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Department of Pharmacology and Toxicology

BENEFICIAL EFFECTS OF 11β-HSD1 INHIBITION ON COGNITIVE PERFORMANCE IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

MASTER'S THESIS

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Hradec Králové 2018

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Hradec Králové .....

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ABSTRACT

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Faculty of Pharmacy in Hradec Králové

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Title: Beneficial Effects of 11β-HSD1 Inhibition on Cognitive Performance in a Mouse Model of Alzheimer's Disease

The increased life expectancy goes hand in hand with ageing-related cognitive impairments. Alzheimer's disease (AD) is the most common type of dementia being an irreversible and progressive brain disorder with loss of cognitive functions. Recent studies suggest that excess of glucocorticoid (GC) action exerts deleterious effects on the hippocampus and causes impaired spatial memory. In addition, it has been demonstrated that aged mice with cognitive deficits show increased gene expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the hippocampus and parietal cortex. The Senescence-Accelerated Mouse Prone 8 (SAMP8) strain is a spontaneous animal model of accelerated ageing. Many studies indicate that SAMP8 harbour the behavioural and histopathological signatures of AD.

In the present study, we evaluated the neuroprotective effects of 11β-HSD1 inhibition by a potent pyrrolidine-based compound RL-118 and/or effects of diet on cognitive performance in different groups of SAMP8 by conducting behavioural and cognitive tests. In mice treated with RL-118, we observed changes in anxiety, improved motivation and social behaviour, as well as ameliorated cognitive performance in both spatial and recognition memories.

Obtained results suggest that the used substance RL-118 is a potent 11β-HSD1 inhibitor with future potential in AD treatment.

ABSTRAKT

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Název práce: Prospěšný vliv inhibice enzymu 11β-HSD1 na kognitivní výkon u myšího modelu Alzheimerovy choroby

Prodloužení délky života jde ruku v ruce s kognitivními poruchami souvisejícími se stárnutím. Alzheimerova choroba je nejčastěji se vyskytující demence. Je to nevratné a progresivní neurodegenerativní onemocnění vyznačující se ztrátou mozkových funkcí. Nedávné studie naznačují, že nadměrné působení glukokortikoidů má škodlivé účinky na hippocampus a způsobuje narušení prostorové paměti. Navíc bylo prokázáno, že starší myši s kognitivním deficitem vykazují zvýšenou genovou expresi enzymu 11β-hydroxysteroiddehydrogenázy typu 1 (11β-HSD1) v oblasti hippocampu a parietální kůry. Kmen myší zvaný Senescence-Accelerated Mouse Prone 8 (SAMP8) je přirozeně se vyskytující zvířecí model zrychleného stárnutí. Mnoho studií naznačuje, že SAMP8 vykazují behaviorální a histopatologické znaky Alzheimerovy choroby.

V rámci této studie jsme hodnotili neuroprotektivní účinky inhibice enzymu 11β-HSD1 potentní sloučeninou s názvem RL-118, která je syntetizována na pyrrolidinovém základě, a/nebo účinky stravy na kognitivní funkce u různých skupin SAMP8 myší, a to provedením série behaviorálních a kognitivních testů. U myší, kterým byla podávána RL-118, jsme pozorovali změny v projevech úzkosti, zvýšenou motivaci a zlepšení sociálního chování, stejně jako zlepšení prostorové i rozpoznávací paměti.

Výsledky studie naznačují, že použitá látka RL-118 je silným inhibitorem 11β-HSD1 s potenciálním využitím v léčbě Alzheimerovy choroby.

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1 INTRODUCTION

The world's population is ageing. It is given by the constantly decreasing natality and at the same time delaying mortality within all age groups. Also, continuous improvements in healthcare in the 20th and 21st centuries play an important role in the contribution to people living longer and healthier lives. Nevertheless, these improvements have also resulted in an increase in the number of patients with non-communicable diseases (NCDs), including Alzheimer's disease (AD) as the most common form of dementia. AD is a serious progressive and irreversible neurodegenerative disease. Slow inconspicuous onset is a distinctive feature of this disease. While its pathogenesis is still not clear to researchers, there are many different hypotheses – AD aetiology is a multifactorial process. With a prevalence of 1% of the population, AD is the fourth to the fifth most frequent cause of death. The estimates from 2016 from Alzheimer Disease International (ADI) indicate that in 2016, nearly 50 million people worldwide were living with dementia and about 10 million more cases arise each year. The predictions say that this number will double or even triple by 2050 and the estimated number of patients with dementia will rise up to 135,5 million. The incidence of AD increases exponentially with age – 3% between the age of 65 to 74 years, 19% between the age of 75 and 84 years, and 47% after reaching 85 years of age. Many cases are left undiagnosed and untreated. That is why AD is sometimes referred to as a "silent epidemy". Dementia and cognitive functions disorders belong to the main factors that lead to invalidity and dependence on other people in the elderly worldwide. It is a costly condition in its social, economic, and health dimensions which is also a problem as nearly 60% of the burden is concentrated in low-income and middle-income countries (Prince et al., 2016; World Health Organization, 2012; Zvěřová, 2017).

Current findings highlight the role of glucocorticoids in cognitive decline, and onset and progression of AD. They are considered to be one of the main biomarkers of AD. By inhibition of their reactivation in brain regions and thus decreasing their CNS levels, we could slow down progression and delay the onset of AD.

The present thesis focuses on this hypothesis of AD onset. We investigated the effects of RL-118, a potent inhibitor of the enzyme 11β-hydroxysteroid dehydrogenase

type 1, on cognition and behaviour of Senescence-Accelerated Mouse Prone 8 (SAMP8), a mouse model of the sporadic AD. This work was carried out at the University of Barcelona by the Neuropharmacology in Aging and Neurodegeneration group.

2 THEORETICAL PART

3.1 Ageing

Ageing can be described as a normal, inevitable process which ends with death. However, its causes remain unclear – even individual cultured cells will age, i.e. after a certain number of cycles, they will stop dividing. Only a few cells do not succumb to this process, it might be said they are immortal, going through unlimited proliferation – germinal, tumour or hemopoietic stem cells to name a few (Silbernagl & Lang, 2012).

The neurobiochemistry of physiological ageing includes changes in the metabolism of monoamines and neuropeptides, as well as it includes immunological and endocrinological changes. Neurotransmitters activity and activity of a number of enzymatic systems is mainly decreasing. What should be noted here is that these physiological changes occurring with ageing are also accompanied by some compensatory mechanisms that balance the decreases. If there are pathological changes involved, the compensatory mechanisms are exhausted and diseases manifest.

