Charles University in Prague
Faculty of Science

Study programme: Biology
Branch of study: Animal Physiology

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Anxiety-like and Depression-like Behaviour in TgF344-AD Rat Model of Alzheimer's Disease

Úzkostné a depresi-podobné chování u TgF344-AD potkaního modelu Alzheimerovy choroby

Diploma thesis

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Prague 2021

Prohlášení:

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V Praze, 9. 6. 2021

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Podpis

Acknowledgments

I would like to thank to my supervisor, RNDr. Jan Svoboda, Ph.D., for his kind attitude towards me, for providing me with helpful advice and comments and last but not least, for the time he invested in me and my work. My thanks also goes to the head and all the members of the lab (Laboratory of Neurophysiology of the Memory, Institute of Physiology, CAS) with whom I worked on my thesis for letting me participate in this project.

Abstract

Patients with Alzheimer disease often report presence of neuropsychiatric symptoms even before the presence of cognitive decline itself. The most reported ones are anxiety, depression, emotional dysregulation, apathy and agitation.

The aim of this work was to investigate the presence of anxiety- and depression-like behaviour, and eventually dysregulation of social behaviour and emotional memory impairment via behavioural approach on the TgF344-AD (tg) rat model.

Results show that tg rats display anxiety-like behaviour in several different tests and parameters. Tg rats of the age of 10 months spent more time around the wall and less in the middle of the arena. Surprisingly, this anxiety-like phenotype has not been demonstrated in the older (14 months) rats. Tg rats spent also less time peeking out from closed arms and looking down from open arms. Moreover, tg rats displayed anxiety-like behaviour in all observed parameters in the Social interaction test. Social deficit expressed as a smaller investment of time into the anogenital and non-anogenital exploration and following of counterparts. In the Forced swim test, tg rats did not spend more time immobile, thus depression-like phenotype has not been demonstrated in these animals. Moreover, 10 months old tg rats spent surprisingly less time immobile. There was no impairment in emotional memory in tg rats of the age of 10 months. Surprisingly, 14 months old tg rats showed enhanced emotional memory in one of the two followed parameters.

TgF344-AD rats have shown anxiety-like phenotype not only by the age of 14, but also by the age of 10 months, regardless of sex. In contrast, a depression-like phenotype was not present in the same cohort of animals according to our data.

Keywords: Alzheimer's disease; animal model; anxiety-like behaviour; depression-like behaviour; TgF344-AD rats

Abstrakt

Pacienti s Alzheimerovou chorobou často reportují přítomnost neuropsychiatrických symptomů, a to ještě před tím, než je u nich zaznamenatelný kognitivní úpadek. Nejčastěji u těchto pacientů pozorujeme úzkost, depresi, emoční dysregulaci, apatii a agitaci.

Cílem této práce bylo pomocí behaviorálního přístupu otestovat přítomnost úzkosti- a depresi-podobného chování, případně dysregulace v sociálním chování a emoční paměti u potkaního modelu TgF344-AD.

Výsledky ukázují, že transgenní (tg) potkani vykazují úzkosti-podobné chování hned v několika různých testech a parametrech. 10. měsíční tg potkani strávili více času u zdi a méně času uprostřed arény. Tento úzkosti-podobný fenotyp překvapivě nebyl prokázán u potkanů starších (14. měsíčních). Tg potkani dále strávili méně času vykukováním z uzavřených ramen a koukáním dolů z otevřených ramen. V testu sociálních interakcí vykazovali tg potkani úzkostné chování ve všech sledovaných parametrech. Sociální deficity se u potkanů projevovali menší investicí času do anogenitálních a non-anogenitálních explorací a pronásledováním partnerů. V testu nuceného plavání tg potkani nestrávili více času imobilní, a tedy depresivní fenotyp u nich dle tohoto testu prokázán nebyl. Co víc, 10. měsíční tg potkani strávili překvapivě méně času imobilní. V 10 měsících nebyla emoční paměť u tg potkanů narušena. 14. měsíční tg potkani vykazovali překvapivě lepší emoční paměť v jednom z dvou sledovaných parametrů.

TgF344-AD potkani vykazovali nejen ve 14, ale i v 10 měsících úzkosti-podobný fenotyp bez rozdílu jak u samic, tak samců. Oproti tomu depresi-podobný fenotyp u tohoto modelu ve stejném věku dle našich dat nebyl zaznamenán.

Klíčová slova: Alzheimerova choroba; animální model; depresi-podobné chování; TgF344-AD potkani; úzkosti-podobné chování

List of abbreviations

AD	Alzheimer disease
ANOVA	analysis of variance
APP	amyloid precursor protein
AS CR	Academy of Sciences, Czech Republic
Aβ	amyloid β
Aβ40	amyloid β with 40 amino acids
Aβ42	amyloid β with 42 amino acids
CFC	contextual fear conditioning
EEG	electroencephalogram
EPM	Elevated plus maze
FST	Forced swim test
GMO	genetically modified organism
HVSs	high voltage spindles
NMDA	N-methyl-D-aspartate
MBI	mild behavioural impairment
MCI	mild cognitive impairment
MWM	Morris water maze
NFTs	neurofibrillary tangles
OFM	Open field maze
OTT	One trial test
PET	positron emission tomography
RRRC	Rat Resource & Research Center
SWRs	Sharp-wave ripples
SIT	Social interaction test
SPT	Sucrose preference test
SSRI	selective serotonin reuptake inhibitors
tg	transgenic
TgF344-AD	transgenic rat model of Alzheimer disease derived from F344
TST	Tail suspension test
WHO	World Health Organization
wt	wild type

Content

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the world as it may contribute to 60-70% of all dementia cases (WHO, 2020). The current prevalence of dementia is estimated around 50 million cases and due to the aging of worldwide population the prevalence is currently even increasing (WHO, 2020). The dramatic increase of the population with diagnosis of AD is expected to continue raising in the next few decades (International, A.s.D, 2015). Therefore, a formation of future direction in AD research is an important issue. Novel and precise diagnostic tools must be established for early detection of this disease. The currently licensed pharmacological treatment is built up on cholinesterase inhibitors prescribed for the patients with any stage of AD dementia, memantine for those with moderate-to-severe AD dementia, and the recently licensed drug Aducanumab (BIIB037) which is a monoclonal antibody with high affinity to epitope on Aβ (Sevigny et al., 2016). However, while the medication seems to enhance quality of patient's life, the decrease rate or course of the disorder remains irreversible (Szeto & Lewis, 2016). Therefore, new therapeutic targets in a drug treatment (alongside the early diagnostics) must be proposed for the purpose of maintaining a successful therapy.

Deficits in memory, language processing, visuospatial and executive functions are commonly manifested among patients with AD (McKhann et al., 2011a). However, what is not so frequently spoken about is that in the early stages of AD dementia progress, or even before the diagnosis, patients often report a kind of psychiatric symptoms. The most commonly reported ones are anxiety, emotional distress, apathy, agitation or depression (Lanctôt et al., 2017). As psychiatric symptoms clearly play a role in the AD, they might be possibly treated as a valid diagnostic tool in the future. Additionally, psychiatric medications or psychotherapy might be more frequently discussed as a therapy option for AD patients to enhance quality of their life.

To better understand the role of manifestation of neuropsychiatric symptoms in AD patients, more animal studies using AD animal models with greater validity must be proposed. Mice models are not always an ideal option, since they do not manifest all the AD pathological features and mice behavioural repertoire is generally poor. In contrast, rats display much richer behavioural patterns, which allows for better modelling neuropsychiatric symptoms. Although rats typically resist genetic manipulations, and therefore transgenic (tg) rat models of AD are much less frequent

than mice. The recently utilized TgF344-AD model is a tg rat model that seems to fulfil the demand of greater validity. It carries a mutation in the gene for an amyloid precursor protein as well as mutation in the genes for presenilin-1. Those mutations then result in progressive plaque and tangle pathogenesis throughout the cortex (Cohen et al., 2013).

Since TgF344-AD model manifests a complete repertoire of AD pathological features, it could be used as a potential model for anxiety- and depression-like behavioural evaluation regarding AD. Several studies (Morrone et al., 2020, Pentkowski et al. 2018, Tournier et al. 2020, Voorhees et al., 2018) have already evaluated this type of behaviour on this model, however, more of these need to be done with the respect for sex and age differences. Hopefully, it will allow us to have a better understanding of how neuropsychiatric symptoms manifest among patients with AD.

2. Alzheimer disease

AD is characterised by neuropathological amyloid plaque deposition, which is associated with the occurrence of neurofibrillary tangles. The whole neuropathological process is further accompanied with widespread cortical neuronal loss. Amyloid plaques are extracellular structures in the brain parenchyma and in the vasculature, whereas phosphorylated tau accumulates intra-neuronally and subsequently forms the neurofibrillary tangles. The aggregation of both pathological structures happens to be a sequential process – at first there is a couple of monomers which subsequently become oligomers and then continue to aggregate into fibrils, from which amyloid plaques and NFTs are made. The presence of these pathological structures later results in a neurodegenerative process in the areas of interest, eventually leading to a neuronal loss (Selkoe, 2001).

2.1. Etiology

There are many hypotheses that aim to explain the progression of AD dementia. Nevertheless, a revision of these is highly recommended since there is still no cure for the disease and there is still a high failure rate of therapeutic drugs in clinical trials (Coric et al., 2015; Farlow et al., 2015; Gilman et al., 2005; Green et al., 2009; Nakao et al., 2019; Siemers et al., 2016; Vellas et al., 2009; Winblad et al., 2012). Failed trials might be pointing out that several invalid concepts occur in the currently proclaimed hypotheses of the etiology of AD. More or less valid, all of the mainstream hypothesis of the AD etiology are briefly described in this section.

Cholinergic hypothesis

The cholinergic hypothesis of AD was formulated over 30 years ago. Acetylcholin is a neuromodulator that plays a major role in cognition. The hypothesis says that AD patients exhibit a lower level of this neurotransmitter. The loss of cholinergic neurons, as a result of the AD pathology, is another feature of this hypothesis. Consequently, the loss of afunctional cholinergic synapses results in the loss of brain volume which affects normal brain function and is mostly recognised as a memory loss (Hampel et al., 2018).

Amyloid hypothesis

The amyloid hypothesis claims that the main feature of AD is a progressive output of amyloids, which consist of a number of diverse proteins that form themselves into these pathological organs. The amyloid plaques are later followed by neuritic and glial cytopathology in the brain regions associated with memory and other cognitive domains. The protein of interest in this hypothesis is the amyloidogenic protein Aβ, the main component of amyloids. Specifically, it is proposed that the amyloid hypothesis operates with AD-causing mutations in amyloid precursor protein (APP) and in presenilins 1 and 2. APP is an integral glycoprotein that is normally present in the brain. When the APP is settled in the cell membrane, it undergoes the process of proteolytic cleavage into the short peptides. A pathological cleavage of APP by the y-secretase enzyme results in the formation of the so-called amyloid plaques. A catalytic subunit of y-secretase is formed by presenilin 1 and 2, proteins responsible for the cleavage of APP. There are two products possibly formable. Aβ40 is a peptide formed by 40 amino acids and is a mainstream product of this cascade. However, when the product of the cascade becomes Aβ42 with the primary structure of 42 amino acids, there is a bigger probability of formation of the pathological Aβ plaques (Musiek & Holtzman, 2015).

This hypothesis directed the potential treatment of AD dementia for a long period as much as it had become a major model of AD pathogenesis. Nevertheless, all of the human trials (as to mention some of them: Coric et al., 2015; Farlow et al., 2015; Nakao et al., 2019; Siemers et al., 2016; Vellas et al., 2009; Winblad et al., 2012) in which APP or presenilin 1 or 2 are used as a target fail to reduce AD symptoms. Moreover, thanks to the recent amyloid imaging advances it was revealed that there are many healthy patients with high levels of amyloid deposits, as well as patients with AD dementia lacking the deposits (Kametani & Hasegawa, 2018). As a result of these findings, the question of whether the deposition of amyloid plaques is or is not a phenomenon of common aging is brought up.

Tau hypothesis

Since all the attempts to use Aβ-targeting drugs to treat AD fail the requirements (Coric et al., 2015; Farlow et al., 2015; Gilman et al., 2005; Green et al., 2009; Nakao et al., 2019; Siemers et al., 2016; Vellas et al., 2009; Winblad et al., 2012), another target in the AD therapy is required. In 1986, Grundke-Iqbal et al. provided a clue that the deposition of tau protein might be an object of future studies. As mentioned above, the

accumulation and deposition of the bundles of microtubule-associated tau protein form pathological structures in the brain, which are referred to as neurofibrillary tangles (NFTs; Brion et al., 2001). Spreading of the tau pathology, which initiates in the entorhinal region and continues to the limbic region and finally to the neocortical areas, is strongly correlated with the extent of cognitive and clinical symptoms (Braak & Del Tredici, 2018).

Results show that the tau pathology could be associated with the Aβ pathology, or even that the Aβ pathology might be a trigger in the AD pathological cascade, whereas the tau accumulation could be responsible for its progression (Kametani & Hasegawa, 2018). On the other hand, results show that the temporal and regional distribution of NFTs and Aβ plaques do not always correlate in AD patients (Bouras, Hof, Giannakopoulos, Michel, & Morrison, 1994). The tau lesion observation often precedes the formation of Aβ lesions themselves. Furthermore, the NFTs formation seems to correlate better with clinical symptoms than with the formation of amyloid plaques (Schönheit, Zarski, & Ohm, 2004).

