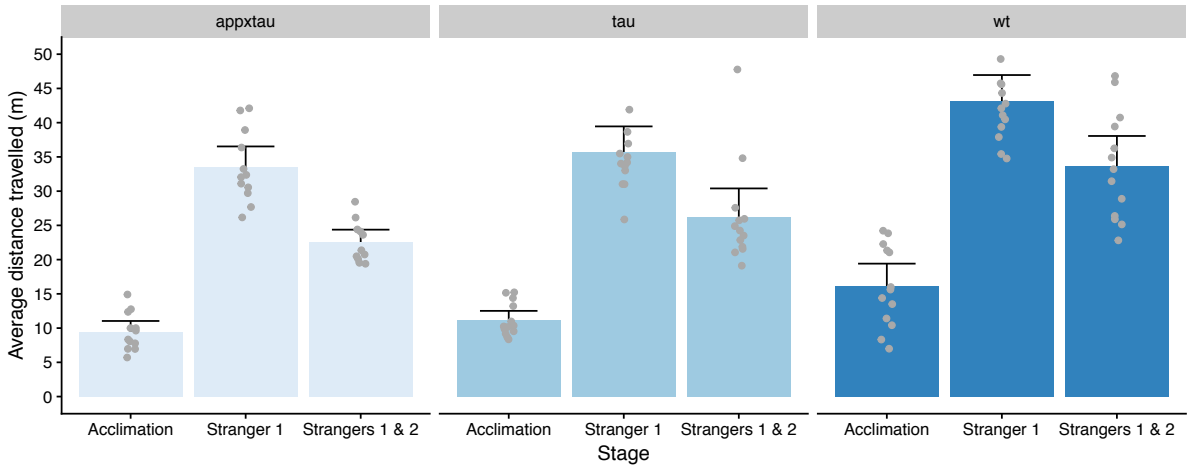

Figure 8. Total distance travelled in the SPSN arena was significantly affected by genotype for each stage ($p = 0.001$ for the acclimation, sociability and social novelty stage). Individual datapoints are presented on the plot.

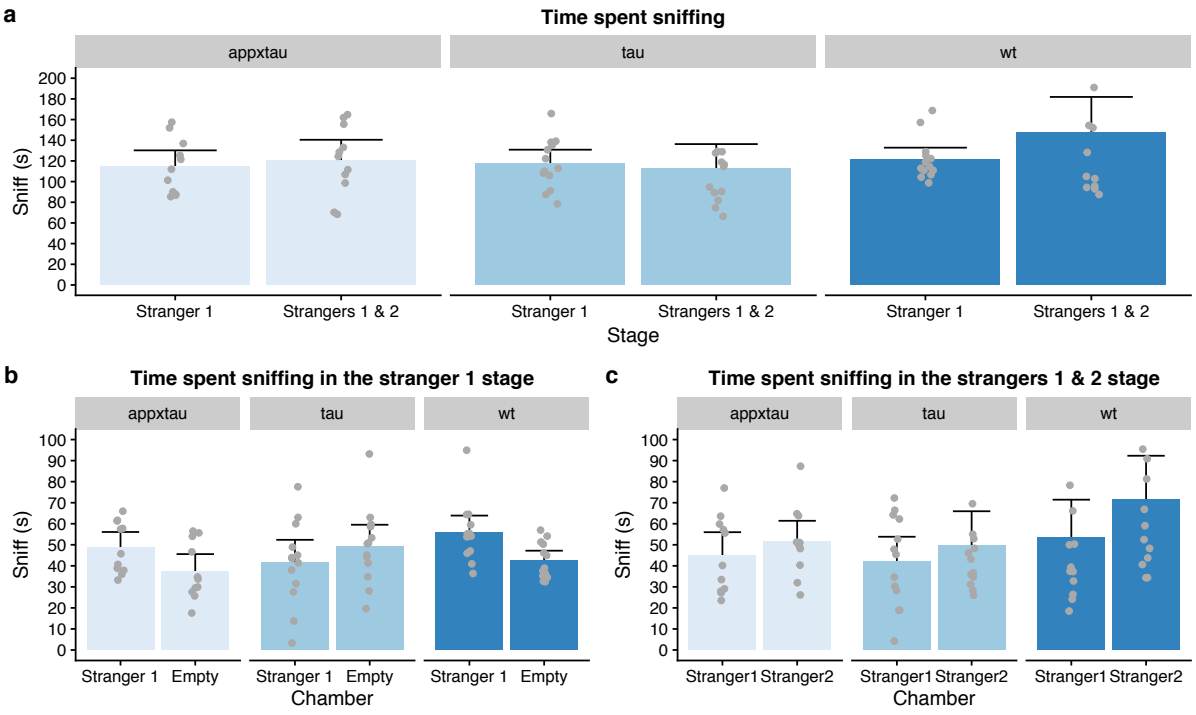

Figure 9. (a) Overall time spent sniffing. There was a significant effect of stage ($p = 0.0368$) and an interaction effect of stage and chamber ($p = 0.0067$) on overall time sniffing. (b) A genotype x chamber interacton significantly affected time spent sniffing in the stranger 1 or sociability stage ($p = 0.0197$). (c) Time spent sniffing in the strangers 1 & 2 or social novelty stage was significantly influenced by the chamber ($p = 0.0325$): animals sniffed the stranger 2 mouse significantly longer. Individual datapoints are presented on the plot.

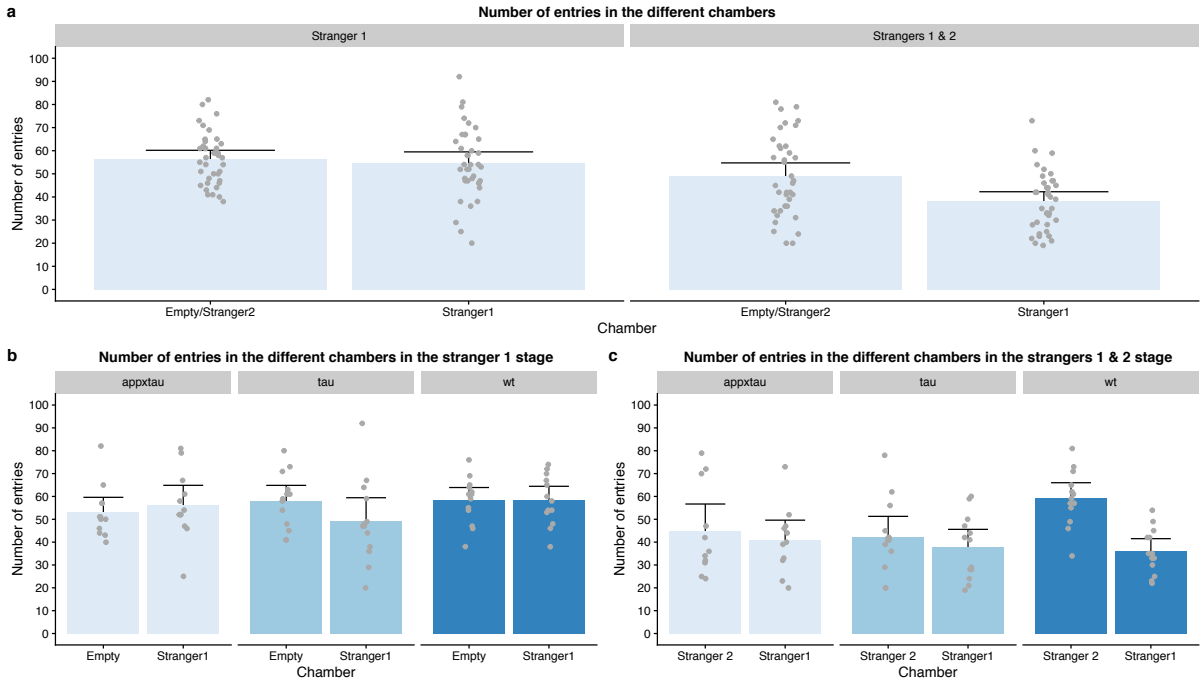

Figure 10. (a) Number of entries in the different chambers. There was a significant effect of stage on the number of entries in the stranger 1 chamber (p<0.000), with more entries in the sociability than in the social novelty stage. (b) - (c) Animals entered the stranger 2 chamber significantly more in the sociability stage than in the social novelty stage (p <0.000). Individual datapoints are presented on the plot.

