ALZHEIMER'S DISEASE HALLMARKS:

BETA-AMYLOID IN THE HIPPOCAMPUS OF TGF344-AD RATS AND ITS RELATION TO COGNITIVE IMPAIRMENT

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ABSTRACT – ENGLISH

Alzheimer's Disease has been known for more than 100 years, affecting more and more people as the human population grows ever older. To this day, the disease remains untreatable and unpreventable, and the full etiology has stayed unknown. Immense amounts of research about Alzheimer's Disease are conducted every year to learn more about the disease that robs people of their memories, their personalities, and their elderly family members. Considerable progress has been made, and brain mapping has come a long way, opening doors for gaining knowledge about human Alzheimer's Disease in vivo. When it comes to development of treatments, however, animal models of the disease are in the front of the field, yet treatment paradigms have failed repeatedly when applied to humans, in spite of working perfectly well in animal models. One possible reason for such dissonance is that the animal models employed may not be complex enough to fully mirror the disease. Granted, such models are invaluable when it comes to learning about the individual features of the disease, but they cannot stand as adequate models for developing broad treatments for Alzheimer's Disease. One very promising, fairly recent animal model, the TgF344-AD rat model, developed by Cohen and colleagues in 2013, may provide a better integrative model, as these rats display more features of Alzheimer's Disease than most other animal models. The model has been investigated with regards to both neuropathology, cognitive impairment, and neuropsychiatric symptoms, and shows great promise as a tool in the process of learning more about the disease that plagues more and more humans. Nonetheless, there is still knowledge to gain about this model. The aim of this thesis is to present current knowledge about Alzheimer's Disease, as well as the TgF344-AD model, and to examine the presence of beta-amyloid in the hippocampus of the rats via immunohistochemistry. Furthermore, the study compares the beta-amyloid levels with impairments in memory, measured on the hippocampus-dependent spatial memory test, Barnes Maze. 8 10-month-old TgF344-AD rats and 8 Fisher 344 wildtype littermates matched for age and gender are employed in the study. A significant difference in beta-amyloid is found between the genotypes, with virtually none being present in the wildtype littermates, whereas on average 9.84% of the hippocampi of transgenic rats is covered in plaques. This is compared with the presence of beta-amyloid in a rat from a previous study, and the

method of immunohistochemistry is discussed. As for memory impairments, no correlation is found with the amount of beta-amyloid present in the hippocampus, which is similar to results found in studies performed on human Alzheimer's Disease patients. Confounding factors, including neuropsychiatric symptoms, are discussed. In sum, the results of this thesis continue to support the promising potential of the TgF344-AD rat model, as its disease course seems to be comparable with that of human Alzheimer's Disease patients on several aspects.

ABSTRACT – DANSK

Alzheimers Demens har været kendt i over 100 år, og flere og flere mennesker rammes eftersom mennesker lever længere og længere. Den dag i dag er sygdommen uhelbredelig og kan ikke forebygges, og dens fulde ætiologi er fortsat ukendt. Enorme mængder forskning om Alzheimers Demens udføres hvert år for at lære mere om sygdommen, som frarøver folk deres minder, deres personlighed og deres ældre familiemedlemmer. Der er gjort betydelige fremskridt, og kortlægning af hjernen er kommet langt, hvilket har ledt til muligheder for at lære om Alzheimer Demens in vivo i mennesker. Når det kommer til udvikling af behandlinger, står dyremodeller derimod forrest i feltet, men alligevel har behandlingsparadigmer fejlet gang på gang når de testes på mennesker, på trods af at fungere helt fint i dyremodeller. En mulig forklaring på denne dissonans er, at dyremodellerne måske ikke er komplekse nok til fuldt ud at repræsentere sygdommen. Disse modeller er ganske nok uvurderlige når det kommer til at lære om sygdommens individuelle aspekter, men de er utilstrækkelige modeller for udviklingen af generel behandling for Alzheimers Demens. En meget lovende og relativt ny dyremodel, TgF344-AD rottemodellen, udviklet af Cohen og kollegaer i 2013, byder muligvis på en mere integrativ model, eftersom disse rotter udviser flere tegn på Alzheimers Demens end de fleste andre dyremodeller. Modellen er blevet undersøgt med hensyn til både neuropatologi, kognitive begrænsninger og neuropsykiatriske symptomer, og udviser stort potentiale som et redskab til at lære mere om den sygdom, som plager flere og flere mennesker. Der er dog stadig viden at opnå om denne model. Formålet med dette speciale er at præsentere aktuel viden om Alzheimers Demens, såvel som om TgF344-AD modellen, og at undersøge tilstedeværelsen af beta-amyloid i rotternes hippocampus via immunohistokemi. Yderligere, studiet sammenligner niveauet af beta-amyloid med hukommelsesproblemer, målt på den hippocampusafhængige spatiale hukommelsestest, Barnes Maze. 8 10 måneder gamle TgF344-AD rotter og 8 Fisher 344 vildtype kuldkammerater matchet for alder og køn er anvendt i dette studie. En signifikant forskel i beta-amyloid blev fundet mellem genotyperne; Hvor virtuelt intet var til stede i vildtyperotterne, var gennemsnitligt 9,84% af de transgene rotters hippocampi dækket af plaques. Dette er desuden sammenlignet med tilstedeværelsen af beta-amyloid i en rotte fra et tidligere studie, og metoden

immunohistokemi diskuteres. Hvad angår hukommelsesproblemer sås ingen korrelation mellem dette og mængden af beta-amyloid i hippocampus, hvilket stemmer overens med resultater fra studier af menneskelige Alzheimers Demens patienter. Konfunderende faktorer, inklusiv neuropsykiatriske symptomer, diskuteres. Samlet set, bakker dette speciales resultater op om TgF344-AD rottemodellens lovende potentiale, eftersom sygdomsforløbet synes at være sammenligneligt med menneskelige Alzheimers Demens patienters sygdomsforløb på flere områder.

TABLE OF CONTENTS

INTRODUCTION

In 1901, the now famous patient Auguste D. started showing symptoms of sleep disorders, memory disturbances, aggressiveness, and confusion. These symptoms were of a progressive type and untreatable, for which reason she was admitted to the Frankfurt Psychiatric Hospital. Here she met the psychiatrist Alois Alzheimer, who took a great curiosity to her case and documented it meticulously. Auguste D. passed away April 8th, 1906. The disease that Auguste D. suffered from was later named Alzheimer's Disease, or Alzheimer's Dementia (AD) (Hippius & Neundörfer, 2003, p. 101).

Dementia, from the latin *de* (meaning apart or away) and *mens* (meaning mind), is a broad term for a group of clinical syndromes presenting with cognitive impairments severe enough to interfere with a patients' daily life activity (Dierckx, 2007, p. 11). AD is a specific type of dementia, and the most prevalent of the dementias, as it is estimated that about 70% of dementia cases follow the pattern of AD (WHO, 2020). Like it was the case for Auguste D., this impairment will eventually be so severe that many AD patients have to receive caregiving from friends and families, from professionals, or even be admitted to specialized care-taking facilities. AD therefore becomes a burden not only to the affected patient, but to everyone around them as well. Hence, it is worrisome that this disease is still untreatable (Alzheimer's Association, 2021, p. 332; p. 350). Other diseases, however, are increasingly becoming treatable, and for this reason humans now live longer than ever before. Thereby, diseases like AD, which have a higher incidence the older the people, are becoming more prevalent (Dierckx, 2007, p. 11). AD is currently the sixth leading cause of death, and the fifth leading cause for those above the age of 65 (Alzheimer's Association, 2021, p. 345). Of the ten diseases that cause the most deaths, AD is the only one that cannot be prevented, nor cured (Bayles & Tomoeda, 2013, p. 48).

For these reasons, research into possible treatments or simply more knowledge about the disease has been growing immensely in the past decades. Although considerable progress has been made, the etiology of the disorder is still to be fully understood, and successful treatment for AD is therefore still some way off (Jellinger, 2006, p. 1604; Zanni et al., 2018, p. 849). The study described in this thesis is exactly an

effort of gaining more knowledge about the disease etiology, particularly by researching one animal model of AD, the TgF344-AD rat model. The model, created by Cohen and colleagues (2013), is fairly recent and studies about it are limited. Nonetheless, the model is one of the most promising animal models for AD research currently existing (Tudela et al., 2019, p. 2). It is therefore interesting to gain further insight into the behavioral and neuropathological development of this model before drafting it into treatment research, which is precisely what this study will explore by looking into the AD neuropathology present in the hippocampus of the rat model at the age of 10 months, and comparing this data with data of cognition and behavior at the same age.

BACKGROUND

To research and evaluate the TgF344-AD model appropriately, it is however first necessary to consider previous research and current knowledge on Alzheimer's Disease, both clinically and neuropathologically, and on various methods for gaining this knowledge, including brain mapping techniques and animal models. This section will consider these topics.

ALZHEIMER'S DISEASE

Following the death of Auguste D. in 1906, Alois Alzheimer performed an autopsy on her, revealing histological alterations in her brain that are now known as beta-amyloid (Aβ) plaques and neurofibrillary tau tangles (NFT) (Hippius & Neundörfer, 2003, p. 106) (cf. *Neuropathology of AD*, p. 24). These neuropathological changes are still the golden standard of AD diagnostication today. The presence of Aβ plaques and NFT is established postmortem, as is the extent of the atrophy that is common in AD patients. However, as technology advances, it is to some extent also possible to establish while the patient is still alive (Rice & Bisdas, 2017, p. 16; Long & Holtzman, 2019, p. 315). Furthermore, a diagnosis of 'probable AD' can be given based on cognitive and behavioral symptoms (WHO, 1994, pp. 41-43).

Currently, about 50 million people suffer from AD worldwide, and this number is increasing (WHO, 2020). About 7.7 million new cases arise each year, and it is estimated that 115 million people will have AD by 2050 (Bayles & Tomoeda, 2013,

p. 48). At age 65 the prevalence of the disease is 0.9%, at age 75 it is 7.4%, and in patients older than 85 the prevalence is as high as 26.8% (Dierckx, 2007, p. 11). AD is progressive and untreatable, and the outcome of the disorder will always, for one reason or another, be death (Gaiteri et al., 2016, p. 413). The course of the disease, after diagnosis, may be very brief and last just a few years, but some patients live for as long as 20 years. On average, patients aged 65 or more survive for four to eight years after receiving a diagnosis of AD, yet the disease is thought to have its onset 20 years or more before symptoms arise and a diagnosis is made (Alzheimer's Association, 2021, p. 328; p. 349).

STAGING OF DISEASE COURSE

Typically, the course of the disease is separated into three stages: preclinical AD, Mild Cognitive Impairment, and dementia due to AD. The last stage is furthermore separated into mild, moderate, and severe, depending on the amount and the severity of cognitive symptoms present (Alzheimer's Association, 2021, p. 330) (figure 1).

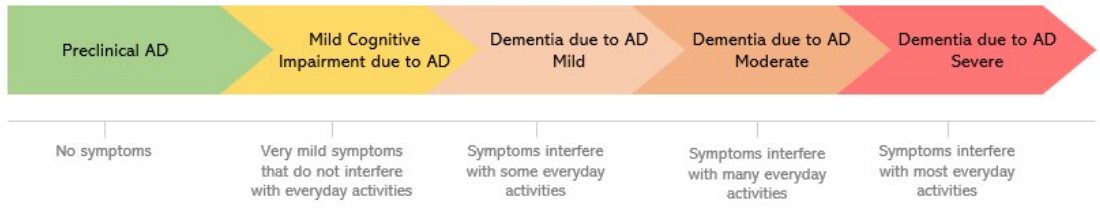

Figure 1 *Stages of AD disease course, model modified from Alzheimer's Association (2021)*

In the preclinical stage of AD, patients have not yet developed cognitive or neuropsychiatric symptoms, although measurable biomarkers, such as beta-amyloid or NFT's, are present (Braak & Braak, 1991, p. 256). AD is rarely discovered at this stage, and even if it is, the presence of biomarkers does not guarantee a development of other AD symptoms, as it is not uncommon to find plaques at autopsy of the brains of deceased individuals, who did not experience severe cognitive deficits while alive (Jellinger, 2006, p. 1618).

Once clinical symptoms present, but the patient has not yet fully developed dementia and is still able to go about their daily life, they are said to have Mild Cognitive Impairment (MCI) (Winblad et al., 2004, p. 243). The term MCI is used to represent any form of cognitive state that is not severe enough to receive a diagnosis but is not a result of 'normal' aging, and MCI can therefore have multiple different causes

apart from AD, including vascular disease, Parkinson's disease, and Lewy Body disease. Notwithstanding, AD is the most frequent cause for MCI (Bayles & Tomoeda, 2013, p. 33).

There are four subtypes of MCI, categorized by whether the patient has amnestic or non-amnestic symptomology, that is, whether the primary impairment is in memory or in another cognitive function, such as visuo-spatial or language abilities, and by whether or not the disease only affects one cognitive domain, or is multimodal (Winblad et al., 2004, p. 244). Amnestic MCI is two to three times more common than non-amnestic MCI, and although estimates of the total prevalence of MCI varies, it seems to be somewhere around 16% after the age of 70. Mitchell and Shiri-Feshki (2009) found an annual conversion rate to AD of 12.2% for amnestic multidomain MCI, 11.7% for amnestic single domain MCI, and 4.1% for non-amnestic MCI (p. 260), implying that patients with amnestic MCI are at a higher risk for conversion to AD. Schneider and colleagues (2009) did not find the same result however, as their study showed that of those who met the pathologic criteria for AD at autopsy, an equal amount had had the non-amnestic and amnestic subtype of MCI (p. 200). Nonetheless, as the subjects in this study had not clinically progressed to AD yet, it may be that the patients with amnestic MCI are simply more likely to develop clinical symptoms alongside their neuropathology (Schneider et al., 2009, p. 200). Whether patients suffering from amnestic MCI are at a higher risk for subsequently developing AD is then unclear, which is mainly due to methodological differences between studies (Bayles & Tomoeda, 2013, pp. 34-36). Either way, the total annual conversion rate is estimated at between 5-10%, and the more domains are affected, and the more severely, the higher the risk of conversion.

It is important to notice though, that after 10 years, more than half of MCI patients will not have developed clinical AD (Bayles & Tomoeda, p. 36f), and 7-10% of MCI patients will not present with progressive neuropathology at autopsy (Smith & Bondi, 2013, p. 97). Therefore, in spite of arguments against the MCI concept, claiming that modern imaging techniques will make the term obsolete because they make it possible to visualize the presence of AD biomarkers in the brain, it nevertheless seems that it might be relevant to continue using the MCI concept in clinical practice (Dubois et al., 2007, p. 734). Following this line of thoughts, Koroelev and

colleagues (2016) found that biomarkers had limited predictive ability on their own, and that the best model for conversion from MCI to AD incorporated MRI measures with cognitive and functional markers (p. 17). In other words, MCI seems to be more than simply early-stage AD (Smith & Bondi, 2013, p. 99).

Once patients develop full-blown dementia, further differentiations can be made not only based on severity, but also on etiology. Additionally, different types of AD seem to have different risk factors.

TYPES OF DEMENTIA AND RISK FACTORS

It is generally agreed upon that AD does not just have one cause, but instead develops as a result of multiple factors. This is with the exception of some familial cases, which typically also have an earlier onset than most other AD cases (Gaiteri et al., 2016, p. 413). It is precisely the age of onset which is used to classify two types of AD, namely early-onset AD (EOAD) which develops before the age of 65 years, and late-onset AD (LOAD) which develops in those older than 65 years of age (Giri et al., 2016, p. 665).

As mentioned, the EOAD cases are typically familial, and are in the majority of cases caused by inherited mutations in the proteins APP (amyloid precursor protein), PSEN1, and PSEN2 (presenilin 1 and 2) (Campion et al., 1999, p. 669). When mutated, these proteins all play a big role in the development of the beta-amyloid plaques characteristic of the AD brain. PSEN1 is estimated to be the culprit in 50% of EOAD cases, and carriers of PSEN2 on average present with clinical symptoms at a slightly older age than PSEN1 carriers (Giri et al., 2016, pp. 667f). An estimation of age of onset depending on the genes a patient carries is illustrated below in figure 2. About 5% of AD patients are estimated to have EOAD (Zanni et al., 2018, p. 849).

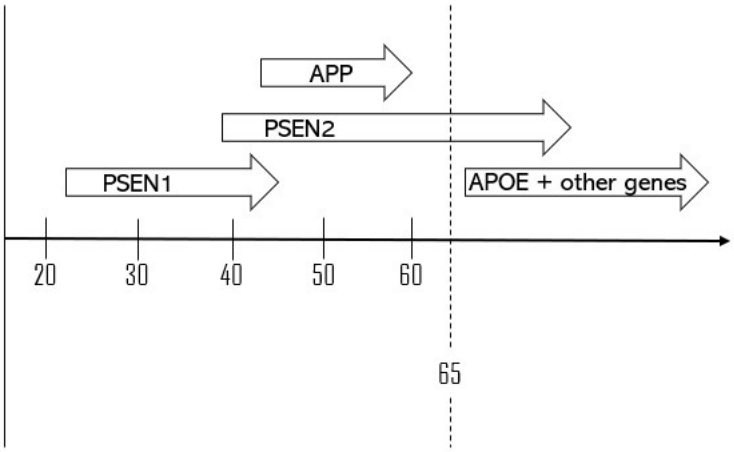

Figure 2 *AD age of onset depending on involvement of different genes, model modified from Bagyinszky et al. (2014)*

The picture becomes blurrier when turning to late-onset sporadic AD. More than 20 genes have been found that may be involved in the development of LOAD, playing a role in a wide variety of functions such as synaptic activity, inflammation, and metabolism – all important for the healthy brain (Gaiteri et al., 2016, p. 413) (figure 2). One of the most investigated potential genetic risk factors for LOAD is the Apolipoprotein E (APOE) gene. APOE is the main cholesterol carrier in the brain, and is involved in inflammation control and synaptic function, amongst other things. It is possible to have three different alleles of the gene: ε2, ε3 and ε4. APOE-ε3 is the most common, whereas being a carrier APOE-ε4 increases the risk of AD, and APOE-ε2 decreases the risk (Giri et al., 2016, pp. 668-670). Noticeably, not all LOAD cases are explained by the presence of APOE-ε4, and other risk factors include smoking, hypercholesterolemia, obesity, diabetes, hypertension, head trauma, depression, exposure to pesticides and solvents, and even lack of social engagement and mental activity. Protective factors include high education, physical and mental activity, and a healthy diet (Rahman et al., 2020, p. 44660). Some other factors that increase the risk of developing AD are being black, Hispanic, or a woman, although there is some discussion as to whether this is due to underrepresentation in the literature, and the fact that women live longer than men. The absolute greatest risk factor for developing AD though, is without a doubt age (Alzheimer's Association, 2021, p. 343; p. 338).

COGNITIVE IMPAIRMENT AND NEUROPSYCHIATRIC SYMPTOMS

Although the risk of developing AD increases with age, some patients do develop the disorder much earlier than others, and age of onset can affect the presentation of symptoms (Barnes et al., 2015, p. 1349). Broadly, the diagnosis of probable AD requires impairment of memory and other cognitive functions, as well as problems with social or emotional behavior, all of this being present for at least 6 months (WHO, 1994, pp. 41-43). The presence of impairments is determined through examination with a broad range of neuropsychological tests. These tests include various tasks on memory, as well as tasks related to language, inhibition, and perceptional abilities, including drawing a clock or recalling certain works (Snowden, 2010, pp. 563-565).