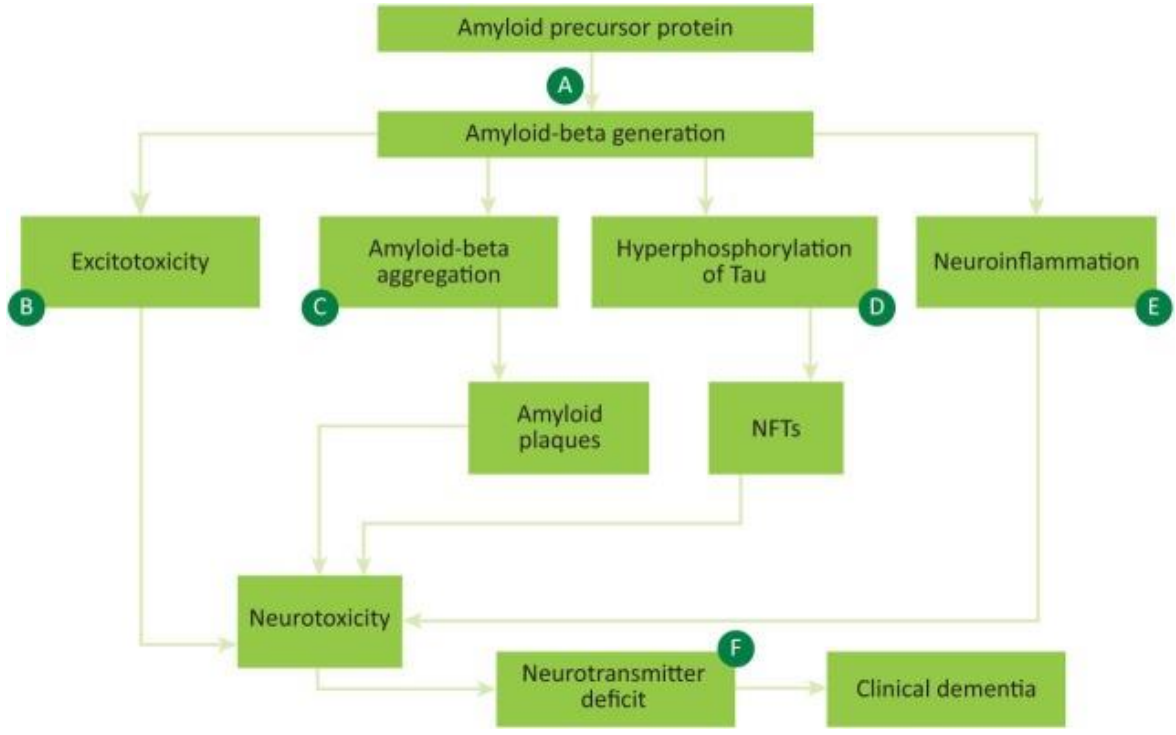

Figure 1: Schema of the cascade of AD etiology *(incorporation of Aβ and Tau pathogenesis and other AD features) with possible therapeutic targets (A-F). A – secretase enzyme inhibitors; B- NMDA receptor modulators (memantine); C – immunotherapy; D – anti-tau therapy; E – anti-inflammatory treatments; F – anticholinesterase inhibitors (donezepil). NFTs = neurofibrillary tangles* (Briggs, Kennelly, & O'Neill, 2016).

Energy metabolism changes, inflammation and microbiome disbalance hypothesis

With the elderly, there is a high energy demand for brain sensitises organism to progressively change the energy fuel and supply the mitochondrial function. The common feature of normal aging is known to fall short in glucose availability and mitotochondrial function that are even accelerated in AD patients (Błaszczyk, 2020). Altered redox status, one of the causes of chronic oxidative stress, might undoubtedly play its role in premature aging of the brain (Liguori et al., 2018). In addition to the oxidative stress, neuroinflammatory changes such as microglial activation or production of the cytokines have been reported in AD patients (W.-Y. Wang, Tan, Yu, & Tan, 2015).

The bioenergetic dysfunction hypothesis operates with many co-dependent brain perspectives. Firstly, there is a metabolic deficit in the brain of AD patients, secondly the neuronal dysfunction appears and lastly a neurodegenerative process manifests itself in full strength (Wilkins, Carl, Greenlief, Festoff, & Swerdlow, 2014). In contrast, neuroinflammatory hypothesis assumes that the driving force of the neurodegeneration process is a microglial activation itself (W.-Y. Wang et al., 2015). Unregulated neuroinflammation induces neurotoxicity which results in the damaging of brain tissue in particular regions. However, a regular neuroinflammatory activity is desirable and important in the normal brain development or process of learning (Chagas et al., 2020).

Nevertheless, growing evidence suggests that redox dysregulation might be a connecting bridge between energy metabolism and neuroinflammatory processes, giving new insight into the perception of AD as a multiple and diverse mechanism dysfunction. As far as these two concepts (energy metabolism and neuroinflammatory) and their bidirectional link (redox control) are considered, etiology of the AD dementia seems to be more complex than being just a result of one single isolated pathology (Yin, Sancheti, Patil, & Cadenas, 2016).

Moreover, concept of the gut microbiome alterations and the consequent inflammation-driven pathogenesis is considered as a promising new area in AD (Sochocka et al., 2019). Enteric bacteria, commensal and pathogenic microorganisms may have a huge impact on immune system and normal brain function, since they produce neuromodulators and neurotransmitters such as serotonine, catecholamine,

kynurenine, or even amyloids. Therefore, dysbiosis in the intestinal microbiome might possibly lead to the metabolic and inflammation changes in the bidirectionally axis connected brain. This is the reason for which the gut is sometimes referred to as the 'second brain.' Increased permeability of gut epithelial barrier is one of the cases of microbiome composition changes (Sochocka et al., 2019).

2.2. Symptoms and diagnostics

Patients with the diagnosis of AD show early and rapid cognitive decline. The deficits are especially alarming in memory (episodic memory impairment, semantic coding impairment), language processing, visuospatial and executive functions (McKhann et al., 2011a).

Nowadays, a neuropsychological assessment is primarily used to detect quality and quantity of the cognitive impairment in AD patients, so it helps with the process of diagnostics as well as with screening of the disease progression. Cognitive impairment is assessed via neuropsychological batteries, which mostly consist of a single domain-specific tests. High-quality neuropsychological tests are especially needed for detection of mild cognitive impairment (MCI). Such a prodromal stage is not characterised by the full-blown dementia syndrome, yet it marks the risk of becoming one (Jongsiriyanyong & Limpawattana, 2018). Diagnosis of the MCI (or dementia) depends on the age and amnestic data of patient in relation to the results of performance tests. Among the manifestation of cognitive impairment, patient has to interfere with the ability to function at work or at usual daily activities, and to represent a decline from previous levels of functioning and performing in order to be diagnosed with AD (McKhann et al., 2011b).

Another criteria AD diagnostics have to meet is a detection of biomarkers in patients. Level of the main constituent of brain plaque (protein Aβ), or of the neurofibrillary tangle tau protein is raised up in the cerebrospinal fluid of those suffering from AD dementia (Andreasen et al., 1998; Frankfort et al., 2008).

The level detection of particular proteins can be sometimes combined with PET imaging techniques, which reveal a deposition of amyloid plaques straight in the brain. More biomarkers are currently being explored in several longitudinal studies (McKhann et al., 2011b).

The combination of many different diagnostic criteria creates a great tool for the diagnostics of AD dementia, yet the heterogeneity of the individual cases of AD makes diagnostics and detection of the progression far from perfect.

2.2.1. Neuropsychiatric symptoms in AD

Until recently, little attention has been paid to neuropsychiatric symptoms in AD patients. Despite the fact that anxiety and depression are common problems in AD population and are associated with poor quality of life and outcomes (Kales, Chen, Blow, Welsh, & Mellow, 2005), there is still no united mental health therapy program for AD patients (Kales, Gitlin, & Lyketsos, 2014). But in fact, affective and emotional symptoms have a noticeable value, since they manifest in the pre-dementia state in the yet cognitively normal adults as well as in the patients with MCI, and predict the amount of cognitive decline afterwards (Geda et al., 2014; Ma, 2020). Furthermore, the treatment of neuropsychiatric symptoms in AD with SSRI is effective in decreasing the symptoms and in improving the quality of life of both patients and caregivers (Moretti, Torre, Antonello, Cazzato, & Bava, 2002). In a prospective study, cognitively normal elderly were treated with the SSRI and data had shown that serotonin signalling had been associated with less Aβ accumulation in those treated with antidepressants compared with those left untreated (Cirrito et al., 2011). Furthermore, the escitalopram (a SSRI antidepressant) seems to attenuate β-amyloid-induced tau hyperphosporylation in hippocampal neurons in rats (Y.-J. Wang et al., 2016).

Mild Behavioural Impairment (MBI) is a dementia syndrome which incorporates symptoms such as depression, anxiety, euphoria, or irritability (Ismail et al., 2017). Display of this complex of symptoms has been considered an at-risk state for AD dementia in the elderly and is a potential manifestation of AD prodromal stage (Barnes et al., 2012; Rosenberg et al., 2013; Steenland et al., 2012). Therefore, neuropsychiatric symptoms should be considered as another clinical variable to predict AD prognosis among the MCI. Targeting MBI symptoms therapeutically may have a potential to delay the onset of dementia. Therefore, a robust research must be done in this field to conclude so. But the fact that assessment of neuropsychiatric symptoms is sometimes difficult because of the overlap between symptoms of anxiety/depression and dementia, and the distinction between anxiety and depression in dementia remains poor, makes the progress of understanding these corelates even harder (Seignourel, Kunik, Snow, Wilson, & Stanley, 2008).

As far as neuropsychiatric symptoms could be considered valuable AD markers, there is still a lack of challenges with the aim to incorporate their screening into clinical practise (diagnostics, prediction of the transition, disease stage identification etc.). For better understanding of the neurobiological and neuropsychological patterns of how neuropsychiatric symptoms pre/co-work with the other symptoms of AD, new animal transgenic AD models may be helpful in research.

2.2.1.1. Depression

Depression is a common and a serious mental illness that negatively affects the way one feels, thinks and acts. Depression causes feelings of sadness and/or a loss of interest in activities once enjoyed (APA, 2020). The prevalence of depression in AD varies from 6% to 42% (Chi et al., 2015).

There has been a growing body of evidence suggesting that emergent depression is a risk factor for dementia (Barnes, Alexopoulos, Lopez, Williamson, & Yaffe, 2006; Kokmen et al., 1991; Rosenberg et al., 2013; Steffens et al., 2004) and/or is a potential prodromal symptom of AD (P. Chen, Ganguli, Mulsant, & DeKosky, 1999; Goveas, Espeland, Woods, Wassertheil-Smoller, & Kotchen, 2011; Singh-Manoux et al., 2017), and/or might be even an accelerating factor in the yet ongoing AD progression (Dafsari, & Jessen, 2020), or can cover all three options at once, as can be seen in the *figure 2*.

Growing evidence further suggests that in a life-course model of contribution of modifiable risk factors for dementia, the elimination of depression, which is possible, is calculated to produce 4% reduction in dementia incidence on the population level, even exceeding the estimated effects of hypertension (2%), diabetes (1,2%), obesity (0,8%), or physical activity (2,6%; Livingston et al., 2017). Yet, the impact of depression in dementia has not been supported with enough scientific data in the WHO guideline for reduction of cognitive decline and dementia (WHO, 2019).

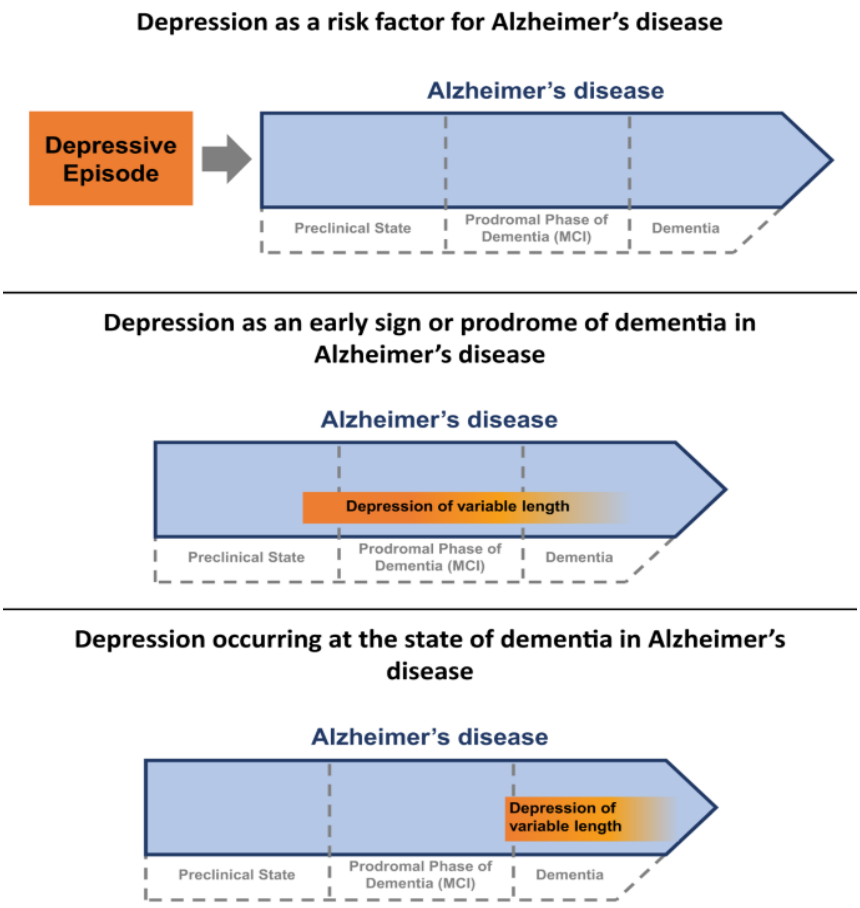

Figure 2: The role of depression in the process of neurodegeneration in AD. *Depression can occur in three different stages in the process of AD neurodegeneration. It can either be a predisposing factor occuring in the yet dementia-free person, or can be an early prodromal symptom, or can show up in the yet advanced AD dementia stage, or all three options (Dafsari, & Jessen, 2020).*

Major depression shares some of the AD anatomical and biochemical characteristics including thinning of the entorhinal cortex (Gerritsen et al., 2011), hippocampal volume reduction (Ballmaier et al., 2008; Gerritsen et al., 2011), and a decrease of Aβ42 in cerebrospinal fluid (Pomara et al., 2012).

In contrast, some studies (Berger et al., 1999; McCutcheon et al., 2016; Wilson et al., 2014) have not found a significant relationship between depressive symptoms and other AD features, such as elevated incidence of amyloid plaques or neurofibrillary tangles. Accordingly, there is still a lack of studies explaining the nature of depression behind the curtain of dementia diseases, such as AD.

2.2.1.2. Anxiety

Anxiety is an emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure. This unpleasant physiological state

occurs as an overreaction to a situation of various character. Nevertheless, as a reaction on the real threat, anxiety may be even beneficial (Zarrindast & Khakpai, 2015). Yet, anxiety as a result of accumulated stressful events, happens to be a risk factor for development of some psychiatric conditions (Räikkönen et al., 2011; Richter-Levin & Xu, 2018; Varese et al., 2012). Different brain regions are involved in the modulation and expression of anxiety, including amygdala, hippocampus, and frontal cortex (Zarrindast & Khakpai, 2015).