Tail suspension

For the data-analysis of the tail suspension test, all tests were divided into two segments of 180 seconds. There was no effect of genotype, nor of test segment on the total time of immobility ($F(2,35) = 0.8975$, $p = 0.417$ and $F(1,35) = 2.9409$, $p = 0.095$). However, there was a significant effect of test segment on number of immobile episodes ($F(1,35) = 38.8036$, p <0.000): there were less immobile episodes in the second half of the test. The main effect of genotype on number of immobile episodes was non-significant ($F(2,35) = 2.4494$, $p = 0.101$). There were no main effects of genotype or test segment on average distance from the center ($F(2,35) = 0.1992$, $p = 0.820$; $F(1,35) = 3.6203$, $p = 0.065$), nor was there a significant effect of genotype on latency to start of the first immobile episode ($F(2,35) = 0.2047$, $p = 0.816$) (Figure 11).

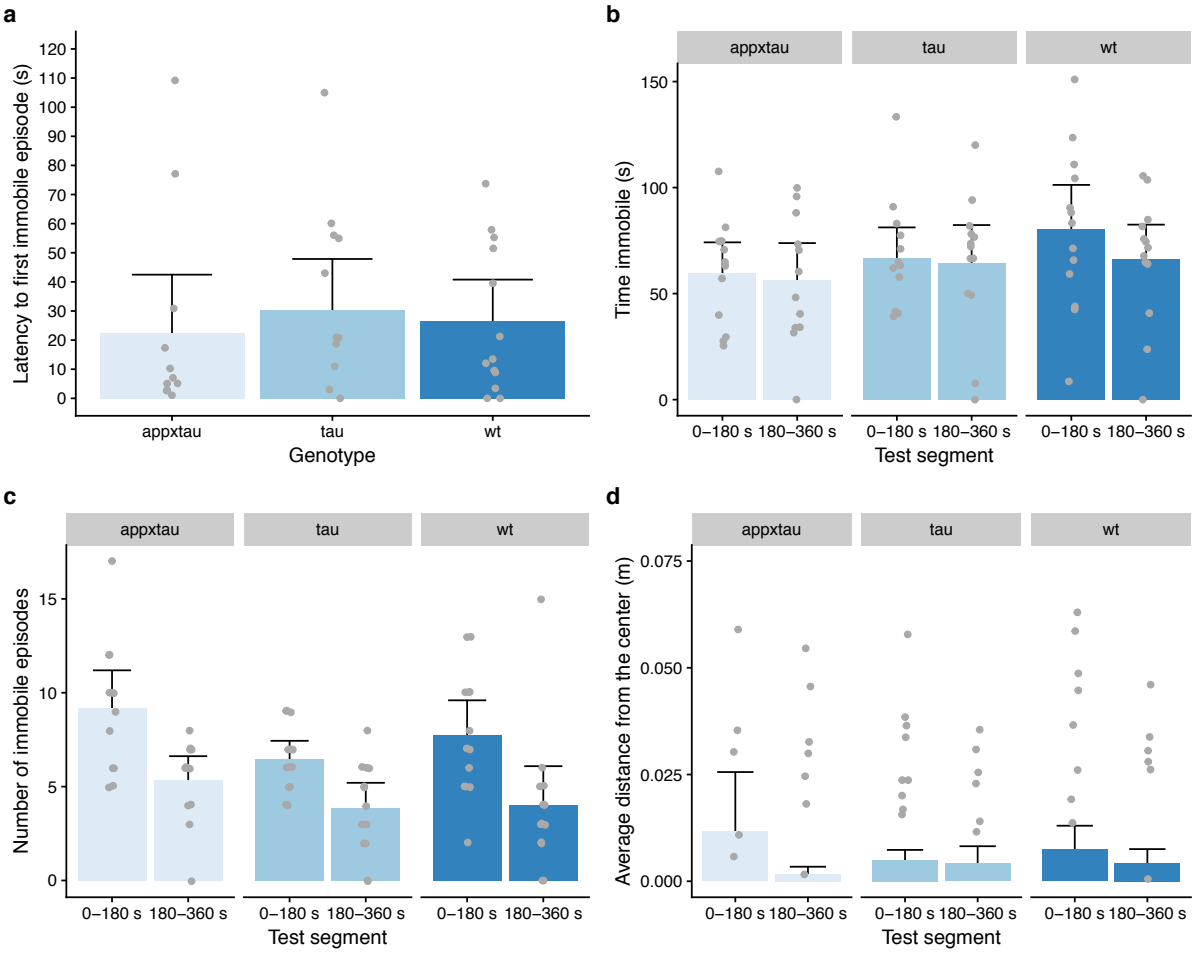

Figure 11. (a) Latency to first immobile episode was independent of genotype. (b) The effect of genotype and test segment on the total time immobile was also non-significant. (c) Number of immobile episodes were affected by test segment (p <0.000), but not by genotype. There were less immobile episodes in the second half of the test. (d) There were no significant differences in average distance from the center. Individual datapoints are presented on the plot.

Marble burying

Inspection of the means reveals small differences in marble burying score between APPxTau mice on the one hand and Tau and wild-type mice on the other hand (M = 7.5833, SD = 5.3676; M = 12.3846, SD = 5.1565 and M = 10.1538, SD = 4.0793 respectively) (see also Figure 12). However, this difference is marginally non-significant (F (2, 35) = 3.013, p = 0.062). A Tukey test revealed a significant difference between

APPxTau and Tau mice ($p = 0.049$), with Tau mice burying significantly more marbles, but no significant differences between wild-type and APPxTau or wild-type and Tau animals.

The assumptions of the statistical model were tested using the Brown-Forsythe test for heteroscedasticity ($p = 0.514$) and Durbin-Watson test for independence ($p = 0.29$). The Shapiro-Wilk test revealed no issues with normality ($p = 0.893$).