Typically, the patient first experiences episodic memory loss, meaning memory loss for everyday events, followed by impairment in other cognitive as well as behavioral functions (Barnes et al., 2015, p. 1349). Of note, a term closely related to memory is learning. The words are often used interchangeably but do have slightly different definitions. Whereas *learning* refers to the process of acquiring new information, *memory* is more related to the ability to store and retrieve that information (Breedlove & Watson, 2017, p. 535). Memory, as well as learning, are particularly related to the medial temporal lobes, which are furthermore closely connected to the hippocampus (Ogden, 2005, p. 12). The hippocampus plays a central role in memory formation, including spatial memory, that is, memory of locations and spatial relations between objects (Knierim, 2015, pp. 1116f). It is therefore not surprising that the hippocampus is generally assumed to be highly involved in AD (Jellinger, 2006, p. 1614). Another brain region central for memory in AD, particularly for episodic memory, is the entorhinal cortex, through which projections between the cortex and the hippocampus go (Paola et al., 2007, p. 779).

Some patients differ from the typical amnestic pattern, and as it turns out people whose cognitive symptoms debut at a younger age are more likely to primarily experience nonmemory cognitive impairment, that is, difficulties with language, visuospatial functions, problem solving, or judgment (Barnes et al., 2015, p. 1351). Visuospatial functions are in particular related to the parietal lobes in the brain, whereas the frontal lobes play a big role in language and executive functions,

including problem solving and judgment (Ogden, 2005, pp. 12f). Another region typically involved in AD is the locus coeruleus (Franzmeier et al., 2020, p. 2). Normally the locus coeruleus synthesizes norepinephrine, which plays a role in enhancing cognitive flexibility and executive function, that is, cognitive abilities associated with the frontal cortex, and also promotes memory consolidation in structures like the hippocampus (Sara & Bouret, 2012, p. 130). In AD, the locus coeruleus is among the first structures to get damaged (Franzmeier et al., 2020, p. 2), suggesting that the frontal cognitive abilities are likely to become impaired early on. The typical amnestic presentation pattern is nevertheless still the most common in all age groups, as only about one third of the EOAD patients present with nonmemory cognitive symptoms as their first cognitive symptom, although this is nonetheless in stark contrast to the sole 6% of LOAD patients who present with these cognitive symptoms first (Koedam et al., 2010, p. 1403).

Also the neuropsychiatric symptoms that are typically present in patients with AD are affected by which type of AD a patient has (Barnes et al., 2015, p. 1352). In general, around 85% of patients are affected by neuropsychiatric symptoms (Nakamura et al., 2017, p. 375), and typical symptoms include depression (about 40% of patients), apathy (about 40%), agitation and aggression, anxiety, disinhibition, irritability, sleep disorders, and most rarely psychosis (about 7.8%) (Li et al., 2014, pp. 2-5; Victoroff et al., 2018, p. 14; Barnes et al., 2015, p. 1350). Psychosis mainly presents in patients in the very late stage of the disease (Li et al., 2014, p. 5) and is more common in patients with older age at onset. On the contrary, depression, disinhibition, irritability, agitation, and sleep disorders are more common in EOAD patients (Barnes et al., 2015, p. 1352). The neuroanatomy of depression has been studied extensively, but findings are still inconsistent (Pandya et al., 2012, p. 634). Several brain regions, including the prefrontal cortex, the amygdala, the hippocampus, the thalamus, and the basal ganglia seem to be involved. Depression might therefore be localized in multiple brain regions, or a separate system might influence several regions, or it may arise due to abnormalities in functional connectivity (Pandya et al., 2012, pp. 635-641). Noticeably, it is still unclear if depression in AD patients arises due to cerebral impairments, or as a psychological response to the diagnosis (Li et al., 2014, p. 3). Neuropsychiatric symptoms related to disinhibition are a bit easier to place and are generally associated with frontal lobe

dysfunction (Ogden, 2005, p. 164). Finally, apathy, the most common neuropsychiatric symptom in AD overall (Barnes et al., 2015, p. 1352), seems to be associated with damage in certain frontal regions also involved in arousal, although the neuroanatomical bases of the symptom are still poorly understood (Huey et al., 2017, pp. 551f). As the locus coeruleus also plays a role in arousal, it might influence the development of apathy in AD patients (Passamonti et al., 2018, p. 17).

Neuropsychiatric symptoms, particularly depression, are also common in MCI (Van der Mussele et al., 2014, p. 323). In general, the presence of neuropsychiatric symptoms is predictive of a more rapid decline of cognitive function and disease course (Li et al., 2014, p. 5). It therefore makes sense, that EOAD patients who are at a higher risk for developing the beforementioned list of neuropsychiatric symptoms also face a more severe disease course along with higher mortality than LOAD patients, even when factors like age at diagnosis, general cognitive function, and physical health are adjusted for (Son et al., 2016, p. 696).

Differential diagnosis

The presence of neuropsychiatric symptoms in AD, and the varying cognitive symptoms, can sometimes make it hard to give the correct diagnosis. For example, dementia and depression are difficult to discriminate, as affective problems and cognitive impairment, such as impaired memory, are common in both disorders in old age (Dierckx, 2007, p. 12). Nonetheless, it seems that one way of differentiating between the two disorders, as well as MCI, is cued recall, since depressed patients will have no issue performing this task, unlike MCI patients, and finally AD patients who obtain the worst results (Dierckx et al., 2007, p. 67). Similarly, untreated sleep disorders and side effects of some medications can also present in similar ways to AD (Alzheimer's Association, 2021, p. 332).

AD can of course also be misdiagnosed as another type of dementia, or vice versa. This includes Vascular Dementia, the second most common type of dementia, being the cause in 15% of dementia cases (O'Brien & Thomas, 2015, p. 1698). Vascular Dementia arises following several strokes which can occur at any place in the brain, meaning that the cognitive impairments can be very different between patients. Depending on the exact etiology, the patient may experience typical AD impairments,

such as memory impairments (Román, 2005, p. 7f), just like MCI can arise due to Vascular Dementia, and it can therefore sometimes be difficult to differentiate between the two diseases (O'Brien & Thomas, 2015, p. 1703). However, it seems that AD patients have more impaired long-term memory retrieval, and that patients suffering from the two disorders, although all exhibiting perseverative behavior, do this in different ways (Traykov et al., 2005, p. 77).

Another type of dementia that can be hard to differentiate from AD is Frontotemporal Dementia. In Frontotemporal Dementia, the frontotemporal brain regions are damaged, whereas posterior regions are spared (Boxer, 2011, p. 145). Since particularly memory is associated with the temporal regions, and since inhibition and impaired judgment, both frontal symptoms, are typical in AD, it is only sensible that these two disorders can be difficult to separate (Ogden, 2005, p. 12; Barnes et al., 2015, p. 1350f). Surely enough, at autopsy a small percentage of cases diagnosed as Frontotemporal Dementia turn out to have actually been AD cases (Boxer, 2011, p. 161), and 10-40% of Frontotemporal Dementia patients will also show some AD pathology (Rice & Bisdas, 2017, p. 20). Particularly patients who develop Frontotemporal Dementia after the age of 65 will often have AD-like symptoms (Alzheimer's Association, 2021, p. 329).

Dementia with Lewy Bodies is another frequent type of dementia (Nagahama et al., 2015, p. 1248). Although there are some pronounced differences between Dementia with Lewy Bodies and AD, including that Dementia with Lewy Bodies patients are likely to experience sleep disturbances earlier in the disease course along with visual hallucinations and visuospatial impairment in the absence of memory impairment, it can sometimes be challenging to tell the diseases apart. Memory symptoms are common in Dementia with Lewy Bodies, and sleep disturbances and visuospatial impairments are not uncommon in AD either (Alzheimer's Association, 2021, p. 329). Furthermore, apraxia, which is common in Dementia with Lewy Bodies, is not unheard of in AD, although Dementia with Lewy Bodies patients tend to present with fluctuations in attention and cognition in general, which is not seen in AD (Nagahama et al., 2015, p. 1248).

Finally, it is not unheard of that some patients have what is termed mixed dementia (Alzheimer's Association, 2021, p. 330). On the contrary, in one study of deceased

AD patients, Kapasi and colleagues (2017) found that only around 3% of the subjects had the brain changes of pure AD, whereas a full 82% showed brain changes of AD and at least one other dementia (p. 173). Mixed dementia is particularly common in those older than 85 (Alzheimer's Association, 2021, p. 330). It is only logical then, that cognitive and behavioral symptoms will overlap in these cases and complicate diagnosis. Nevertheless, correct differential diagnosis is important for caretaking and potential treatments (Snowden, 2010, p. 561). Alongside cognitive and behavioral symptoms, brain mapping can be useful for differentiating between various dementias, and between dementia and other causes for cognitive and behavioral impairment.

BRAIN MAPPING

Brain mapping has been defined as *"the study of the anatomy and functions of the brain and spinal cord through the use of imaging, immunohistochemistry, molecular and optogenetics, stem cell and cellular biology, engineering, neurophysiology, and nanotechnology"* (Sagar et al., 2019, p. 639). In other words, brain mapping consists of a long row of techniques making it possible to see what is happening in the brain. This is an interesting ability for neurological research in healthy people in general, but particularly relevant when it comes to diseased patients, who are likely to show divergence from the neurotypical brain (Sagar et al., 2019, p. 639). It is important to remember though, that patients' clinical presentations may differ vastly even when their brain scans show similar results (Parsons et al., 2015, p. 74). Nonetheless, the various techniques can lend a useful hand in gaining knowledge in diseases like AD (Sagar et al., 2019, p. 639).

IN VIVO - IMAGING TECHNIQUES AND CSF MEASURES

With technology advancing, it has become possible to view the AD brain while the patient is still alive (Parsons et al., 2015, p. 74). This is done through a diverse range of techniques including, but not limited to, imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) (Sagar et al., 2019, pp. 640-642), and by measuring biomarkers of AD in the cerebrospinal fluid (CSF) (Pannee et al., 2016, p. 139).

MRI, which was introduced in 1979, is a structural imaging technique, meaning that it makes it possible to see brain structures, whereas functional imaging techniques make it possible to examine brain activity (Parsons et al., 2015, p. 79; Raichle, 2008, p. 119). It is frequently applied to examine cerebral atrophy in AD patients (Rice & Bisdal, 2017, pp. 19). A magnetic field is created around the head of the patient which makes the protons in various tissue align in parallel with this field. Radio frequency pulses then disturb this state, and a coil measures the change in voltage that follows this disturbance. Different types of tissue have different characteristics, which affect the time-dependent changes in voltage in different ways. From this information, it can then be induced what kind of tissue is being examined (Raichle, 2008, p. 121).

Functional imaging using PET was introduced not long after MRI, in 1982 (Raichle, 2008, p. 119). Functional use of PET takes advantage of the differences in metabolism at more or less active brain sites, by measuring the uptake of an injected tracer (Parsons et al., 2015, p. 83). By using a tracer, PET becomes an invasive method. Additionally, it has a very high sensitivity, which leads to a poor signal-to-noise ratio. Both the temporal and spatial resolution is poor (Sagar et al., 2019, p. 641). Nevertheless, PET is still a highly useful tool. Several different tracers exist, and two central tracers for AD are fluorodeoxyglucose (FDG) and Pittsburg compound B (PiB) (Perani, 2014, p. 405; Lockhart, 2007, p. 2607). FDG measures cerebral glucose metabolism, and PET_{FDG} is therefore a useful measure of neurodegeneration (Rice & Bisdas, 2017, p. 20). PiB on the other hand binds to Aβ, and PET_{PiB}, also termed amyloid-PET, thereby allows imaging hereof (Lockhart, 2007, p. 2607).

Another method increasingly applied when working with AD is measurement of the biomarkers, such as Aβ and tau, in the patient's cerebrospinal fluid (CSF) (Blennow et al., 2010, p. 131). In AD patients, this method can play a role in confirming the suspected underlying pathology, since, as previously mentioned, the presence of Aβ and tau is obligatory for a final diagnosis of AD (Galasko & Shaw, 2017, p. 131). This method furthermore detects Aβ earlier than PET_{PiB} (Kern et al., 2018, p. 1683). However, as will be seen below (cf. *Neuropathology of AD*, p. 24), the amount of Aβ

in the cortex and CSF may not always be the ideal measurement for AD disease course, and the method therefore has its limitations.

POSTMORTEM – IMMUNOHISTOCHEMISTRY

A technique allowing for a look into the presence of Aβ, tau, inflammation and much more in subjects' brains postmortem instead of in vivo, is immunohistochemistry. This method is useful both in research, and in patients in order to determine if the underlying pathology was AD as suspected or something else (Brüel et al, 2016, p. 54). Unlike the aforementioned methods, this process is performed postmortem, as it requires the removal of parts of the brain followed by fixation in specific chemicals between two glass slices (Thermo Fisher Scientific). Antibodies binding to specific antigens, depending on the research interest, are applied to tissue sections, which results in colored areas, stains, of the relevant antigens which can then be seen through a microscope and quantified (Brüel et al, 2016, pp. 54-56). The method is fairly common and has been applied in studies since 1942 (Thermo Fisher Scientific).

The process starts with the collection of tissue samples, which must be preserved right away in order to prevent them from breaking down (Brüel et al, 2016, p. 49). This is often done via perfusion, sometimes in vivo on anesthetized animals. Subsequently, the tissue is fixated, most often in formalin. This prevents the tissue from decaying. The next step is the sectioning of the tissue, that is, cutting it into very thin slices. Once the tissue is sectioned, the actual staining can begin. Protocols vary widely but have in common that antibodies are applied to the sections alongside some sort of coloring agent (Thermo Fisher Scientific). In indirect immunohisto-chemistry, a more sensitive version of the method, a primary antibody first reacts with the antigen of interest, and a secondary antibody then reacts with the primary antibody, thereby reinforcing the reaction (Brüel et al, 2016, p. 56). Ultimately, tissue sections are mounted on glass and sealed for preservation. The samples can now be visualized by light or fluorescence microscopy (Thermo Fisher Scientific).

The obvious disadvantage of immunohistochemistry in AD is that it cannot be used as a diagnostics tool while the patient is still alive (Thermo Fisher Scientific). Noticeably, none of the previously mentioned techniques should be used as diagnostics tool on their own either, but they can play a role alongside

neuropsychological tests (Parsons et al., 2015, p. 84). Immunohistochemistry is, on the other hand, very useful in research and drug development, as it creates the possibility of easily visualizing almost any antigen in the brain and other tissue (Thermo Fisher Scientific).

Together, all of the abovementioned techniques play a big role in understanding more about AD, and in particular the disease's neuropathology.

NEUROPATHOLOGY OF AD

As previously mentioned, the diagnosis of probable AD is given based on the patient's cognitive impairment profile (WHO, 1994, pp. 41-43), whereas a final diagnosis requires the presence of certain neuropathological features. These features are still the same as Alzheimer originally proposed in 1906, namely beta-amyloid (Aβ) plaques and neurofibrillary tau tangles (NFT) (Rice & Bisdas, 2017, p. 16). This section will explore findings related to these neuropathological changes in AD, as well as their strengths and limitations in explaining the etiology and development of AD, followed by an exploration of two other possible neuropathological factors.

BETA-AMYLOID PLAQUES

AD is, to put it simply, generally characterized by Aβ plaques, followed by accumulation of tau pathology, then hippocampal volume loss, and finally cognitive impairment (Long & Holtzman, 2019, p. 313; Jellinger, 2006, p. 1618). Virtually all AD patients show progressive, enhanced Aβ deposition which, furthermore, as shown by CSF measurements, develop years before the onset of clinical symptoms (Selkoe & Hardy, 2016, p. 595; p. 600). Patients with abundance of Aβ also show more NFT, and a faster NFT accumulation (Franzmeier et al., 2020, p. 3). Aβ is estimated to be present in about one fifth of elderly without dementia (Kern et al., 2018, p. 1683). Cases also exists presenting solely with Aβ, and no tau pathology (Hippius & Neundörfer, 2003, p. 106), and finally progressive tau deposition has not been shown to induce Aβ in humans (Selkoe & Hardy, 2016, p. 600). Therefore, in 1992, the Amyloid Cascade Hypothesis was proposed. According to the Amyloid Cascade Hypothesis, Aβ is the initiating step of the AD pathology, and it is Aβ that leads to the characteristic tau deposition, as well as the neuronal and synaptic loss often seen in AD patients (Long & Holtzman, 2019, p. 315). The hypothesis is

supported by the findings of genetic mutations, previously accounted for, that affect amyloid: APP, PSEN1, and PSEN2 (Jellinger, 2006, p. 1616) (cf. *Types of dementia and risk factors*, p. 15). When mutated, these genes increase the deposition of Aβ in the brain, and cleavage hereof can additionally be inhibited (Crews et al., 2009, p. 6). This increased deposition is suggested to activate enzymes involved in programmed cell death, which includes the formation of NFT (Jellinger, 2006, p. 1618). Before following the exploration of AD etiology further, a consideration of Aβ itself is however warranted.

Amyloid plaques in the brain mainly consist of insoluble Aβ peptide fibrils, that is, misfolded amyloid proteins (Vassar, 2005, p. 93; Glabe, 2005, p. 168). There are several types of plaques, which can be categorized into three main subtypes: *diffuse deposits* are not aggregated into fibrils; *primitive deposits* in which the Aβ is aggregated; and *classic deposits* in which the Aβ is aggregated to an extent that it forms a central amyloid core surrounded by neurites (Armstrong, 2011, p. 72). The plaque aggregation starts out as an intracellular process, but as the disease advances, the plaques increasingly become extracellular – possibly due to apoptosis in overly burdened neurons, which then release their aggregates. These aggregates then bind to receptors normally involved in amyloid clearance, thereby exacerbating the process (Crowther et al., 2011, p. 61f).

Amyloid is also present in neurotypical brains of elderly without cognitive impairment (Jellinger, 2006, p. 1618). What makes the difference, apart from the progressive accumulation, is the type of amyloid peptide found in the brains. Particularly relevant is the ratio between the two amino acid peptides $A\beta_{40}$ and $A\beta_{42}$. Healthy individuals mainly have $A\beta_{40}$, which is less aggregatory, allowing for more effective clearing and thereby less accumulation. AD patients instead show increased concentrations of $A\beta_{42}$, which is more prone to aggregate, either total or relative to $A\beta_{40}$ (Crowther et al., 2011, p. 59). Particularly mutations in PSEN1 lead to an increase in $A\beta_{42}$ and at the same time a decrease in $A\beta_{40}$ (Giri et al., 2016, p. 667).

The distribution of amyloid plaques varies widely both within brain regions and between patients, and it is therefore difficult to define stages of the disease based on plaque load (Braak & Braak, 1991, p. 239). With this in mind, it is nonetheless still possible to look at a general and typical spatial pattern (Thal et al., 2004, p. 2). At

first, low densities of amyloid are seen in the neocortex, particularly the basal parts of the frontal, temporal, and occipital lobes, while the hippocampus is largely spared (Braak & Braak, 1991, pp. 242). PET_{PiB} studies indicate that especially the frontal cortex seems to have a high amyloid burden (La Joie et al., 2012, p. 16270). Later in the disease course, slightly higher densities of amyloid are present in almost all neocortical association areas, although the primary sensory and motor areas remain devoid or only mildly affected by amyloid. The hippocampus is mildly affected at this point. At the end of the disease course, the whole brain is affected, but the hippocampus is generally not worse off than in the middle stage (Braak & Braak, 1991, pp. 242f).