Even though anxiety is less studied than depression in regard to AD dementia, increasing evidence suggests that anxiety is common in AD dementia population, with the prevalence estimates of 39% (Zhao et al., 2016). However, not many population studies on the presence of anxiety symptoms in patients with AD have been done yet. Rather, most of the studies have been done on the population with any kind of dementia, not specifically the AD one. This is the reason why the sample of these studies is excessively heterogenic. AD patients usually cover majority of the sample though. Regardless of this methodological handicap, we can say that people diagnosed with dementia have a bigger probability of having anxiety than those not having dementia (Porter et al., 2003) and conversely, people with anxiety have a bigger probability of having dementia than those not suffering from anxiety (Santabárbara et al., 2020). At the same time, anxiety in dementia patients correlates with worse quality of life (Hoe, Hancock, Livingston, & Orrell, 2006) and worse neuropsychological performance (Porter et al., 2003).

In the review by Gimson et al. (2018) it is concluded that clinically significant anxiety in midlife is associated with increased risk of developing dementia over an interval of at least 10 years, suggesting that anxiety may be a risk factor for late-life dementia onset with the exclusion of anxiety related to prodromal cognitive decline (Gimson, Schlosser, Huntley, & Marchant, 2018).

Anxiety is also linked to worse cognitive performance and cognitive decline in clinically healthy elderly (Gallacher et al., 2009; Sinoff & Werner, 2003). At the same time, anxiety symptoms are considered as risk factor for adults with MCI, since patients displaying these have a higher conversion rate to manifestation of AD dementia (Mah, Binns, & Steffens, 2015). Therefore, the results suggest that display of anxiety has a predictive validity in AD similar to depression (Palmer et al., 2007) and may be related to the neuropathological mechanism in AD.

Concerning subjects with the MCI, abnormal concentration of t-tau and Aβ42 in cerebrospinal fluid was associated with anxiety symptoms, which at the same time were not related to symptoms of depression or apathy (Ramakers et al., 2013). In the study by Mah et al. (2015), researchers have found that anxiety is associated with increased rate of atrophy throughout the entorhinal cortex. This suggests that anxiety may be an accelerating factor contributing to the conversion from MCI into AD, particularly via the direct or indirect degrading effect on the regions of the entorhinal cortex.

In contrast, some of the studies (Devier et al., 2009; Robert et al., 2008) found no association between anxiety symptoms in MCI and a significantly higher risk of conversion to AD dementia. Because of the discrepancy in results, further longitudinal research with larger samples needs to be done, so that the conclusions about the role of anxiety within and even before the manifestation of AD dementia in patients could be made.

2.2.1.3. Social dysregulation

Socially dysfunctional behaviour of patients suffering from AD dementia remains a significant burden for patients, families as well as caregivers. One of the sources of such behaviour might be apathy, a symptom which is defined as a loss of interest and motivation in different domains in everyday life functioning. The apathy often results in social withdrawal or general deficits in social behaviour in patients with AD dementia (Nobis & Husain, 2018). The study assuming that social dysregulation in AD could be resulting from apathy produces an interesting finding. Apathetic AD patients were investigated to assess attentional bias via nonverbal visual scanning task. Eye tracking results showed that apathetic AD patients spent less time than their non-apathetic counterparts fixating on social, but not neutral images (Chau, Chung, Herrmann, Eizenman, & Lanctôt, 2016). Another socially relevant symptoms often reported in AD patients are aggression and agitation. Antipsychotic mediations constitute the first-line pharmacological strategy of treating these symptoms (Ballard & Corbett, 2013).

Even though social behaviour is clearly pathologically altered in patients with AD, a lack of attention still crosses this important topic in the research.

2.3. Therapy and treatment

Current pharmacological therapy offers only a few medication options available for patients diagnosed with AD. As far as the cholinergic hypothesis of AD is considered, cholinesterase inhibitors such as donepezil (Birks & Harvey, 2018; Howard et al., 2012), rivastigmine (Hansen et al., 2008), or galantamine (Hansen et al., 2008) are officially licensed as a therapy for AD patients. However, these drugs only work as a symptomatic treatment (as you can see in the *figure 1*), while the ultimate prognosis remains unchanged.

The second class of AD medication represents memantine with the activity of non-competitive N-methyl-D-aspartate receptor antagonist, especially for those who have attention deficits. However, memantine as well as cholinergic inhibitors only work as attenuators of the cognitive symptoms of AD (Howard et al., 2012; X. Wang, Blanchard, Grundke-Iqbal, & Iqbal, 2015).

On June 7/2021, a new drug Aducanumab (BIIB037) was officially licensed as a treatment of AD. BIIB037 is a monoclonal antibody which binds to a conformational epitope on Aβ with high affinity. Mice studies suggest that chronic dosing of this drug indeed significantly reduces plaques of all sizes (Sevigny et al., 2016), however many controversies surround the approval of this medication by the authorities.

The deficiency of vitamin D is considered a risk factor, therefore its supplementation is used as an alternative method of the treatment of AD (Gangwar et al., 2015). Management of cardiovascular deficits contributes to overall brain health, and it works as a protective factor in neurodegenerative diseases in general. Much attention goes to omega-3-fatty acid supplements, as the results show that it strengthens cognitive functioning in MCI (Bo et al., 2017).

For the cognitive decline, training of cognitive skills is recommended as it delays manifestation of full-blown dementia syndrome in those yet suffering from MCI (Nousia et al., 2018). Physical exercise is recommended as well (Okonkwo et al., 2014; Smith et al., 2014).

For those experiencing neuropsychiatric symptoms associated with the MCI or AD, psychotherapy or other psychological interventions are considered, however, the exact

behavioural manual is about yet to be published (Forstmeier, Maercker, Savaskan, & Roth, 2015).

Future direction

Nonetheless, none of the therapies currently available for AD patients work amazingly. It is a great challenge of the current research to find one that does. Modern pharmacological research deals with the option of targeting AD-associated pathological structures, such as the neurofibrillary tangles or senile plaques. As suggested above, one of the studied classes of medication are monoclonal antibodies working as a passive immunotherapy agent. However, the removal of the plaques or tangles by these agents generally does not seem to improve cognitive outcomes of progressed AD patients (Gauthier et al., 2016; Salloway et al., 2014).

Neural circuitry restoration is also currently under the investigation as a potential tool for treating of AD (Busche & Konnerth, 2016; Nakazono, Jun, Blurton-Jones, Green, & Igarashi, 2018).

3. Testing anxiety-like and depression-like behaviour in animal models

The use of rodents, such as of rats or mice, in research has been immensely helpful. It allows us to look for the causes of, and treatments for psychiatric/neurological illnesses and diseases (Lezak, Missig, & Carlezon, 2017).

It is possible because some behavioural features of emotional and/or motivational dysregulation, such as of anxiety or depression, are similarly detectable in rodents as in humans. Nevertheless, with rodents we may not talk about neither anxiety nor depression. Instead, we use terms such as 'anxiety-like behaviour' and 'depression-like behaviour.' The reason for this is that we cannot conclude from such subjective manners in animals, since animals are not able to express their feelings explicitly. That is why researchers in the laboratory environment have to rely on basic ethologically relevant behavioural paradigms (Lezak, Missig, & Carlezon, 2017).

3.1. Anxiety-like behaviour

Anxiety in rodent produces several similar behavioural responses to fear, freezing and increased vigilance and/or general hypoactivity, elevated heart rate, suppressed food consumption included (Walker, Toufexis, & Davis, 2003). Anxiety-like behaviour, unlike fear, is defined as a behavioural response to aversive stimuli, which is diffuse, unpredictable, distal, or of long duration (Lezak, Missig, & Jr, 2017)

Some traditional tests of unconditioned anxiety in rats include the Open-field (OF), Elevated plus-maze (EPM), or Social interaction test (SIT; Belzung & Griebel, 2001; Lezak et al., 2017).

3.1.1. Open Field Maze

The OF test was originally developed by Hall in 1934 for the purpose of assessing emotional behaviour in rodents. This was possible because some behaviour (etc. locomotor activity and defecation) refers to animal emotional state (Hall, 1934). OF offers an easy way to assess behaviour without previous training. The OF maze consists of a wall-enclosed square area. In the beginning of the test an animal is placed at the centre of the apparatus. Duration of the test session is usually settled at 5 minutes. The anxiety paradigm presumes that movement in the open box is mainly result of an

exploratory drive (similar to the entrance and time spent in an open arm in the EPM). An ethological event which is characterised by a rodent standing against the wall is referred to as thigmotaxis, which is an anxious-related behaviour. As a result, the less anxious animals spend relatively more time in the centre of the maze, while animals which prefer the perimeter area more are considered more anxious (Seibenhener & Wooten, 2015). Anxiety related drugs such as diazepam have shown an anti-anxious effect in rodents, since the time in the centre of arena is magnified in the diazepam-treated animals (J. Crawley & Goodwin, 1980).

3.1.2. Elevated Plus Maze

The EPM, originally proposed by Pellow et al. (1985), is a widely used behavioural survey of rodents. The EPM has been validated to assess anxiety as well as anti-anxiety effects. The apparatus consists of four arms radiating from a central platform forming a plus sign shape. Walls of the two opposed arms are elevated whilst the two other arms remain open. The test starts with the animal placed in the junction of the maze's four arms, facing one of the open arms. Ethological parameters such as duration and/or entries in every single arm are recorded on a video tape. The whole session typically takes 5 minutes (Pellow et al., 1985).

The principal outcome of this test is that it generates conflict between the drive to approach novel areas and, simultaneously, to avoid potential threat therein. It is expected that rodents with a higher level of anxiety spend relatively less time in the open arms and relatively more time in the closed ones because the need to avoid a potential threat within is dominant over the drive to approach novel areas in their case (Arantes, Tejada, Bosco, Morato, & Roque, 2013; Carobrez & Bertoglio, 2005). The administration of anti-anxiolytic drugs, such as of diazepam, promotes exploratory behaviour, thus the diazepam-treated rats spend relatively more time in the open arms (Sharon Pellow & File, 1986).

3.1.3. Social Interaction Test

Social behaviour is by definition a term describing activities of at least two individuals of the same species (Sokolowski, 2010). It is presumed that an altered social behaviour is possible to observe as a change in the frequency of social interactions which arise from anxiety-like conditions. Thus, psychiatric and/or neurological conditions can

derive from an animal's behavioural interaction in relation to other specimen (J. N. Crawley, 2007; Hanks, Dlugolenski, Hughes, Seymour, & Majchrzak, 2013).

When psychiatric symptoms are considered, researchers follow the type of an animal's behaviour which is neither aggressive, nor sexual, nor territorial in general. The type of social behaviour which they follow in this case may be referred to as 'friendly encounters' (Peleh, Ike, Wams, Lebois, & Hengerer, 2019). Often reported friendly encounters include following, anogenital and non-anogenital contact, allo-grooming, huddling etc. (Peleh et al., 2019).

Following: One subject is walking/running after another subject. It is different from an aggressive following which is named chasing.

Contact: Two subjects being in close proximity to each other. Social contact may be either nose-to-nose referred to as non-anogenital contact, or nose-to-anogenital referred to as anogenital contact.

Allo-grooming: One subject is licking and cleaning the fur of another subject.

Huddling: Subjects sleeping close together.

The SIT, originally developed by File and Hyde in 1978, is usually used for the purpose of social behaviour testing in rats in the laboratory environment. In the SIT, two unfamiliar rodents (usually rats) are tested without the need of previous training session. They are placed in a neutral environment and are allowed to interact freely without any additional stimulus for 10 minutes (File & Hyde, 1978).

The session is being recorded the whole time. In one case, a pair of animals may be assessed as one unit, since the social behaviour is directly reciprocally impacted by each animal. Or, if there is a necessity to follow behaviour of only one animal in the experimental design, the other animal serves as a control (Campos et al., 2013).

The more animal interacts with the other (the more 'friendly encounters' animal makes), the more is its behaviour considered anxiolytic-like. Conversely, the less time the animal spends engaging in social interactions indicate anxiogenic-like behaviour (Campos et al., 2013).

3.1.4. Fear conditioning

Fear memory is a widely studied form of rodent's memory. To an extent, hippocampus greatly participates in the formation of fear-conditioned memories (Fastenrath et al., 2014; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999; Sotres-Bayon, Sierra-Mercado, Pardilla-Delgado, & Quirk, 2012), the disruption of its process is expected to be found in AD patients, since one of the first brain region which undergoes the neurodegenerative process in AD is in fact the hippocampal formation (Hibar et al., 2017).

Fear conditioning is a fundamental form of learning, thanks to which an animal can predict aversive events. Within this type of learning, an initially neutral stimuli generates fear because it is recognised as a threat through pairing with an aversive fear-forming stimulus. Fear conditioning can be cue directed, in which a specific cue called conditioned stimuli (usually an auditory stimulus) is presented in the training session and is associated with unconditioned aversive stimuli (usually foot shocks). Or the second classical (Pavlovian) procedure to assess the emotional memory formation is contextual fear conditioning (CFC). In the CFC, the training compartment itself is a conditioned stimuli as it is directly connected to unconditioned aversive stimuli (no additional stimulus is presented in the task). A standard fear-like response to conditioned stimuli (conditioned response) is freezing behaviour, a generalized immobility caused by a generalized tonic response of the skeletal musculature except the breathing muscles (Izquierdo, Furini, & Myskiw, 2016).

Contextual Fear Conditioning

In the CFC, an animal is placed in a box with rectangular stainless steel during the training session. After 3-10 minutes the animal receives series of foot shocks (1-3) from which it cannot escape. If there are no additional stimuli, the animal learns to react with a fear-like response just by its exposition to the box (which is the learned context). During the test session, no foot shock is given to the animal, yet it reacts by freezing as it learned that the box is an environment in which the animal is about to be given a foot shock just like in the training session (Izquierdo et al., 2016). High degree of emotional arousal is known to enhance memory consolidation (Cahill & McGaugh, 1998) and reconsolidation (Akirav & Maroun, 2013), via which emotional dysregulation takes its part as it highly influence an animal behaviour in the CFC.

3.2. Depression-like behaviour

While there are some features of depressive syndrome specific for humans, including guilt, suicidality, or sad mood, other features can be laboratory measured in animals and can be even ameliorated with an antidepressant treatment in both species. The symptoms of depressive syndrome observable in animals include helplessness, anhedonia, behavioural despair and neurovegetative changes, such as alteration in sleep cycles and appetite patterns (Krishnan & Nestler, 2011). As we cannot reflect state of mind in rodents, behavioural paradigms are used to assess specific depression-like phenotype, which is stereotypical and species-dependent and to some point, analogical to depressive symptomatic in humans.