As previously mentioned, changes in the metabolism of brain neurotransmitters start occurring with ageing – acetylcholine, noradrenaline, serotonin, dopamine, GABA and others. Alterations in responses to stimuli are also caused by changes in sensitivity and density of their respective receptors. The overall balance of all the bodily systems and the sustainability of homeostasis also play a role.

Overall, the neuroanatomical changes found in aged people closely relate to the neurobiological changes that occur in dementia (Pidrman, 2007).

3.1.2 Normal Cognitive Ageing

Change in cognition as a normal process of ageing is a well-known phenomenon. Various cognitive abilities, e.g. vocabulary, are resilient to brain ageing and may even improve with age. However, over time, other abilities, such as conceptual reasoning,

memory and processing speed, gradually decline. Significant heterogeneity among the elderly in the rate of decline in some abilities, such as measures of perceptual reasoning and processing speed, are existent (Harada et al., 2013).

Considering the severity of the changes occurred, cognitive ageing can be classified according to these three following groups:

- Successful ageing - maintaining functional abilities, intact cognition, performance comparable to middle age. No memory, behaviour or motor impairment.
- Normal ageing – physiological changes in cognitive and other mental abilities, these being minor abnormalities that occur in most of the healthy population. These may be described as benign states of forgetfulness with no progression. We speak of Age-Associated Cognitive Decline – AACD.
- Pathological ageing – pathological changes in the brain leading to cognitive and other mental impairments and thus manifestation of diseases like dementia (Pidrman, 2007).

It has been proved that with age, volume, size, and weight of the brain decrease. These changes also occur in dementia, namely in AD (Koukolík & Jirák, 1998).

3.2 Dementia

According to the WHO International Classification of Diseases ICD-10, dementia is defined as:

"Dementia (F00-F03) is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgment. Consciousness is not clouded. The impairments of cognitive function are commonly accompanied, and occasionally preceded, by deterioration in emotional control, social behaviour, or motivation. This syndrome occurs in Alzheimer disease, in cerebrovascular disease, and in other conditions primarily or secondarily affecting the brain." (World Health Organization, 2012).

Dementia can be understood as an acquired disturbance of cognitive functions that is so severe that it has fundamental effects on other bodily functions and thus on the patient's life. Symptoms of dementia can be divided into three basal groups of disturbances called A-B-C:

- activities of daily life
- behaviour
- cognition

The impairments presented in these groups are all connected and influence one another. The third group is the most important in regard to dementia. In dementia, decrease of cognitive functions, memory and intellect in particular, is the basal and most characteristic attribute. It is the fundament of the disease. Also, diseases that manifest as dementia are often the underlying of death.

Dementia as a group of diseases that appears only after the creation of the bases of cognitive functions, which is approximately in the scope of 2 to 5 years of age. Thus, there are rare cases of childhood dementia. However, the occurrence of dementia increases with age and dementia as a whole is predominantly a disease of old age (Jirák & Koukolík, 2004; Pidrman, 2007).

3.3 Alzheimer's Disease

As previously mentioned in the introduction, AD is a progressive and irreversible neurodegenerative disease. The clinical picture of the disease is due to cortical degeneration with characteristic anterograde amnesia (patients cannot retain new knowledge and memories). AD is classified as primary dementia located cortico-subcortically. This means that its symptoms are the result of the combination of mainly cortical and partially subcortical impairments.

The pathogenesis of this disease is still not known. However, the main aetiopathological agents seem to be brain atrophy, neuronal loss and decrease of synaptic plasticity accompanied by a set of pathological changes.

The neurobiology of this disease includes:

- macroscopic cortico-subcortical atrophies
- pathological production and deposition of β-amyloid (formation of plaques)
- neuronal tau-protein degeneration and formation of neurofibrillary tangles
- toxic effects of β-amyloid oligomers
- inflammation
- oxidative stress
- mitochondrial metabolism impairment
- decreased nerve growth factor production
- and other neuropathological mechanisms leading to decreased neuronal plasticity (Koukolík & Jirák, 1998; Zvěřová, 2017).

Also, impairments in neurotransmitter systems occur in AD. Acetylcholinergic system is very important for cognition and is the first one disrupted. In later stages of dementia, glutamatergic and other transmitter systems (somatostatin, serotonin, noradrenaline, GABA, substance P and neuropeptide Y) are also afflicted (Zvěřová, 2017).

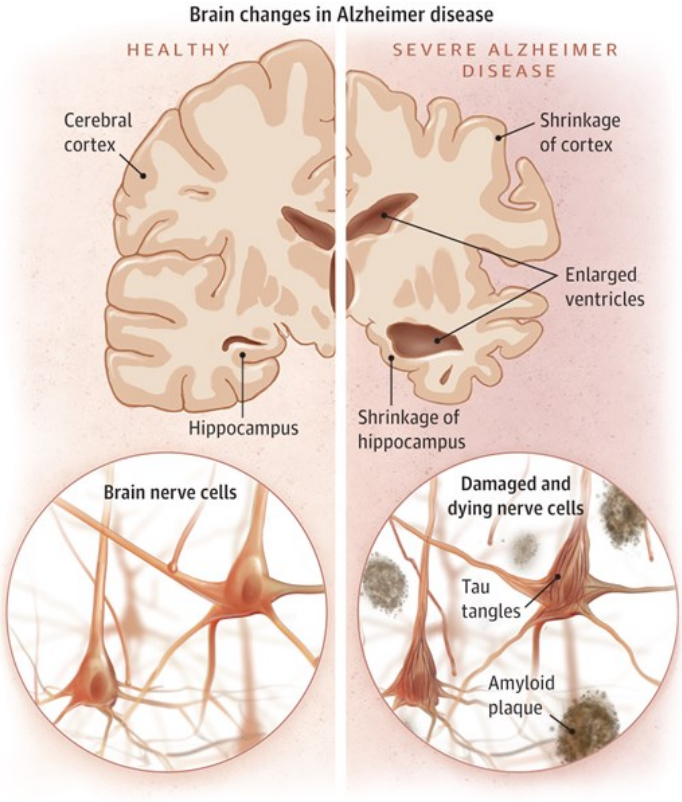

Figure 1 *Brain changes in AD - comparison to a healthy brain* (Jin, 2015).