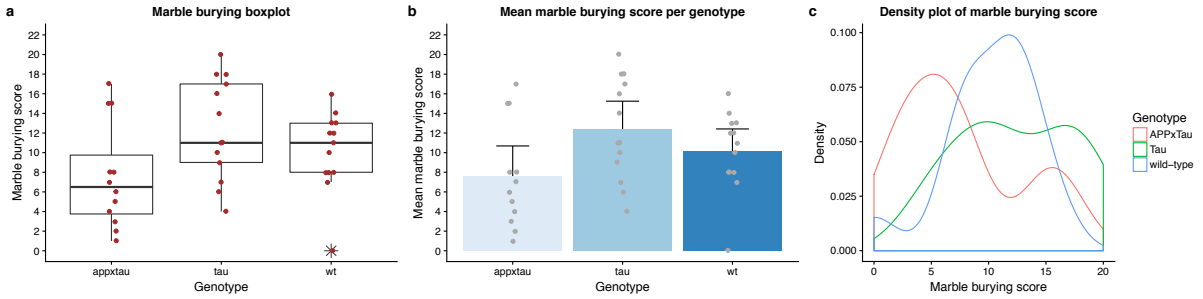

Figure 12. (a) Marble burying boxplot. Individual datapoints are represented on the plot. * indicates outliers. (b) Mean marble burying scores per genotype. There is no significant difference between genotypes ($p = 0.062$). Nonetheless, a Tukey test revealed a significant difference between APPxTau and Tau mice ($p = 0.049$), but no significant differences between wild-type and APPxTau or wild-type and Tau animals. Individual datapoints are represented on the plot. (c) Density plot of marble burying score for each genotype. The distributions are different across groups, indicating different score patterns.

Morris water maze

Acquisition A linear mixed-effects model indicated a significant main effect of acquisition day on the average path length ($F(1,1285.73) = 312.211$, $p < 0.000$). Post-hoc T-testing showed a significant difference between the first and second acquisition week: path length was shorter in the second week ($t = 12.129$, $p < 0.000$) (Figure 13, panel a).

Escape latency was significantly influenced by acquisition day as well ($F(1,1326.30) = 109.756$, $p < 0.000$), with post-hoc T-testing indicating a decrease in escape latency ($t = 5.5166$, $p < 0.000$). Despite the lack of effect of genotype, Dunnett's test revealed a significant difference between both Tau and wild-type and APPxTau and wild-type mice

(p <0.000 and $p = 0.0051$): wild-type mice had a higher escape latency (Figure 13, panel b).

Finally, a linear mixed-effects model revealed a significant main effect of acquisition day on mean velocity ($F(1,1325.42) = 198.397$, p <0.000): mean velocity actually decreased in the second week. Moreover, Dunnett's test again indicated a significant difference between Tau and wild-type and APPxTau and wild-type mice (both p <0.000), despite the lack of main effect of genotype in the model (Figure 13, panel c).

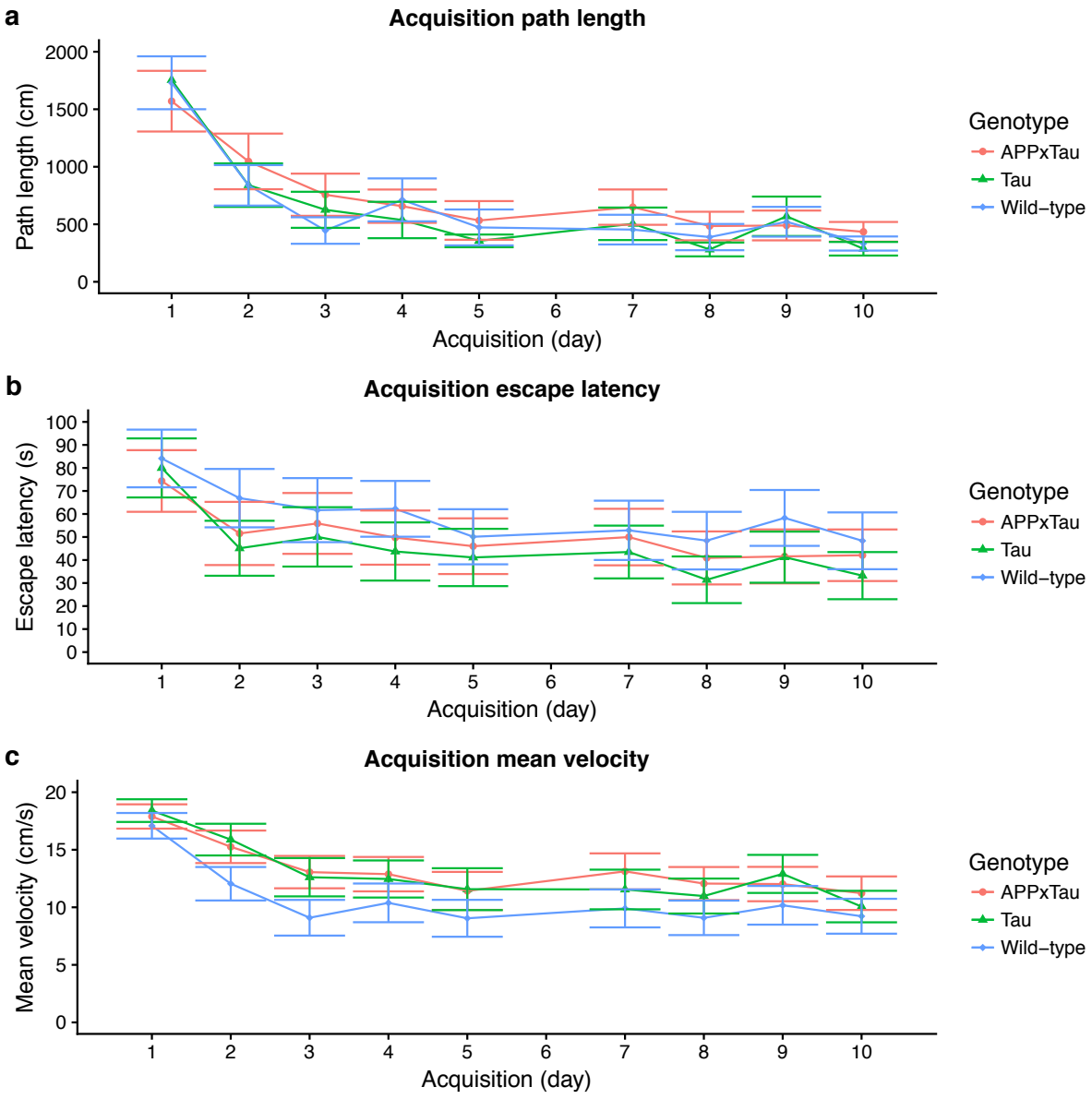

Figure 13. (a) Average path length in the Morris water maze. There was a main effect of acquisition day (p <0.000). (b) Escape latency was significantly influenced by acquisition day (p <0.000). (c) There was a main effect of acquisition day on mean velocity (p <0.000), albeit in the opposite direction of our expectations: animals became slower over time. Note: there are no data for the 6th acquisition day.

Acquisition probes For the probe trials, there was a significant effect of the quadrant ($F(3,432) = 9.2508$, p <0.000), indicating that mice spent more time in the target quadrant. However, there was a significant probe day x quadrant interaction ($F(3,432) = 4.3087$, $p = 0.0052$). Therefore, the linear mixed-effects model was analysed separately

for the first and the second probe day. On the first probe day, there was a significant effect of the quadrant on time ($F(3,140) = 12.3978$, $p < 0.000$). The same main effect emerged on the second probe day ($F(3,140) = 17.2922$, $p < 0.000$), but the interaction effect of genotype and quadrant was significant as well ($F(6,140) = 2.3073$, $p = 0.0373$). Overall, mice spent significantly more time in the target quadrant than in the adjacent quadrants (both $p < 0.000$), but there was no significant difference in the time spent in the target quadrant and the opposite quadrant ($p = 0.8202$) (Figure 14, panel a).