In laboratory conditions, an exposition to stress is usually used to induce depression-like state in rodents. For this purpose, animal models of acute stress are a quick and easy way to test depression-like behaviour. The most widely used are the Forced swim test (FST), Tail suspension test (TST), Sucrose preference test (SPT), the learned helplessness model etc. (Krishnan & Nestler, 2011).

3.2.1. Forced Swim Test

FST is a rodent behavioural test, which was originally developed in 1978 by Porsolt and his colleagues. The original application of this model was predicting clinical efficacy of antidepressant drugs (Porsolt, Anton, Blavet, & Jalfre, 1978). Nowadays, a modified version is commonly used to assess depression-like behaviour in rodents (Voorhees et al., 2018).

The basic FST contains two session. First session takes 15 minutes, during which an animal is placed into a cylinder with water of constant temperature. First session (pre-test session) is a stressor by itself, because the lack of choice to escape from water induces a state of behavioural despair and possibly subsequent passive stress coping strategy characterised by general immobility of the animal. The observable immobility of an animal during the second session (test session), which usually takes 5 minutes, is therefore recognised as a passive depression-like behaviour analogical to behavioural despair (Slattery & Cryan, 2012). However, the basic FST two-session paradigm is mainly used for antidepressant drug efficacy testing. A modified shortened one-session paradigm is possible to use in the GMO testing as it is in the case of our study (for more information see *6.2.*).

3.2.2. Tail Suspension Test

In the TST, an animal is suspended by its tail and the time of the overall immobility of animal is measured. It is expected that the less depression-like state animal experiences, the less feeling of behavioural despair the animal experiences, and the more mobility it reports. However, the TST is exclusively used to study depression-like behaviour in mice rather than rats due to the differences in the overall size of these two species (Can et al., 2012).

3.2.3. The Learned Helplessness Model

Other animal models of depression follow a symptom of learned helplessness as another depressive feature to be possibly assessed in animals. Exposure to an inescapable and uncontrollable stress due to consistent re-exposition to the same electric shocks develop a state of helplessness in animals. Finally, when the animal is allowed to escape the shocks, it displays either increased escape latency, or completely fails to escape (Seligman, Rosellini, & Kozak, 1975).

3.2.4. Sucrose Preference Test

Another symptom commonly assessed in animals as a feature of depression-like state is anhedonia, a loss of the ability to derive pleasure from an activity that usually produces it (Söderlund & Lindskog, 2018).

It is generally expected that animals prefer sweetened water or food over the unsweetened. In the SPT, a lost preference for sweet water is interpreted as anhedonia. A 2-bottle choice procedure for assessment of the sucrose preference in water is mostly used with rodents. Preference is measured by volume and/or weight of liquid consumed daily. The volume and/or weight is afterwards converted to a percent of preference compared to an original baseline period consisting only of water. Depression-like behaviour is characteristic by reduction of the sweet preference and is reversed with an antidepressant treatment (Eagle, Mazei-Robison, & Robison, 2016).

4. Animal Models of Alzheimer Disease

In the AD research, we use either mice or rat models that can be both, transgenic and non-transgenic.

4.1. Non-transgenic Animal Models

When transgenic animal models were not yet been approachable, non-transgenic models had been trending in the AD research. One of them is a toxin-induced Scopolamine experimental model, a neuropharmacological animal model used in the neuroscience-related research regarding AD. Scopolamine is a nonselective, competitive muscarinic receptor antagonist. Thanks to the muscarinic antagonistic effect, an AD-related cholinergic dysfunction often appears in animals. Moreover, the increased amyloid-β deposition seems to be another hallmark of the scopolamine application (W. N. Chen & Yeong, 2020).

Another pharmacological model commonly used in the AD research is Streptozotocin model. Streptozotocin is a glucosamine-nitrosourea compound of which the intracerebroventricular administration produces impairment in cognition. Streptozotocin mediates neuroinflammation which is closely associated with the overall pathology of AD because its presence precedes progressive plaque and tangle formation (Kamat, 2015).

4.2. Transgenic Animal Models

Until recently, transgenic AD mice models with the Aβ-overproduction have been dominantly used in the field of AD dementia research. However, as the amyloid cascade hypothesis alone fails to explain all the etiopathological features of and events in AD and as invented medication, which works in the effective way in mice AD models, fails to show a significant effect in the human trials, new AD animal models are called for. The main handicap of the transgenic AD mice models is the absence of demonstration of the robust tauopathy and neuronal loss among the Aβ-overproduction (Drummond & Wisniewski, 2017).

Furthermore, rats, compared to mice, show less territorial behaviour and are less aggressive toward others and at the same time they show a wider range of ‘friendly’ social interactions, both in nature and laboratory environment. Considering the

complex and collaborative nature of human social behaviour, rats appear to be a better option for the purpose of modelling the AD dementia in research, especially when neuropsychiatric symptoms are evaluated (Ellenbroek & Youn, 2016).

4.2.1. TgF344-AD rat model

Recently, the attention has been brought to a new transgenic rat model that manifests many of the age-dependent AD pathologies as well as an appropriate cognitive impairment. The relatively new generation of TgF344-AD rats has been generated on a Fischer 344 background as the rat pronuclei was co-injected with two exclusively human genes driven by the mouse prion promotor. TgF344-AD line bears the so-called 'Swedish' mutant human APP and exon 9 mutant human presenilin-1 (Cohen et al., 2013).

In the original study conducted by Cohen et al. (2013), a complex behavioural, histological, biochemical and immunohistochemical analysis was done with TgF344-AD rats. Data demonstrated that tg AD rats manifest the complete repertoire of AD pathological features. Among other things, such as the progressive accumulation of amyloid plaques, tauopathy and neurodegeneration, the neuronal loss was confirmed in this model. It is noteworthy that the neuronal loss in cortical and hippocampal regions was registered to be much wider than in other AD rat models, and even was marked as an age dependent. What also seems to be age-dependent in this model is the cognitive impairment, which was confirmed via behavioural analysis (Cohen et al., 2013). As the result of behavioural, histological, biochemical and immunohistochemical analysis, TgF344-AD rat model with only two mutant human transgenes, each separately independent causative-factors for early-onset of the familial AD, seems to be sufficient to mimic the full spectrum of AD pathological features, therefore may be used in future research. However, more studies must be done on this model to conclude about the translational potential of this model more vividly.

4.2.1.1. Cognitive functioning in TgF344-AD rat model

So far, a few studies evaluating cognitive impairment associated with AD dementia have been done with the TgF344-AD rats. Reference memory as well as reversal have been found to be impaired in the tg rats older than 6 months (Berkowitz, Harvey, Drake, Thompson, & Clark, 2018; Rorabaugh et al., 2017) in the water maze, where

parameter was latency to platform. However, reference memory has not yet been impaired in rats of a little younger age (4-6 months; Berkowitz et al., 2018; Pentkowski et al., 2018). Therefore, the results show an age-dependent cognitive impairment trend in AD. As expected, according to another results from the water maze test, in 24 months old rats there was an impairment in the reversal as well as in the reversal probe, where parameter was a time to platform (Voorhees et al., 2018). Nevertheless, in the same study, there was not an impairment in the reference memory in rats of the same age (Voorhees et al., 2018).

In the Barnes maze with a parameter of the number of errors, rats older than 6 months shown impairment in the reversal, whereas in the acquisition there was an impairment in rats older than 15, but not younger than 6 months (Cohen et al., 2013). When a parameter of the latency was used in the same behavioural paradigm, there had been no impairment in the acquisition even if the rats were 15-16 months old (Morrone et al., 2020; Voorhees et al., 2018). On the other hand, rats of the age of 12-15 months had displayed impairment in the reversal (Morrone et al., 2020).

In the study with longitudinal follow-up by Muñoz-Moreno et al. (2018), working memory performance was evaluated by means of the delayed nonmatch-to-sample task with 5 months old TgF344-AD rats. These rats exhibited progressive cognitive impairment in spatial learning and memory as they had to accomplish more trials to meet the criterion to gain food. In the same study, the hypothesis of AD as of a disconnection syndrome was tested. The hypothesis assumes that functional and/or structural interactions between brain regions are responsible for the cognitive impairment in AD, rather than alterations in single brain areas. Indeed, the structural connectome analysis results indicated different organization of the whole-brain network in the tg rats. Detected changes such as the decrease in global and local efficiency and clustering are coherent with changes of the network characteristics in human studies. However, functional networks were not different in the tg rats and controls (Muñoz-Moreno, Tudela, López-Gil, & Soria, 2018). The observed relation between cognitive performance and structural network metrics, but not functional connectivity, in the TgF344-AD rats has been recently replicated by the same team of authors (Muñoz-Moreno, Tudela, López-Gil, & Soria, 2020).

Left-right discrimination task in the T-maze was used to assess reference memory in the study by Tournier et al. (2020), besides that a percentage of spontaneous

alternations in the Y-maze had been used to assess spatial working memory in the same cohort of animals. The results showed that 9-10 months old tg rats had performed worse than controls in the T-maze, but not in the Y-maze, therefore had poorer performance in reference memory, but not spatial working memory (Tournier et al., 2020). The performance in the T-maze was also impaired as soon as the rats were 6 months old according to study by Saré et al. (2020).

To probe place discrimination, the Novel object recognition test was used in the study by Morrone et al. (2020). Time spent with a novel against a familiar one has been analysed for each rat. Tg rats performed worse in disambiguating between familiar and novel stimuli, in other words they exhibited deficits in learning and memory (Cohen et al., 2013; Morrone et al., 2020).

Understanding of the neural basis of cognitive deficits in AD is important, because it offers a link for identifying new biomarkers suitable for AD diagnostics in the early stage. With this aim, hippocampal and cortical oscillatory network activities were observed in TgF344-AD rats. Significantly higher occurrence of cortical HVSs and impaired interaction between HVSs and SWRs were found in the study by Stoiljkovic et al. (2019). It is suggested that accumulation of Aβ and tauopathy, major hallmarks of AD pathology, is associated with complex disturbances in synaptic and neuronal function, which leads to an impairment in coordination of activity in the neural networks being responsible for the appropriate function of memory and cognition. Alterations can range from subtle changes in rhythmic activity, such as EEG signal pathology to the more observable ones. Indeed, impairment of hippocampal theta oscillation and hippocampal theta-gamma coupling precedes the cognitive deficits in tg AD animals and these changes even seem to be age-dependent (Stoiljkovic et al., 2019).

Together the results indicate that above all, there is a cognitive impairment in the TgF344-AD rats, which even show an age-dependent deteriorating effect. However, the detection of initial deficits depends on which test and/or parameter we actually use.

4.2.1.2. Anxiety-like and Depression-like behaviour in TgF344-AD rat model

Until nowadays, they have looked for anxiety- and depression-like phenotypes in TgF344-AD rat model only in four studies. It seems that the age-related decline depended on the choice of behavioural method to assess the phenotypes.

The studies using the EPM paradigm have found enhanced anxiety-like behaviour in TgF344-AD animals in majority of cases (Pentkowski et al., 2018; Tournier et al., 2020b), as soon as the animals were 4-6 months old, regardless sex. The anxiety-like behaviour was even detectable in the yet cognitively normal animals as there was normal spatial memory in the same cohort of animals at that time (Pentkowski et al. 2018). In contrast to the results from the EPM, no overall difference was observed in the OFM in the study by (Morrone et al., 2020), even in much older (12-13 months) animals than it was in the case of the EPM design.

A single study evaluated depression-like behaviour in the FST on the TgF344-AD rat model and they had found that regardless sex tg rats showed more passive (depression-like) strategy characterised by more of the time spending immobile and again, the passivity was present even before the cognitive impairment had occurred (Voorhees et al., 2018). However, anhedonia, a major depressive symptom, was not present in 9 months old tg animals in the study by Tournier et al. (2020).

Nonetheless, I dig more deeply into the analysis of anxiety- and depression-like behaviour in TgF344-AD model in discussion, where I compare the selected data with data from our experiments.

EXPERIMENTAL PART

5. Aims

According to human studies, neuropsychiatric symptoms such as anxiety, or depression are closely related to AD, however there is still a lack of knowledge about the precise mechanisms of this relationship, both in humans and animals.

The aim of this study was to evaluate neuropsychiatric symptoms, specifically - anxiety- and depression-like behaviour as well as social dysregulation and emotional memory impairment, in the relatively new TgF344-AD rat model, which is supposed to mimic all the neuropathological features of AD in humans. Moreover, the design of our study (which was conducted to test both, 10 and 14 months, old animals) allowed us to follow a potential progressive trend in the onset of each neuropsychiatric-like symptom. We prospectively even aimed to look at the sex differences, since we tested both males and females.

We evaluated the neuropsychiatric-like behaviour via behavioural methods. We assessed anxiety-like behaviour in the Open field maze (OFM), Elevated plus maze (EPM) and Social-interaction test (SIT), and depression-like behaviour in the Forced swim test (FST). The dysregulation of emotional memory was assessed via a task using contextual fear conditioning (CFC) paradigm.

6. Materials and methods

6.1. Subjects

For the evaluation of behavior in the battery of anxiety-like tests (consisting of the OF test, EPM test, SIT and CFC), 50 TgF344-AD (tg) and 45 wild type Fischer 344 (wt) rats were obtained from Institute of Physiology AS CR where they have been bred for 4-5 generations. The original resource however is the Rat Resource & Research Center (RRRC), Missouri, USA. Some of the animals (5) were excluded from further analysis due to various physical conditions (lung tumors, and inflammation of the eye). Tg as well as wt subjects were approximately counterbalanced for sex and age. Subjects were tested between 10 and 14 months of age. The testing took place in several separate runs. List of subjects for battery of anxiety-like tests is summarized in the *Fig. 3*.

	10 months				14 months			
	males		females		males		females	
	tg	wt	tg	wt	tg	wt	tg	wt
RUN1	7	5	4	6				
RUN2	6	0	3	8	1	3	3	1
RUN3	4	4	2	2	4	1	3	5
RUN4					4	8	5	1
TOTAL	17	9	9	16	9	12	11	7

Figure 3: *Table shows a summary of the list of subjects for the battery of anxiety-like tests.*

The assessment of behavior in the FST was exclusively accomplished on a different group of subjects. 38 tg rats and 30 wt rats were obtained in this case. The reason for the use of a unique group of subjects was that the FST was added to the experimental study design additionally. Therefore, the test could not be included in the yet ongoing anxiety-like tests experimental design. List of subjects for FST is summarized in the *Fig. 4*.