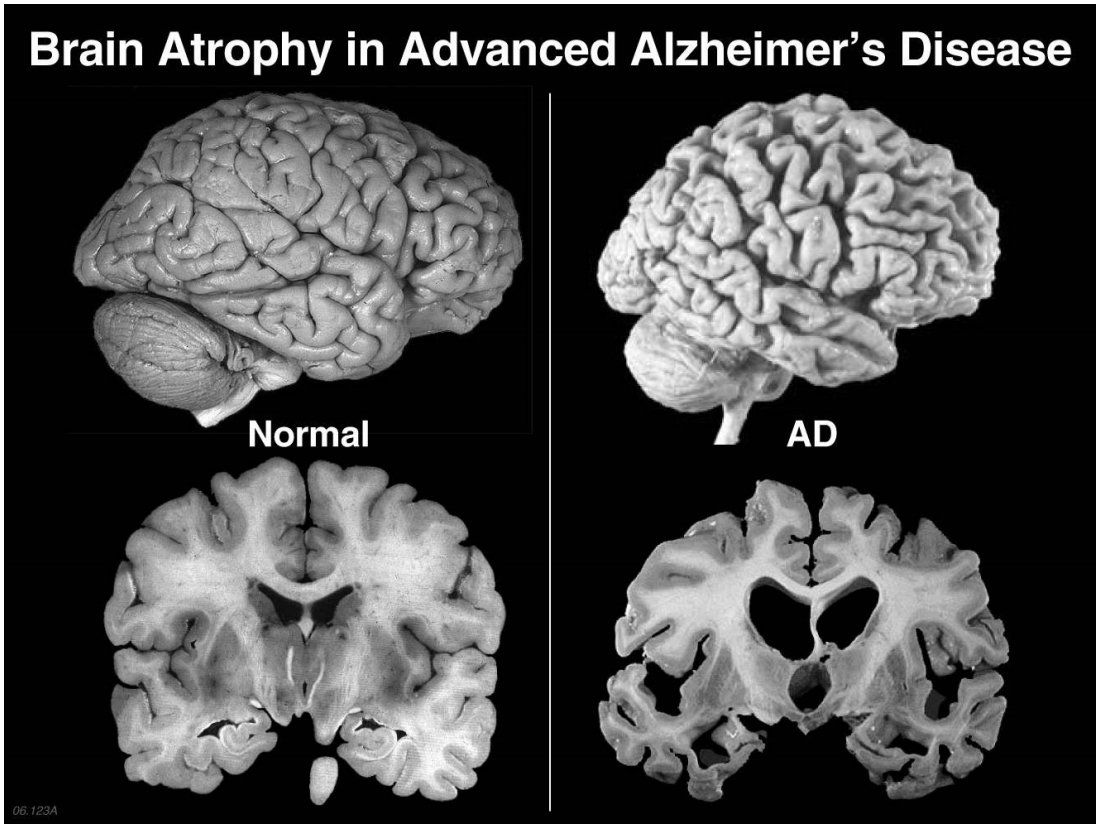

Figure 2 *Demonstration of brain atrophy in advanced AD compared to a healthy brain. Retrieved from https://www.brightstarcare.com.*

Macroscopical and microscopical changes can be found in AD brains. Macroscopical alterations include cortex shrinkage, enlarged ventricles and sulci as well as significant atrophy in the hippocampus. Microscopical alterations found are both extra- and intracellular – extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFT).

Aβ is an insoluble peptide originating from amyloid precursor protein (APP), which is a transmembrane protein that is broken down by α-secretase to short fragments containing 40 amino acids. These fragments are fully soluble and have their physiological roles. Most likely, they play a role in neuroprotection and influence neuroplasticity of brain tissue. However, under pathological changes, APP, is cleaved by β- and γ-secretase to longer, insoluble fragments containing 42 or more amino acids. These fragments then aggregate into heavily neurotoxic oligomers and afterwards into longer fibrils. These coagulate in the extracellular matrix in cortex, and polymerase into β-amyloid. Sterile inflammation can be found around these plaques accompanied by a release of cytokines, free radicals and other neurotoxic products. Aβ is also believed to cause intraneuronal damage affecting mitochondrial and other functions.

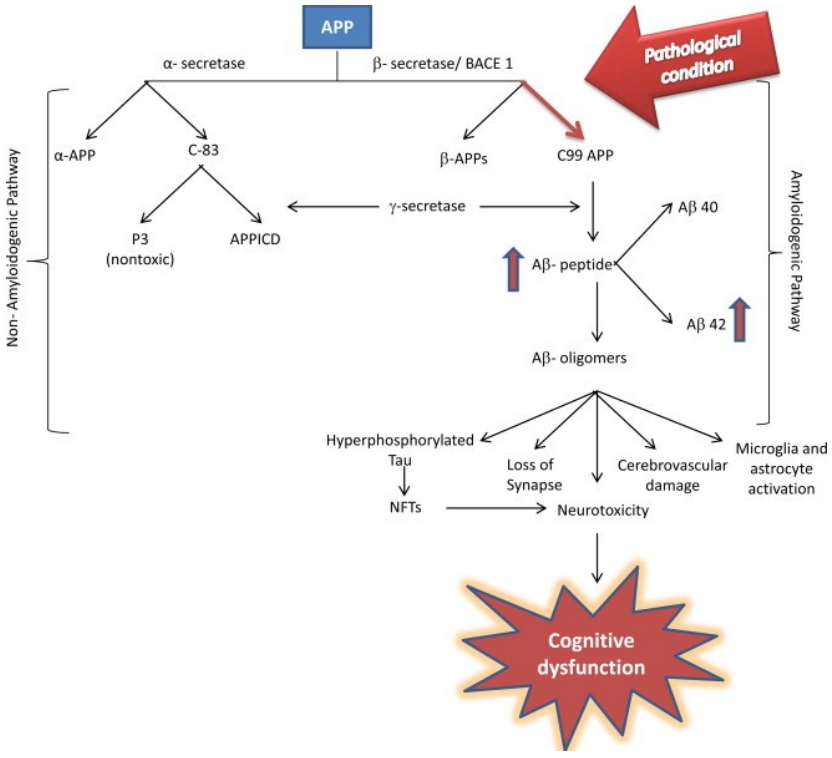

Figure 3 *Presentation of APP processing pathways* (Kumar & Singh, 2015).