Number of entries in the quadrants was also influenced by the quadrant and a probe day x quadrant interaction ($F(3,397) = 8.8036$, $p < 0.000$ and $F(3,397) = 8.7868$, $p < 0.000$, respectively). Consequently, the linear mixed-effects model was again analysed separately for the first and the second probe day. For both probe days, there was a main effect of quadrant on number of entries ($F(3,105) = 4.3299$, $p = 0.0064$ and $F(3,105) = 10.8484$, $p < 0.000$). Post-hoc T-tests revealed a significant difference between the target and the opposite quadrant ($p = 0.0222$) and the target and the adjacent 1 quadrant ($p < 0.000$), but not between the target and the adjacent 2 quadrant ($p = 0.1033$). Dunnett's test again showed a significant difference between wild-type and Tau ($p = 0.0066$) and wild-type and APPxTau mice ($p = 0.0349$), although the main effect of genotype was non-significant ($p = 0.3250$) (Figure 14, panel b).

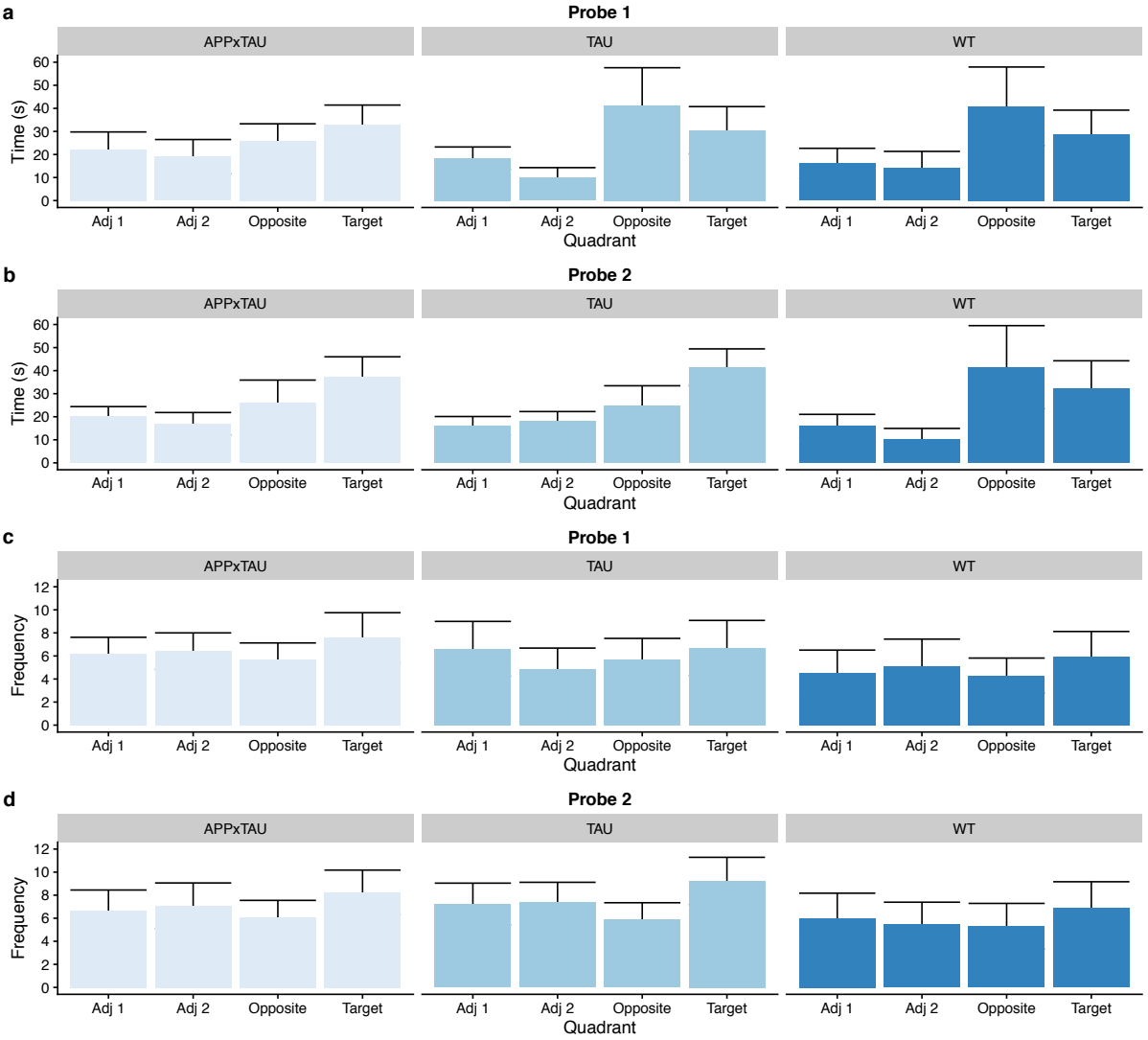

Figure 14. (a) Time spent in each quadrant during the first probe trial (day 6). There was a significant effect of quadrant on time (p <0.000). (b) Time spent in each quadrant during the second probe trial (day 11). There was a main effect of quadrant (p <0.000), but the genotype x quadrant interaction was significant as well ($p = 0.0373$). (c) Number of entries to each quadrant during the first probe trial. There was a main effect of quadrant on the number of entries ($p = 0.0064$). (d) Number of entries to each quadrant during the second probe trial is significantly influenced by quadrant (p <0.000).

Reversal During the third week, the platform was placed in the opposite quadrant as before to assess the flexibility of the mice's search strategy. Path length was significantly influenced by genotype ($F(2,151.95) = 8.273$, $p = 0.0004$) and reversal day

($F(1,719.75) = 117.425$, $p < 0.000$): path length gradually became shorter. There was a significant genotype x reversal day interaction ($F(2,719.74) = 4.136$, $p = 0.0164$). Post-hoc T-tests revealed a significantly shorter path length for wild-type than APPxTau mice ($p < 0.000$) and for Tau as compared to APPxTau mice ($p = 0.0006$) (Figure 15, panel a).

There were also main effects of genotype ($F(2,187.43) = 3.158$, $p = 0.0448$) and reversal day ($F(1,719.17) = 142.820$, $p < 0.000$) on escape latency: over time, the mice escaped faster. Post-hoc testing showed that APPxTau mice escape significantly slower than wild-type animals ($p = 0.0030$) (Figure 15, panel b).

Finally, reversal day and the genotype x reversal day interaction had a significant effect on the mean velocity ($F(1,716.18) = 107.204$, $p < 0.000$ and $F(2,716.17) = 3.861$, $p = 0.0215$). Mean velocity decreased over time, indicating the mice swam slower over time (Figure 15, panel c).