Although Aβ is present in several diseases, as well as in healthy individuals, this pattern is distinctive even in mild AD, and can therefore play a role in differentiating AD from other underlying diseases, such as Vascular Dementia in which amyloid burden is also common (Thal et al., 2004, pp. 2-6). PET_{PiB} has also been shown to be a sensitive and specific measure for distinguishing Frontotemporal Dementia from AD (Rice & Bisdas, 2017, p. 20). The total amount of Aβ is also useful for differentiating between healthy individuals and AD patients in general, no matter if the amyloid distribution follows the above-described distribution pattern or not, as Aβ is present at significantly higher levels in the brains of AD patients in all age groups, although this differentiation is better in younger patients, specifically around 70 years of age as opposed to 85+ years (Middleton et al., 2011, p. 1742). That it becomes difficult to differentiate at older age and later disease stage is also the case when it comes to differentiation between AD and other amyloid diseases, as most of these diseases eventually show a similar pattern with the entire brain severely affected (Thal et al., 2004, pp. 6). Interestingly, and in support of the Amyloid Cascade Hypothesis, the spatial pattern of NFT appears to follow that of diffuse deposits within the frontal and temporal cortex (Armstrong, 2011, pp. 76f).

Following this, it is again relevant to point out that increased levels of Aβ are suggested to be enough to drive tau pathology (Long & Holtzman, 2019, p. 315). However, not all results support this notion. For starters, amyloid deposits are as mentioned also common in healthy people, and it therefore seems unlikely to be the only driving factor of AD (Jellinger, 2006, p. 1618). Furthermore, some AD patients

present with what is termed the *plaque-predominant* subtype of AD, in which NFT are not abundant (Jellinger, 2006, p. 1614). This is also the case in many transgenic animal models, which, in spite of developing amyloid plaques and cognitive impairment typical of AD following a mutation in APP, do not develop NFT (Vassar, 2005, p. 93). Finally, some studies report a dissociation between the spatial distribution of Aβ and tau pathology in AD patients (Armstrong, 2011, p. 77). Noh and colleagues (2017) found a gradual increase and association between amyloid and tau in LOAD patients, whereas the development of the two biomarkers was more abrupt and independent in EOAD patients (p. 3). It might be, then, that the inconsistent results arise due to research on what is actually different etiologies of AD, and that the involvement of different AD-genes like APP will interact differently with the development of NFT. Therefore, the Amyloid Cascade Hypothesis might hold water in some cases, whereas evidence against it is also present and cannot be ignored (Busfield & Goate, 1995, pp. 71f).

Another such argument against the Amyloid Cascade Hypothesis is the fact that plaque burden does not seem to reflect disease severity in terms of cognitive impairment (Edison et al., 2007, p. 501). Middleton and colleagues (2011) found no difference in plaque load between different age groups in a postmortem study (p. 1739), and Ossenkoppele and colleagues (2015), using PET_{PiB}, even found a decrease in Aβ in AD patients between the ages of 50 and 90, especially in APOE ε4 carriers (p. 1339). This is in stark contrast to the cognitive abilities of AD patients, which progressively worsen (Rice & Bisdas, 2017, p. 17). Being positive for Aβ, measured by PET_{PiB}, instead seems to play a role in conversion from MCI to AD, as these patients are significantly more likely to develop full blown AD within two years, than those who are negative for Aβ, in spite of no overall increase in plaque load. The presence of amyloid does not predict a shorter conversion time though (Rice & Bisdas, 2017, pp. 17-19). Aβ load is therefore likely to contribute to the cognitive impairment in AD but is unlikely to explain it on its own (Edison et al., 2007, p. 507). Interestingly, Aβ might additionally play a role in the development of neuropsychiatric symptoms, as correlations are shown between PET_{PiB} measurements in the frontal cortex and apathy in AD patients (Mori et al., 2014, p. 451).

One potential reason for why Aβ load and cognitive impairment is not found to correlate significantly, might be that most research has focused on diffuse plaques, as opposed to oligomers, which are toxic, bigger aggregates than simple amyloid fibrils (Crowther et al., 2011, p. 60). Oligomers are shown to induce tau pathology (Selkoe & Hardy, 2016, p. 596), as well as decrease synapse density by destroying the dendritic spines, and reduce synaptic transmission (Crews et al., 2009, p. 6). When injected into the brains of healthy rats, these start showing memory impairments. Furthermore, although healthy individuals often show Aβ, the relative amount of oligomers is much lower than in AD patients (Selkoe & Hardy, 2016, p. 596f). Yet it is still unclear if the oligomers are sufficient to cause the cognitive impairment seen in AD on their own, as they might be less toxic in vivo than they are in vitro, which is how most studies on oligomers have been conducted. It is also somewhat uncertain, then, if oligomers in vivo are able to drive tau pathology (Long & Holtzman, 2019, p. 315; Glabe, 2005, pp. 172-174).

NEUROFIBRILLARY TAU TANGLES

As mentioned above, the Amyloid Cascade Hypothesis predicts that it is Aβ that induces tau pathology, which is assumed to play a bigger role in cognitive impairment than Aβ itself (Long & Holtzman, 2019, p. 315; Jellinger, 2006, p. 1618). Tau in itself is not dangerous, but rather plays an important role in maintaining a healthy brain. Microtubule associated protein (MAP) tau is involved in assembly and stabilization of microtubules, which are part of cell cytoskeletons. In brief, tau establishes and maintains neuronal morphology (Armstrong, 2011, p. 72). In AD, on the other hand, the tau becomes hyperphosphorylated and aggregated (Long & Holtzman, 2019, p. 316). Instead of building neurons, it forms intracellular neurofibrillary tangles (Armstrong, 2011, p. 72). Interestingly, aggregated tau is also found in cognitively healthy individuals without Aβ pathology, in some temporal lobe regions including the hippocampus (Pontecorvo et al., 2017, p. 757). Already in their twenties, 10% of people show a small amount of NFT, and in healthy elderly they are frequent (Nunomura et al., 2009, p. 104). Notwithstanding, Thal and colleagues (2004) point out that the level of NFT is much lower and present in fewer brain regions in healthy individuals than in AD patients (p. 2). Jack and colleagues hypothesize, that tau and Aβ pathology might develop independently, but that Aβ

accelerates tau pathology to a clinically relevant level thereby starting the spread of NFT beyond the temporal lobe (*in* Pontecorvo et al., 2017, pp. 749-758).

Unlike Aβ, NFT show a clear and common spatial pattern that follows six stages, called *Braak stages* (Braak & Braak, 1991, p. 239). Stages I and II are called the *transentorhinal stages* and represent the preclinical part of the disease (Braak & Braak, 1991, p. 256). In these stages, only the transentorhinal region of the brain is affected by NFT. The following two stages, III and IV, are characterized by higher affection of the transentorhinal region, as well as the entorhinal cortex and locus coeruleus (Braak & Braak, 1991, pp. 245-247; Franzmeier et al., 2020, p. 2). These are called the *limbic stages*, and the last two stages, V and VI, are called the *isocortical stages* (Braak & Braak, 1991, p. 256). In these stages, virtually all parts of the hippocampus are involved alongside the full neocortex (Braak & Braak, 1991, p. 247). The presence of NFT seems to be able to seed further misfolding of tau, and thereby exacerbating the presence of NFT in the brain (Franzmeier et al., 2020, p. 2). Interestingly though, the spread of tau is predominantly related to functional, rather than structural, connectivity in brains, which means that regions "working together" with NFT-infested regions are also more likely to become NFT-infested themselves (Franzmeiner et al., 2020, p. 3).

In all age groups, the clinical diagnosis is strongly associated with the presence of NFT – both the amount and the location (Middleton et al., 2011, p. 1739). As previously mentioned, there is some difference between LOAD and EOAD patients with regards to clinical symptoms (cf. *Cognitive impairment and neuropsychiatric symptoms*, p. 17). LOAD patients typically present with memory symptoms, whereas EOAD patients are more likely to experience difficulties with language, visuospatial functions, problem solving, and judgment (Barnes et al., 2015, pp. 1349-1351). Adequately, NFT accumulation is mainly confined to the temporal lobes of LOAD patients, whereas it is additionally widespread in the prefrontal, premotor, and inferior parietal areas in EOAD patients, which overlaps well with the cognitive symptoms present in the two forms of the disease (Schöll et al., 2017, p. 2289). Furthermore, EOAD patients in general have a higher NFT load than LOAD patients (Schöll et al., 2017, p. 2292), which can play a role in explaining the often more aggressive disease course seen in EOAD patients (Son et al., 2016, p. 696).

In spite of this, there are still some phenomena in AD that tau pathology cannot explain. This includes the plaque-only cases, where patients show clinical symptoms in spite of a lack of NFT presence (Hippius & Neundörfer, 2003, p. 106), and the patients without Aβ pathology who also show cognitive impairment, but whose NFT presence does not differ from that of cognitively healthy individuals (Pontecorvo et al., 2017, p. 757). Furthermore, the older the AD patient, the worse is the correlation between NFT and cognitive impairment (Middleton et al., 2011, p. 1739). Something else seems to be at play as well. Multiple studies indicate that NFT drives neurodegeneration, or atrophy, in AD (Long & Holtzman, 2019, p. 316). This atrophy largely follows the spatial distribution of NFT, and just like with NFT, atrophy is also more likely in regions functionally connected to already atrophied regions (Franzmeier et al., 2020, p. 12). Despite this, the neuronal loss exceeds the NFT presence. Additionally, whereas turning off tau expression stops the neuronal loss, it does not stop the accumulation of NFT once started, which suggests that NFT are not the only factor responsible for the atrophy seen in AD (Nunomura et al., 2009, pp. 104f).

Loss of neurons and synapses

To complicate matters even further, the loss of neurons correlates much better with cognitive impairment than NFT load does (DeKosky et al., 1996, p. 417). Although NFT may play a role in driving neurodegeneration, as well as in cognitive symptoms in AD, it therefore cannot explain the whole disease on its own (Long & Holtzman, 2019, p. 316; Jellinger, 2006, p. 1616). Additionally, neuronal loss does not correlate with the presence of Aβ, which also speaks against the Amyloid Cascade Hypothesis proposed idea of Aβ inducing tau, which then induces neuronal loss (La Joie et al., 2012, p. 16270).

As the neurons die, a loss of synapses is of course also seen. However, this loss exceeds that of neurons, and might therefore even precede the neuronal loss (Serrano-Pozo et al., 2011, p. 11). Interestingly, the dendrites seem to grow to cover more area as the synapses are lost, which might limit the cognitive impairment in the beginning of the disease, thereby playing a role in keeping the disease preclinical for years. Eventually, as the disease spreads, this will not be enough to keep the brain fully functioning, and clinical symptoms will set in or worsen (DeKosky et al., 1996,

p. 419). A decline in synapses may also be due to a mechanism interfering with neurogenesis (Crews et al., 2009, p. 5).

Loss of synapses and neurons is particularly seen in the hippocampus, which as previously considered is an important brain region in AD (Perez et al., 2013, p. 1). As for the rest of the brain, there is some disagreement. La Joie (2012) states that whereas atrophy, as shown by PET_{FDG}, is pronounced in the hippocampus, it is less common although still significant in the posterior association cortex, and low in frontal areas (p. 16270). Rice and Bisdas (2017) on the other hand, also using PET_{FDG}, show notable atrophy in both the frontal, temporal, and parietal regions with the medial temporal lobe (including the hippocampus) as the first affected region (p. 20). They also believe this pattern to be useful for differential diagnosis between different types of dementia, specifically AD and Dementia with Lewy Bodies, as only Dementia with Lewy Bodies patients show additional atrophy in the occipital lobe (Rice & Bisdas, 2017, pp. 21f). Noh and colleagues (2017) go a step further and separate AD into EOAD and LOAD, thereby finding that in EOAD the picture of loss largely follows that of tau pathology, whereas in LOAD – recall that 95% of AD cases are of this type – there is no correlation between the two patterns (p. 3; Zanni et al., 2018, p. 849). This further makes sense when considering that neuron and synapse loss is the pathology most associated with cognitive impairment, as EOAD and LOAD patients show different cognitive profiles. True enough, AD patients seem to have different patterns of atrophy depending on their age of onset, with LOAD patients mainly experiencing loss in the medial temporal lobe and EOAD patients mainly in the neocortex as well as to a greater extent than LOAD patients (Schöll et al., 2017, p. 2287). Loss in locus coeruleus furthermore seems to correlate with tau pathology in patients with amnestic symptoms, whereas this is not the case for patients with non-amnestic symptoms (Oliviera et al., 2019, p. 1349). Additionally, the amount of synaptic and neuronal loss in the entorhinal cortex correlates well with cognitive impairment in general (Long & Holtzman, 2019, p. 313).

Atrophy is not just present in full blown AD, but also in MCI patients, who show intermediate loss of neurons and synapses in the hippocampus. A correlation is seen between amount of loss and conversion from MCI to AD, and hippocampal atrophy

as measured by MRI not only shows who is at risk, but also predicts a shorter time until disease progression (Rice & Bisdal, 2017, pp. 19-21). Furthermore, hippocampal atrophy, alongside atrophy in the cortex and more specifically in locus coeruleus, is associated with depression in AD patients as these patients show greater loss than non-depressed AD patients (Victoroff et al., 2018, p. 15; Morris, 1995, p. 217). Atrophy is not found correlated to other neuropsychiatric symptoms, like apathy, agitation, anxiety, or sleep disorders (Victoroff et al., 2018, pp. 16-18).

Due to this very variable and still debated pattern, and because atrophy can also be present following other diseases, just like it can be present in healthy elderly, atrophy is not diagnostic for AD (Serrano-Pozo et al., 2011, p. 3; Thal et al., 2004, p. 2). Add in the previously explored counterarguments for Aβ and tau pathology, and it is easy to see that much is still left to be understood about AD (Jellinger, 2006, p. 1604). More recently, research has therefore turned towards neuroinflammation as a possible explaining factor in AD (Walters et al., 2016, p. 25).

NEUROINFLAMMATION

Chronic neuroinflammation is a common feature of AD neuropathology (Delatour et al., 2011, p. 144). Nondemented individuals who show high AD-related neuropathology still have less inflammation in their brain than AD patients, and it has therefore been proposed that inflammation might play a role in the development of the disease (Serrano-Pozo et al., 2011, p. 13; Bernhardi, 2009, p. 140). The accumulation of Aβ and NFT is, in AD patients, followed by a so-called recruitment of microglia which entail inflammation when activated, which then further worsens the already present AD pathology (Heneka et al., 2015, p. 388). This happens when microglia release cytokines, which in extreme concentrations can trigger hyperphosphorylation of tau and reduce the removal of pathological Aβ, hereby allowing Aβ to aggregate further. The cytokines also affect synaptic loss. These processes are however not yet fully understood (Rojo et al., 2009, pp. 128f; Bernhardi, 2009, pp. 140-142).

Neuroinflammation may therefore bring us closer to a whole model of AD pathology. Nevertheless, there is no doubt that pathological Aβ, NFT, and cerebral atrophy do play a role in the development of the disease, for which reason it is still relevant to

gain further understanding hereof (Jellinger, 2006, p. 1616). Furthermore, adequate models for studying inflammation should also exhibit the other pathological hallmarks of AD, to be as accurate for human AD as possible (Delatour et al., 2011, p. 137). Such relevant models include animal models, and in particular the promising TgF344-AD rat model of which a better understanding is sought through this thesis.

ANIMAL MODELS

In spite of regular discussions about animal research, animal models are applied in a wide range of research fields, not only that of neurodegenerative disorders, and have been for a long time. This is because the use of animal models is key for gaining knowledge of different factors leading to various diseases, how these play together, and what leads to which symptoms, as well as testing potential disease treatments (Pasquali, 2018, pp. 144f). Furthermore, animal models allow for studying the preclinical stages of various diseases (Tudela et al., 2019, p. 2).

Transgenic mouse models have been studied in relation to AD for around twenty years. Most of the models have induced mutations of APP and PSEN1, and are thereby models of familial EOAD (Delatour et al., 2011, p. 137). It is much more difficult to model sporadic LOAD, since the etiology of this form of AD is still not fully known (Rahman et al., 2020, p. 44660). Models hereof are therefore not nearly as established as EOAD animal models (Delatour et al., 2011, p. 137), in spite of LOAD being the AD type present in 95% of the cases (Zanni et al., 2018, p. 849). As previously shown, LOAD and EOAD patients present with somewhat different neuropathology and clinical impairments (Schöll et al., 2017, p. 2289; Barnes et al., 2015, p. 1351), yet some overlap in symptoms is also seen (Koedam et al., 2010, p. 1403). It is therefore still uncertain how much relevance these animal models have for LOAD cases (Delatour et al., 2011, p. 137).

Most mouse models show pathological Aβ accumulation, but not tau pathology. They thereby offer an opportunity to look into the role of Aβ in AD, without other pathology as confounding factors (Delatour et al., 2011, p. 139). For example, many of these mouse models still show hippocampal atrophy, even at only a few months of age, as well as neuroinflammation, in spite of a lack of tau pathology (Delatour et al., 2011, pp. 140-145). The accumulation of Aβ in mice seems to be quite similar to that

of humans, both with regards to location and intracellular aggregates turning extracellular – although recall that there is not one clear distribution pattern of Aβ in human AD patients, as is the case with NFT. Even so, studies of the spread of Aβ in mice are fairly limited. (Delatour et al., 2011, p. 141). Additionally, the inflammatory patterns in mice seem to present with slightly different stages and severity than in humans (Delatour et al., 2011, p. 145). Finally, in spite of the advantages of being able to study Aβ on its own, the lack of NFT in mouse models has led to widespread critique of the models as inadequate of AD (Delatour et al., 2011, p. 139).

Because of the limitations of mouse models, it has been hypothesized that rats might make for better transgenic AD models. After all, rats are about 4-5 million years closer to humans than mice are, in an evolutionary sense (Cohen et al., 2013, p. 6246). True enough, not long ago a rat model expressing both pathological amyloid accumulation, neuronal loss, and tau pathology was developed by Cohen and colleagues (2013). This model is named the TgF344-AD model.

THE TGF344-AD RAT MODEL

The rat model was generated on a background of Fischer 344 rats. These were injected with mutant human APP as well as mutant human PSEN1, which led to the overexpression of Aβ – both intraneuronal $A\beta_{42}$ and oligomers (Cohen et al., 2013, p. 6246). The transgenic rats were tested for neurological reflexes, visual and tactile responses, and locomotor activity; all of which were normal with the exception of increased age-dependent hyperactivity, assumed to be due to disinhibition following hippocampal or cortical damage related to AD pathology (Cohen et al., 2013, pp. 6246-6249).