Researchers suppose that Aβ induces degeneration of tau-protein which is a soluble intraneuronal phosphoprotein whose biological activity is regulated by its phosphorylation degree (normal tau-protein consists of 2-3 mol of phosphate for a mol of protein). This degeneration leads to accumulation of tau-protein intracellularly and to the formation of NFT which is believed to be the most serious intraneuronal change. Tau is a microtubule-associated protein that is dephosphorylated or partially phosphorylated state binds to actin filaments of the microtubules and thus stabilises them. Changes in the structure or amount of tau-protein can affect its stabilising role as well as its interaction with microtubules leading to attenuation of axonal transport and afterwards to retrograde degeneration manifesting as dementia. Therefore, when pathological alterations occur, tau cannot bind to microtubules and the levels of free tau are increased. Thus, the probability of aggregation and fibrillization of tau-protein also increases. The key to the cascade of NFT formation is a split-off of terminal amino acids from tau-protein, its hyperphosphorylation and fibrilization. Intracellular NFT depositions disrupts the cell's cytoarchitecture and thus causes neuronal death. It was discovered that AD patients' brains contain three to four times more phosphorylated tau-protein than healthy brains (Koukolík & Jirák, 1998; Zvěřová, 2017).

In addition to the aforementioned, free radicals and reactive oxygen also contribute to neuronal degeneration by peroxidation of lipids of the cellular membrane . (Zvěřová, 2017) Neuroinflammation is one another feature of AD. This term refers to the intrinsic cellular response in CNS associated with cell neurodegeneration. It was suggested that the inflammatory signals induce microglia to an activated state which leads to morphological changes and secretion of pro-inflammatory factors like interleukin 1, 6 and tumour necrosis factor. These factors maintain inflammation and favour the production of reactive oxygen species. This inflammatory cycle may trigger NFT deposition and neuronal death (Pasqualetti et al., 2015). Also, as far as metabolism of lipids is concerned, it has been hypothesised that presence of apolipoprotein E4 (ApoE4), a mediator of lipid metabolism, plays a role in Aβ polymerisation. Moreover, APOE ε4 allele occurrence is one of the main genetic risk factors for the sporadic AD with late onset. Another one of AD biomarkers is cortisol (Zvěřová, 2017).

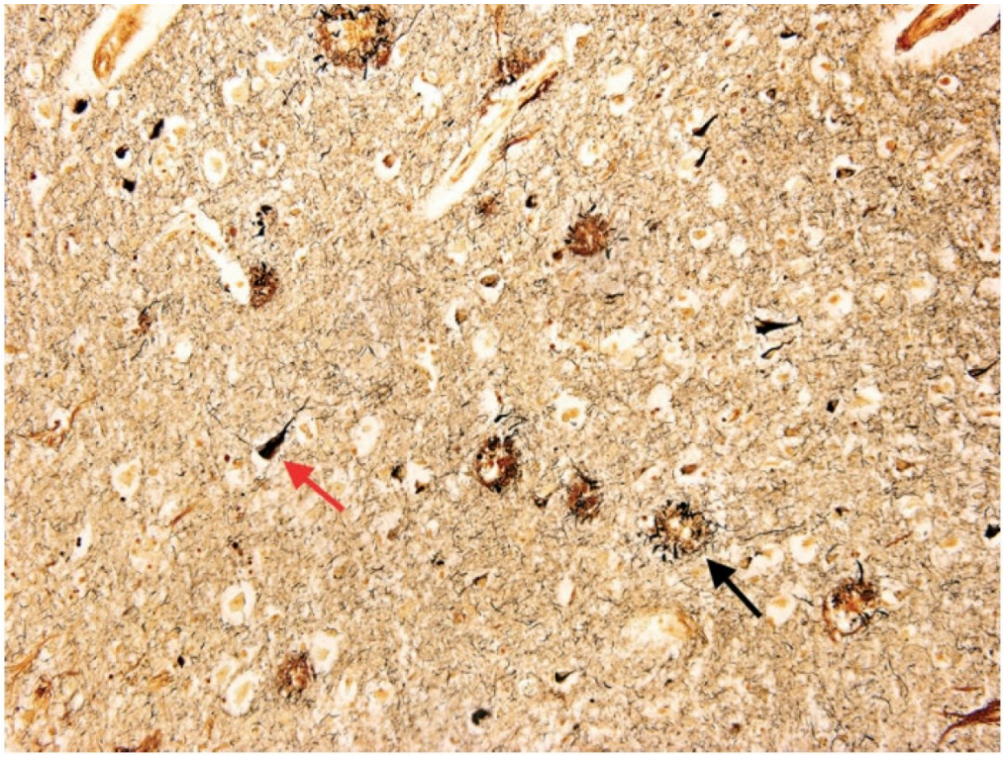

Figure 4 *Amyloid plaques (black arrow) and neurofibrillary tangles (red arrow) in the temporal cortex* (Perl, 2010).

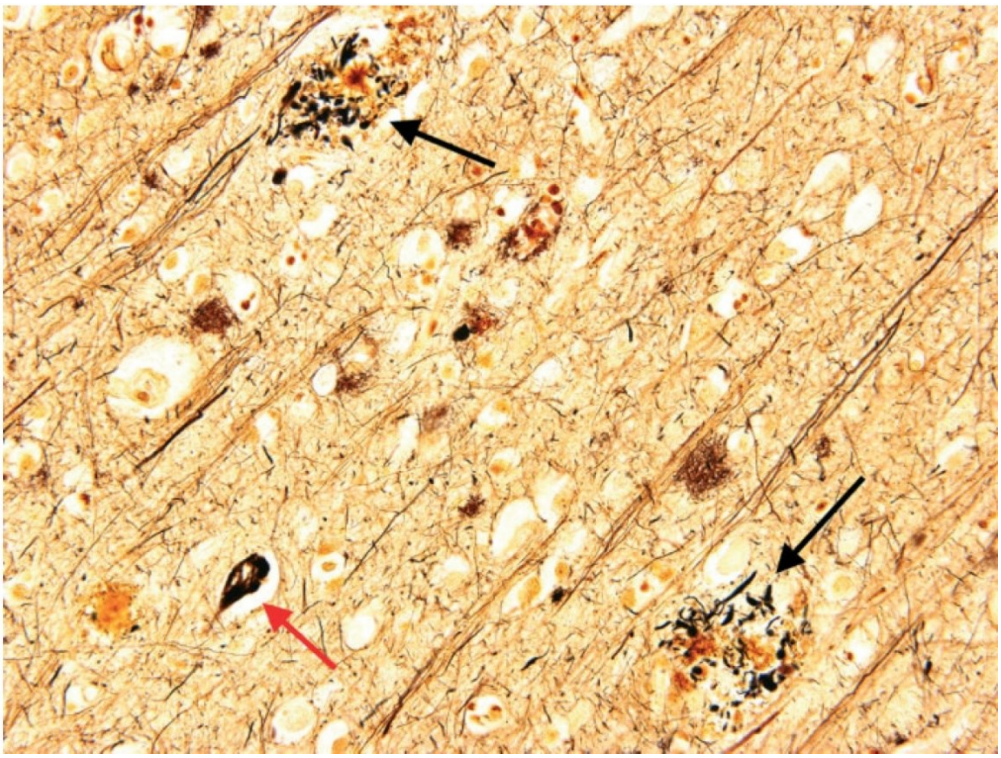

Figure 5 *Amyloid plaques (black arrow) and neurofibrillary tangles (red arrow) in the temporal cortex* (Perl, 2010).