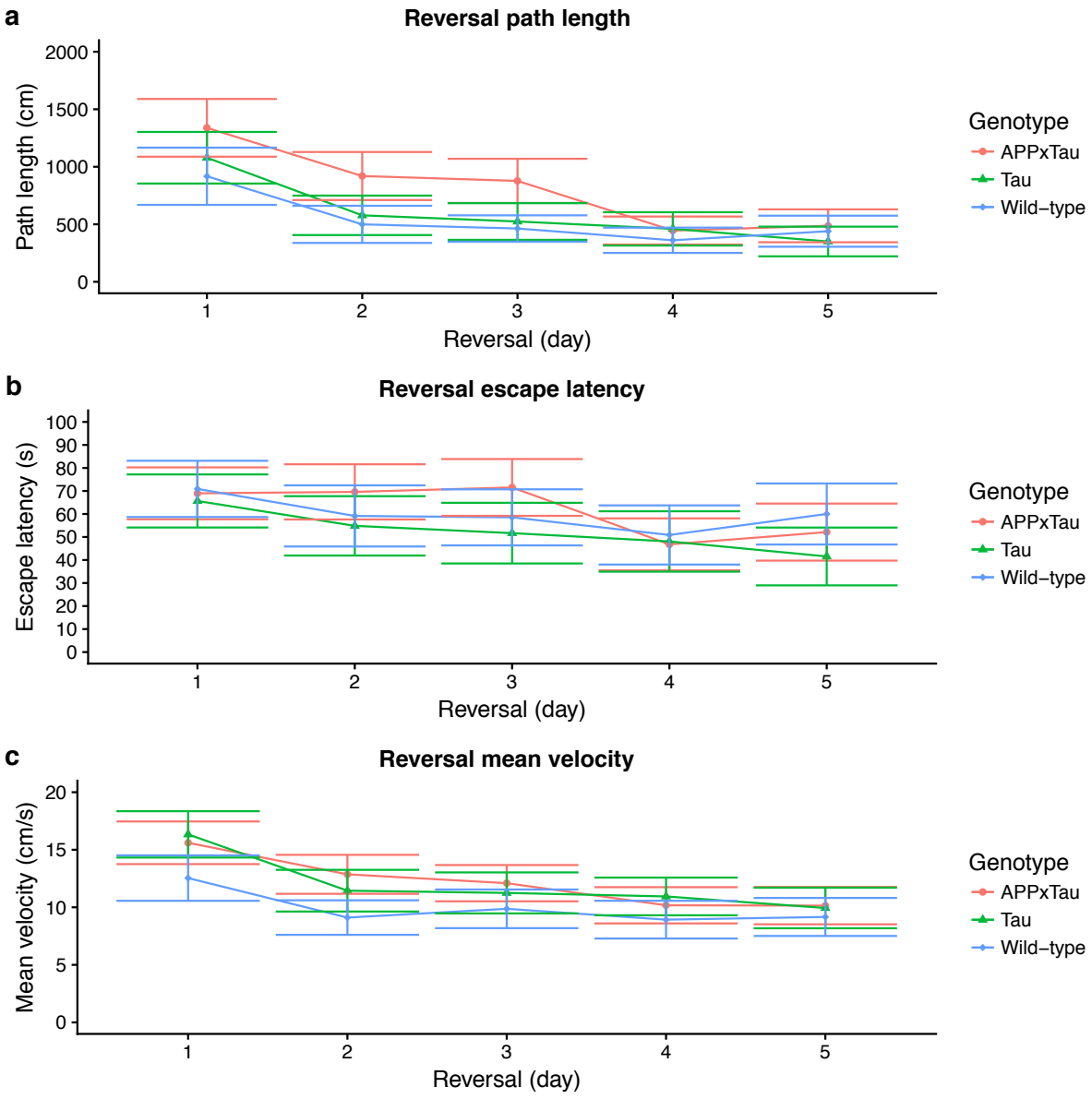

Figure 15. (a) Path length in the reversal trials. Path length was significantly affected by genotype ($p = 0.0004$) and reversal day ($p < 0.000$): path length became shorter over time. APPxTau animals had a significantly longer path length than Tau ($p = 0.0006$) and wild-type animals ($p < 0.000$). (b) There were main effects of genotype ($p = 0.0448$) and reversal day ($p < 0.000$) on escape latency: over time, mice escaped faster. (c) Mean velocity was significantly affected by reversal day ($p < 0.000$) and the genotype x reversal day interaction ($p = 0.0215$).

Reverse probe Quadrant had a significant effect on time in the reverse probe trial ($F(3,140) = 10.7058$, $p < 0.000$). A post-hoc T-test revealed a significant difference between time in the reverse-target quadrant and time in the adjacent 1 quadrant ($p < 0.000$) (Figure 16, panel a).

A linear mixed-effects model also revealed a significant effect of quadrant on the number of entries in each quadrant ($F(3,105) = 10.7006$, $p < 0.000$). Post-hoc T-tests showed a significant difference between the reverse-target and the adjacent 1 quadrant ($p < 0.000$), the reverse-target and the adjacent 2 quadrant ($p = 0.0009$), and the reverse-target and the opposite quadrant ($p = 0.0129$) (Figure 16, panel b).

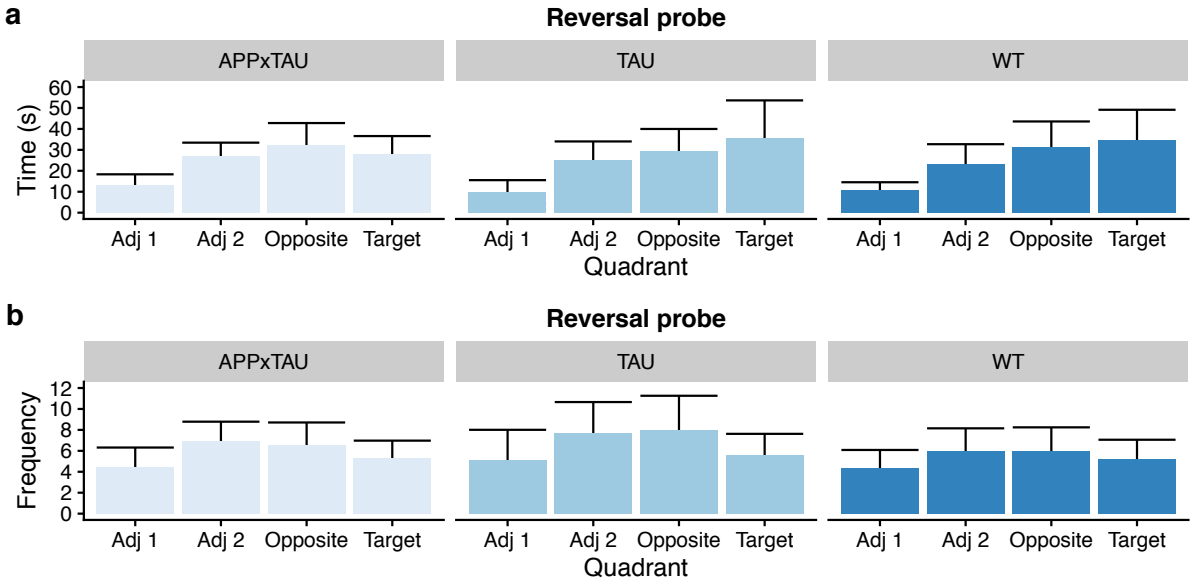

Figure 16. (a) Time in each quadrant on the reverse probe trial (day 16) was significantly influenced by quadrant ($p < 0.000$). (b) Number of entries in each quadrant was also significantly affected by quadrant ($p < 0.000$).

Context- and cue-dependent fear conditioning

Fear conditioning A linear mixed-effects model revealed a significant effect of genotype, stage and the interaction between genotype and stage on percentage freezing ($F(2, 35.103) = 8.845$, $p < 0.000$, $F(3, 104.297) = 256.612$, $p < 0.000$ and $F(6, 104.295) = 4.082$, $p = 0.0010$ respectively). Post-hoc T-tests indicated a significant higher level of freezing during fear conditioning than during habituation ($p < 0.000$) and during context test and cued fear test than during fear conditioning (both $p < 0.000$). There was no

significant difference in percentage freezing between the context test and cued fear stage ($p = 0.2087$). There was a significant effect of genotype on the percentage freezing during habituation ($\chi^2 = 8.1226$, $p = 0.0172$), fear conditioning before presentation of the CS ($\chi^2 = 8.6248$, $p = 0.0134$), the context test ($F(2, 35) = 9.144$, $p < 0.000$) and the cued fear test ($F(2, 35) = 5.188$, $p = 0.0106$): overall, APPxTau and Tau animals had a higher percentage freezing than wild-type mice (Figure 17, upper panel).