Studies of the model are yet to become abundant, but the ones that do exist have found virtually no Aβ pathology in the hippocampus at age 6 months, 1.3% plaque coverage at 9 months, about 3% at 16 months and about 5% at 26 months. At 9 months an average of 0.6% coverage was seen in cortical regions, with higher accumulation in the entorhinal cortex (Joo et al., 2017, p. 4; Cohen et al., 2013, p. 6248). These results were found using immunofluorescence, staining with Thioflavin S (Joo et al., 2017, p. 2). Another study looked into two hippocampal sections of a 10-month-old TgF344-AD rat, using immunofluorescence, staining with Thioflavin

T, on one and immunohistochemistry with DAB staining on the other, and found a 2.27% and 5.43% coverage respectively (Jensen et al., 2019), p. 12).

As for tau pathology, at as early as 6 months hyperphosphorylated tau was found in the locus coeruleus of the rats, which emerged prior to NFT in the entorhinal cortex and the hippocampus (Tudela et al., 2019, p. 2). At 16 months the rats had fully formed NFT, in spite of the fact that no mutated human tau genes were introduced to the model (Cohen et al., 2013, p. 6251).

At age 6 months, transgenic rats had not yet experienced neuronal loss, whereas they had a 36% decrease in cell count in the hippocampus at 16 months, and a 45% decrease at 26 months (Cohen et al., 2013, p. 6252).

Turning to behavioral impairment, mainly hippocampus-dependent memory functions have been tested in animal models of AD (Delatour et al., 2011, p. 146). Using the Barnes Maze, a hippocampus-dependent spatial learning and memory test, the TgF344-AD rats showed no memory or learning impairments at 6 months, whereas both memory and learning impairment was present at 15 months, and even more so at 24 months (Cohen et al., 2013, p. 6249). A very recent study of memory impairments in the model shows impairment in memory, but not yet in learning, at age 10 months (Christensen, 2021, p. 17).

Finally, only very few studies have assessed neuropsychiatric symptoms in the rat model, especially at young ages before significant cognitive impairment sets in. One study did nevertheless find increased anxiety in rats between 4 and 6.5 months of age, which did not show cognitive nor locomotor deficits (Pentkowski et al., 2018, pp. 173f).

All of this makes the TgF344-AD rat model for AD one of the most promising models for learning more about AD, as well as for testing potential treatments of AD (Tudela et al., 2019, p. 2). Before the model can be fully used for this, it is nevertheless still necessary to learn more about it.

PURPOSE AND HYPOTHESES

To learn more about the development of typical AD pathology in the TgF344-AD rat model is exactly the purpose of this thesis. In particular, the focus will be on Aβ plaques in the hippocampus of 10-month-old rats compared with a wildtype control group. It might as well have been on NFT, atrophy, or neuroinflammation, which have been argued highly relevant for AD and possibly more relevant than Aβ for cognitive and behavioral symptoms in AD patients (cf. *Neuropathology of AD*, p. 24). However, Aβ does play a role in AD (Edison et al., 2007, p. 507), and is also highly relevant for differentiation between AD and other dementias, such as Vascular Dementia and Frontotemporal Dementia (Thal et al., 2004, pp. 2-6; Rice & Bisdas, 2017, p. 20). To gain a thorough understanding of the disease – and the model as a model of AD in particular – it is therefore still necessary to examine this biomarker.

Ideally, it will eventually be possible to create an overview of the development of plaques in the TgF344-AD rat model over time, but so far various studies have applied different staining techniques (Joo et al., 2017; Cohen et al., 2013). One previous study has applied immunohistochemical staining to the TgF344-AD rat in an effort to determine if this method is appropriate for identifying plaque load (Jensen et al., 2019, p. 4). Whereas the authors found that this was the case, they did not examine the actual plaque load, except for reporting the plaque load in one section of one rat at age 10 months (Jensen et al., 2019, p. 18; p. 23). This thesis then in part seeks to confirm, or at least to further investigate, the results obtained in that study.

Specifically, the main hypothesis of this study is that the transgenic (TG) rats will present with more Aβ plaques in their hippocampus than their wildtype (WT) littermates, that is, have a higher plaque coverage. It is expected that the plaque coverage of the hippocampus will be similar to the one found by Jensen and colleagues (2019).

Furthermore, the results from this study will be compared with results from Christensen (2021), a study about the behavior of the very same rats, to explore if there is any association between the plaque coverage in the hippocampus and the rats' performance on the hippocampus-dependent spatial memory test Barnes Maze.

METHODS AND MATERIALS

Before considering whether the results of this study are in line with the hypotheses, it is first relevant to go through the methods applied to obtain these results. This section will describe both the behavioral testing of the rats and tissue perfusion, sectioning, and staining, as well as the analysis of the stained sections. Before this, it is however relevant to briefly turn to the rat model itself once again.

RESEARCH ANIMALS

The 8 TgF344-AD (TG) rats used in the study were provided by the *Rat Resource and Research Center* P400OD011062. Both a hemizygous and a homozygous genotype of this strain exists, however only the homozygous type is employed in this study, as Jensen and colleagues (2019) have found this type to have more pronounced AD pathology than the hemizygous equivalent. 8 Fisher 344 wildtype (WT) rats were employed as control. Additionally, I was kindly given access to one image of the hippocampus of a 10-month-old rat, which had undergone the same procedure as the rats in the study at hand, but two years prior in the study by Jensen and colleagues (2019).

BARNES MAZE

All 16 rats in this study had previously undergone behavioral testing on Barnes Maze, a hippocampus-dependent spatial memory test for rodents (Cohen et al., 2013, p. 6249). The test consists of a round table with 18 holes. One of these, the target hole, allows for the rat to climb into it and hide from the bright light being shone on the table. The other holes are fakes, merely 1 cm deep (Christensen, 2021, p. 11).

Using this test, the abilities for learning and memory were assessed in both TG and WT rats by teaching them the location of the hole they can hide in. Following a habituation period, the rats were placed on the Barnes Maze and guided to this hole. They hereafter underwent 5 learning trials over the span of two days, during which several measurements were made. These included, but were not limited to, measurements regarding the time it took them to first find the hole (primary latency), the time spent in the target quadrant, and the amount of nose pokes in fake holes before finding the target hole (primary errors). On the third and the tenth day, the rats were given 3 minutes on Barnes Maze to find the target hole, which was now

covered and looked like all the other holes. The same measurements as mentioned above were noted. The *learning* phase then refers to the process of achieving knowledge about the location of the hole, that is, the process happening on the training days 1 and 2 in Barnes Maze, whereas *memory* refers to what the rats had learned and exhibited on days 3 and 10 (Christensen, pp. 11f).

The results from this part of the study, alongside a more in-depth description of the test and a discussion of the use of this test, are further elaborated in a previous project (Christensen, 2021), and will be drawn upon in the discussion of the thesis at hand.

PERFUSION PROCEDURE AND TISSUE SECTIONING

Sacrificing of the animals was performed in compliance with the Animals Welfare Act (Miljø- og Fødevarministeriet, 2018), and approved by the Animal Ethics Inspectorate of Denmark (nr. 2019-15-0201-00215). The rats were treated as gently as possible to avoid unnecessary stress up until the sacrificing. Sacrificings took place the day after the last behavioral testing.

The rats were sedated by a mixture of hypnorm and dormicum by the animal caretakers at the animal laboratory at Aalborg University and checked for the toe-pinch reflex before any procedure was begun. The rats then had their chests opened, and a perfusion with potassium-phosphate-buffered saline (KPBS) was performed until all blood had been washed out of the body. Immediately after, a 3.7% formalin solution was injected into the left side of the heart of the rat. The head was removed and stored in KPBS at 4 degrees Celsius until further use. Due to the COVID-19 lockdown, the wildtype rats had to stay in KPBS longer than first anticipated, and for this reason 0.02% sodium acid was added to the solution to prolong the possible storage time.

Following this, the brains were removed from the skulls, and put in a 30% sucrose solution for a minimum of three days at 4 degrees Celsius until they had sunk. This was done for cryopreservation of the brain after perfusion. Tissue sectioning was then carried out at -21 degrees Celsius in a Leica CM3050 S microtome cryostat. Each brain was divided into 7 series, A-G, A being the most anterior and G being the most posterior. Every series holds 6 glasses containing 12 sections of 40 µm

respectively. Subsequently, the sections were transferred to antifreeze solution for storage at -19 degrees Celsius.

IMMUNOHISTOCHEMICAL STAINING FOR BETA-AMYLOID

The full protocol in Danish can be found attached in Appendix A. All 8 TG-rats were employed in this part of the study, alongside 2 WT-rats as control.

Series D is the main series containing the hippocampus, as calculated via Paxinos' and Watsons Rat Brain Atlas (2002). Therefore, D2 was chosen for staining for Aβ. To prepare the sections for the staining, they were removed from the antifreeze solution and washed in KPBS thrice. Following this, they were incubated for 60 minutes in an incubation buffer consisting of 3% porcine serum, KPBS, and 0.3% Triton X-100. This helps clean up the sections, thereby removing background noise in later analysis. Once the hour had passed, one section from rat TG54 (female), rat TG58 (male), rat WT70 (female), and rat WT74 (male) respectively were separated from the main sample as negative controls. The remaining sections from these rats as well as from the six other transgenic rats employed in the study were incubated overnight with the primary antibody, purified anti-beta-amyloid 1-16 antibody 803001 biolegend Mouse-anti-Human, dissolved in the incubation buffer. This antibody binds to the amyloid in the sections. The reason it is anti-human, and not anti-rat as one might assume, is because the amyloid has developed in the brains of the rat following a human mutation, as the rats were injected with mutated human APP and PSEN1 (cf. *The TgF344-AD rat model*, p. 34*)*.

The next morning, all sections including the negative controls were once again washed three times in KPBS to remove excess primary antibody, and then incubated for 60 minutes in biotinylated secondary antibody, DAKO E0354 biotin anti-mouse, dissolved in incubation buffer. The secondary antibody binds to the primary antibody. Following this, the sections were washed twice in washing buffer, which is diluted incubation buffer, and once in KPBS. The sections were then incubated for 30 minutes in Avidin-Biotin Complex solution, which binds to the secondary antibody and itself, thereby increasing the locations where the coloring substance can later bind, and then once again washed in KPBS, twice, followed once by Tris/HCl with a pH=7.6. DAB solution, the coloring substance, was added and sections were

incubated for 9 minutes, after which they were washed once in Tris/HCl, followed by KPBS twice. The now stained sections were moved to a petri dish with a gelatin solution in it, from which they could be transferred onto 76x26 mm Superfrost object glass slides (Hounisen Laboratorieudstyr A/S). After a night of drying, the sections were sealed with Pertex and covered by 24x60 mm cover glasses (Hounisen Laboratorieudstyr A/S).

DATA ANALYSIS

All raw data, and a detailed descriptions of settings in the data analysis program ImageJ can be found in Appendix B and Appendix C.

Once the sections were sealed and dried, images were taken using a Zeiss Axioplan 2 microscope. Depending on the amount of whole hippocampus sections available, between 3 and 15 sections were photographed per transgenic rat, using 2.5x magnification. The wildtype rats each had one section photographed, and between 3 and 4 images were taken of the two negative controls. In most cases the hippocampus sections were too large to fit into a single image, for which reason multiple images had to be taken. These were later edited together to create one image per section. However, the edges of the images taken with the microscope are darker than the middle, which sometimes left a mismatch between the image pieces and left dark wedges in the middle of some of the edited images.

From each transgenic rat, two edited images were chosen for further analysis. This choice depended solely on the light-darkness mismatch of the images, so that the edited images with the smoothest transitions between the original photos were picked. Images of one negative control from the TG and WT groups respectively, as well as of the WT sections, were also chosen. These images where then analyzed for quantification of plaque load in ImageJ software. The image from Jensen and colleagues (2019) was also re-analyzed, to ensure that results would be comparable. Images were first converted to 8-bit black and white, and the scale was then set according to calculations from Jensen and colleagues (2019). Note that this means that the *absolute* area measurements may not be completely accurate, as there may be a difference between the number of pixels in their microscopy pictures and the ones in this project. Nonetheless, this was done for a lack of more accurate measurement

and since the *relative* area measurements, which are of interest here, will still be accurate.

First, the total area desired for quantification was measured. This was done manually and was therefore done three times in order to reach a more accurate average measure. Hereafter, the brightness/contrast of the images were adjusted to give the most accurate outcome image once converted to binary. Following the conversion to binary only plaques can be seen, and plaques outside of the area of interest were removed manually from the images. The threshold was set to 105, and the area of plaques were measured. From this, a percentage of plaque coverage could be calculated. These percentages were averaged across sections within the same rat, leaving one average plaque coverage score per rat. Figure 3 shows the process.

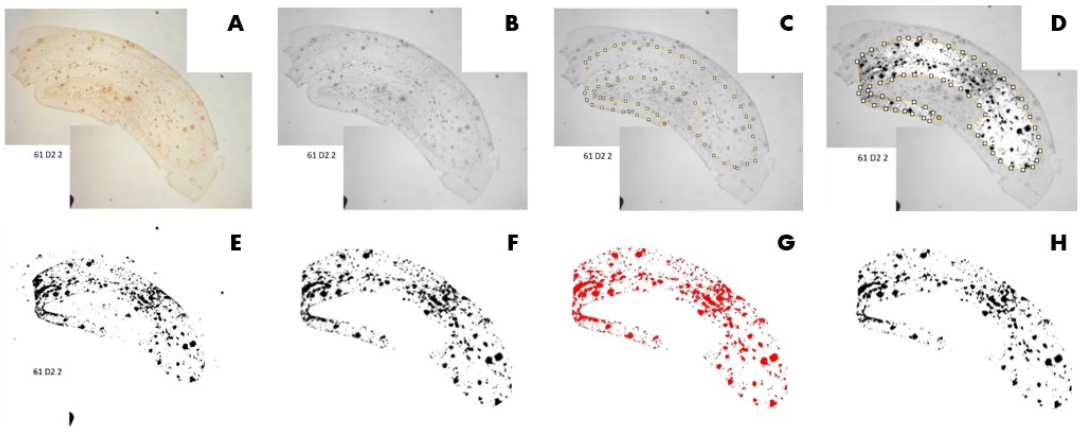

Figure 3 *Stages of image analysis using ImageJ, TG61: (A) edited image; (B) 8-bit converted image; (C) measuring of total area; (D) brightness/contrast adjusting; (E) conversion to binary; (F) binary image with plaques outside area of interest removed; (G) thresholding; (H) final plaque amount.*

Statistical analysis was conducted in Jamovi 1.2.2. Shapiro-Wilk tests were performed to examine normality (p<0.05). Only one violation was seen, namely for the measure of time spent in the target quadrant on Barnes Maze. However, as the p-value was 0.042 and the Q-Q plot still looked rather acceptable, and since parametric tests are quite robust for deviations (Field, 2018, p. 283; p. 250), it was decided to nevertheless use the parametric tests: independent t-test and linear regression, for statistical analysis. All tests are two-tailed, and the significance level was set at 5% (α=0.05).

RESULTS

A fairly small group of animals have been examined in this project due to ethical considerations. Furthermore, for each rat the final plaque coverage percentage is calculated on the basis of data from just two hippocampal sections from the TG-rats, or one section from the WT-rats, negative controls, and the rat made available by Jensen and colleagues (2019). Nonetheless, the data does contribute with preliminary knowledge about the model, and the results presented below will therefore without restraint be based on this available data.

DESCRIPTIVES

8 TgF344-AD rats, 4 female and 4 male, were used in this study. Furthermore, 2 wildtype rats, one female and one male, were employed as control. All rats were 10 months old. T-tests were performed to examine possible gender differences, but no significant differences were seen ($p>0.05$). The genders were therefore grouped together within the genotypes for further analysis. Additionally, access was given to a single image of one other 10-month-old TG-rat from a previous study, gender unknown (Jensen et al., 2019). The data from this image was not grouped with the data of the other TG-rats, but rather used for comparison herewith.

The plaque coverage percentages of the TG-rats hippocampal sections varied from 3.31% (TG54) to 16.67% (TG57). The lowest average plaque coverage across sections for individual TG-rats was 6.39%, and the highest was 12.78 (M = 9.84, SD = 2.62). For the WT-rats, the lowest plaque coverage as 0.088%, and the highest was 0.114% (M = 0.101, SD = 0.019). Although Jensen and colleagues (2019) originally found a plaque coverage of 5.43%, re-analysis of their image following the exact same procedure as for the rats employed in this study yielded a coverage of merely 3.19%. All raw data can be found in Appendix C.

NEGATIVE CONTROL

A negative control was made for each genotype, in order to check that the secondary antibody or the coloring agent did not bind to the beta-amyloid or anything else in the brain sections. One of these negative controls is shown on figure 4. Analysis with ImageJ showed a coverage of 0.041% in the TG negative control, and 0.005% for the WT negative control, most likely as a result of dirt or smudges. With these very low

percentages in mind, it can be assumed that coverage in the stained sections can be seen as a measure of Aβ plaques, and not of other entities.

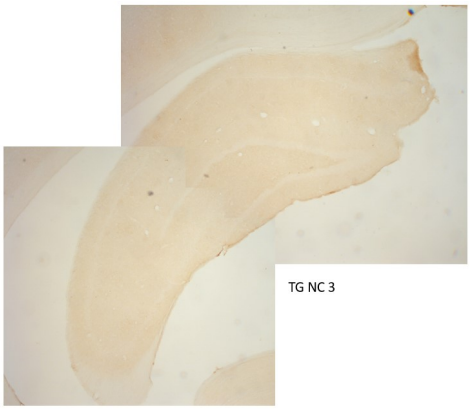

Figure 4 *Negative control of a hippocampal section from one of the TG- rats. No plaques can be seen.*

HYPOTHESIS 1 – PLAQUE COVERAGE

It was expected that the TG-rats would present with more Aβ plaque coverage than their WT littermates. Additionally, it was expected that the TG-rats would present with a similar plaque coverage to that of the rat from the 2019 study. Figure 5 shows hippocampal sections from a TG- and a WT-rat, and figure 6 illustrates the average plaque coverage found within the two genotypes, as well as plaque coverage in the rat from the 2019 study.