Lastly, it should be mentioned that some researchers have begun to refer to AD as type 3 diabetes. This is due to the revealed information that impairments in cerebral glucose utilisation and energy metabolism represent very early abnormalities preceding or accompanying the initial stages of cognitive decline. This led to the concept that impaired insulin signalling has an important role in the pathogenesis of AD and the hypothesis that AD is in fact diabetes type 3 (de la Monte, 2008).

3.3.1 Forms of Alzheimer's Disease

As of now, there are two main forms of AD described: familial and sporadic with either early or late onset.

Familial form is a hereditary subtype of AD when at least 2 family generations had an occurrence of AD. Less than 5% of patients have early-onset form (in patients below 65 years of age) and between 15% and 25% late-onset familial form. Four genes mutations have been described to lead to early-onset familial AD (eFAD) – gene for APP, presenilin 1, presenilin 2 and tau-protein.

The sporadic form is a non-hereditary form, i.e. a form with no family occurrence. Although this form might also be due to genetics and previously mentioned mutations, this form is mainly connected to non-genetic risk factors. 75% of all the AD patients have late-onset (after the age of 65) sporadic form of AD (LOAD). Findings say that the later the onset of AD, the more ageing, environmental and lifestyle factors are thought to dominate, and the smaller the patient's genetic predispositions appear to be. Therefore, age, gender (women are more likely to suffer from AD than men), medical history, and mainly lifestyle choices such as high-fat diet, dyslipidaemia, lack of exercise, stress or head injuries are dangerous risk factors related to onset of AD (Strobel; Zvěřová, 2017).

3.4 Elevated Levels of Cortisol

Glucocorticoids (GC) are one of the biomarkers of AD that are traceable in peripheral blood (Zvěřová, 2017).

Glucocorticoid hormones, namely cortisol in humans and corticosterone in rodents, are hormones synthesised from cholesterol in zona fasciculata in adrenal cortex. In plasma, cortisol is bound to corticosteroid-binding globulin (CBG) and the free (biologically active) fraction makes up about 3% of the plasmatic pool. Cortisol follows a circadian rhythm of release. Daily production of cortisol is 10 to 20 mg, maximum is reached between 7AM and 9AM (250-650 nm/l) and its minimum is reached between 4PM to 6PM (50-280 nmol/l) (Lara et al., 2013; Zvěřová, 2017).

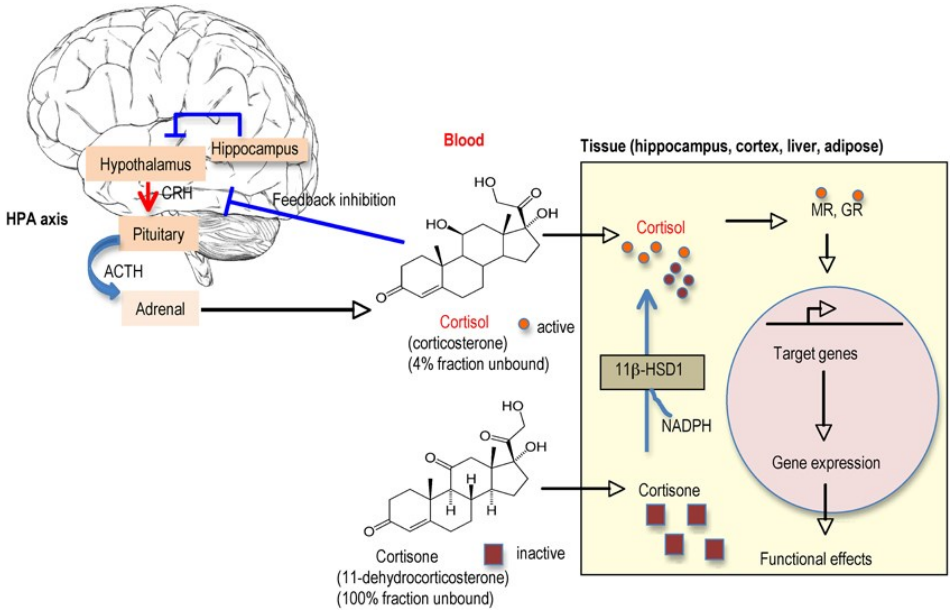

Figure 6 *Regulation of GC levels in blood and tissues* (Yau & Seckl, 2012).

Production of cortisol is regulated by the HPA axis and the main stimuli for its acute release are stress, hypoglycaemia, pyrogens, pain etc. Therefore, GC hormones play essential role in stress adaptation, metabolism regulation, inflammatory responses of the organism, modulation of the immune system, increasing cerebral perfusion, enhancing cardiovascular input and redistributing blood flow, and even intermediary metabolism of proteins, glucose and lipids (Lara et al., 2013; Wu et al., 2007; Zvěřová, 2017). Besides hypothalamus and pituitary, the hippocampus has also been implicated in the regulation of GC activity (Lara et al., 2013). GC are steroid hormones and as such,

they cross the blood-brain barrier. Afterwards, they bind to glucocorticoid (GR) and mineralocorticoid (MR) receptors. Activity of GR and MR is necessary for a normal metabolic activity of the cell and is crucial for many CNS functions with learning and memory being one of them (Green, 2006).

With advancing age, changes of hormonal regulation originating in the HPA axis occur. The ample evidence implicates HPA axis dysfunction, a state which is reflected by markedly elevated basal levels of circulating cortisol. This dysfunction only seems to be relevant in the early stages of AD as studies show that it did not worsen over time. Other findings established that early sporadic AD patients display elevated levels of circulating cortisol which causes faster progression of the disease (Lara et al., 2013; Puigoriol-Illamola et al., 2018). It has been implicated that higher levels of cortisol are linked to Aβ and tau pathology and as such are associated with impaired declarative memory and cognitive decline even in people without signs of dementia. High concentrations of cortisol have been observed in individuals with hippocampal atrophy and cognitive decline (Lara et al., 2013). Therefore, increased glucocorticoids are believed to be a risk factor for AD alongside a number of other environmental and genetic factors or may exacerbate existing AD pathologies (Green, 2006).