Retention The linear mixed-effects model showed a significant effect of genotype, stage and the interaction between genotype and stage on percentage freezing during the retention phase ($F(2, 35) = 25.8108$, $p < 0.000$, $F(1, 35) = 13.4432$, $p < 0.000$ and $F(2, 35) = 5.1106$, $p = 0.0113$ respectively). There was a significant effect of genotype on the percentage freezing during the context test ($F(2, 35) = 21.05$, $p < 0.000$) and the cued fear test, both with and without CS present ($F(2, 35) = 18.26$, $p < 0.000$, $F(2, 35) = 5.753$, $p = 0.0069$ and $\chi^2 = 14.97$, $p = 0.0006$). Wild-type animals again had a lower percentage freezing than APPxTau and Tau mice (Figure 17, lower panel).

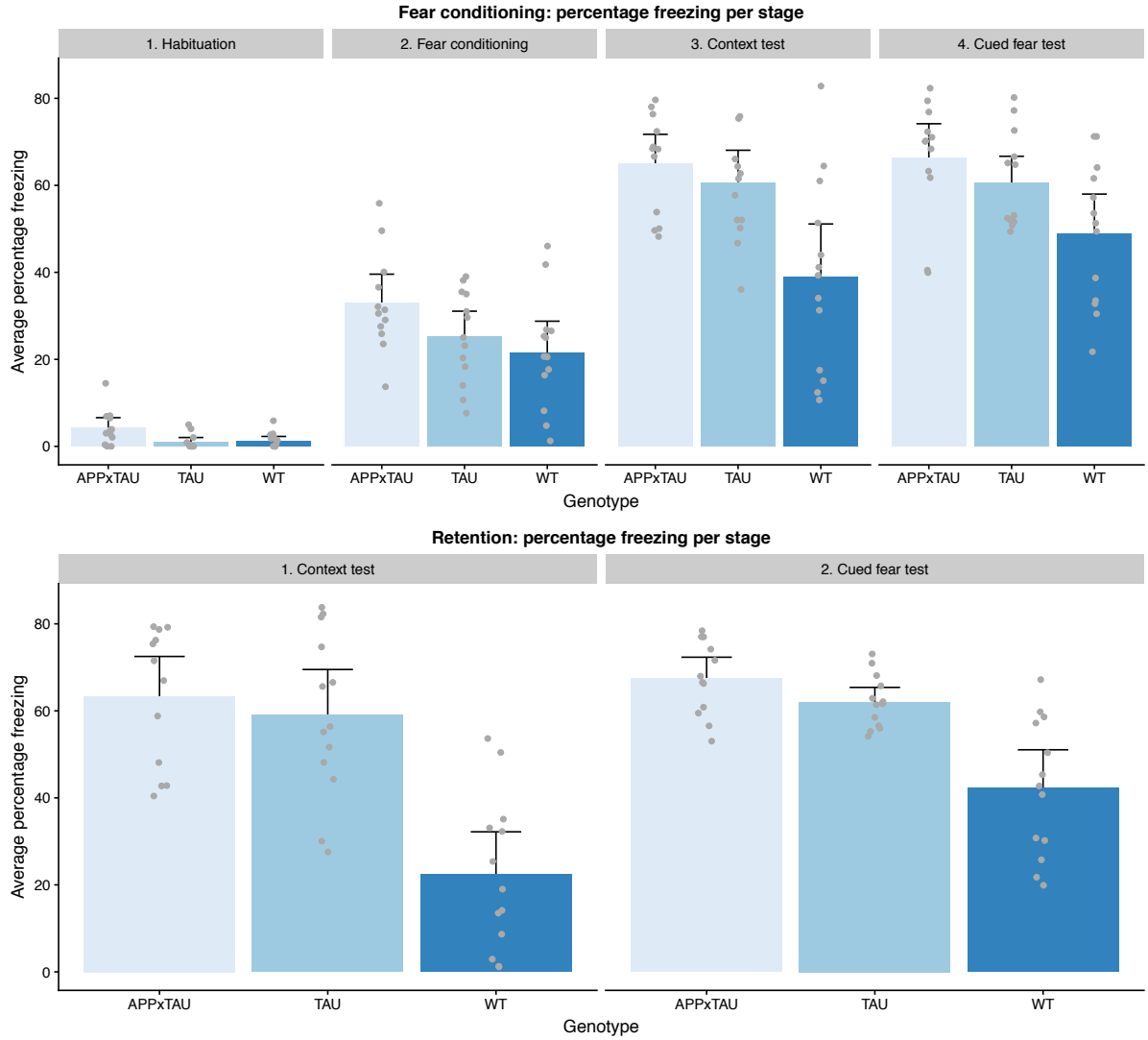

Figure 17. Percentage freezing per genotype and stage for fear conditioning and retention. During the fear conditioning, there was a significant effect of genotype (p <0.000) and stage (p <0.000) on percentage freezing. The interaction term of genotype and stage was significant as well (p = 0.0010). During retention, genotype and stage, as well as a genotype x stage interaction, significantly influenced percentage freezing (p <0.000, p <0.000 and p = 0.0113, respectively). Individual datapoints are presented on the plot.

Tail withdrawal

Reaction to thermal nociceptive stimuli was operationalised as the latency to tail withdrawal. There was an expected significant effect of temperature ($F(3,105)$ = 49.439, p <0.000): higher temperatures led to faster tail withdrawal (Figure 18). Post-hoc T-tests

revealed a significant difference between tail withdrawal at 47°C and 49°C (p <0.000), 47°C and 51°C (p <0.000), 47°C and 53°C (p <0.000), 49°C and 51°C (p = 0.0397) and 49°C and 53°C (p = 0.0081). The difference between 51°C and 53°C was non-significant (p = 0.7498). There was no effect of genotype on the latency of tail withdrawal (F(2,35) = 0.8322, p = 0.4435), nor an interaction effect between genotype and temperature (F(6,105) = 1.697, p = 0.1288) (Figure 19).

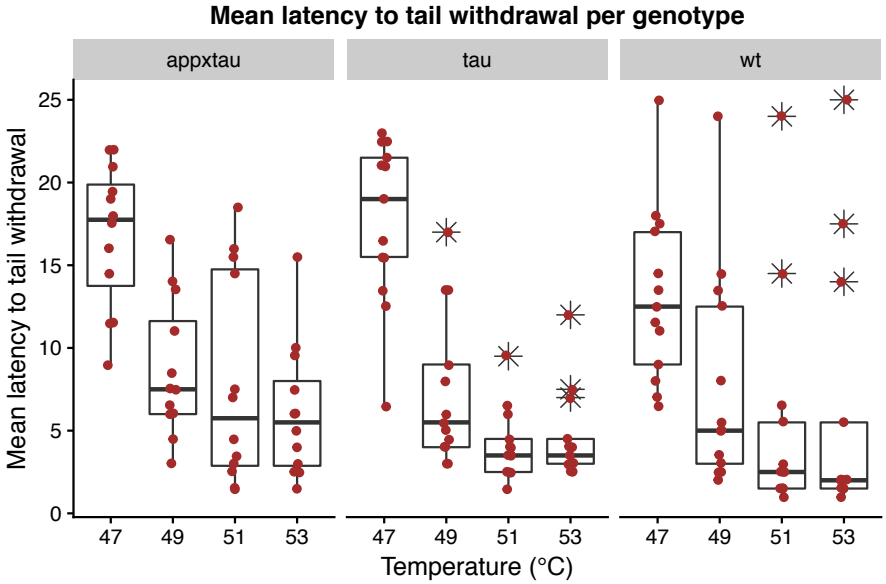

Figure 18. Mean latency to tail withdrawal per genotype. The boxplot shows an expected significant effect of temperature (p <0.000): higher temperatures led to faster tail withdrawal. * indicates outliers.