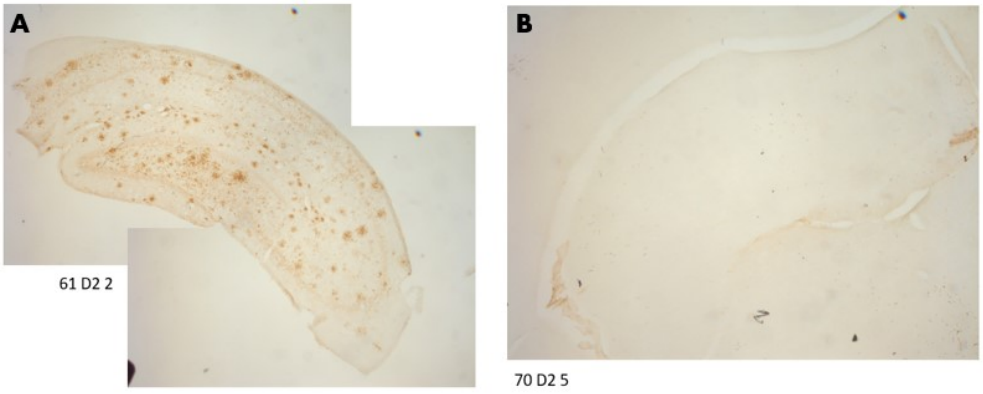

Figure 5 *Hippocampal sections from: (A) a TG-rat; (B) a WT-rat.*

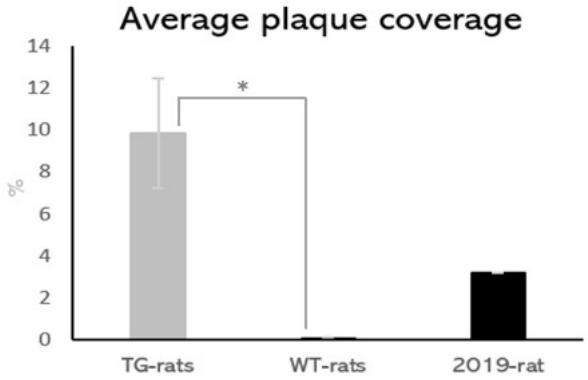

Figure 6 *Average plaque coverage in TG-rats, WT-rats, and 2019-rat. Standard deviation is displayed. * indicates p<0.05.*

A clear difference can be seen between the images of the hippocampal sections of the TG- and WT-rats, and on the average plaque coverage as well. As expected, a t-test shows a significant difference between the two groups: $t(8) = -5.02$, $p=0.001$; Cohen's d = 7.38. As for the 2019-rat, there seems to be some difference between the plaque coverage of this rat, and both the WT-rats and the TG-rats of the same age. Performing a t-test is not possible due to the single data point, but further discussion will ensue below (cf. *Discussion of hypothesis 1 – Plaque coverage*, p. 45).

HYPOTHESIS 2 – PLAQUES AND BEHAVIOR

Using the hippocampus-dependent Barnes Maze, Christensen (2021) found a difference between TG-rats and WT-rats at age 10 months with regards to cognition when looking into memory, but not learning, using scores of primary latency, time spent in target quadrant, and number of primary errors on day 10. It is therefore relevant to explore the possible association between these measures of hippocampal memory and the plaque coverage in the hippocampus. Figure 7 shows the patterns of these associations.

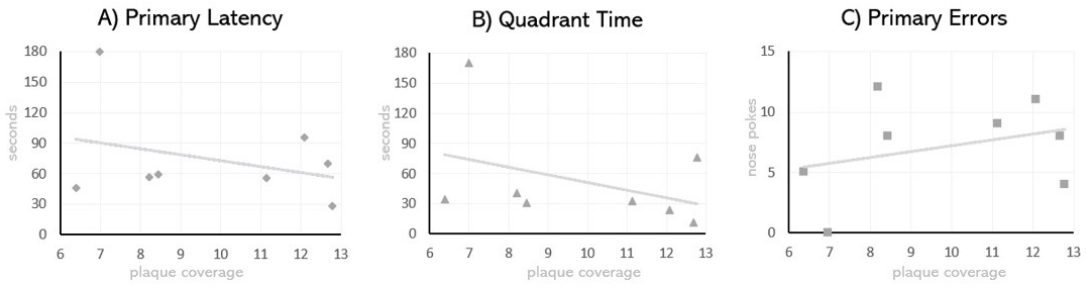

Figure 7 *Associations between plaque coverage and measures of memory on the Barnes Maze, specifically (A) primary latency; (B) time spent in target quadrant; (C) primary errors.*

For all three measurements, the data points are widely spread across the graphs, suggesting non-significant correlations. True enough, correlation analysis shows no significant correlation between plaque coverage and primary latency, $r(6) = 0.323$ (p=0.436), nor between plaque load and time spent in the target quadrant, $r(6) = 0.386$ (p=0.345), and also not between plaque load and primary errors $r(6) = 0.317$ (p=0.444). There therefore seems to be no correlation between the plaque coverage and the memory impairments in the 8 TG-rats employed in this study.

DISCUSSION

The above presented results will, in the coming sections, be further discussed in relation to the previously presented current knowledge about AD. Following this, there will be a consideration of the study itself, and of potential future avenues of research related to the TgF344-AD rat model.

DISCUSSION OF HYPOTHESIS 1 – PLAQUE COVERAGE

As expected, the 10-month-old TG-rats presented with a significantly higher Aβ plaque coverage in their hippocampus than the WT-rats of the same age. Furthermore, the effect size as measured by Cohen's d was 7.38, which is medium-large, indicating a strong difference between the two genotypes. No significant gender differences were found with regards to plaque coverage, which is consistent with previous studies of the model (Cohen et al., 2013, p. 6246). Similarly, human studies do not report gender differences in plaque load (Franzmeier et al., 2020; Jellinger, 2006; Crowther et al., 2011). There seems to be some difference between the TG-rats with regards to plaque coverage though, as the average coverage spans from 6.39% in the rat with the lowest coverage (TG54), to double this, 12.78%, in the rat with the highest coverage (TG58). Also within the same rat, a variance between the coverage of different hippocampal sections was found, as measures span a difference in plaque percentage from 1.42 in rat TG55 up to 9.18 in rat TG57. This wide variance between individuals in the amount of Aβ present in the hippocampus is also found in humans (Braak & Braak, 1991, p. 239). It seems then, that this rat model may also give an (at least to some extent) accurate representation of the human Aβ accumulation with regards to this aspect.

In spite of the variance, most AD patients do nevertheless show some Aβ deposition, and often even years before the onset of clinical symptoms (Selkoe & Hardy, 2016, p. 595; p. 600). Braak and Braak (1991) found, that plaques were typically present in the hippocampus at middle and late stages of the disease, but not at early stages (p. 242f). The widespread presence of plaques in the rats employed in this study would suggest, then, that TgF344-AD rats are either in the middle or late stage of their disease course at age 10 months. Additionally, with regards to behavioral results, the 10-month-old rats show somewhat, but not fully, impaired cognition, and seem to show some neuropsychiatric symptoms as well (Christensen, 2021, pp. 17-19). Considering this, it appears likely that the 10-month-old rats are somewhere in the middle stage of their disease course. It is noteworthy however that the rats are models of the familial form, EOAD, rather than LOAD, since their disease develops on the basis of human AD genes (Delatour et al., 2011, p. 137; Cohen et al., 2013, p. 6246). Therefore their disease course is likely to be more aggressive, and therefore not completely comparable with, the disease course of most human patients (Son et al., 2016, p. 696; Zanni et al., 2018, p. 849). Knowledge about clinical and neuropathological presentation at various stages can nonetheless still provide relevant information, even if they set in earlier than one would normally expect.

The presence of Aβ in the hippocampus of the TgF344-AD rats, as well as their cognitive impairments presented in this and several other studies (Cohen et al., 2013, p. 6249; Christensen, 2021, p. 17), following injection of mutant human APP and PSEN1 genes and nothing else supports the Amyloid Cascade Hypothesis insofar that these genes can lead to AD neuropathology and clinical pathology (Long & Holtzman, 2019, p. 315). Furthermore, it seems that these mutated genes not only lead to an abundance of Aβ and to memory impairment in these rats, but also to the presence of NFT and neuronal loss (Cohen et al., 2013, p. 2651f), and to some neuropsychiatric symptoms (Pentkowski et al., 2018, pp. 173f; Christensen, 2021, p. 19) – all of these common parts of AD pathology (cf. *Background*, p. 12). These findings further support the Amyloid Cascade Hypothesis. In previous animal models, the injection of these genes has not led to NFT, but as rats are closer to humans evolutionarily speaking, they might serve as a better model which could explain the presence of NFT in this model, where it is vacant in others (Cohen et al., 2013, p. 6245f).

Apart from the TG-rats showing a higher plaque load than the WT-rats, it was also expected that the rats would present with a similar plaque coverage to the 2019-rat, that is, the 10-month-old rat examined by Jensen and colleagues (2019). Some comments are to be tied to the comparison with this rat. First of all, Jensen and colleagues (2019) used three different dilutions of the primary antibody: 1:5,000, 1:10,000, and 1:20,000 (p. 6), whereas this study only used the dilution 1:20,000. It is not known which of these dilutions were applied to the 2019-rat, and it is therefore possible that it is not the same as the rats employed in this study. However, Jensen and colleagues (2019) claim that the three dilutions provide similar stainings (p. 11), for which reason it will be considered in this study after all. Second, only a single section is available from the 2019-rat, and the plaque coverage might therefore not be representative of the whole hippocampus of this rat. The fact that only one section is available leads to another problem, namely that of statistical comparison. As it is not possible to perform a t-test with this data, instead the 95% confidence interval of the average plaque coverage of the TG-rats employed in this study was calculated. This is because the confidence interval provides an idea about the limits within which the mean will fall, thereby allowing for some comparison with the 2019-rat: If the 2019-rat's plaque coverage falls inside the confidence interval of the TG-rats' plaque coverage, it is likely to be similar to that of the TG-rats, whereas the opposite is true if it falls outside the confidence interval (Field, 2018, p. 70). The mean average plaque coverage was found to be 9.84%, with a 95% confidence interval from 8.02–11.66. The 2019-rat yielded a plaque coverage of 3.19%, which is then outside the confidence interval, suggesting a difference between this rat and the other 10-month-old TG-rats. However, 3.19% is not very far from the lowest plaque coverage in any of the hippocampal sections from the TG-rats, which was 3.31% (TG54), and in general the variance between plaque coverage is high both between and within rats. It is therefore quite possible, that although a difference is seen, this could be explained by a coincidence of which exact section was chosen in the 2019 study. The best way to eliminate these concerns would be to collect more data on the rats employed in this study, and the study by Jensen and colleagues (2019).

DISCUSSION OF HYPOTHESIS 2 – PLAQUES AND BEHAVIOR

Like in humans, then, the progressive accumulation of Aβ in the hippocampus is present in the TgF344-AD model. As is the progressive impairment of memory and

learning, as further discussed in Christensen (2021, p. 17). Drawing on the results from that study, a potential association between hippocampal memory impairment and plaque load was examined, but not found in this study. In fact, the correlation was nowhere near significant, and not even a trend was seen. Although there is some discussion as to the sensitivity of the various measures for memory using the Barnes Maze, particularly the number of primary errors is seen as a sensitive measure (Gawel et al., 2018, p. 9). This measurement however is, like the other two, not correlated with plaque coverage, and it therefore seems that in these rats, at least at age 10 months, there is no direct association between plaque load and memory performance. This finding then does not seem to support the Amyloid Cascade Hypothesis, which anticipates that plaque load would lead to cognitive impairment. It is nevertheless not a surprising finding, as it confirms the results of several studies performed with humans (Middleton et al., 2011; Edison et al., 2007; Ossenkoppele et al., 2015).

Nonetheless, it is relevant to consider some potential confounding factors which might explain (part of) the lack of association. First of all, the rats in this study underwent less training on the Barnes Maze than is typically the case, which was done to avoid potential overtraining. More training might have led to slightly different final outcomes. However, all TG-rats did show learning, as well as an unimpaired memory relative to the WT-rats on day 3. Moreover, after the last of the learning trials all of the TG-rats had found and entered the target hole within the time limit, which implies that they had no issues performing the task at the end of the training. It is therefore deemed unlikely that fewer learning trials affected the final outcome to such an extent, that it would change the association between plaque load and memory impairment.

A more likely confounding factor is that of neuropsychiatric symptoms, which are common in AD patients and therefore are likely to also be common in animal models of AD. It is once again to be stressed that studies within this field are limited, but Pentkowski and colleagues (2018) did find increased anxiety in TgF344-AD rats as young as 4 months old. Furthermore, the 10-month-old TG-rats employed in this study seemed to show increased defecation and urination relative to the WT-rats, which is a sign of stress in animals (Christensen, 2021, p. 19). Finally, as discussed

above (cf. *Cognitive impairment and neuropsychiatric symptoms*, p. 17), depression in AD seems to be related to hippocampal dysfunction among other things (Pandya et al., 2012, pp. 635f), and it is clear from the results of this study that these rats do show hippocampal damage. Additionally, depression is more common in EOAD patients than LOAD patients (Barnes et al., 2015, p. 1352), and the TgF344-AD rats are models are EOAD (Delatour et al., 2011, p. 137). All of this is important because neuropsychiatric symptoms in AD patients seem to exacerbate the disease course and are related to worse cognitive deterioration in humans (Li et al., 2014, p. 5). It should be considered, then, if the same picture emerges in the TgF344-AD rats. At age 4-6.5 months, Pentkowski and colleagues (2018) found that increased anxiety did not impair the rats' spatial learning abilities. As for age 10 months, depression, anxiety, or stress has not been directly assessed. Nonetheless, since McHail and colleagues (2018) argue that time spent deliberating before initial movement can be used as a measure of anxiety (p. 144), it is possible to examine the effect of anxiety by this measure post-hoc. Figure 8 illustrates the average deliberation time before initial movement on each trial.

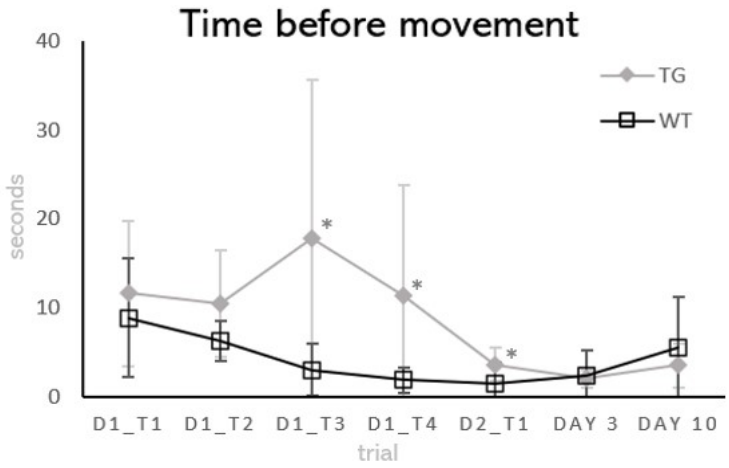

Figure 8 *Average deliberation time of TG- and WT-rats before initial movement on each trial. Standard deviation is displayed. * indicates $p<0.05$.*

As it turns out, there is no significant difference between TG- and WT-rats with regards to deliberation time on day 3 and 10, in which memory is assessed. A significant difference is however observed on the three last of the five trials assessing learning. This might imply that both groups are anxious when the test first begins (an understandable reaction), but whereas the WT-rats quickly adjust, this is not the case for TG-rats who need a longer time to adjust. One might argue that these results are

in favor of more learning trials, but since there is no difference in deliberation on day 3, it seems that five learning trials could still be adequate. Either way, some anxiety seems to be present some of the days, and as anxiety is related to decreased cognitive performance there is a risk that this plays a role in the lack of association between the rats' memory performance and hippocampal plaque load. It is difficult to say how big this role is though. Since the correlations between the measures are very far from significant, and since there is no difference between the genotypes on the measure for anxiety on day 10 of the Barnes Maze testing, it is still quite possible that even when taking neuropsychiatric symptoms into account, no correlation between memory impairment and plaque load would be found.

Another consideration relates to the testing of memory itself, and whether or not it is the cognitive impairment of most relevance to look into for these rats. Memory impairment and hippocampal dysfunction are the symptoms most characteristic of AD, and for this reason most of the animal research on AD has focused on this aspect (Delatour et al., 2011, p. 146). But as mentioned above, the TgF344-AD rats have familial EOAD, and at least in humans, patients suffering from EOAD are much more likely than LOAD patients to present with non-memory cognitive symptoms (Barnes et al., 2015, pp. 1349-1351). Furthermore, NFT, which in some studies are found to follow the distribution of Aβ, are more widespread in the frontal and parietal lobes in EOAD patients (Jellinger, 2006, p. 1618; Schöll et al., 2017, p. 2289). It might be then, that the association between cognitive impairment and plaque load is much stronger – that is, exists at all – when examining other cognitive abilities and other areas of the brain in these rats. However, according to Noh and colleagues (2017), Aβ and NFT accumulation are not correlated in EOAD, which may therefore suggest that no correlation will be found when examining other brain regions either. Additionally, two thirds of EOAD patients still do present with memory symptoms first (Koedam et al., 2010, p. 1403), and the rats in this study definitely have both memory impairments and plaque abundance in the hippocampus. Additionally, Noh and colleagues (2017) found that in EOAD, unlike in LOAD, the spread of the biomarkers Aβ and NFT is not correlated (p. 3). Since the distribution of NFT is highly correlated with cognitive impairment (Long & Holtzman, 2019, p. 315), it does seem logical that memory impairment and plaque load are not associated in the rats employed in this study.

METHODOLOGICAL CONSIDERATIONS AND FUTURE RESEARCH

Although the lack of correlation between plaque load and cognitive memory impairment is well established, more recent research has started looking into the role of amyloid oligomers, rather than diffuse plaques, and found that these induce tau pathology in vitro. Additionally, whereas Aβ is commonly present in healthy individuals, it is diffuse plaques rather than oligomers that dominate these cases (Selkoe & Hardy, 2016, p. 596f). The role of oligomers in vivo is still a debated topic, but nevertheless a consensus is emerging that it is oligomers, rather than diffuse plaques, that are the amyloid culprits of AD (Long & Holtzman, 2019, p. 315; Glabe, 2005, pp. 172-174). It is therefore a limitation of this study that no differentiation is made between the type of amyloid in the hippocampi of the rats – especially since it is known that both $A\beta_{42}$ and oligomers are present in these rats (Cohen et al., 2013, p. 6246). Similarly, the relative presence of diffuse, primitive, or classic plaques (Armstrong, 2011, p. 72) has not been investigated, nor is a differentiation made between intracellular and extracellular plaques (Crowther et al., 2011, p. 61). Finally, the relative presence of $A\beta_{40}$ and the more toxic $A\beta_{42}$ has not been examined. Although this study nonetheless provides valuable results, a more nuanced picture of the presentation of amyloid in the hippocampus of the rat model might in the future provide a clearer and more accurate idea of AD etiology and course.

Part of the reason why there has been no differentiation with regards to the types of amyloid plaques is due to the immunohistochemical method employed in this study, which is not sensitive to these concerns. Some further comments can be tied to the use of this method. Immunohistochemistry is a complex process with many steps that can go wrong, from fixation of the brain to interpretation of the results (Taylor & Levenson, 2006, pp. 413-415). Each of these steps involve human actions, and after all, as the famous saying goes, "*to err is human*" (Alexander Pope). Noticeably, this does not mean that errors will discredit the results. Rather, it means that there might be small differences between the procedure of this study, and of past and future studies. This includes how well-performed the fixation was, how many sections were lost during sectioning, the exact time between when different solvents were added in the staining process, and perhaps most of all variances in the image analysis. Particularly, there are the dark wedges on the edited images to consider, as these can

interfere with how many plaques are counted as plaques, and how many are miscategorized as dark background, or vice versa. Furthermore, the settings in ImageJ, such as brightness or threshold, in the end all come down to a personal evaluation of what seems the most appropriate. These details can, and will have, affected the final results. Nonetheless, by taking precautions in practicing fixation beforehand and to check that no sections are lost during sectioning, and by thoroughly following and documenting the staining protocol and analysis procedure, these potential issues can be strongly limited, if not completely circumvented. For this reason, these precautions were taken, and the appendix of this thesis includes the staining protocol (Appendix A), alongside a documentation of the settings applied in ImageJ during image analysis (Appendix B).