The hippocampus is a brain structure particularly vulnerable to ageing and is a prime target for GCs, prolonged exposure compromises hippocampal electrophysiology, structure and function. It is sensitive to the deleterious actions of chronic GC excess and it is believed that this potentiates neurotoxicity, dendritic atrophy, hippocampal atrophy and perhaps neuronal loss. GC levels in the blood tend to increase with ageing and correlate with impaired spatial memory and learning in ageing rodents and humans (Yau & Seckl, 2012).

Glucocorticoid-related genetic susceptibility to AD has been identified through the gene for 11β-hydroxysteroid dehydrogenase type 1 which is an enzyme that activates glucocorticoids (Green, 2006). This enzyme is widely expressed throughout the CNS and elevated hippocampal and neocortical concentrations are observed with ageing and the causes of cognitive decline (Wyrwoll et al., 2011). The magnitude of GC action within tissues depends on their local metabolism by 11β-HSD1, among others (Yau & Seckl, 2012).

3.5 11β-Hydroxysteroid Dehydrogenase Type 1

The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) belongs to the short-chain dehydrogenase/reductase superfamily. 11β-HSD1 is a microsomal enzyme expressed mainly in peripheral tissues, notably liver, adipose tissue and widely expressed throughout the adult central nervous system (multiple regions of the brain such as the cortex, cerebellum and hippocampus) (MacLullich et al., 2012; Mohler et al., 2011; Odermatt & Nashev, 2010; Wyrwoll et al., 2011). Referring to MacLullich et al., 2012, 11β-HSD1 has been implicated to have activity in cognitive decline. Importantly for our study, the expression of 11β-HSD1 mRNA increases in aged mouse brains. Thus, expression of the enzyme through mRNA transcription is also increased and higher levels of 11β-HSD1 in older animals result in worse cognitive functions.

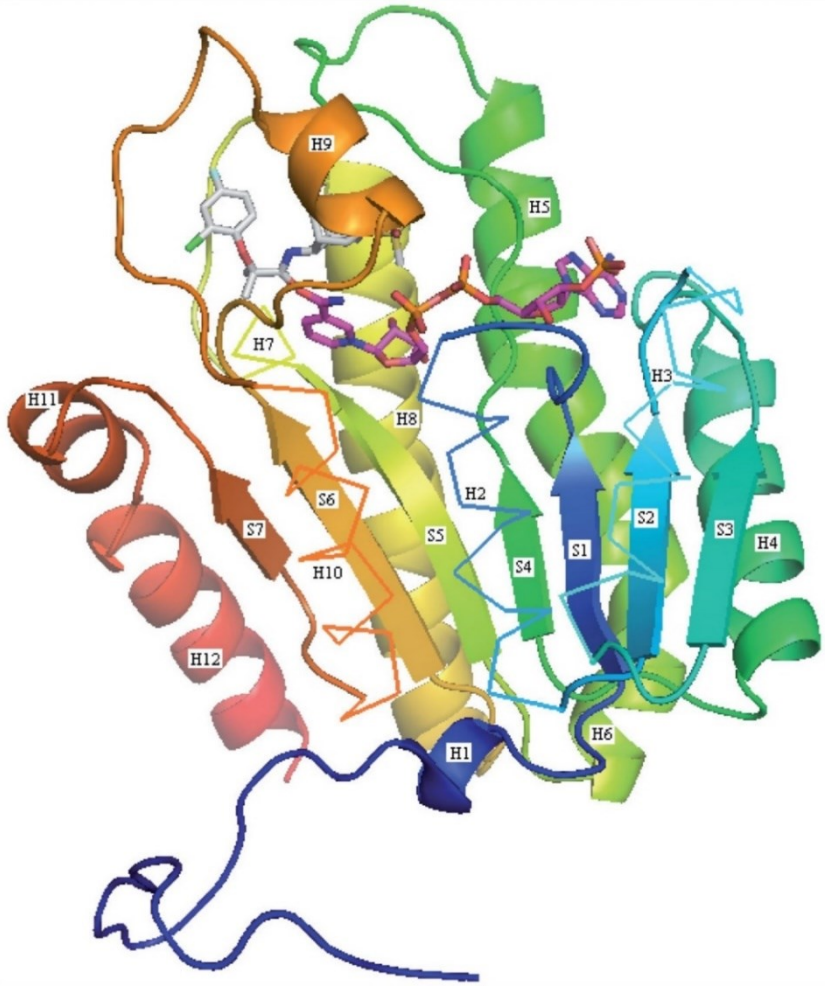

Figure 7 *The secondary and tertiary structure of a human 11β-HSD1* (Thomas & Potter, 2011).

11β-HSD1 is an enzyme responsible for regulation and catalysation of the reversible enzymatic conversion of glucocorticoids from their inactive forms (i.e. cortisone in humans, 11-dehydrocorticosterone in rodents) to their active forms (cortisol and corticosterone, respectively), while its isoenzyme 11β-hydroxysteroid dehydrogenase type 2 catalyses the opposite reaction, i.e. the conversion from active metabolites to their inactive forms. 11β-HSD1 is a NADPH-dependent reductase while its isoenzyme is a NAD^+-dependent oxidase. Both isoenzymes, therefore, play an important role in cortisol/corticosterone metabolism, intracellular concentrations of active and inactive metabolites, and their clearance (Mohler et al., 2011; Štambergová, 2013; Tomlinson et al., 2004).

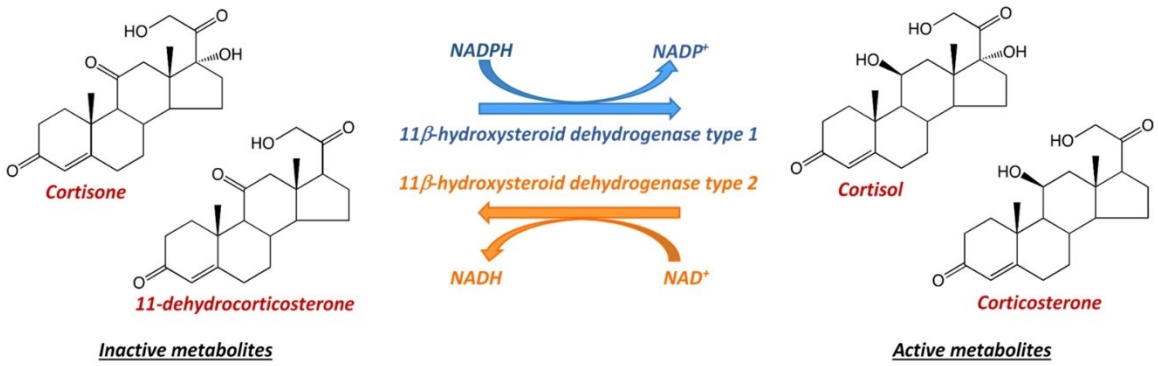

Figure 8 *Conversion of cortisone and 11-dehydrocorticosterone to cortisol and corticosterone, respectively by 11β-hydroxysteroid dehydrogenase type 1 and vice versa by type 2. Retrieved from http://www.fgu.cas.cz.*

As previously mentioned, 11β-HSD1 is expressed in the brain, namely in the cerebral cortex, hippocampus, hypothalamus and pituitary, among others. These sites within the brain are responsible to the negative feedback actions of glucocorticoids. This fact suggests that 11β-HSD1 may be a key regulator of the HPA axis (Wyrwoll et al., 2011).