	10 months					14 months			
	males		females			males		females	
	tg	wt	tg	wt		tg	wt	tg	wt
RUN2	5	5	6	4	RUN1	8	8	7	3
RUN3	5	6	5	4	RUN3			2	

Figure 4: *Table shows a summary of the list of subjects for the depression-like test.*

All subjects were housed in an accredited animal room with controlled temperature (23 ± 2 ◦C) and were kept on a 12-hour light/dark cycle with lights off at 06:00 a.m. Tg and wt subjects were housed separately in pairs in the cages. The access to food and water was provided *ad libitum* throughout the duration of the study. Particular attention was paid so that illumination did not exceed 50 Lux to prevent retinal degeneration that is observed in TgF344-AD rats housed under standard illumination (Tsai et al., 2014). All experiments and housing conditions and care of the rats were approved by a resort Committee of Animal Welfare with a protocol (136/2016). All animal manipulation was done in accordance with Czech legislation and appropriate directive of European Council (2010/63/EU).

6.2. Experimental Design

After the acclimatization at the Institute of Physiology AS CR, all subjects were handled for 5 days (they were habituated to human touch, holding, and manipulation by the experimenter for 5-10 min per day for each animal) prior to the experimental session. Battery of anxiety-like test was then conducted to assess the behavioral phenotype in this order: OFM, EPM, SIT, Morris water maze (MWM), One trial test (OTT) and CFC. The whole experimental design lasted 3 working weeks (14 days) for each cohort of animals. *Figure 5* shows timing of the experimental steps. Within this design, a simple MWM task was only used to detect whether any progressive changes in vision had occurred in subjects at the time of their testing. Therefore, MWM data would not occur in the analysis. Neither would the data from OTT. The data from OTT were excluded from further analysis in this thesis, since the OT paradigm does not assess anxiety-, nor depression-, or any other psychiatric-like conditions. The whole experimental procedure had always been maintained in the same order. Subjects were tested in 4 different runs according to their birthdate. The age of the animals ranged from 10 to 14 months at the start of their testing. Each subject was placed in a group according to its genotype (tg/wt), sex (male/female) and age (10/14 months) for statistical analysis.

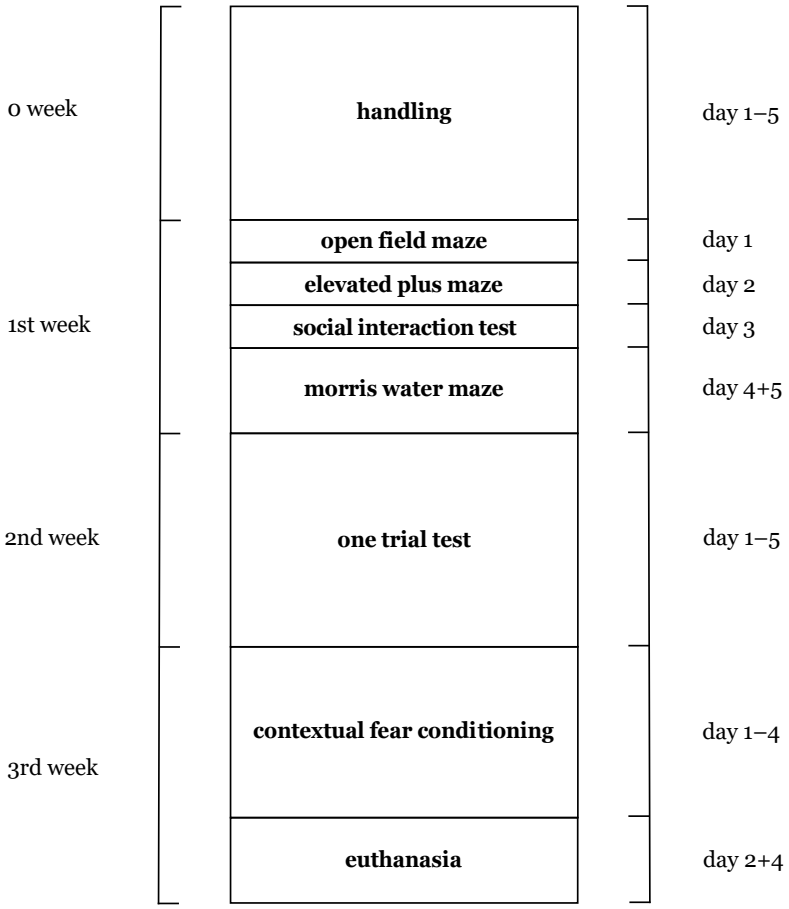

Figure 5: *Scheme shows the timing of experimental steps in the battery of anxiety-like tests.*

I: Battery of anxiety-like tests

Experiment 1: Open Field Maze

The locomotor activity of animals was assessed via standard OFT (originally proposed by Hall, 1934; for more information see chapter *3.1.1.*). The OF apparatus consisted of a white wood box measuring 70 x 70 x 40 cm. The box was cleaned before the first run of the day, between subjects, and after the last run of the day using tap water and ethanol. On the day of the testing, animals in their home cages were brought into the experimental room 30 min prior to the testing. The level of illumination in the experimental room was set at 5-10 lux during the testing. The tested animal was placed in the center of the apparatus and was allowed to freely explore for 5 min. The time spent in the middle (which was defined 50 x 50) and the periphery and the distance moved in the middle and periphery, specifically their ratio, were used as parameters for a statistical analysis. All trials were video recorded, and the data was obtained using the automatic video analysis in program Ethovision (Noldus).

Figure 6: Open Field Maze apparatus. *(photo by Kristýna Malenínská).*

Experiment 2: Elevated Plus Maze

One day after the OF task, all animals were tested for anxiety-like behavior in the EPM. The used protocol was originally proposed by Pellow et al. (1985; for more information see chapter *3.1.2.*). The EPM apparatus consisted of 4 black Plexiglas arms arranged in a cross, elevated 60 cm above the floor. Each arm was 11 cm wide and 50 cm long, and each arm was joined at the center by a 11 x 11 cm square black platform. Two arms remained 'open' without walls, while two opposite 'closed' arms contained 30 cm tall orange opaque sides. The maze was cleaned before the first run of the day, between subjects, and after the last run of the day using tap water and ethanol. On the day of the testing, animals in their home cages were brought into the experimental room 30 min prior to the testing. The level of illumination in the experimental room was set at 5-10 lux during the testing. The tested animal was placed in the center of the apparatus facing 1 of the 2 open arms. Each subject was tested for 5 minutes. All test trials were video recorded and subsequently analyzed using the behavioral analysis software Boris (*version 7.9.7.)*. The ratio of time (duration) spent in open vs. closed arms was manually measured for each subject. As the secondary parameter, duration of peeking out (from closed arms) and looking down (from open arms) was manually measured for each subject.

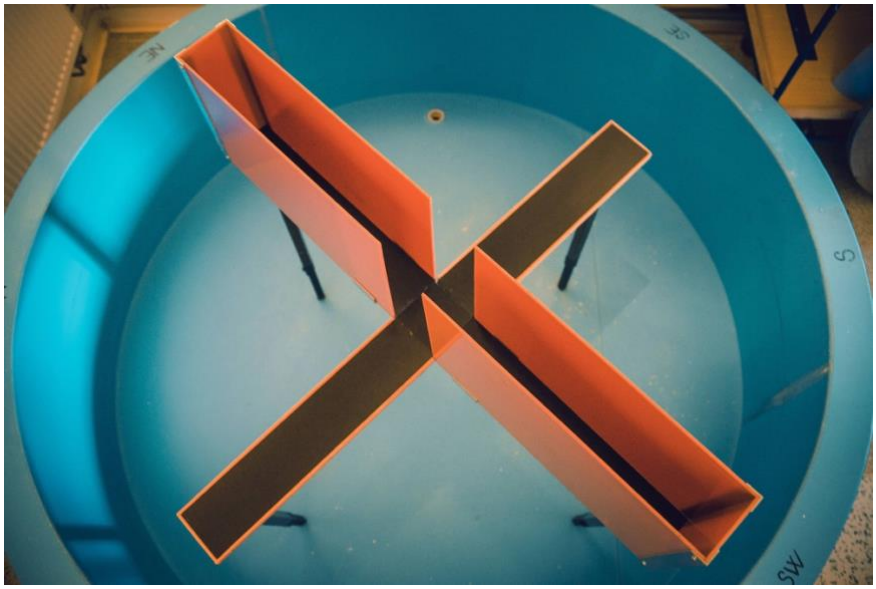

Figure 7: Elevated Plus Maze apparatus. *(photo by Kristýna Malenínská).*

Experiment 3: Social Interaction Test

For the purpose of testing social interactions between subjects (for more information see *3.1.3.*), the same apparatus as in the OF task was used (white wood box measuring 70 x 70 x 40 cm). The box was cleaned before the first run of the day, between subjects, and after the last run of the day using tap water and ethanol. On the day of testing, animals in their home cages were brought into the experimental room 30 min prior to the testing. The level of illumination in the experimental room was set at 5-10 lux during the testing. Each experimental animal was put in the box with its partner which it had never interacted with before. The partner animal was put to the further corner facing the wall and the very next moment, the tested experimental animal was put to the opposite corner facing the wall too. The partner animal was marked by non-washable marker on fur so it could be easily distinguishable from the experimental animal. Animals were allowed to freely interact in the box throughout a 10 min session. The time spent by socializing of the experimental animal toward the partner animal was the subject of behavioral analysis. Specific parameters, such as the time spent by anogenital (nose of the experimental animal-to-anogenital of the partner animal) exploration, non-anogenital (nose-to-nose) exploration, following a conspecific (experimental animal moves toward moving partner animal) were used in statistical analysis (for more information see chapter *3.1.3.*). All test trials were video recorded and manually evaluated by the experimenter in the Boris behavioral software (*version 7.9.7.*).

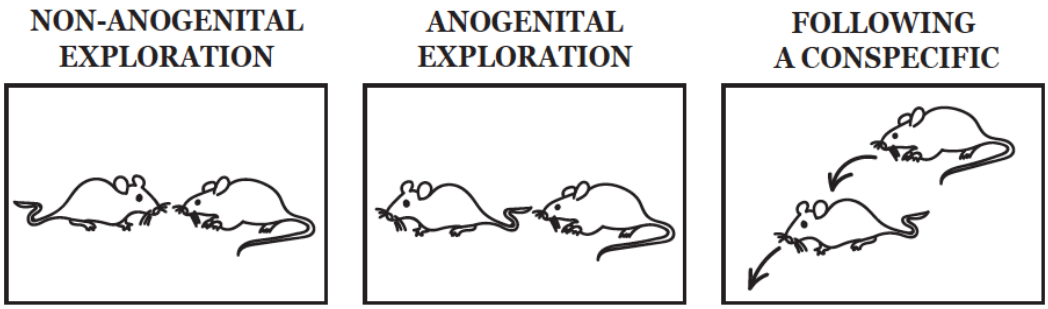

Figure 8: *Scheme shows parameters that we observed in the Social interaction test.*

Experiment 4: Morris Water Maze

Within battery of anxiety-like tests, a simple MWM task was only used to detect whether any progressive changes in vision had occurred in the subjects at the time of their testing. However, the data from the MWM task were not a subject of our study and therefore were not included in the statistical analysis, neither were the exact experimental protocol discussed here. I would just propose that we used a visible platform paradigm with 4 different default points from where the tested animal was put in the water. On the first day, an animal underwent a training session – the animal was let to look for the visible platform until the maximum of one minute had passed. In the case the animal not finding the platform, the experimenter had guided the animal. On the second day, it was expected that the animals without progressive vision changes would find the platform within one minute in maximum. In the matter of results from MWM, none of the animals were excluded from further analysis due to vision conditions.

Experiment 4: One Trial Test

After that the animals underwent the OT experimental procedure, which took 5 days (throughout the week 2). However, since the OT paradigm does not measure anxiety-, nor depression-like behavior, the protocol is not further described here, and the analysis of its data is not included in results.

Experiment 5: Contextual Fear Conditioning Test

Emotional memory, which is associated with the overall psychological-like state, was assessed via the CFC paradigm. Because of the overall invasiveness of fear conditioning, the CFC was proceeded as the final experiment in the battery of anxiety-

like test prior to euthanasia. The CFC took place in a different experimental room than the other experiments in the same experimental design described above. The apparatus was a square conditioned chamber with Plexiglas walls (45 x 40 cm), all of them were transparent and were located in a sound-attenuating box (TSE systems, Germany). The bottom of the chamber consisted of an insulated shock grid floor via which the animal received a series of foot shocks. The apparatus was cleaned before the first run of the day, between subjects, and after the last run of the day using tap water and ethanol. The experiment took two days. On the first day, an animal underwent the pretest trial and the day after, an animal underwent the test trial. The cage with a tested animal was brought to the experimental room prior to testing (only a single animal was present in the experimental room at one time). At the start of the pretest trial on day 1, the tested animal was put to the center of the chamber. The whole pretest took 5,7 min and it consisted of 7 parts. The first part was a 3-minutes habituation session in the apparatus. The second, fourth and sixth part were represented by a short series of 3 foot-shocks of 0.8 mA, which the animal could not escape from, and which were interleaved with series of 3 after-shocks pauses. After that, the animal was left in the conditioning chamber for another 30 sec and then it was placed in its home cage. On day 2, the procedure was similar as on day 1 – the tested animal was brought to the experimental room in a transport cage immediately prior to the testing. After the cleaning of the apparatus, an animal was put in the center of the chamber and was allowed to freely explore for 5 min. There were no foot shocks present at that time, neither was any additional stimuli (as the conditioned stimuli was perceived as the environment of the chamber itself). Both trials (pretest and test) were video recorded. The parameter used for statistical analysis was the time (duration) of freezing behavior during the test session (which was defined as the complete absence of body movements, except for those necessary for respiration) in contrast to the duration of freezing in the habituation part. Freezing behavior was coded and scored automatically in the TSE Fear Conditioning System software.