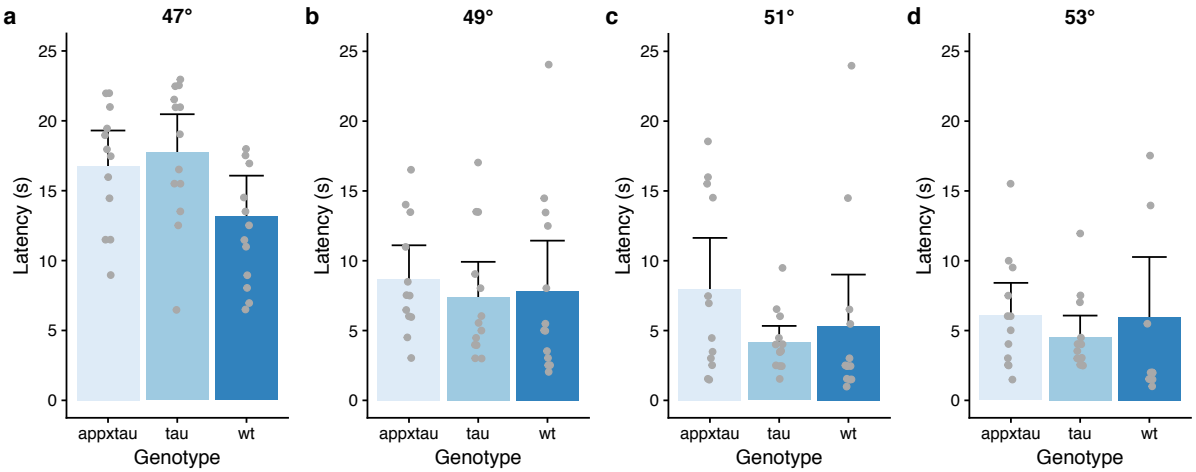

Figure 19. Mean latency to tail withdrawal for each temperature. There was no main effect of genotype on the latency of tail withdrawal ($p = 0.4435$), nor an interaction effect between genotype and temperature ($p = 0.1288$). Individual datapoints are presented on the plot.

Discussion

In the present study, we aimed to identify early behavioural and cognitive deficits in two transgenic mouse models of AD, more specifically Tau.P301L (TPLH mouse model) and APP.V717I x Tau.P301L (biAT mouse model) mice. To achieve this goal, the performance of transgenic mice in several behavioural and cognitive tasks was compared to that of C57BL/6J wild-type animals at the age of three months. As explained in the introduction of this thesis, the tasks were chosen to detect changes in behavioural traits that resemble preclinical cognitive and non-cognitive impairments in humans as well as impairments that arise in later stages of the disease.

Deficits in episodic memory, caused by pathology in the hippocampal area, arise very early and are a key characteristic of preclinical AD (Webster et al., 2014). In mice, deficits in hippocampus-dependent spatial learning and memory typically emerge after three to six months, depending on the mouse model (Webster et al., 2014). Consequently, reference memory and working memory were assessed using the MWM. During the acquisition days, there was a significant difference in escape latency: transgenic mice escaped faster than age-matched wild-type animals. However, analysis of the swimming speed revealed that in general, transgenic mice swam faster than wild-type animals. Overall, there was no difference between genotypes in average path length, indicating no difference in spatial learning. During the probe trials, transgenic mice entered the target quadrant more often than wild-type mice. Yet, it is important to note that transgenic mice entered all quadrants more often than wild-type mice. During the reversal trials, which evaluated cognitive flexibility, wild-type animals had a shorter path length than transgenic mice and they escaped faster than APPxTau animals. These findings indicate that transgenic, especially APPxTau mice, were less flexible in their search strategy than wild-type mice. This finding corresponds to the early deficits in executive functioning found in humans (Bäckman et al., 2005), yet, the animals did not display deficits in hippocampus-dependent learning. However, two important considerations need to be taken into account when interpreting the results above. First of all, on top of the main effects described, there are also many interaction effects, which make it more difficult to interpret these main effects. Therefore, one has to be very careful in drawing conclusions. Secondly, four animals, of which three wild-types and one Tau, displayed severe floating behaviour, defined as spending at least 10% of the trial floating on the water. These an-

imals were treated as outliers and removed from additional analyses for the probe trials, but the results remained largely similar. Therefore, the outlying mice were included in the final analyses.

Hippocampal learning and memory was also assessed using the context- and cue-dependent fear conditioning protocol (Paradee et al., 1999). Overall, during the fear conditioning, transgenic mice displayed a higher level of freezing than control mice. This pattern of increased freezing was also present in the retention phase. These results cannot be explained by differences in pain perception, since the tail withdrawal test revealed no effect of genotype on nociceptive behaviour. Consequently and in line with a study by Wang, Dineley, Sweatt and Zheng (2004), fear learning was not impaired in transgenic mice. On the other hand, these findings indicate increased anxiety in transgenic mice, which is an early symptom of preclinical AD.

In general, preclinical AD is characterised by an increased prevalence of late-life psychiatric symptoms, especially anxiety and mood disorders (Steenland et al., 2012). Consequently, this study specifically assessed anxiety-related and depressive behaviour in mice. Decreased exploration of the open arms in the elevated plus maze, for example, indicates anxiogenic behaviour. Transgenic mice spent significantly less time in the open arms than age-matched control animals, which is indicative of increased anxiety in these transgenic mice. Nevertheless, this finding was not replicated in the open field task, in which wild-type animals were further away from the center on average than transgenic mice, indicating an opposite conclusion. Digging activity and anxiety-like behaviour, assessed with marble burying, does not seem altered in transgenic mice either, as there was no significant difference in marble burying scores between genotypes. However, in contrast to the scores of Tau and wild-type animals, the marble burying scores in APPx-Tau mice seem to be bimodally distributed. Therefore, the effect of rearing environment, more specifically the cage in which animals grew up, was checked as a covariate, but did not influence the scores. Together, these findings provide substantive but somewhat inconclusive evidence that anxiety-related behaviour in transgenic mice is increased as compared to controls. Depression-like behaviour, measured by the tail suspension test, on the other hand, did not differ between wild-type and transgenic mice. There were no significant differences in immobility time, number of immobile episodes or latency to first immobile episode.

Alzheimer's disease is often characterised by social withdrawal in both humans and mice (Filali, Lalonde, & Rivest, 2011) and this social withdrawal is already present before AD diagnosis (Jost & Grossberg, 1996). In the SPSN sociability stage, Tau mice spent significantly more time sniffing the empty cage than the cage with the stranger 1 mouse, while the reverse was true for APPxTau and wild-type mice. During the social novelty stage, all mice spent more time sniffing the novel stranger mouse (stranger 2) than the stranger 1 mouse. There was a significant difference between genotypes in the number of entries to the chambers, but the time spent inside the chambers did not differ between transgenic and wild-type mice, indicating that they all spent a similar amount of time with the stranger mice. Thus, wild-type and transgenic mice did not differ in their preference for social novelty, but Tau mice showed a decreased sociability as compared to APPxTau and wild-type mice.