It has been discovered that 11β-HSD1 knockout mice (i.e. mice with genetically engineered DNA that do not express particular proteins) have decreased corticosterone levels within the hippocampus despite elevated circulating corticosterone levels. This indicates impairment in glucocorticoid reactivation (Tomlinson et al., 2004). For instance, aged 11β-HSD1 knockout mice resist cognitive impairment seen in wild-type

(WT) mice, which occurs in various cognitive tests such as Morris water maze (MWM), novel object recognition (NORT); yet these mice seem to be protected from the deficits seen in wild type animals. Thus, these findings have been hypothesised as important in the explanations of improved age-related memory impairments in comparison with controls (Tomlinson et al., 2004; Wyrwoll et al., 2011).

Available data suggest that changes in 11β-HSD1 show more significant impact with ageing. It has also been implicated that for the effects of manipulations with 11β-HSD1 to impact noticeably on cognitive functions, ageing by itself is required. Furthermore, it has been hypothesised that elevated activity of the enzyme is sufficient to produce cognitive decline with ageing. Studies suggest that even short-term treatment (as long as 2 weeks) with a 11β-HSD1 selective inhibitor, provided it is CNS-active, improves cognitive function in aged mice. Thus, it can be concluded that the 11β-HSD1 associated part of the GC contribution to cognitive ageing appears not to be entirely irreversible. Lastly, age-related rise in GC levels has been implied to potentially further amplify the deleterious effects they have in the CNS by inducing 11β-HSD1 expression (Wyrwoll et al., 2011).

Some performed preclinical studies demonstrate that 11β-HSD1 inhibition does indeed improve cognition and the severity of hallmarks of AD. These findings suggest neuroprotective effect of the enzyme inhibition (Puigoriol-Illamola et al., 2018).

3.6 SAMP8 Model

Senescence-Accelerated Mouse (SAM) is a complex model of ageing developed by Professor Takeda at Kyoto University through phenotypic selection. It derives from AKR/J breeding colony, where a number of mice showed characteristics of rapid ageing. By selective breeding, two series of SAM were obtained – senescence-prone SAMP (short-lived mice) and senescence-resistant SAMR (long-lived mice). These series have several sublines, one of them being SAMP8 or the Senescence-Accelerated Mouse Prone 8, which is a subline that, given its characteristics, was chosen for our study (Morley, 2002; Nomura & Okuma, 1999; Takeda et al., 1997).

SAMP8 exhibit age-related behavioural and neuropathological phenotypes as well as changes in biological markers of ageing (Carter et al., 2005; Yanai & Endo, 2016). Deficits in learning and memory, emotional disorders (for example reduced anxiety-like behaviour), immune dysfunction in lifespan and abnormal circadian rhythms were observed. Alterations in cognitive behaviour (in tests such as Morris water maze and novel object recognition), as well as in non-cognitive emotional behaviour (in tests such as elevated plus maze, open field and swimming forced tests) were found in SAMP8 when compared to SAMR1 (a widely used control for SAMP8 lacking accelerated senescence), and that as early as 2 months of age (Akiguchi et al., 2017; Carter et al., 2005; Morley et al., 2012).

Table 1 *Comparison of hallmarks in AD and SAMP8 mouse* (Morley et al., 2012).

	Alzheimer's disease	SAMP8
Overproduction of amyloid-β	Yes	Yes
Amyloid plaques	Yes	Late[a]
Phosphorylated tau	Increased	Increased
Cerebral amyloid angiopathy	Yes	Yes
Neuron loss	Yes	Yes
Synaptic dysfunction	Yes	Yes
Dendritic spine loss	Yes	Marked
Gliosis	Yes	Yes
Cholinergic deficit	Yes	Yes
Learning and memory impaired	Yes	Yes
Circadian rhythm disturbances	Yes	Yes
Oxidative damage	Yes	4 months

[a] Occur at 16 to 18 months.

SAMP8 brains do not show the morphological changes as are the pathological hallmarks of AD, such as NFT and senile plaques (SP). Instead, SAMP8 brains show several pathophysiological signatures of AD – overproduction of amyloid precursor protein (APP), abnormal β-amyloid protein accumulation in the hippocampus and its impaired clearance, tau-protein hyperphosphorylation, increased oxidative stress and gliosis (Akiguchi et al., 2017; Morley et al., 2012).

The study of Nomura & Okuma, 1999 as well as studies of different authors suggest that SAMP8 is a useful and pertinent model used for the investigation of mechanisms of brain-ageing in senile dementia and an excellent model for AD-like pathology and sporadic form of AD (Morley et al., 2012).