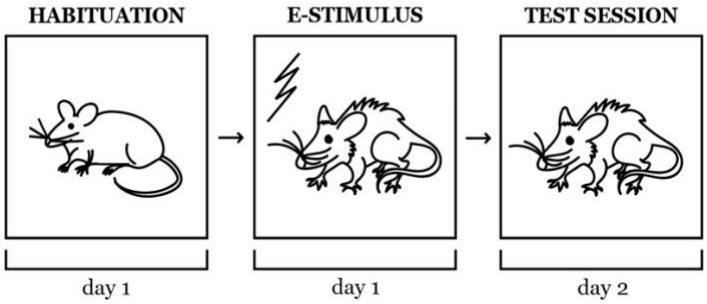

Figure 9: *Schema shows timing of the contextual fear conditioning experiment in our design.*

Figure 10: Contextual Fear Conditioning chamber. *Photo taken from official website of TSE systems.*

II: Depression-like test

Experiment 1: Forced Swim Test

A modified version of the FST without pretest (for more information see *3.2.1.*) was used for the purpose of assessment of depression-like behavior on exclusively different group of animals than in a case of battery of anxiety-like tests design. Apparatus for the FST was a transparent cylindrical swim tank measuring 15 cm in diameter and it was 50 cm tall. The tank was filled with water of room temperature (22-23 °C) to 30 cm. On the day of testing, animals in their home cages were brought into the experimental room 30 min prior to the testing. The level of illumination in the experimental room was set at 5-10 lux during the testing. The tank was filled with clean water between testing of each animal. The tested animal was placed in the tank and its

behavior was video recorded. Further analysis was manually conducted via Boris behavioral software (*version 7.9.7.*). The time (duration) of immobility of the animal was used as the main and only parameter for statistical analysis. Immobility was defined as a state of an animal when it is floating with the absence of any movement except for those necessary for keeping the nose above water.

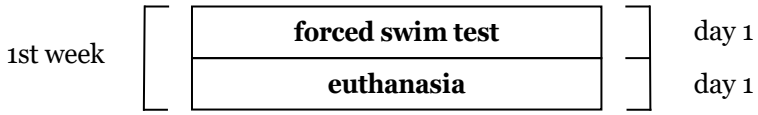

Figure 11: *Scheme shows the timing of experimental steps in the Depression-like test experimental design.*

6.3. Data analysis

Using GraphPad Prism 8, a two-way ANOVA with the factors of sex and genotype was conducted, followed by a Sidak multiple comparisons *post-hoc* test when appropriate. Statistical significance was set at $p<.05$. All data are presented as a group mean ± standard error of mean.

7. Results

I: Battery of anxiety-behavior tests

Open Field Maze

We followed two parameters in the OFM test. We looked for the ratio of distance moved around the wall/in the middle of the arena (wall/middle; distance; Fig. 12A, B), and we also looked for the ratio of time spent around the wall/in the middle of the arena (wall/middle; time; Fig. 12C, D). Statistical analysis showed that there is no difference in the ratio of the moved distance between 10 months old tg and wt rats (Fig. 12A) [(F (1,47) = 3.358, (p=0.0732)] with no difference in sex (Fig. 12A) [(F (1,47) = 0.08418, (p=0.7730)]. However, a *post-hoc* test found a significant difference between tg and wt genotype in males of the age of 10 months in the same parameter ($p<.05$; Fig. 12A). Additionally, there was a significant difference between tg and wt of the same age considering the ratio of time spent around the wall/in the middle (Fig. 12C), as tg rats spent more time around the wall and less time in the middle of the arena [(F (1,47) = 5.954, ($p<.05$)] with no difference in sex [(F (1,47) = 0.01380, (p=0.9070)]. A *post-hoc* test found a significant difference between tg and wt animals in males only ($p<.05$; Fig. 12C). Surprisingly, there was no difference in 14 months old rats in the ratio of distance moved around the wall/in the middle of the arena (Fig. 12B) [(F (1,35) = 0.2536, (p=0.6177)] regardless of sex (Fig. 12B) [(F (,135) = 1.597, (p=0.2146)], or time spent around the wall/in the middle of the arena (Fig. 12D) [(F (1,35) = 0.7035, (p=0.4073)] and again, regardless sex (Fig. 12D) [(F (1,35) = 1.755, (p=0.1938)].

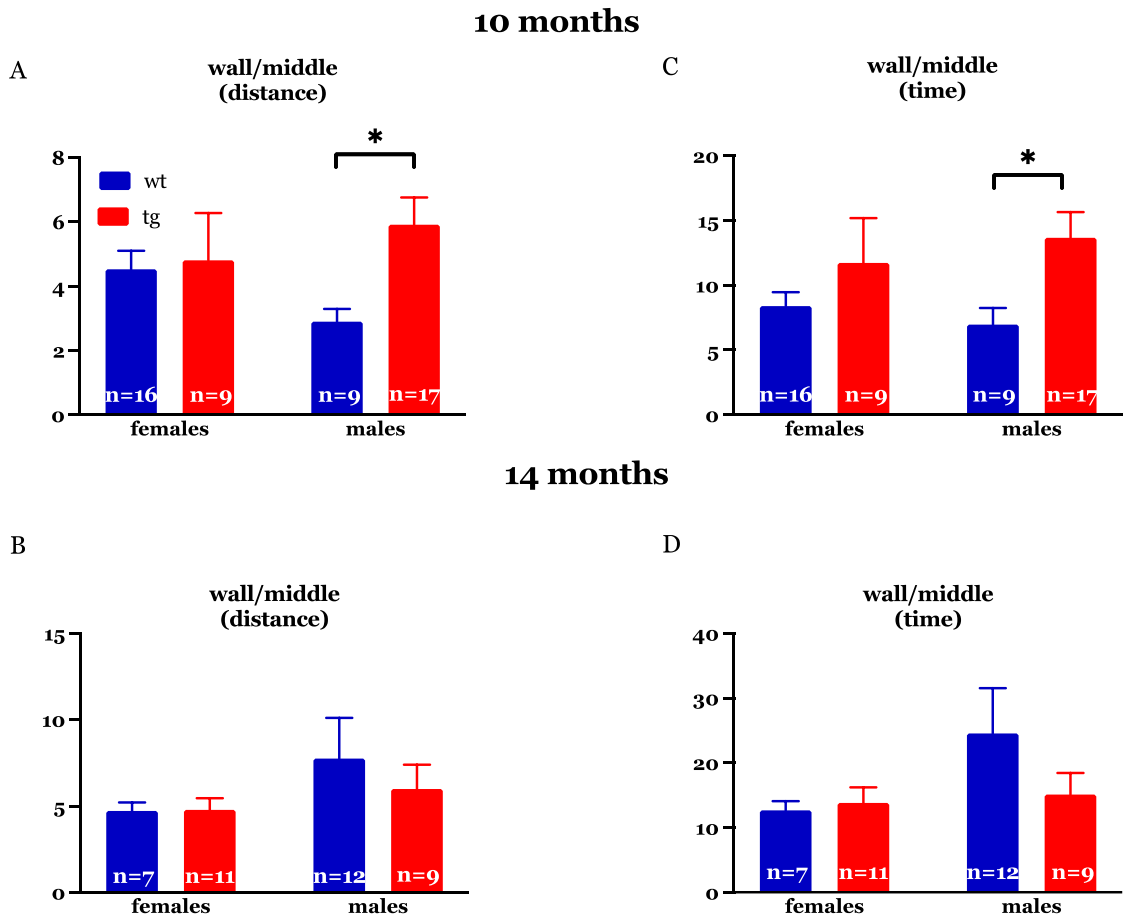

Figure 12: Open Field Maze test*: The graphs show results of Open field maze test in both observed parameters – the ratio of moved distance around the wall/in the middle of the arena (A, B) and the ratio of time spent around the wall/in the middle of the arena (C, D). There was an effect of genotype in 10 months old rats (C) (p<.05) considering the time spent around the wall/in the middle of the arena. A post-hoc test revealed difference only in males (C) (p<.05). Considering the parameter of moved distance in the 10 months old rats (A) no overall effect of genotype was found there, but a post-hoc analysis revealed the effect of genotype in males only (A) (p<.05). *p<.05.*

Elevated Plus Maze

There was a trend toward tg rats spending relatively less time in the open arms and more time in the closed ones in contrast to wt rats, however in 10 months old rats (Fig.13A) [(F (1,47) = 1.506, (p=0.2259)] and concordantly, neither in 14 months old rats (Fig. 13B) [(F (1,35) = 0.8137, (p=0.3732)] was this effect found significant. Unlike that, a significant difference between tg and wt genotypes was found in the 10 months old rats in the case of peeking out (Fig. 13C) [(F (1,47) =8.042, (p<.01)] as well as looking down (Fig. 13E) [(F (1,47) = 6.692, (p<.05)] parameter. However, a *post-hoc* test revealed that there was a difference only in males in both peeking out (p<.05; Fig. 13C) and looking down (p<.05; Fig. 13E). Surprisingly, no effect of genotype was found to be significant considering these two parameters in 14 months old rats (Fig. 13D, F) [(F (1,35) = 1.291 (p=0.2635); F (1,35) = 0.3059, (p= 0.5837)], although the data trend

is similar to the 10 months old rats as we can see in the Fig.13D, F. None of the parameters in groups of both ages revealed any effect of sex, except for the peeking out parameter in the rats of the age of 10 months (Fig. 13C), where males spent more time peeking out than females [(F (1,47) = 5.732, (p<.05)]. As said, there was no effect of sex in 10 months old in the open/closed arms (Fig. 13A) [(F (1,47) = 0.4760, (p=0.4936)], or looking down (Fig. 13E) [(F (1,47) = 0.06353, (p=0.8021)] parameter, neither was any effect of sex found in 14 months old rats in any of the three parameters [(Fig. 13B) open/closed arms; F (1,35) = 0.5028, (p=0.4830); (Fig. 13D) peeking out: F (1,35) = 0.2189, (p=0.6428); (Fig. 13F) looking down F (1,35) = 0.6981, (p=0.4091)].

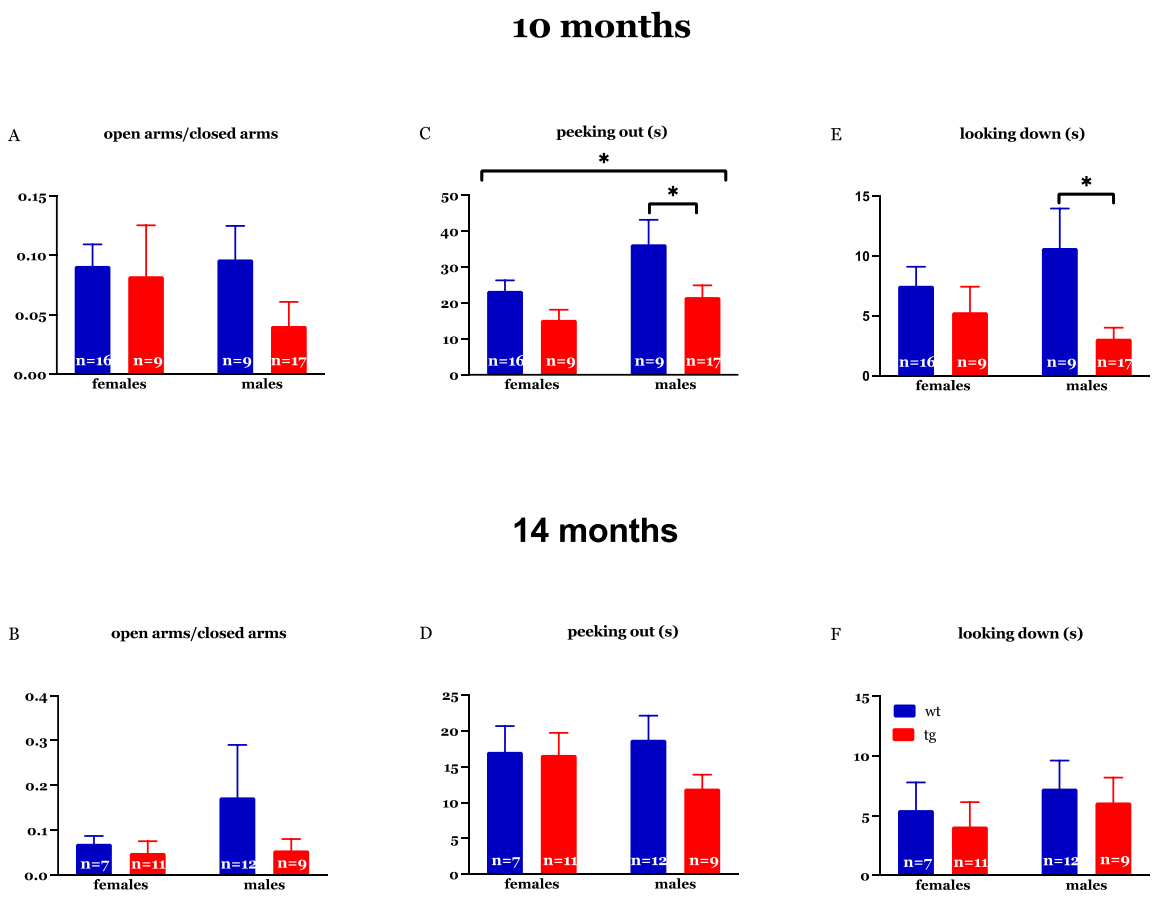

Figure 13: Elevated Plus Maze Test*: The graphs show results of Elevated plus maze test in all three parameters –the ratio of time spent in open arms vs. closed arms (A, B), duration of peeking out (C, D) and duration of looking down (E, F). In the 10 months old rats, there was an effect of genotype considering peeking out (C) (p<.01). A post-hoc test found a difference only in males (C) (p<.05). There was also an effect of sex (C) (p<.05). 10 months old tg rats also spent less time looking down (E) (p<.05) but again, a post-hoc test revealed that the difference was only in males (E) (p<.05). Surprisingly, no significant differences were detected in the 14 months old rats. However, looking at the graphs there is a trend towards tg animals spending less time by peeking out (D), looking down (F), or in the open arms (B). * p<.05.*

Social Interaction Test

Statistical analysis of data from the SIT revealed several straightaway effects in both, 10 months and 14 months old rats. In the 10 months old rats, there was an effect of genotype in the amount of time spent by anogenital explorations favoring wt rats (Fig. 14A) [(F (1,37) = 9.857, (p<.01)] with no difference between sexes (Fig. 14A) [(F (1,37) = 2.209, (p=0.1457)]. A *post-hoc* test revealed a difference only in males (p<.05; Fig. 14A). In the 14 months old rats, the effect of genotype was even detectable at a lower level of significance for anogenital explorations (Fig. 14B) [(F (1,32) = 14.04, (p<.001)]. Additionally, a *post-hoc* test found the effect in both sexes separately as well (p<.05; Fig. 14B), and again, there was no effect of sex (Fig. 14B) [(F (1, 32) = 2.911, (p=0.0977)]. As the data suggest, there was also a difference between tg and wt 10 months old rats in the amount of time invested into non-anogenital interactions, as the tg rats spent less of the time engaging in those (Fig. 14C) [(F (1,37) = 4.382, (p<.05)], but no sex difference was present (Fig. 14C) [(F (1,37) = 0.1080, (p=0.7443)]. A *post-hoc* test found the difference only in female rats (p<.05; Fig. 14C). Additionally, a significant difference between tg and wt rats was found in the 14 months old rats in the non-anogenital parameter (Fig. 14D) [(F (1,32) = 9.909, (p<.01)], but a *post-hoc* test found that only females showed a significant reduction in the time spent by non-anogenital interactions (p<.05; Fig. 14D), but at the same time there was no significant effect of sex (Fig. 14D) [(F (1,32) = 0.7741, (p=0.3855)]. The analysis of the last parameter (time spent by following a conspecific) did not reveal any effect of genotype in 10 months old rats (Fig. 14E) [(F (1, 37) = 3.832, (p=0.0578)]. Nevertheless, the effect was significant in the 14 months old rats (Fig. 14F) [(F (1,32) = 16.51, (p<.001)]. However, a *post-hoc* test found the effect only in females (p<.001; Fig. 14F). Additionally, it must be highlighted that there was even an effect of sex, with females spending relatively more time by following a conspecific than males (Fig. 14F) [(F (1,32) = 8.736, (p<.01)]. The effect of interaction between sex and genotype was also present [(F (1,32) = 8.882, (p<.01)] favoring wt female rats spending more of the time by following a conspecific.