The number of rats employed in the study is quite small, and so is the number of analyzed hippocampal sections from each rat. This was in part due to the mentioned dark wedges, and in part due to time restrictions. Since the plaque coverage varies widely even within the individual rat, as shown above, analyzing only two sections can possibly skew the results. Nevertheless, it does give an indication of the total plaque load. This study also enhances the credibility of applying this particular staining protocol, as it is now shown to consistently detect the plaque coverage, and differences herein, in several 10-month-old rats. The next step, then, is to apply this method on TgF344-AD rats of other ages. As for the number of rats employed, this is of course due to practical and ethical limitations. However, as the animal model, unlike humans, is bred and raised for the purpose, the variability between cases will be much lower than in humans, for which reason much fewer subjects are required when working with transgenic animal models (Hansen, 2005, p. 18).

To sum up, the TgF344-AD model seems to be a very promising model of human AD, and the results of the study performed in relation to and presented in this thesis further support this notion. It would therefore be prudent that future research on the model, apart from looking into plaque load, also consider the development of NFT, neuronal and synaptic loss, and especially the most recent focus of AD-research: neuroinflammation – both in the hippocampus, but also in other various relevant brain regions, which have been touched upon in this thesis, such as the frontal and parietal cortices, as well as the entorhinal cortex and the locus coeruleus (cf.

Background, p. 12). These regions are particularly relevant, because the rats are, as previously mentioned, a model of EOAD, and these brain regions are often more involved in EOAD than in LOAD. In general the rats show limited neuropathology at age 6 months, whereas the disease seems to have taken its hold at age 16 months (Tudela et al., 2019, p. 2; Cohen et al., 2013, p. 6251f). As AD is usually discovered in humans somewhere in-between these two stages, it will also be highly relevant to explore the rat model at various ages between 6 and 16 months, as this time period is likely to be the one that future treatments will have to be aimed towards. Additionally, as technology advances, it might become of interest to apply in vivo brain mapping to learn more about the rat model and its disease course.

Apart from further examining the rats' neuropathology, the neuropsychiatric symptoms are also not to be forgotten as they do play a tremendous role in human AD, exacerbating the disease, and as they are likely to influence cognitive test scores (Li et al., 2014, p. 5). Cohen and colleagues (2013) themselves state that these rats might suffer from disinhibition (p. 6249), and as previously considered there seems to be some evidence for anxiety in the rats as well (cf. *Discussion of hypothesis 2 – Plaques and behavior*, p. 47). Apart from further examining these symptoms, apathy could also be particularly relevant to look into. It is present in close to half of human AD cases (Li, Hu, Tan, Yu & Tan, 2014, p. 4), yet no studies have been found that consider the role hereof in the animal model, even in spite of the fact that Aβ – which is shown to definitely be present in these rats – in the frontal cortex seems to correlate with apathy in humans (Mori et al., 2014, p. 451). Apart from the frontal cortex, also the locus coeruleus seems to play a role in the development of apathy (Passamonti et al., 2018, p. 17). Neuronal loss in this brain region additionally correlates with tau pathology, which is present here relatively early in the disease course (Franzmeier et al., 2020, p. 2). There is still a lot, then, to learn about the TgF344-AD model, and that it can in turn teach us.

CONCLUSION

Alzheimer's Disease is a major problem in today's society. Gaining knowledge about it gets more important every year as more and more people receive this diagnosis, so that this knowledge might lead to the development of possible prevention and

treatment strategies. One important step on the way to developing preventive methods and treatments is animal models. In order to adequately develop and evaluate such animal models, it is important to learn about both the clinical and neuropathological symptoms of AD, as well as about the disease course in general. This thesis has sought to give an understanding of the current knowledge within the field. Furthermore, the thesis has presented a study of the seemingly highly relevant animal model for AD: the TgF344-AD rat model.

Although a long and exciting path of research on the TgF344-AD model still lies in the future, it is exactly the relevance of the model that makes this path an interesting avenue to follow. And the relevance of the model is just what this thesis has sought to elaborate on, particularly by focusing on the presence of beta-amyloid in the hippocampus of the rat-model. Previously, studies have examined the hippocampal plaque load at 6 months, 9 months, 16 months, and 24 months of age (Joo et al., 2017; Cohen et al., 2013). The findings of these studies suggest that the amyloid pathology seems to begin somewhere around 9 months of age, for which reason it was particularly of interest to learn more about what is happening right around that point in the disease course. Therefore, 10-month-old rats were examined in this thesis.

The major goal of the study was to determine the hippocampal amyloid plaque load in the rats, which was found to be widely present throughout the hippocampus in the transgenic rats, but not in their wildtype littermates. The rats show similarities to human Aβ accumulation, both with regards to no significant gender differences being found, and with regards to the wide variation in Aβ both within and between rats.

A further aim of this thesis was to explore if any correlation between plaque load and memory impairment, as measured by Barnes Maze, would be seen. This was, just like in humans, not the case. Interestingly, the rat model starts exhibiting memory impairment, but not learning impairment between ages 6 and 10 months. Neuropsychiatric symptoms are also seen at this point (Christensen, 2021; Pentkowski et al., 2018). An argument has therefore been made for the 10-month-old rats being past the preclinical disease stage, but not yet in the severe stage – ergo somewhere in the middle, whereas 6 months of age might serve as the preclinical stage. Humans do typically not show high plaque coverage in the hippocampus in the

early stages of the disease course, whereas it is present in middle stages. As the TgF344-AD model also does not show plaque coverage at age 6 months, but does at age 10 months, this is another way in which it presents as an appropriate model for human AD.

Finally, this study employs immunohistochemical staining of hippocampal sections of TgF344-AD rats, thereby becoming the second study known to the author applying this method on this rat model. In combination with the results from the previous study by Jensen and colleagues (2019), this thesis then helps create an argument for immunohistochemical staining being applicable when examining accumulation of Aβ in the hippocampus of the TgF344-AD rat model.

Tudela and colleagues (2019) deem the model "*one of the most suitable and promising animal models for AD research*", and the results of the study performed in relation to this thesis further support this statement.

REFERENCES

Alzheimer's Association (2021) 2021 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, *17*(3).

Armstrong, R. A. (2009). The Spatial Patterns of β-Amyloid (Aβ) Deposits and Neurofibrillary Tangles (NFT) in Late-Onset Sporadic Alzheimer's Disease. In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments*. Nova Science Publishers, Incorporated.

Barnes, J., Dickerson, B., Frost, C., Jiskoot, L., Wolk, D., & van der Flier, W. (2015). Alzheimer's disease first symptoms are age dependent: Evidence from the NACC dataset. *Alzheimer's & Dementia*, *11*(11).

Bayles, K., & Tomoeda, C. (2014). *MCI and Alzheimer's Dementia: Clinical Essentials for Assessment and Treatment of Cognitive-Communication Disorders* (1st ed.). Plural Publishing, Inc. Chapter 2 & 3

Bernhardi, R. (2009). Participation of Glial Cells in the Pathogenesis of AD: A Different View on Neuroinflammation. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease*. Springer.

Blennow, K., Hampel, H., Zetterberg, H., & Weiner, M. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature Reviews. Neurology*, *6*(3).

Boxer, A. L. (2011). Frontotemporal Dementia. In Budson, E. & Kowall, N. W., *The Handbook of Alzheimer's Disease and Other Dementias*. Wiley-Blackwell.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, 82(4).

Breedlove, S. M. & Watson, N. V. (2017). Behavioral Neuroscience (8th Ed.). Sinauer Associates. Chapter 3 & 17.

Brüel, A., Geneser, F., & Geneser, F. (2016). *Genesers histologi.* (1st ed.). Munksgaard.

Busfieldm F. & Goate, A. M. (1995). The genetics of Alzheimer's disease and mutations in the amyloid β-protein precursor gene. In Jenner, P., Goate, A., Ashall, F., & Ashall, R. *Pathobiology of Alzheimer's Disease*. Elsevier Science & Technology.

Campion, D., Dumanchin, C., Hannequin, D., Dubois, B., Belliard, S., Puel, M., Thomas-Anterion, C., Michon, A., Martin, C., Charbonnier, F., Raux, G., Camuzat, A., Penet, C., Mesnage, V., Martinez, M., Clerget-Darpoux, F., Brice, A., & Frebourg, T. (1999). Early-Onset Autosomal Dominant Alzheimer Disease: Prevalence, Genetic Heterogeneity, and Mutation Spectrum. *American Journal of Human Genetics*, *65*(3).

Christensen, V. K. (2021). Alzheimer og Adfærd - TgF344-AD Rotters Spatiale Lærings- og Hukommelsesevner. *Aalborg Universitet*.

Cohen, R., Rezai-Zadeh, K., Weitz, T., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y., Vasilevko, V., Glabe, C., Breunig, J., Rakic, P., Davtyan, H., Agadjanyan, M., Kepe, V., Barrio, J., Bannykh, S., Szekely, C., Pechnick, R., & Town, T. (2013). A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric aβ, and frank neuronal loss. *The Journal of Neuroscience*, *33*(15).

Crews, L., Spencer, B. & Masliah, E. (2009) Role of Aβ Degrading Enzymes in Synaptic Plasticity and Neurogenesis in Alzheimer's Disease. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease*. Springer.

Crowther, D. C., Page, R. M., Luheshi, L. & Lomas, D. A. (2009) How and Where Does Aβ Exert its Toxic Effects in Alzheimer's Disease? In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments*. Nova Science Publishers, Incorporated.

DeKosky, S., Scheff, S., & Styren, S. (1996). Structural Correlates of Cognition in Dementia: Quantification and Assessment of Synapse Change. *Neurodegeneration* (London, England), 5(4).

Delatour, B., Le Cudennec, C., Tayara, N. E. T. & Dhenain, M. (2009). Transgenic Models of Alzheimer's Pathology: Success and Caveats. In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments*. Nova Science Publishers, Incorporated.

Di Paola, M., Macaluso, E., Carlesimo, G., Tomaiuolo, F., Worsley, K., Fadda, L., & Caltagirone, C. (2007). Episodic memory impairment in patients with Alzheimer's disease is correlated with entorhinal cortex atrophy: A voxel-based morphometry study. *Journal of Neurology*, 254(6).

Dierckx, E., & Osterwalder, H. (2007). *Early detection of Alzheimer's disease : a neuropsychological approach* . VUBPress. Chapter 0.

Dierckx, E., Engelborghs, S., De Raedt, R., De Deyn, P. P., & Ponjaert-Kristoffersen, I. (2007). Differentiation between Mild Cognitive Impairment, Alzheimer's disease and depression by means of cued recall. Psychological Medicine, 37.

Dubois, B., Feldman, H., Jacova, C., DeKosky, S., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P., & Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria. *Lancet Neurology*, *6*(8).

Edison, P., Archer, H., Rossor, M., Brooks, D., Hinz, R., Hammers, A., Pavese, N., Tai, Y., Hotton, G., Cutler, D., Fox, N., & Kennedy, A. (2007). Amyloid, hypometabolism, and cognition in Alzheimer disease : An [11C]PIB and [18F]FDG PET study. *Neurology*, 68(7).

Field, A. (2018). Discovering Statistics Using IBM SPSS Statistics (5 ed.). Sage Publishers.

Franzmeier, N., Neitzel, J., Rubinski, A., Smith, R., Strandberg, O., Ossenkoppele, R., Hansson, O., & Ewers, M. (2020). Functional brain architecture is associated with the rate of tau accumulation in Alzheimer's disease. *Nature Communications*, 11(1).

Gaiteri, C., Mostafavi, S., Honey, C., De Jager, P., & Bennett, D. (2016). Genetic variants in Alzheimer disease - molecular and brain network approaches. *Nature Reviews. Neurology*, *12*(7).

Galasko, D., & Shaw, L. (2017). Alzheimer disease: CSF biomarkers for Alzheimer disease - approaching consensus. *Nature Reviews. Neurology*, *13*(3).

Gawel, K., Gibula, E., Marszalek-Grabska, M., Filarowska, J. & Kotlinska, J. H. (2018). Assessment of spatial learning and memory in the Barnes maze task in rodents – methodological consideration. Naunyn-Schmiedeberg's Archives of Pharmacology, 392.

Giri, M., Zhang, M., & Lü, Y. (2016). Genes associated with Alzheimer's disease: an overview and current status. *Clinical Interventions in Aging*, *11*.

Glabe, C. C. (2005). Amyloid Accumulation and Pathogensis of Alzheimer's Disease: Significance of Momomeric, Oligomeric and Fibrillar Aβ. In Harris, J., & Fahrenholz, F. *Alzheimer's Disease: Cellular and Molecular Aspects of Amyloid*. Springer Science+Business Media, Inc.

Hansen, A. K. (2005). Planlægning og evaluering af dyreforsøg. In Krohn, T. C. *Forsøgsdyrhåndbogen: Håndbog for Kategori B Kursus* (pp. 15-18). Laboratorium for forsøgsdyrkundskab og -velfærd.

Heneka, M., Carson, M., Khoury, J., Landreth, G., Brosseron, F., Feinstein, D., Jacobs, A., Wyss-Coray, T., Vitorica, J., Ransohoff, R., Herrup, K., Frautschy, S., Finsen, B., Brown, G., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., … Kummer, M. (2015). Neuroinflammation in Alzheimer's disease. *Lancet Neurology*, 14(4).

Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. *Dialogues in clinical neuroscience*, *5*(1).

Huey, E., Lee, S., Cheran, G., Grafman, J., & Devanand, D. (2017). Brain Regions Involved in Arousal and Reward Processing are Associated with Apathy in Alzheimer's Disease and Frontotemporal Dementia. *Journal of Alzheimer's Disease*, 55(2).

Jellinger, K. (2006). Alzheimer 100 – highlights in the history of Alzheimer research. *Journal of Neural Transmission*, *113*(11).

Jensen, K. P., Andersen, M. R., Larsen, S., & Hansen, J. L. (2019). Genotype Dependent Aggravation of Alzheimer's Disease in a TgF344-AD Rat Model. Aalborg Universitet.

Joo, I., Lai, A., Bazzigaluppi, P., Koletar, M., Dorr, A., Brown, M., Thomason, L., Sled, J., McLaurin, J., & Stefanovic, B. (2017). Early neurovascular dysfunction in a transgenic rat model of Alzheimer's disease. *Scientific Reports*, 7(1).

Kapasi, A., DeCarli, C., & Schneider, J. (2017). Impact of multiple pathologies on the threshold for clinically overt dementia. *Acta Neuropathologica*, *134*(2).

Kern, S., Zetterberg, H., Kern, J., Zettergren, A., Waern, M., Höglund, K., Andreasson, U., Wetterberg, H., Börjesson-Hanson, A., Blennow, K., & Skoog, I. (2018). Prevalence of preclinical Alzheimer disease: Comparison of current classification systems. *Neurology*, 90(19).

Knierim, J. (2015). The hippocampus. *Current Biology*, 25(23).

Koedam, E., Lauffer, V., van der Vlies, A., van der Flier, W., Scheltens, P., & Pijnenburg, Y. (2010). Early-Versus Late-Onset Alzheimer's Disease: More than Age Alone. *Journal of Alzheimer's Disease*, *19*(4).

Korolev, I., Symonds, L., & Bozoki, A. (2016). Predicting Progression from Mild Cognitive Impairment to Alzheimer's Dementia Using Clinical, MRI, and Plasma Biomarkers via Probabilistic Pattern Classification. *PloS One*, *11*(2).

La Joie, R., Perrotin, A., Barré, L., Hommet, C., Mézenge, F., Ibazizene, M., Camus, V., Abbas, A., Landeau, B., Guilloteau, D., de La Sayette, V., Eustache, F., Desgranges, B., & Chételat, G. (2012). Region-specific hierarchy between atrophy, hypometabolism, and β-amyloid (Aβ) load in Alzheimer's disease dementia. *The Journal of Neuroscience*, 32(46).

Li, X., Hu, N., Tan, M., Yu, J., & Tan, L. (2014). Behavioral and Psychological Symptoms in Alzheimer's Disease. *BioMed Research International*, *2014*.

Lockhart, A., Lamb, J., Osredkar, T., Sue, L., Joyce, J., Ye, L., Libri, V., Leppert, D., & Beach, T. (2007). PIB is a non-specific imaging marker of amyloid-beta (Aβ) peptide-related cerebral amyloidosis. *Brain (London, England : 1878)*, *130*(10).

Long, J., & Holtzman, D. (2019). Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell (Cambridge)*, *179*(2).

McHail, D. G., Valibeigi, N. & Dumas, T. (2018). A Barnes maze for juvenile rats delineates the emergence of spatial navigation ability. Learning and Memory.

Middleton, L., Grinberg, L., Miller, B., Kawas, C., & Yaffe, K. (2011). Neuropathologic features associated with Alzheimer disease diagnosis: Age matters. *Neurology*, 77(19).

Miljø- og Fødevareministeriet (2018). Bekendtgørelse af dyreværnsloven. Accessed 06.05.21. at 11:22 from Dyreværnsloven (retsinformation.dk)

Mitchell, A., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia meta-analysis of 41 robust inception cohort studies. *Acta Psychiatrica Scandinavica*, *119*(4).

Mori, T., Shimada, H., Shinotoh, H., Hirano, S., Eguchi, Y., Yamada, M., Fukuhara, R., Tanimukai, S., Zhang, M., Kuwabara, S., Ueno, S., & Suhara, T. (2014). Apathy correlates with prefrontal amyloid β deposition in Alzheimer's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, 85(4).

Nagahama, Y., Okina, T., & Suzuki, N. (2015). Impaired imitation of gestures in mild dementia: comparison of dementia with Lewy bodies, Alzheimer's disease and vascular dementia. *Journal of Neurology, Neurosurgery & Psychiatry*, 86(11).

Nakamura, K., Kumai, K., Takada, J., Chida, K., Kato, Y., Takahashi, Y., Takahashi, K., Nakatsuka, M., Yamaguchi, S., & Meguro, K. (2017). Behavioral and psychological symptoms of dementia (BPSD) in Alzheimer disease is "cognitive" symptoms? The Osaki-Tajiri Project. *Alzheimer's & Dementia*, *13*(7).

National Institute on Ageing (2021). What is Dementia? Symptoms, Types, and Diagnosis. Accessed 12.04.21 at 10:45 from What Is Dementia? Symptoms, Types, and Diagnosis | National Institute on Aging (nih.gov)

Noh, Y., Na, H., Seo, S., Lee, S., Jeong, H., Okamura, N., Ido, T., & Na, D. (2017). Relationship among tau, amyloid burden and brain atrophy in early-onset and late-onset Alzheimer's disease. *Alzheimer's & Dementia*, 13(7).

Nunomura, A., Takeda, A., Moreira, P. I., Castellani, R. J., Lee, H., Zhu, X., Smith, M. A. & Perry, G. (2009). Neurofibrillary Tangle Formation as a Protective Response to Oxidative Stress in Alzheimer's Disease. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease*. Springer.

O'Brien, J., & Thomas, A. (2015). Vascular dementia. *The Lancet (British Edition)*, *386*(10004).

Ogden, J.A. (2005). *Fractured Minds: A Case Study Approach to Clinical Neuropsychology* (2nd ed.) Oxford University Press. Chapters 1 & 9.