Preclinical AD and MCI are mainly characterised by cognitive symptoms (Bateman et al., 2012) and motor skills are often affected in later stages of the disease. Nonetheless, exploration and motor activity were assessed in several behavioural tasks. The number of crossings in the elevated plus maze is indicative of the exploratory behaviour of the mice. Wild-type mice crossed the infrared beams in the maze significantly more often than Tau or APPxTau mice, which indicates reduced exploratory behaviour in the transgenic mice, even at an early age. The total distance travelled in the open field task also differed significantly between genotypes, with transgenic mice showing reduced exploration as compared to wild-type mice. The same finding emerged in the SPSN test, where wild-type mice consistently travelled further in all stages (acclimation, sociability and social novelty) than the transgenic mice. In the spontaneous activity task, all animals showed the expected first exploratory peak and three nocturnal peaks. However, the first two peaks were less strong for APPxTau and Tau animals than for wild-type animals, again indicating less exploratory behaviour. Motor coordination was assessed using the accelerating rotarod. In our sample, there was no significant effect of genotype on the latency to fall. To conclude, these tasks revealed a significant and robust decrease in exploratory behaviour and a small but significant drop in the first nocturnal peak in young transgenic mice compared to controls. However, they do not display any changes in motor coordination.

Finally, two nesting tasks were used to assess natural murine behaviour, which can

be translated to activities of daily living (ADLs) in humans (Deacon, 2012). ADLs like bathing, dressing oneself and eating, are typically affected in later stages of AD (Alzheimer Association, 2016), although subtle differences in instrumental activities of daily living (IADLs) might already present up to ten years before diagnosis (Fuentes, 2012). In the first nesting task, using paper as nesting material, APPxTau mice scored significantly worse than both Tau and wild-type mice, whose scores were very similarly distributed. Yet, this finding was not replicated in the second nesting task, using Cocoon nestlets, in which there was no effect of genotype on scores at all.

Although mice have shown strong preferences for and increased nesting behaviour with certain nesting materials over others (Van de Weerd et al., 1997), an immediate comparison between paper and Cocoon has not been made. Therefore, it is unclear if preference for nesting material contributes to the difference in results in the nesting and nesting 2.0 test. If Cocoon is preferred over paper, this could explain the increased nesting behaviour and quality of nests in all groups. However, the results could also be influenced by the difficulty of the nesting material. We hypothesize that making nests with suboptimal material like paper is more difficult and therefore also influenced by motor coordination and activity level, which was affected in transgenic mice in this study. However, a learning effect could also account for the differences in performance and cannot be excluded. Finally, the difference in results could be due to experimenter differences: even though the nests were rated by two individual experimenters, both blind to the genotype of the animals, it is possible that rating the paper nests was more difficult and ambiguous than rating the Cocoon nests.

The significant changes in behaviour reported above are supported by reduced long-term potentiation (LTP) in three to five month old Tau.P301L and APP.V717I x Tau.P301L mice, albeit a different batch. Schreurs and colleagues (manuscript in preparation) performed field excitatory postsynaptic potential (fEPSP) recordings in acute brain slices and measured basal synaptic transmission, paired-pulse responses and synaptic plasticity in the prefrontal cortex, hippocampal CA1 and dentate gyrus. LTP was induced by high-frequency stimulation to the tissue and was recorded for at least two hours. Both Tau and APPxTau mice showed impaired LTP in CA1 and the prefrontal cortex, regions that are involved in memory retrieval and higher cognitive functions. CA1 also showed altered paired-pulse responses and more epileptiform activity in the transgenic mice (Figure S6).

These electrophysiology findings are in accordance with the behavioural changes found in this study, and provide additional evidence for early preclinical changes in these AD mouse models.

Previous research found differences in cognitive performance between transgenic Tau mouse models and transgenic APP models (Lo et al., 2013). Therefore, we wanted to compare Tau mice with APPxTau mice, since the APPxTau model more closely mimics human AD pathology. Other than the increased cognitive inflexibility en decreased nesting behaviour in the first nesting task in APPxTau, we found no differences in behavioural and cognitive impairments between the two transgenic models. This is surprising, as the presence of Aβ oligomers in APPxTau would be expected to accelerate tau pathology (Selkoe & Hardy, 2016) and cognitive impairment (Webster et al., 2014; Spires-Jones & Hyman, 2004). Consequently, the data in this thesis do not support the amyloid cascade hypothesis (Selkoe & Hardy, 2016).

Mouse models have been developed for a wide variety of diseases, including AD, and are in many cases preferable over *in vivo* or *in vitro* human studies since they make it possible to mimic a specific pathology and study therapeutic interventions extensively. Nonetheless, there are some limitations to this study and studies with animal models in general. First, mice do not display the full range of human behaviour and mouse models cannot model all aspects of human behaviour (Webster et al., 2014). Furthermore, no animal model can fully simulate the pathology of human AD. Moreover, as mentioned before, most mouse models are based on genetic mutations found in the familial variant of AD, which accounts for only 5% of human AD (Drummond & Wisniewski, 2017). Consequently, mouse models should always be selected with consideration for their application and the goals of the study. For future studies, it could be interesting to repeat this study with other transgenic mouse models as well as knock-in mouse models. Knock-in mouse models, for example APPNLGF, show less overexpression of APP in general and therefore have a more natural phenotype that is closer to human AD (Latif-Hernandez et al., 2016). In general, it is difficult to directly compare different transgenic mouse strains (Götz et al., 2004), yet, future research should try to replicate the current findings with other mouse models in order to develop a robust taxonomy of preclinical behavioural and social changes in mice. Finally, we did not investigate Aβ and tau pathology in this sample, so we are unable to directly correlate the cognitive and behavioural impairments

to specific brain changes in this batch. Yet, we would not expect large differences in neuropathology between batches, so it is possible to indirectly relate behavioural observations to underlying pathology. Future research should however strive to collect both behavioural and physiological data in the same sample to uncover the temporal mechanisms of early AD.

Conclusion

Alzheimer's disease is one of the most common diseases in older age and its worldwide prevalence is expected to increase drastically in the near future. Research has shown that AD develops over many years, with changes in the brain present long before the onset of the typical AD symptoms. Moreover, several preclinical behavioural changes might indicate conversion to AD, thereby enabling earlier diagnosis and more efficient interventions. This study aimed to identify preclinical changes in the cognitive and social behaviour of two transgenic mouse models for AD, Tau.P301L (TPLH mouse model) and APP.V717I x Tau.P301L (biAT mouse model), in order to lay the foundations for better detection of preclinical AD in at-risk individuals. We uncovered differences in natural murine behaviour (nesting), exploratory behaviour (elevated plus maze, open field and SPSN), anxiety-like behaviour and fear learning (elevated plus maze and CFR) and inflexibility in hippocampus-dependent learning. These findings can be indicative of small but significant changes that are present in older adults with preclinical AD as well. Consequently, the findings presented here are important in the advancement of early diagnosis of AD. However, follow-up research is necessary to identify clear behavioural markers of preclinical AD that are applicable in clinical settings.

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