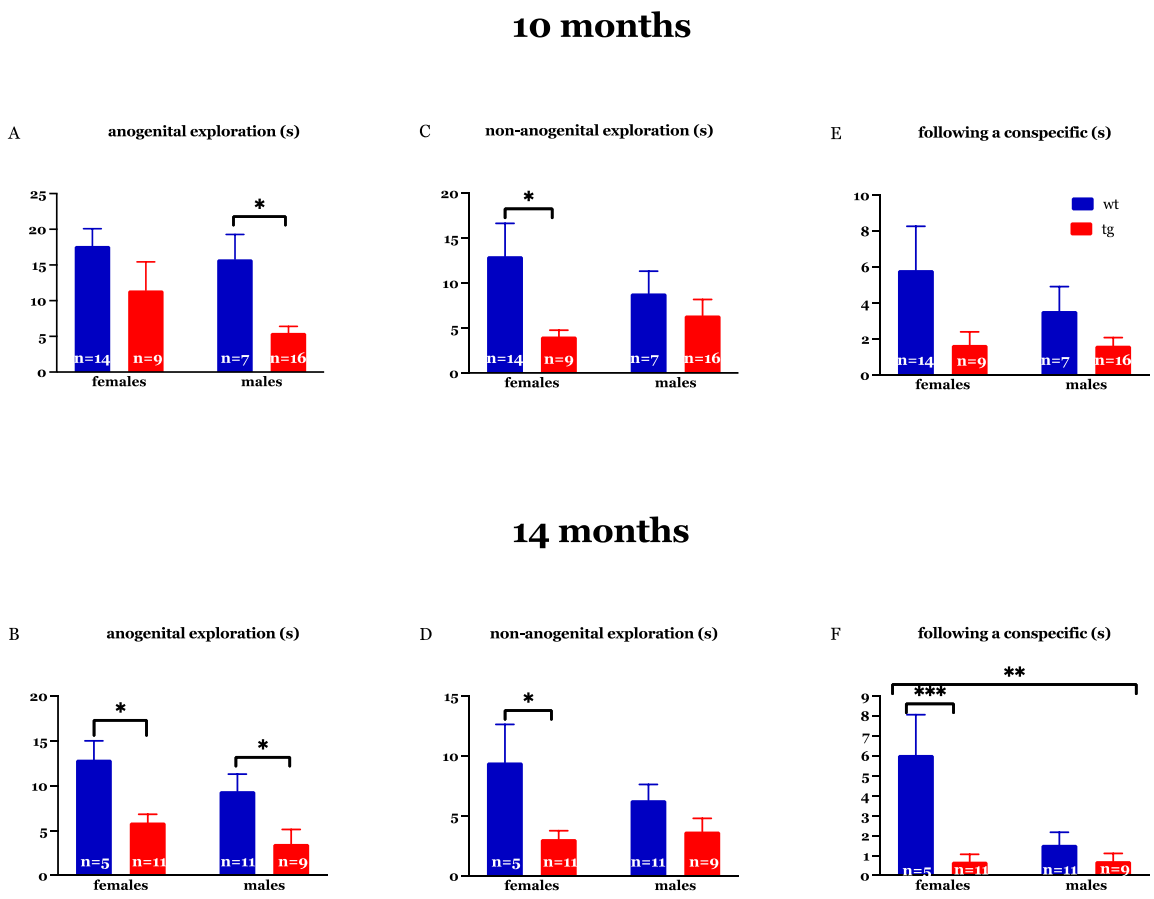

Figure 14: Social Interaction Test: *The graphs show results of Social interaction test in all three parameters – (A, B) anogenital exploration (s), (C, D) non-anogenital exploration (s), (E, F) following a conspecific (s). Statistical analysis revealed several effects. The effect of genotype was present in the 10 months old rats as they spent less time by anogenital explorations (A) (p<.01) with a post-hoc test finding difference only in males (A) (p<.05), and non-anogenital explorations (C) (p<.05) with a post-hoc test finding difference only in females (C) (p<.05). 14 months old tg rats spent less time by anogenital explorations (B) (p<.001), according to a post-hoc test, the difference was in both males and females (B) (p<.05) and females (B) (p<.05). Furthermore, there was an effect of genotype in non-anogenital explorations (D) (p<.01) with a post-hoc test revealing the difference only in females (D) (p<.05). Finally, the effect of genotype was found in the following a conspecific parameter for the 14 months old rats as well (F) (p<.001). A post-hoc test found the difference only in females (F) (p<.001). * p<.05, ** p<.01, *** p<.001.*

Contextual Fear Conditioning Test

We used two parameters for the statistical analysis of data from the CFC test. We used the difference between time of the freezing during the test session and time of the freezing during the habituation (Fig. 15A, B). Apart from that, we used the time of freezing just in the test session (Fig. 15C, D). In the 10 months old animals, there was no difference between tg and wt rats in the first (Fig. 15A) parameter [(F (1,25) = 1.204, (p=0.2829)], nor it was in the second (Fig. 15C) parameter [(F (1,25) = 0.0008724, (p=0.9767)]. No effect of sex was present in either of parameters (Fig. 15A) [(F (1,25) = 0.1043, (p=0.7494)]; (Fig. 15C) [(F (1,25) = 0.4264, (p=0.5197)]. The same goes for 14 months old rats considering the time of test-habituation freezing parameter (Fig.

15B), as there was no difference between tg and wt rats [(F (1,35) = 3.611, (p=0.0657)], nor it was any effect of sex [(F (1,35) = 1.238, (p=0.2735)]. However, there was a significant difference between 14 months old tg and wt rats (Fig. 15D) when the parameter used was only freezing during the test session [(F (1,35) = 8.362, (p<.01)]. A *post-hoc* test found a significant difference only in females (p<.05; Fig. 15D). There was also an effect of sex (Fig. 15D) as the males spent more time freezing than females [(F (1,35) = 0.0364, (p<.05)].

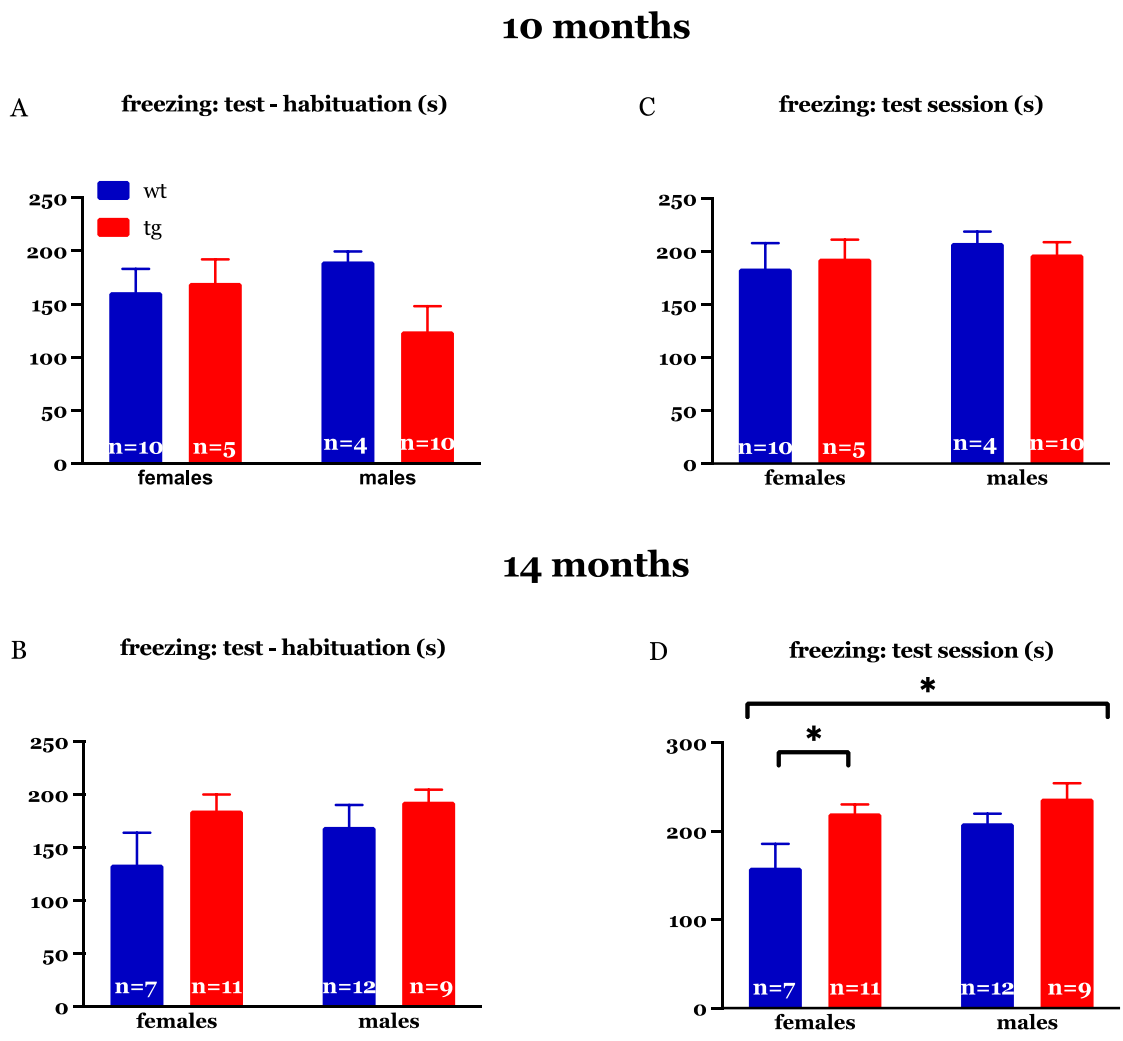

Figure 15: Contextual Fear Conditioning Test*: The graphs show results of Contextual fear conditioning test in both of the two used parameters – (A, B) freezing: test - habituation (s), (C, D) freezing: test session (s). Statistical analysis revealed the effect of genotype in 14 months old rats (D) (p<.01) as they spent more time freezing during the test session. However, a post-hoc test found the effect to be significant only in females (p<.05). There was an effect of sex as well (D). * p<.05.*

II: Depression-like test

Forced Swim Test

The time of immobility was used as a parameter in the FST. Surprisingly, there was a significant effect of genotype (Fig. 16A) in rats of the age of 10 months, that favors tg rats spending relatively less of the time immobile [(F (1,37) = 6.848, (p<.05)]. Additionally, the effect of sex (Fig. 16A) was present, as females spent relatively less of the time immobile in contrast to males [(F (1,37) = 35.79, (p<.0001)]. In the 14 months old rats, there was no difference between genotypes (Fig. 16B) [(F (1,24) = 0.8404, (p=0.3684)], but there was a difference between males and females (Fig. 16B), as females spent relatively less time immobile compared to males [(F (1,24) = 7.728, (p<.05)].

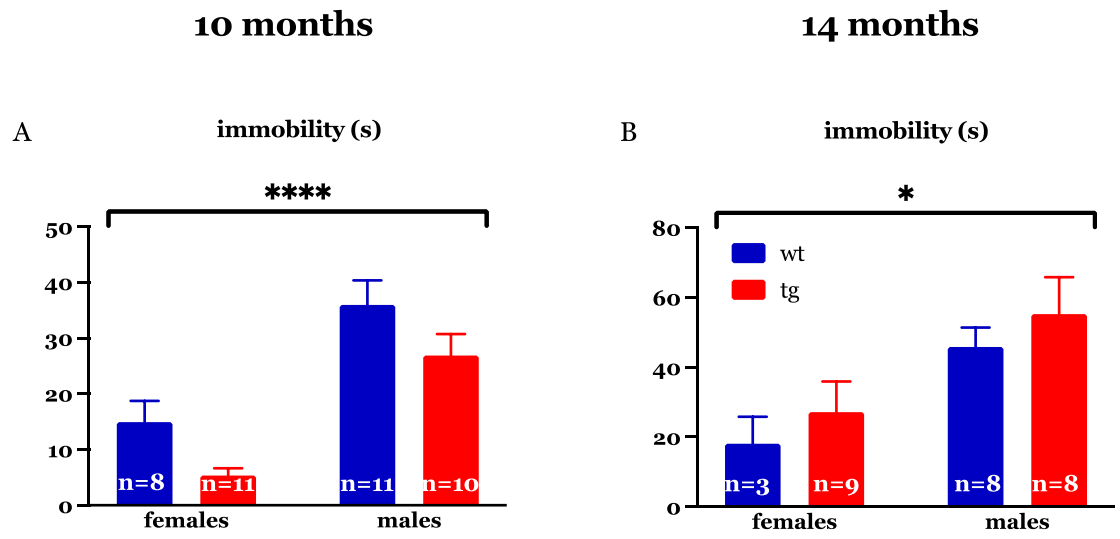

Figure 16: Forced Swim Test: *The graphs show results of the Forced swim test. Time of immobility (s) was used as a parameter for statistical analysis (A, B). There was an effect of genotype as 10 months wt rats spent more time immobile (A) (p<.05). The effect of sex was also present as males spent more time immobile in both ages (p<.0001; p<.05). * p<.05. **** p<.0001.*

8. Discussion

TgF344-AD is a rat model that mimics all the human neuropathological hallmarks of AD, as to mention some of the main: progressive accumulation of amyloid plaques, tauopathy, neurodegeneration and neuronal loss (Cohen et al., 2013). The studies looking for cognitive decline in this model are thoroughly summarized in the chapter *4.2.1.1*. However, less studies had been done with the aim of looking for the presence of neuropsychiatric symptoms in the same model.