Olivieri, P., Lagarde, J., Lehericy, S., Valabrègue, R., Michel, A., Macé, P., Caillé, F., Gervais, P., Bottlaender, M., & Sarazin, M. (2019). Early alteration of the locus coeruleus in phenotypic variants of Alzheimer's disease. *Annals of Clinical and Translational Neurology*, 6(7).

Ossenkoppele, R., Jansen, W., Rabinovici, G., Knol, D., van der Flier, W., van Berckel, B., Scheltens, P., & Visser, P. (2015). Prevalence of Amyloid PET Positivity in Dementia Syndromes A Meta-analysis.

Pandya, M., Altinay, M., Malone Jr, D., & Anand, A. (2012). Where in the Brain Is Depression? *Current Psychiatry Reports*, 14(6).

Pannee, J., Portelius, E., Minthon, L., Gobom, J., Andreasson, U., Zetterberg, H., Hansson, O., & Blennow, K. (2016). Reference measurement procedure for CSF amyloid beta (Aβ)1–42 and the CSF Aβ1–42/Aβ1–40 ratio – a cross-validation study against amyloid PET. *Journal of Neurochemistry*, *139*(4).

Parsons, M. E., Jones, S. E. & Krewson, T. (2015). Structural and functional brain imaging for the neuropsychologist. In Parsons, M. W. & Hammeke, T. A., *Clinical Neuropsychology: A Pocked Handbook for Assessment* (3rd ed.). American Psychological Association.

Pasquali, P. (2018). The importance of animal models in research. *Research in Veterinary Science*, 118.

Passamonti, L., Lansdall, C., & Rowe, J. (2018). The neuroanatomical and neurochemical basis of apathy and impulsivity in frontotemporal lobar degeneration. *Current Opinion in Behavioral Sciences*, 22.

Paxinos, G., & Watson, C. (2002). *The rat brain in stereotaxic coordinates* (4. ed.). Academic Press.

Pentkowski, N., Berkowitz, L., Thompson, S., Drake, E., Olguin, C., & Clark, B. (2018). Anxiety-like behavior as an early endophenotype in the TgF344-AD rat model of Alzheimer's disease. *Neurobiology of Aging*, 61.

Perani, D. (2014). FDG-PET and amyloid-PET imaging: the diverging paths. *Current Opinion in Neurology*, *27*(4).

Perez, P., Hall, G., Kimura, T., Ren, Y., Bailey, R., Lewis, J., Febo, M., & Sahara, N. (2013). In vivo functional brain mapping in a conditional mouse model of human tauopathy (tauP301L) reveals reduced neural activity in memory formation structures. *Molecular Neurodegeneration*, 8(1).

Pontecorvo, M., Devous, S., Navitsky, M., Lu, M., Salloway, S., Schaerf, F., Jennings, D., Arora, A., McGeehan, A., Lim, N., Xiong, H., Joshi, A., Siderowf, A., & Mintun, M. (2017). Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain* (London, England : 1878), 140(3).

Rahman, M., Rahman, M., Uddin, M., Mamum-Or-Rashid, A., Pang, M., & Rhim, H. (2020). Emerging risk of environmental factors: insight mechanisms of Alzheimer's diseases. *Environmental Science and Pollution Research International*, *27*(36).

Raichle, M. (2008). A brief history of human brain mapping. *Trends in Neurosciences (Regular Ed.)*, *32*(2).

Rice, L., & Bisdas, S. (2017). The diagnostic value of FDG and amyloid PET in Alzheimer's disease—A systematic review. *European Journal of Radiology*, 94.

Rojo, L. E., Fernández, J., Jiménez, J., Maccioni, A. A., Sekler, A., Kuljis, R. O. & Maccioni, R. B. (2009). Central Nervous System Inflammation and Cholesterol Metabolism Alterations in the Pathogenesis of Alzheimer's Disease and Their Diagnostic and Therapeutic Implications. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease*. Springer.

Román, G. C. (2005). Clinical Forms of Vascular Dementia. In Paul, R., Cohen, R., Ott, B., & Salloway, S., *Vascular Dementia: Cerebrovascular Mechanisms and Clinical Management* (pp. 7-21). Humana Press Inc.

Sagar, S., Sagar, S., Rick, J., Rick, J., Chandra, A., Chandra, A., Yagnik, G., Yagnik, G., Aghi, M., & Aghi, M. (2019). Functional brain mapping: overview of techniques and their application to neurosurgery. *Neurosurgical Review*, *42*(3).

Sara, S., & Bouret, S. (2012). Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. *Neuron* (Cambridge, Mass.), 76(1).

Schneider, J., Arvanitakis, Z., Leurgans, S., & Bennett, D. (2009). The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Annals of Neurology*, *66*(2).

Schöll, M., Ossenkoppele, R., Strandberg, O., & Palmqvist, S. (2017). Distinct 18F-AV-1451 tau PET retention patterns in early- and late-onset Alzheimer's disease. *Brain* (London, England : 1878), 140(9).

Selkoe, D., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine*, 8(6).

Serrano-Pozo, A., Frosch, M., Masliah, E., & Hyman, B. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*, 1(1).

Smith, G., & Bondi, M. (2013). *Mild cognitive impairment and dementia : definitions, diagnosis, and treatment* . Oxford University Press. Chapter 4.

Snowden, J. (2010). The neuropsychological presentation of Alzheimer's disease and other neurodegenerative disorders. In Gurd, J., Kischka, U. & Marshall, J. (2nd ed), Handbook of Clinical Neuropsychology. Oxford University Press.

Son, S., Chang, K., Noh, J., Roh, H., Oh, B., Kim, D., Jang, M., Kim, S., Choi, S., Lee, K., & Hong, C. (2016). Mortality after a diagnosis of early-onset Alzheimer's disease versus late-onset Alzheimer's disease: A propensity score matching analysis. *Alzheimer's & Dementia*, *12*(7).

Taylor, C., & Levenson, R. (2006). Quantification of immunohistochemistry-issues concerning methods, utility and semiquantitative assessment II. *Histopathology*, *49*(4).

Thal, D. R., Tredici, K. D. & Braak, H. (2004). Neurodegeneration in Normal Brain Aging and Disease. *Science of Aging Knowledge Environment*, 23.

Thermo Fisher Scientific. Overview of Immunohistochemistry (IHC). Accessed 14.04.21 at 14:49 from Overview of Immunohistochemistry | Thermo Fisher Scientific - FR

Traykov, L., Baudic, S., Raoux, N., Latour, F., Rieu, D., Smagghe, A., & Rigaud, A. (2005). Patterns of memory impairment and perseverative behavior discriminate early Alzheimer's disease from subcortical vascular dementia. *Journal of the Neurological Sciences*, *229*.

Tudela, R., Muñoz-Moreno, E., Sala-Llonch, R., López-Gil, X. & Soria, G. (2019). Resting State Networks in the TgF344-AD Rat Model of Alzheimer's Disease are Altered from Early Stages. *Frontiers in Aging Neuroscience*, 11.

Van der Mussele, S., Marien, P., Saerens, J., Somers, N., Goeman, J., De Deyn, P., & Engelborghs, S. (2014). Behavioral Syndromes in Mild Cognitive Impairment and Alzheimer's Disease. *Journal of Alzheimer's Disease*, *38*(2).

Vassar, R. (2005). β-Secretase, APP and Aβ in Alzheimer's Disease. In Harris, J., & Fahrenholz, F. *Alzheimer's Disease: Cellular and Molecular Aspects of Amyloid*. Springer Science+Business Media, Inc.

Victoroff, J., Lin, F., Coburn, K., Shillcutt, S., Voon, V., & Ducharme, S. (2018). Noncognitive Behavioral Changes Associated With Alzheimer's Disease: Implications of Neuroimaging Findings. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *30*(1).

Walters, A., Phillips, E., Zheng, R., Biju, M., & Kuruvilla, T. (2016). Evidence for neuroinflammation in Alzheimer's disease. *Progress in Neurology and Psychiatry* (Guildford), 20(5).

WHO (1994) ICD-10: Psykiske lidelser og adfærdsmæssige forstyrrelser. Munksgaard.

WHO (2020). Dementia. Accessed 12.04.21 at 11:04 from Dementia (who.int)

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., De Leon, M., Decarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., … Petersen, R. (2004). Mild cognitive impairment - Beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *Journal of Internal Medicine*, *256*(3).

Zanni, R., Garcia-Domenech, R., Galvez-Llompart, M., & Galvez, J. (2018). Alzheimer: A Decade of Drug Design. Why Molecular Topology can be an Extra Edge? *Current Neuropharmacology*, 16(6).

CURRICULUM (1405 PAGES)

PREVIOUSLY EXAMINED CURRICULUM (293 PAGES)

Breedlove, S. M. & Watson, N. V. (2017). Behavioral Neuroscience (8th Ed.). Sinauer Associates. Chapter 3 & 17.

Cohen, R., Rezai-Zadeh, K., Weitz, T., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y., Vasilevko, V., Glabe, C., Breunig, J., Rakic, P., Davtyan, H., Agadjanyan, M., Kepe, V., Barrio, J., Bannykh, S., Szekely, C., Pechnick, R., & Town, T. (2013). A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric aβ, and frank neuronal loss. *The Journal of Neuroscience*, *33*(15), 6245–6256.

Dierckx, E., Engelborghs, S., De Raedt, R., De Deyn, P. P., & Ponjaert-Kristoffersen, I. (2007). Differentiation between Mild Cognitive Impairment, Alzheimer's disease and depression by means of cued recall. Psychological Medicine, 37, 747-755.

Field, A. (2018). Discovering Statistics Using IBM SPSS Statistics (5 ed.). Sage Publishers. 64-71, 283, 250.

Galasko, D., & Shaw, L. (2017). Alzheimer disease: CSF biomarkers for Alzheimer disease - approaching consensus. *Nature Reviews. Neurology*, *13*(3), 131–132.

Gawel, K., Gibula, E., Marszalek-Grabska, M., Filarowska, J. & Kotlinska, J. H. (2018). Assessment of spatial learning and memory in the Barnes maze task in rodents – methodological consideration. Naunyn-Schmiedeberg's Archives of Pharmacology, 392, 1-18.

Hansen, A. K. (2005). Planlægning og evaluering af dyreforsøg. In Krohn, T. C. *Forsøgsdyrhåndbogen: Håndbog for Kategori B Kursus* (pp. 15-18). Laboratorium for forsøgsdyrkundskab og -velfærd.

Jensen, K. P., Andersen, M. R., Larsen, S., & Hansen, J. L. (2019). Genotype Dependent Aggravation of Alzheimer's Disease in a TgF344-AD Rat Model. Aalborg Universitet, 1-27.

Li, X., Hu, N., Tan, M., Yu, J., & Tan, L. (2014). Behavioral and Psychological Symptoms in Alzheimer's Disease. *BioMed Research International*, *2014*, 927804–927809.

McHail, D. G., Valibeigi, N. & Dumas, T. (2018). A Barnes maze for juvenile rats delineates the emergence of spatial navigation ability. Learning and Memory, 138-146.

Miljø- og Fødevareministeriet (2018). Bekendtgørelse af dyreværnsloven. Accessed 06.05.21. at 11:22 from Dyreværnsloven (retsinformation.dk)

Ogden, J.A. (2005). *Fractured Minds: A Case Study Approach to Clinical Neuropsychology* (2nd ed.) Oxford University Press. Chapters 1 & 9.

Pentkowski, N., Berkowitz, L., Thompson, S., Drake, E., Olguin, C., & Clark, B. (2018). Anxiety-like behavior as an early endophenotype in the TgF344-AD rat model of Alzheimer's disease. *Neurobiology of Aging*, 61, 169–176.

Rahman, M., Rahman, M., Uddin, M., Mamum-Or-Rashid, A., Pang, M., & Rhim, H. (2020). Emerging risk of environmental factors: insight mechanisms of Alzheimer's diseases. *Environmental Science and Pollution Research International*, *27*(36), 44659–44672.

Serrano-Pozo, A., Frosch, M., Masliah, E., & Hyman, B. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*, 1(1), 1-23.

Snowden, J. (2010). The neuropsychological presentation of Alzheimer's disease and other neurodegenerative disorders. In Gurd, J., Kischka, U. & Marshall, J. (2nd ed), Handbook of Clinical Neuropsychology (pp. 561-584). Oxford University Press.

Tudela, R., Muñoz-Moreno, E., Sala-Llonch, R., López-Gil, X. & Soria, G. (2019). Resting State Networks in the TgF344-AD Rat Model of Alzheimer's Disease are Altered from Early Stages. *Frontiers in Aging Neuroscience*, 11, 1-14.

WHO (2020). Dementia. Accessed 12.04.21 at 11:04 from Dementia (who.int)

NEW CURRICULUM (1112 PAGES)

Alzheimer's Association (2021) 2021 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, *17*(3), 327–406.

Armstrong, R. A. (2009). The Spatial Patterns of β-Amyloid (Aβ) Deposits and Neurofibrillary Tangles (NFT) in Late-Onset Sporadic Alzheimer's Disease. In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments* (pp. 71-82). Nova Science Publishers, Incorporated.

Barnes, J., Dickerson, B., Frost, C., Jiskoot, L., Wolk, D., & van der Flier, W. (2015). Alzheimer's disease first symptoms are age dependent: Evidence from the NACC dataset. *Alzheimer's & Dementia*, *11*(11), 1349–1357.

Bayles, K., & Tomoeda, C. (2014). *MCI and Alzheimer's Dementia: Clinical Essentials for Assessment and Treatment of Cognitive-Communication Disorders* (1st ed.). Plural Publishing, Inc. Chapter 2 & 3

Bernhardi, R. (2009). Participation of Glial Cells in the Pathogenesis of AD: A Different View on Neuroinflammation. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease* (pp. 139-157). Springer.

Blennow, K., Hampel, H., Zetterberg, H., & Weiner, M. (2010). Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature Reviews. Neurology*, *6*(3), 131–144.

Boxer, A. L. (2011). Frontotemporal Dementia. In Budson, E. & Kowall, N. W., *The Handbook of Alzheimer's Disease and Other Dementias* (pp. 145-178). Wiley-Blackwell.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, 82(4), 239–259.

Brüel, A., Geneser, F., & Geneser, F. (2016). *Genesers histologi.* (1st ed.). Munksgaard. 49-56.

Busfieldm F. & Goate, A. M. (1995). The genetics of Alzheimer's disease and mutations in the amyloid β-protein precursor gene. In Jenner, P., Goate, A., Ashall, F., & Ashall, R. *Pathobiology of Alzheimer's Disease* (pp. 59-78). Elsevier Science & Technology.

Campion, D., Dumanchin, C., Hannequin, D., Dubois, B., Belliard, S., Puel, M., Thomas-Anterion, C., Michon, A., Martin, C., Charbonnier, F., Raux, G., Camuzat, A., Penet, C., Mesnage, V., Martinez, M., Clerget-Darpoux, F., Brice, A., & Frebourg, T. (1999). Early-Onset Autosomal Dominant Alzheimer Disease: Prevalence, Genetic Heterogeneity, and Mutation Spectrum. *American Journal of Human Genetics*, *65*(3), 664–670.

Christensen, V. K. (2021). Alzheimer og Adfærd - TgF344-AD Rotters Spatiale Lærings- og Hukommelsesevner. *Aalborg Universitet*, 1-55.

Crews, L., Spencer, B. & Masliah, E. (2009) Role of Aβ Degrading Enzymes in Synaptic Plasticity and Neurogenesis in Alzheimer's Disease. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease* (pp. 3-12). Springer.

Crowther, D. C., Page, R. M., Luheshi, L. & Lomas, D. A. (2009) How and Where Does Aβ Exert its Toxic Effects in Alzheimer's Disease? In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments* (pp. 59-70). Nova Science Publishers, Incorporated.

DeKosky, S., Scheff, S., & Styren, S. (1996). Structural Correlates of Cognition in Dementia: Quantification and Assessment of Synapse Change. *Neurodegeneration* (London, England), 5(4), 417–421.

Delatour, B., Le Cudennec, C., Tayara, N. E. T. & Dhenain, M. (2009). Transgenic Models of Alzheimer's Pathology: Success and Caveats. In Boyd, M. *Alzheimer's Disease Diagnosis and Treatments* (pp. 137-168). Nova Science Publishers, Incorporated.

Di Paola, M., Macaluso, E., Carlesimo, G., Tomaiuolo, F., Worsley, K., Fadda, L., & Caltagirone, C. (2007). Episodic memory impairment in patients with

Alzheimer's disease is correlated with entorhinal cortex atrophy: A voxel-based morphometry study. *Journal of Neurology*, 254(6), 774–781.

Dierckx, E., & Osterwalder, H. (2007). *Early detection of Alzheimer's disease : a neuropsychological approach* . VUBPress. Chapter 0.

Dubois, B., Feldman, H., Jacova, C., DeKosky, S., Barberger-Gateau, P., Cummings, J., Delacourte, A., Galasko, D., Gauthier, S., Jicha, G., Meguro, K., O'Brien, J., Pasquier, F., Robert, P., Rossor, M., Salloway, S., Stern, Y., Visser, P., & Scheltens, P. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria. *Lancet Neurology*, *6*(8), 734–746.

Edison, P., Archer, H., Rossor, M., Brooks, D., Hinz, R., Hammers, A., Pavese, N., Tai, Y., Hotton, G., Cutler, D., Fox, N., & Kennedy, A. (2007). Amyloid, hypometabolism, and cognition in Alzheimer disease : An [11C]PIB and [18F]FDG PET study. *Neurology*, 68(7), 501–508.

Franzmeier, N., Neitzel, J., Rubinski, A., Smith, R., Strandberg, O., Ossenkoppele, R., Hansson, O., & Ewers, M. (2020). Functional brain architecture is associated with the rate of tau accumulation in Alzheimer's disease. *Nature Communications*, 11(1), 1-17.

Gaiteri, C., Mostafavi, S., Honey, C., De Jager, P., & Bennett, D. (2016). Genetic variants in Alzheimer disease - molecular and brain network approaches. *Nature Reviews. Neurology*, *12*(7), 413–427.

Giri, M., Zhang, M., & Lü, Y. (2016). Genes associated with Alzheimer's disease: an overview and current status. *Clinical Interventions in Aging*, *11*, 665–681.

Glabe, C. C. (2005). Amyloid Accumulation and Pathogensis of Alzheimer's Disease: Significance of Momomeric, Oligomeric and Fibrillar Aβ. In Harris, J., & Fahrenholz, F. *Alzheimer's Disease: Cellular and Molecular Aspects of Amyloid* (pp. 167-177). Springer Science+Business Media, Inc.

Heneka, M., Carson, M., Khoury, J., Landreth, G., Brosseron, F., Feinstein, D., Jacobs, A., Wyss-Coray, T., Vitorica, J., Ransohoff, R., Herrup, K., Frautschy, S., Finsen, B., Brown, G., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz,

E., Halle, A., … Kummer, M. (2015). Neuroinflammation in Alzheimer's disease. *Lancet Neurology*, 14(4), 388–405.

Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. *Dialogues in clinical neuroscience*, *5*(1), 101–108.

Huey, E., Lee, S., Cheran, G., Grafman, J., & Devanand, D. (2017). Brain Regions Involved in Arousal and Reward Processing are Associated with Apathy in Alzheimer's Disease and Frontotemporal Dementia. *Journal of Alzheimer's Disease*, 55(2), 551–558.