Anxiety-like phenotype in TgF344-AD rat model

One of such symptoms, which according to human studies might have an undeniable role in the AD pathology, is anxiety. Some studies had evaluated anxiety-like behavior via analysis of locomotor activity in the OFT. At first, Cohen et al. (2013) had shown that 15 months old TgF344-AD rats display hyperactivity, which may result from disinhibition associated with hippocampal or cortical injury. The phenotype was operationalized as increased numbers of beam breaks and rears. However, the data trend (although it was there) was non-significant in 6 months old tg rats suggesting an age-dependent trend in hyperactivity, not to say anxiety in tg animals. In contrast, no overall differences were observed in a percentage of time spent in the centre of the OFM, nor locomotor activity (distance moved during the task) in the 12-13 months old tg rats in the study conducted by Morrone et al. (2020). What our study found is similar to the results of study by Morrone et al. (2020), as we found that there is no difference between tg and wt 14 months old rats in the ratio of distance moved around the wall/in the middle of the arena as well as in the ratio of time spent around the wall/in the middle of the arena. However, we also found that there is a difference between tg and wt 10 months old animals in one of the two observed parameters. 10 months old tg animals showed enhanced anxiety-like behaviour compared to wt animals of the same age, as they spent relatively less of the time in the middle and more by the wall of the arena.

In a few studies, anxiety-like behaviour was assessed via the EPM test. Results from the study by Pentkowski et al. (2018) indicate that regardless sex, enhanced anxiety-like behaviour represents an early-stage behavioural marker in the 4-6 months old tg rats. An interesting finding is that the rats of the same age had not shown a significant impairment in spatial memory at the time anxiety had already shown up. The EPM test

was afterwards used to assess anxious behaviour in older (9 months) tg rats too. The time spent in an open arm of tg animals was indeed significantly reduced (Tournier et al., 2020b). Therefore, results replicated the outcome of the previous study by Pentkowski et al. (2018). Our study only partly replicates the findings from the previous EPM studies. We found that 14 as well as 10 months old tg rats indeed display anxiety-like behaviour, however in different parameters than previously used in the study by Pentkowski et al. (2018) and Tournier et al. (2020b). In our study, tg rats of both ages and both sexes spent less time looking down from open arms of the plus maze and they also showed reduction in the time spent by peeking out from closed arms of the maze. It seems like there is a trend in tg animals of both ages spending relatively less time in open arms and more time in the closed ones in contrast to wt, however the statistical analysis had not found the effect to be significant.

As far as my knowledge goes, our study is the first to evaluate social behavior via SIT on the TgF344-AD rat model, and we find the final results interesting. We had chosen three parameters (anogenital exploration, non-anogenital exploration, and following) to observe during the free exploration of an animal with its counterpart. Then we separately measured time spent by each of the three types of chosen interactions. The analysis showed that both 10 and 14 months old tg rats spent less time participating in anogenital as well as non-anogenital interactions. Furthermore, the effect of genotype was even more vivid in 14 months old tg rats in contrast to the 10 months old suggesting a progressive deterioration. Additionally, the 14, but not 10, months old tg rats spent less time by friendly following of a conspecific. The amount of time spent by friendly social interactions refers to an overall anxiety-like state of an animal. It is generally assumed that the less an animal spends time by socializing, the more anxious the animal is. These results, besides the finding of the presence of the general social dysregulation, therefore indicate the presence of an anxiety-like state in the TgF344-AD model. However, more studies need to be done to verify these findings.

We were also the first one to test emotional memory impairment in TgF344-AD rats. For that purpose, we used the CFC test. The difference between time of the freezing during test session and habituation was same in both 10 and 14 months old animals considering genotype. In the 14 months old animals, there was only difference between tg and wt rats when one of the two parameters were considered (freezing just in the test session). However, the effect was of the opposite direction than we expected, as

the tg animals were freezing more, therefore showed smaller emotional memory impairment.

Depression-like phenotype in TgF344-AD rat model

In contrast to the results in anxiety-like tests battery, the FST results in our study did not provide any evidence about the occurrence of depression-like phenotype in the TgF344-AD model. It seemed that in the 10 months old animals there was an opposite effect than we expected, as tg animals spent less time immobile compared to wt animals. However, there was no difference in immobility in 14 months old animals. But interestingly, an analysis of the effect of sex in 10 as well as in 14 months old animals revealed that females spent less time immobile than did the males. In contrast to our study, results from study by Voorhees et al. (2018) suggest that both, male and female tg rats, show depression-like behavior in the same task, as tg rats spent more time immobile compared to their wt counterparts. However, this study was run with animals a bit older (15 months) than in our case. What is also interesting in this study is that the depressive phenotype had occurred earlier than did the cognitive deficit in the same cohort of animals, consistently with human studies (Dafsari & Jessen, 2020). TgF344-AD 9 months old rats did not show the reduction in the ability to experience pleasure as they were exposed to the Sucrose preference test in the study conducted by Tournier et al. (2020). Only when the total volume intake was considered, there was a difference in genotype as tg animals showed enhanced anhedonia (Tournier et al., 2020).

I would point out the study of Pentkowski et al. (2018) and Voorhees et al. (2018). In the first study, they did not only found the presence of anxiety-like phenotype in tg rats, but they also found out that this phenotype had occurred even before the cognitive deficits were detectable in the same animals. The same goes for the study by Voorhees et al. (2018), where they found that depression-like phenotype had occurred in the yet cognitively normal rats. Therefore, in the future research there should be more studies done evaluating depression- and anxiety-like behaviour simultaneously with the observation of cognitive performances in the same animals. It would allow us to dig more deeply into the chronology and onset of neuropsychiatric symptomatology in AD dementia etiology. Nowadays, we still cannot conclude whether the neuropsychiatric symptoms behave as a risk factor, or a prodromal symptom, or an accelerating factor in the AD pathology. More findings could provide more evidence with the aim of

incorporating the neuropsychiatric symptomatology in future AD research, or even clinical practise (diagnostics, prediction of the transition, disease stage identification etc.). Moreover, considering the fact that we were the first to assess social interactions in TgF344-AD, and we found out that tg rats spent less time with those, more studies should incorporate the testing of social behaviour on this model. This demand is even valid because of the fact that social dysregulation comes up as an everyday burden of both, patients as well as caregivers.

Last but not least, anxiety- and depression-like symptoms should continue being compared with other AD markers, such as with changes in functional connectivity of brain or with detection of the brain plaque Aβ protein, or the neurofibrillary tau protein to see whether there are some relationships between those.

9. Conclusions

We aimed to look for anxiety- and depression-like phenotypes in the TgF344-AD rat model. Using a behavioural approach we found out that tg animals of both ages showed anxiety-like phenotype in the EPM test and SIT, while in the OFM test only 10 months old tg rats showed enhanced anxiety-like behaviour compared to wt rats in just one of the two parameters. The most substantial were the results of SIT which, as far as my knowledge goes, we were the first to measure behaviour of the TgF344-AD model at. In contrast to the anxiety-like test results, no depression-like phenotype was found in the tg rats according to the results from FST, nor emotional memory impairment was present in tg rats. There was no difference between sexes in the results in majority of parameters.

As we found out that the anxiety-like phenotype is present not only in the TgF344-AD rats of the age of 14 months, but also in those of the age of 10 months, we suggest that there should be continue looking for the character of the display of anxiety-like behaviour in this model in future research. Additionally, the results should be interrelated with the onset of cognitive functioning deficits, or other AD markers, such as with changes in functional connectivity, or with the detection of Aβ, or tau protein level. Hopefully, it will allow us to dig deeper into the understanding of how neuropsychiatric symptoms manifest among patients with AD, and eventually it will allow us to use the knowledge in clinical practise.

10. References

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Appendix

Table 1: Open field: a statistical analysis results

Parameter	**age**	**factors**	**% of total variation**	**F value**	**P value**
Wall/middle (time}	10 months	interaction	1,236	F (1, 47) = 0,6730	P=0,4161
		sex	0,02534	F (1, 47) = 0,01380	P=0,9070
		genotype	10,93	F (1, 47) = 5,954	P=0,0185
Wall/middle (distance)	10 months	interaction	4,406	F (1, 47) = 2,328	P=0,1338
		sex	0,1594	F (1, 47) = 0,08418	P=0,7730
		genotype	6,356	F (1, 47) = 3,358	P=0,0732
Wall/middle (time)	14 months	interaction	2,897	F (1,35) = 1,134	P=0,2942
		sex	4,482	F (1,35) = 1,755	P=0,1938
		genotype	1,797	F (1,35) = 0,7035	P=0,4073
Wall/middle (distance)	14 months	interaction	0,7869	F (1,35) = 0,2947	P=0,5907
		sex	4,265	F (1,35) = 1,597	P=0,2146
		genotype	0,6771	F (1,35) = 0,2536	P=0,6177

Table 2: Elevated plus maze: a statistical analysis results

Parameter	**age**	**factors**	**% of total variation**	**F value**	**P value**
Open arms/closed arms	10 months	interaction	1,584	F (1, 47) = 0,8008	P=0,3754
		sex	0,9414	F (1, 47) = 0,4760	P=0,4936
		genotype	2,978	F (1, 47) = 1,506	P=0,2259

Peeking out (s)	10 months	interaction	1,174	F (1, 47) = 0,6857	P=0,4118
		sex	9,815	F (1, 47) = 5,732	P=0,0207
		genotype	13,77	F (1, 47) = 8,042	P=0,0067
Looking down (s)	10 months	interaction	3,614	F (1,47) = 2,028	P=0,1611
		sex	0,1132	F (1,47) = 0,06353	P=0,8021
		genotype	11,93	F (1,47) = 6,692	P=0,0128
Open arms/closed arms	14 months	interaction	1,091	F (1,35) = 0,4044	P=0,5290
		sex	1,356	F (1,35) = 0,5028	P=0,4830
		genotype	2,194	F (1,35) = 0,8137	P=0,3732
Peeking out (s)	14 months	interaction	2,667	F (1,35) = 1,003	P=0,3234
		sex	0,5818	F (1,35) = 0,2189	P=0,6428
		genotype	0,2635	F (1,35) = 1,291	P=0,2635
Looking down (s)	14 months	interaction	0,006047	F (1,35) = 0,002190	P=0,9629
		sex	1,927	F (1,35) = 0,6981	P=0,4091
		genotype	0,8445	F (1,35) = 0,3059	P=0,5837

Table 3: Social interaction test: a statistical analysis results

Parameter	**age**	**factors**	**% of total variation**	**F value**	**P value**
Anogenital exploration (s)	10 months	interaction	1,084	F (1, 37) = 0,5983	P=0,4441
		sex	4,000	F (1, 37) = 2,209	P=0,1457
		genotype	17,85	F (1, 37) = 9,857	P=0,0033
Non-anogenital exploration (s)	10 months	interaction	3,242	F (1, 37) = 1,433	P=0,2389
		sex	0,2442	F (1, 37) = 0,1080	P=0,7443

		genotype	9,912	F (1, 37) = 4,382	P=0,0432
Following a conspecific (s)	10 months	interaction	1,196	F (1,37) = 0,5207	P=0,4751
		sex	1,285	F (1,37) = 0,5596	P=0,4592
		genotype	8,804	F (1,37) = 3,832	P=0,0578
Anogenital exploration (s)	14 months	interaction	0,2254	F (1,32) = 0,1055	P=0,7474
		sex	6,217	F (1,32) = 2,911	P=0,0977
		genotype	29,99	F (1,32) = 14,04	P=0,0007
Non-anogenital exploration (s)	14 months	interaction	4,068	F (1,32) = 1,729	P=0,1979
		sex	1,822	F (1,32) = 0,7741	P=0,3855
		genotype	23,32	F (1,32) = 9,909	P=0,0035
Following a conspecific (s)	14 months	Interaction	15,73	F (1,32) = 8,882	P=0,0055
		sex	15,47	F (1,32) = 8,736	P=0,0058
		genotype	29,23	F (1,32) = 16,51	P=0,0003

Table 4: Contextual fear conditioning test: a statistical analysis results

Parameter	**age**	**factors**	**% of total variation**	**F value**	**P value**
Freezing: test-habituation (s)	10 months	interaction	7,173	F (1, 25) = 2,067	P=0,1629
		sex	0,3620	F (1, 25) = 0,1043	P=0,7494
		genotype	4,179	F (1, 25) = 1,204	P=0,2829
Freezing: test session (s)	10 months	interaction	0,6437	F (1, 25) = 0,2193	P=0,6437
		sex	0,5197	F (1, 25) = 0,4264	P=0,5197
		genotype	0,9767	F (1, 25) = 0,0008724	P=0,9767
Freezing: test-	14 months	interaction	1,222	F (1,35) = 0,4820	P=0,4921

habituation (s)		sex	3,137	F (1,35) = 1,238	P=0,2735
		genotype	9,153	F (1,35) = 3,611	P=0,0657
Freezing: test session (s)	14 months	interaction	2,522	F (1,35) = 1,178	P=0,2852
		sex	10,14	F (1,35) = 4,736	P=0,0364
		genotype	17,90	F (1,35) = 8,362	P=0,0065

Table 5: Forced swim test: a statistical analysis results

Parameter	**age**	**factors**	**% of total variation**	**F value**	**P value**
Immobility (s)	10 months	interaction	0,005327	F (1, 37) = 0,004409	P=0,9474
		sex	43,24	F (1, 37) = 35,79	P<0.0001
		genotype	8,274	F (1, 37) = 6,848	P=0,0128
Immobility (s)	14 months	interaction	0,0005747	F (1, 24) = 0,0001878	P=0,9892
		sex	23,64	F (1, 24) = 7,728	P=0,0104
		genotype	2,571	F (1, 24) = 0,8404	P=0,3684