Jellinger, K. (2006). Alzheimer 100 – highlights in the history of Alzheimer research. *Journal of Neural Transmission*, *113*(11), 1603–1623.

Joo, I., Lai, A., Bazzigaluppi, P., Koletar, M., Dorr, A., Brown, M., Thomason, L., Sled, J., McLaurin, J., & Stefanovic, B. (2017). Early neurovascular dysfunction in a transgenic rat model of Alzheimer's disease. *Scientific Reports*, 7(1), 1-15.

Kapasi, A., DeCarli, C., & Schneider, J. (2017). Impact of multiple pathologies on the threshold for clinically overt dementia. *Acta Neuropathologica*, *134*(2), 171–186.

Kern, S., Zetterberg, H., Kern, J., Zettergren, A., Waern, M., Höglund, K., Andreasson, U., Wetterberg, H., Börjesson-Hanson, A., Blennow, K., & Skoog, I. (2018). Prevalence of preclinical Alzheimer disease: Comparison of current classification systems. *Neurology*, 90(19), e1682–e1691.

Knierim, J. (2015). The hippocampus. *Current Biology*, 25(23), R1116–R1121.

Koedam, E., Lauffer, V., van der Vlies, A., van der Flier, W., Scheltens, P., & Pijnenburg, Y. (2010). Early-Versus Late-Onset Alzheimer's Disease: More than Age Alone. *Journal of Alzheimer's Disease*, *19*(4), 1401–1408

Korolev, I., Symonds, L., & Bozoki, A. (2016). Predicting Progression from Mild Cognitive Impairment to Alzheimer's Dementia Using Clinical, MRI, and Plasma Biomarkers via Probabilistic Pattern Classification. *PloS One*, *11*(2), 1-25.

La Joie, R., Perrotin, A., Barré, L., Hommet, C., Mézenge, F., Ibazizene, M., Camus, V., Abbas, A., Landeau, B., Guilloteau, D., de La Sayette, V., Eustache, F., Desgranges, B., & Chételat, G. (2012). Region-specific hierarchy between atrophy, hypometabolism, and β-amyloid (Aβ) load in Alzheimer's disease dementia. *The Journal of Neuroscience*, 32(46), 16265–16273.

Lockhart, A., Lamb, J., Osredkar, T., Sue, L., Joyce, J., Ye, L., Libri, V., Leppert, D., & Beach, T. (2007). PIB is a non-specific imaging marker of amyloid-beta (Aβ) peptide-related cerebral amyloidosis. *Brain (London, England : 1878)*, *130*(10), 2607–2615.

Long, J., & Holtzman, D. (2019). Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell (Cambridge)*, *179*(2), 312–339.

Middleton, L., Grinberg, L., Miller, B., Kawas, C., & Yaffe, K. (2011). Neuropathologic features associated with Alzheimer disease diagnosis: Age matters. *Neurology*, 77(19), 1737–1744.

Mitchell, A., & Shiri-Feshki, M. (2009). Rate of progression of mild cognitive impairment to dementia meta-analysis of 41 robust inception cohort studies. *Acta Psychiatrica Scandinavica*, *119*(4), 252–265

Mori, T., Shimada, H., Shinotoh, H., Hirano, S., Eguchi, Y., Yamada, M., Fukuhara, R., Tanimukai, S., Zhang, M., Kuwabara, S., Ueno, S., & Suhara, T. (2014). Apathy correlates with prefrontal amyloid β deposition in Alzheimer's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, 85(4), 449–455.

Nagahama, Y., Okina, T., & Suzuki, N. (2015). Impaired imitation of gestures in mild dementia: comparison of dementia with Lewy bodies, Alzheimer's disease and vascular dementia. *Journal of Neurology, Neurosurgery & Psychiatry*, 86(11), 1248–1252.

Nakamura, K., Kumai, K., Takada, J., Chida, K., Kato, Y., Takahashi, Y., Takahashi, K., Nakatsuka, M., Yamaguchi, S., & Meguro, K. (2017). Behavioral and psychological symptoms of dementia (BPSD) in Alzheimer disease is

"cognitive" symptoms? The Osaki-Tajiri Project. *Alzheimer's & Dementia*, *13*(7), P375–P376.

National Institute on Ageing (2021). What is Dementia? Symptoms, Types, and Diagnosis. Accessed 12.04.21 at 10:45 from What Is Dementia? Symptoms, Types, and Diagnosis | National Institute on Aging (nih.gov)

Noh, Y., Na, H., Seo, S., Lee, S., Jeong, H., Okamura, N., Ido, T., & Na, D. (2017). Relationship among tau, amyloid burden and brain atrophy in early-onset and late-onset Alzheimer's disease. *Alzheimer's & Dementia*, 13(7), P2–P3.

Nunomura, A., Takeda, A., Moreira, P. I., Castellani, R. J., Lee, H., Zhu, X., Smith, M. A. & Perry, G. (2009). Neurofibrillary Tangle Formation as a Protective Response to Oxidative Stress in Alzheimer's Disease. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease* (pp. 103-113). Springer.

O'Brien, J., & Thomas, A. (2015). Vascular dementia. *The Lancet (British Edition)*, *386*(10004), 1698–1706.

Olivieri, P., Lagarde, J., Lehericy, S., Valabrègue, R., Michel, A., Macé, P., Caillé, F., Gervais, P., Bottlaender, M., & Sarazin, M. (2019). Early alteration of the locus coeruleus in phenotypic variants of Alzheimer's disease. *Annals of Clinical and Translational Neurology*, 6(7), 1345–1351.

Ossenkoppele, R., Jansen, W., Rabinovici, G., Knol, D., van der Flier, W., van Berckel, B., Scheltens, P., & Visser, P. (2015). Prevalence of Amyloid PET Positivity in Dementia Syndromes A Meta-analysis. 1939-1949.

Pandya, M., Altinay, M., Malone Jr, D., & Anand, A. (2012). Where in the Brain Is Depression? *Current Psychiatry Reports*, 14(6), 634–642.

Pannee, J., Portelius, E., Minthon, L., Gobom, J., Andreasson, U., Zetterberg, H., Hansson, O., & Blennow, K. (2016). Reference measurement procedure for CSF amyloid beta (Aβ)1–42 and the CSF Aβ1–42/Aβ1–40 ratio – a cross-validation study against amyloid PET. *Journal of Neurochemistry*, *139*(4), 651–658.

Parsons, M. E., Jones, S. E. & Krewson, T. (2015). Structural and functional brain imaging for the neuropsychologist. In Parsons, M. W. & Hammeke, T. A., *Clinical Neuropsychology: A Pocked Handbook for Assessment* (3rd ed.) (pp. 74-89). American Psychological Association.

Pasquali, P. (2018). The importance of animal models in research. *Research in Veterinary Science*, 118, 144–145.

Passamonti, L., Lansdall, C., & Rowe, J. (2018). The neuroanatomical and neurochemical basis of apathy and impulsivity in frontotemporal lobar degeneration. *Current Opinion in Behavioral Sciences*, 22, 14–20.

Paxinos, G., & Watson, C. (2002). *The rat brain in stereotaxic coordinates* (4. ed.). Academic Press.

Perani, D. (2014). FDG-PET and amyloid-PET imaging: the diverging paths. *Current Opinion in Neurology*, *27*(4), 405–413.

Perez, P., Hall, G., Kimura, T., Ren, Y., Bailey, R., Lewis, J., Febo, M., & Sahara, N. (2013). In vivo functional brain mapping in a conditional mouse model of human tauopathy (tauP301L) reveals reduced neural activity in memory formation structures. *Molecular Neurodegeneration*, 8(1), 1–10.

Pontecorvo, M., Devous, S., Navitsky, M., Lu, M., Salloway, S., Schaerf, F., Jennings, D., Arora, A., McGeehan, A., Lim, N., Xiong, H., Joshi, A., Siderowf, A., & Mintun, M. (2017). Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. *Brain* (London, England : 1878), 140(3), 748–763.

Raichle, M. (2008). A brief history of human brain mapping. *Trends in Neurosciences (Regular Ed.)*, *32*(2), 118–126.

Rice, L., & Bisdas, S. (2017). The diagnostic value of FDG and amyloid PET in Alzheimer's disease—A systematic review. *European Journal of Radiology*, 94, 16–24.

Rojo, L. E., Fernández, J., Jiménez, J., Maccioni, A. A., Sekler, A., Kuljis, R. O. & Maccioni, R. B. (2009). Central Nervous System Inflammation and Cholesterol Metabolism Alterations in the Pathogenesis of Alzheimer's Disease and Their Diagnostic and Therapeutic Implications. In Maccioni, R. B. & Perry, G., *Current Hypothesis and Research Milestones in Alzheimer's Disease* (pp. 125-138). Springer.

Román, G. C. (2005). Clinical Forms of Vascular Dementia. In Paul, R., Cohen, R., Ott, B., & Salloway, S., *Vascular Dementia: Cerebrovascular Mechanisms and Clinical Management* (pp. 7-21). Humana Press Inc.

Sagar, S., Sagar, S., Rick, J., Rick, J., Chandra, A., Chandra, A., Yagnik, G., Yagnik, G., Aghi, M., & Aghi, M. (2019). Functional brain mapping: overview of techniques and their application to neurosurgery. *Neurosurgical Review*, *42*(3), 639–647.

Sara, S., & Bouret, S. (2012). Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. *Neuron* (Cambridge, Mass.), 76(1), 130–141.

Schneider, J., Arvanitakis, Z., Leurgans, S., & Bennett, D. (2009). The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Annals of Neurology*, *66*(2), 200–208.

Schöll, M., Ossenkoppele, R., Strandberg, O., & Palmqvist, S. (2017). Distinct 18F-AV-1451 tau PET retention patterns in early- and late-onset Alzheimer's disease. *Brain* (London, England : 1878), 140(9), 2286–2294.

Selkoe, D., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine*, 8(6), 595–608.

Smith, G., & Bondi, M. (2013). *Mild cognitive impairment and dementia : definitions, diagnosis, and treatment* . Oxford University Press. Chapter 4.

Son, S., Chang, K., Noh, J., Roh, H., Oh, B., Kim, D., Jang, M., Kim, S., Choi, S., Lee, K., & Hong, C. (2016). Mortality after a diagnosis of early-onset

Alzheimer's disease versus late-onset Alzheimer's disease: A propensity score matching analysis. *Alzheimer's & Dementia*, *12*(7), P696–P696.

Taylor, C., & Levenson, R. (2006). Quantification of immunohistochemistry-issues concerning methods, utility and semiquantitative assessment II. *Histopathology*, *49*(4), 411–424.

Thal, D. R., Tredici, K. D. & Braak, H. (2004). Neurodegeneration in Normal Brain Aging and Disease. *Science of Aging Knowledge Environment*, 23, 1-11.

Thermo Fisher Scientific. Overview of Immunohistochemistry (IHC). Accessed 14.04.21 at 14:49 from Overview of Immunohistochemistry | Thermo Fisher Scientific - FR

Traykov, L., Baudic, S., Raoux, N., Latour, F., Rieu, D., Smagghe, A., & Rigaud, A. (2005). Patterns of memory impairment and perseverative behavior discriminate early Alzheimer's disease from subcortical vascular dementia. *Journal of the Neurological Sciences*, *229*, 75–79.

Van der Mussele, S., Marien, P., Saerens, J., Somers, N., Goeman, J., De Deyn, P., & Engelborghs, S. (2014). Behavioral Syndromes in Mild Cognitive Impairment and Alzheimer's Disease. *Journal of Alzheimer's Disease*, *38*(2), 319–329.

Vassar, R. (2005). β-Secretase, APP and Aβ in Alzheimer's Disease. In Harris, J., & Fahrenholz, F. *Alzheimer's Disease: Cellular and Molecular Aspects of Amyloid* (pp. 79-103). Springer Science+Business Media, Inc.

Victoroff, J., Lin, F., Coburn, K., Shillcutt, S., Voon, V., & Ducharme, S. (2018). Noncognitive Behavioral Changes Associated With Alzheimer's Disease: Implications of Neuroimaging Findings. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *30*(1), 14–21.

Walters, A., Phillips, E., Zheng, R., Biju, M., & Kuruvilla, T. (2016). Evidence for neuroinflammation in Alzheimer's disease. *Progress in Neurology and Psychiatry* (Guildford), 20(5), 25–31.

WHO (1994) ICD-10: Psykiske lidelser og adfærdsmæssige forstyrrelser. Munksgaard. 41-43

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., De Leon, M., Decarli, C., Erkinjuntti, T., Giacobini, E., Graff, C., Hardy, J., … Petersen, R. (2004). Mild cognitive impairment - Beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *Journal of Internal Medicine*, *256*(3), 240–246

Zanni, R., Garcia-Domenech, R., Galvez-Llompart, M., & Galvez, J. (2018). Alzheimer: A Decade of Drug Design. Why Molecular Topology can be an Extra Edge? *Current Neuropharmacology*, 16(6), 849–864.

APPENDIX A – IHC STAINING PROTOCOL (IN DANISH)

Udarbejdet i februar 2021 af MF

Immunofarvning free floating med DAB (beta-amyloid rotter)

Inkubationsbuffer:	3 % svineserum i KPBS + 0,3 % Triton X-100
	50 ml: 1,5 ml svineserum + 150 µl Triton X-100 + 48,4 ml KPBS
Vaskebuffer:	Inkubationsbufferen fortyndes i 1:50 i KPBS
Antistoffer:	Primære AB: Purified anti-beta-amyloid 1-16 antibody 803001 biolegend Mus anti-Human 1 : 20.000 på køl 4F
	Sekundære AB: DAKO E0354-biotin 1:200 på køl 1 B

ABComplex Vectastain (ABC): 1 dråbe A + 1 dråbe B + 10 ml KPBS. Blandes 30 min. før brug.

DAB: 1 rør med 1 ml DAB + 9 ml Tris-HCl buffer 0,05 M pH = 7,6 + 3,3 µl H_2O_2 umiddelbart før brug.

Dag 1:

1. Vævsnittene vaskes 3x5 min i KPBS
2. Tilsæt 1 ml inkubationsbuffer til hvert glas og inkuberer/blokerer i 60 min ved stuetemperatur
3. Inkubationsbuffer hældes fra så der ikke er mere tilbage
4. Husk at tage et par snit fra til negativ kontrol.
5. Inkuber med 1 ml primær antistof natten over ved 4 C.

Dag 2:

1. Skyl 3x5 min i vaskebuffer
2. Inkuber med 1 ml biotinyleret sekundært antistof i 60 min ved stuetemperatur
    a. Fortynd sekundært antistof i inkubationsbuffer
3. Fremstil ABC opløsning (10 ml KPBS + 1 dråbe af A og B) 30 min før brug
4. Vask 2x5 min i vaskebuffer
5. Vask 1x5 min i KPBS
6. Tilsæt 1 ml ABC til hvert glas og inkubere i 30 min ved stuetemperatur
7. Skyl 2x5 min i KPBS og 1x5 i 0,05 M Tris/HCl pH = 7,6
8. Tilsæt 1 ml DAB-opløsning i stinkskab
9. Inkuber MAX 10 min ved stuetemperatur
10. Skyl 1x5 i Tris/HCl og 2x5 min i KPBS
    a. Spildet fra første hold skyllevand opsamles i affaldsgruppe H.
11. Snittene flyttes til en petriskål med gelatineopløsning og opsamles efterfølgende på objektglas. Efter tørring forsegles snittene med Pertex og dækglas sættes på.

APPENDIX B – IMAGEJ PROTOCOL AND SETTINGS

1) Images were converted to 8-bit.

2) Scale was set, so 20 μm equals 7.75 pixels.

Measuring of total area

3) Area of relevance was manually drawn in 3 times, yielding an average sum in μm^2.

Measuring of plaque coverage

3) Area of relevance was manually drawn

4) Brightness/Contrast was set to 182-213 in every case except a few, where this setting did not in any way result in any way result in an accurate binary representation. Instead, the setting 163-213 as applied for 5 of the 16 TG sections (56_1, 56_2, 57_1, 57_2, 58_2), and one WT section (70). Furthermore, Brightness/Contrast was set to 100-213 for both negative control sections analyzed.

5) Images were converted to binary, and plaques seen outside of the relevant area were manually removed from the images.

6) Threshold was set to 105.

7) Plaque area was calculated in μm^2.

APPENDIX C – RAW DATA

Table 1 Descriptives

	Genotype	Gender
#54	TgF344-AD	Female
#55	TgF344-AD	Female
#56	TgF344-AD	Female
#57	TgF344-AD	Female
#58	TgF344-AD	Male
#59	TgF344-AD	Male
#60	TgF344-AD	Male
#61	TgF344-AD	Male
#70	F344	Female
#71	F344	Female
#72	F344	Female
#73	F344	Female
#74	F344	Male
#75	F344	Male
#76	F344	Male
#77	F344	Male
2019	TgF344-AD	Unknown

Table 2 Hippocampal measurements.

	1st section			2nd section			Average
	Total area	Plaque area	Area %	Total area	Plaque area	Area %	Area%
#54	520513.35	17242.14	3.31	418029.00	39545.42	9.46	6.39
#55	335659.24	25160.35	7.50	394267.08	35176.70	8.92	8.21
#56	347515.26	29495.84	8.49	383114.25	20978.04	5.48	6.98
#57	340523.07	25526.61	7.50	402789.32	67156.75	16.67	12.08
#58	316064.10	52385.42	16.57	460986.47	41430.26	8.99	12.78
#59	307688.38	28789.96	9.36	405442.11	30607.72	7.55	8.45
#60	274081.17	33072.21	12.07	331037.39	33838.12	10.22	11.14
#61	340489.77	46744.57	13.73	387802.71	45126.44	11.64	12.68
#70	788918.21	692.62	0.088	-	-	-	0.088
#74	617767.60	705.93	0.114	-	-	-	0.114
NC_{TG}	438239.057	179.81	0.041	-	-	-	0.041
NC_{WT}	518737.73	26.64	0.005	-	-	-	0.005
2019	1705929.66	54376.64	3.19	-	-	-	3.19

Table 3 Results from memory measures on Barnes Maze, day 10, TG-rats

	Primary latency (s)	Quadrant time (s)	Primary errors
#54	46	34	5
#55	57	41	12
#56	180	170	0
#57	96	24	11
#58	28	76	4
#59	59	31	8
#60	56	33	9
#61	70	11	8

Table 4 Deliberation time for TG rats in seconds

Trial:	d1_t1	d1_t2	d1_t3	d1_t4	d2_t1	Day 3	Day 10
#54	2	18	6	8	3	2	2
#55	15	7	4	3	1	1	9
#56	10	10	10	3	3	3	5
#57	8	3	10	9	3	1	2
#58	28	21	56	11	5	3	2
#59	16	8	18	41	7	2	2
#60	10	9	7	4	2	1	2
#61	4	8	32	12	5	3	4

Table 5 Deliberation time for WT rats in seconds

Trial:	d1_t1	d1_t2	d1_t3	d1_t4	d2_t1	Day 3	Day 10
#70	5	5	1	1	1	1	5
#71	6	5	2	3	1	1	0
#72	4	6	1	1	0	0	4
#73	17	6	2	1	2	1	3
#74	6	5	2	2	1	1	6
#75	22	11	10	5	7	9	19
#76	6	4	3	1	0	3	3
#77	5	8	3	1	0	3	4