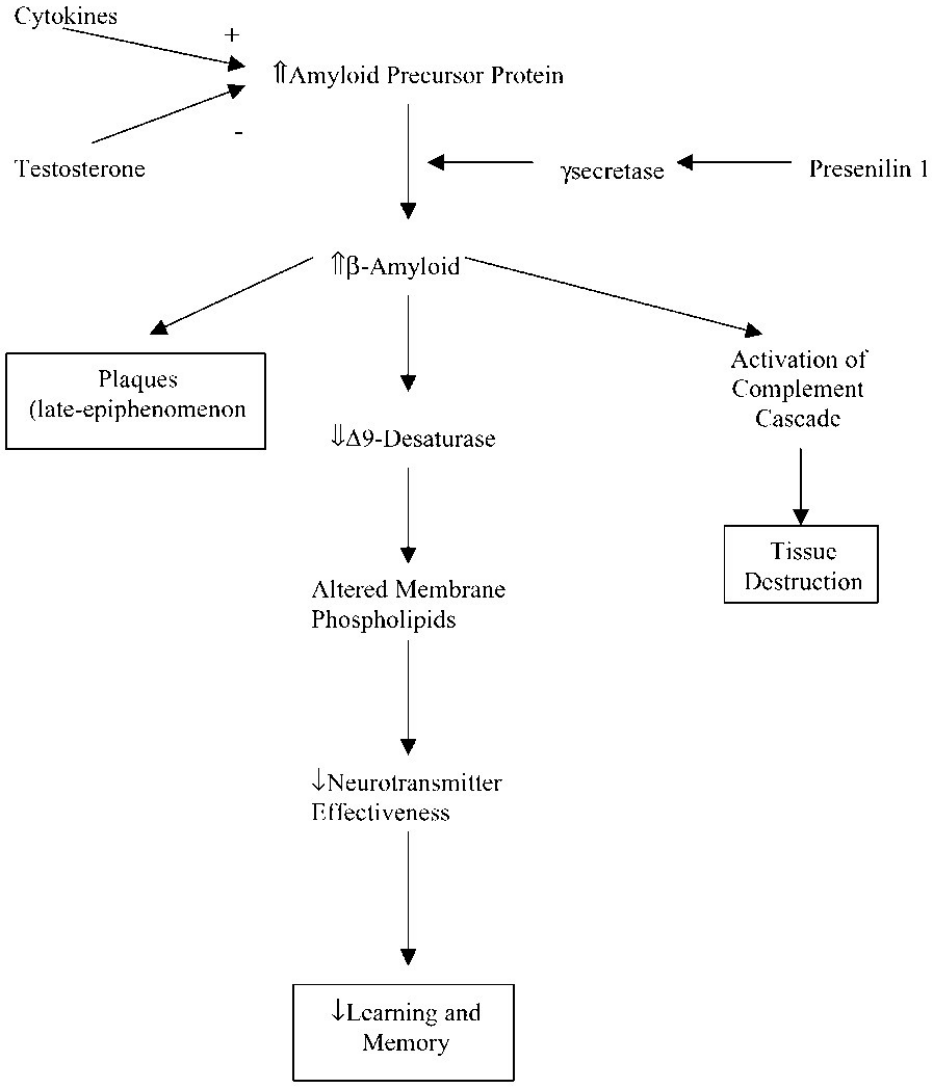

Figure 9 *Demonstration of the pathophysiology of the memory deficit in SAMP8* (Morley, 2002).

4 HYPOTHESIS AND OBJECTIVES

Hypothesis:

Increased levels of circulating cortisol are detected in patients with AD which causes faster disease progression. Also, there is a connection between onset of AD and lifestyle choices, such as sedentary lifestyle and high-fat diet (HFD). Aged mice with cognitive deficits show overexpression of 11β-hydroxysteroid dehydrogenase type 1 in the hippocampus and parietal cortex which is linked to premature memory decline. Thus, in line with some preclinical studies that demonstrate improved cognition and hallmarks of AD after 11β-hydroxysteroid dehydrogenase type 1 inhibition, administration of such inhibitor might represent a new potential way leading to neuroprotection, prevention and possibly even treatment of AD.

Objectives:

- Study the behavioural and cognitive effects of 11β-HSD1 inhibition by a pyrrolidine-based potential drug for AD, RL-118.
- Study the correlation between cognitive performance and high-fat diet.
- Study the correlation between high-fat diet and 11β-HSD1 inhibition by RL-118.

5 EXPERIMENTAL PART

5.1 Study Subjects

Our subjects were female SAMP8 mice obtained from Harlan Laboratories. These mice were housed at the animal facility of the Faculty of Pharmacy and Food Sciences, University of Barcelona, maintained under standard temperature 22 ± 2 °C, 12-h light/dark cycle (300 lx/0 lx) at a relative humidity of 55%. The animals were housed 3-6 per home cage with water *ad libitum*, fed either a standard chow for rodents, or were put on a high-fat diet which consisted of 60% of fats. This study and animal handling were performed in accordance with the European Community Council Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC) and the institutional guidelines established by the Ethical Committee for Animal Experimentation at the University of Barcelona.

After 21 days of weaning with their mothers, the mice were split into 4 groups: control; control treated with RL-118; high-fat diet (HFD); high-fat diet treated with RL-118. After 21 days, designated groups started being fed HFD. At 5 months of age, the treatment with RL-118 of the respective groups started, and at 6 months of age, we began performing behavioural tests.

At the end of the study, triglycerides determination together with the glucose tolerance test were performed following 12 h of fasting. Three days after, the mice were anesthetised with 80 mg/kg of sodium pentobarbital, a sample of their blood was obtained intracardially and the mice were sacrificed by decapitation. Afterwards, their brains were dissected and separated sagitally into two hemispheres, samples of both hippocampi, both cortices and a sample of thigh muscle were obtained, chilled on dry ice and stored at -80 °C for protein and RNA extraction and immunohistochemistry.

5.2 Tested Substance

As the hypothesis of this study suggests, a 11β-hydroxysteroid dehydrogenase type 1 inhibitor was used for treatment of the subject mice. A group of these pyrrolidine-based inhibitors was synthesised at the laboratories of University of Barcelona. For our study, we used a compound with the working name RL-118, chemical structure [(4-azatetracyclo[5.3.2.$0^{2,6}$.$0^{8,10}$]dodec-4yl)(cyclohexyl)methanone].

It was administered to the treated mice groups orally by gavage at 21 mg per kilogram of body weight daily for 4 weeks before behavioural tests were performed. 2-hydroxypropyl-β-cyclodextrin serving as vehicle for RL-118 was administered as placebo to the untreated mice on HFD and normal diet (ND) control groups.

The prepared solution or RL-118 that was administered to the mice consisted of 52,5 mg of RL-118, 5 g of 2-hydroxypropyl-β-cyclodextrin as vehicle in 25 ml of distilled water. For the placebo, 5 g in 25 mL of distilled water were used. Both were kept refrigerated at the temperature of 4-8 °C at the animal facility.

5.3. Methods

5.3.1 Novel Object Recognition (NORT)

The novel object recognition test (NORT) is a recognition test often used for psychopharmacological studies in rodents (Stolerman, 2010). Novelty is defined as a new object or environment (Leger et al., 2013). NORT is a behavioural test used to study memory and learning, the preference for novelty, the influence of different brain regions in the process of recognition and the study of different drugs and their effects. This test is evaluated by the differences in the exploration time of novel and familiar objects (Antunes & Biala, 2012).

A symmetric plastic L-shaped apparatus of black colour, 25 cm in length and 20 cm in height, was used for this test. Any outer visual intervention coming from the room was prevented by a black curtain encircling the testing area. Before conducting the test, the apparatus was properly cleaned with 70% ethanol to minimise olfactory cues and eliminate other contaminants, such as urine and faeces, that might interfere with the conduct of the experiment, i.e. influence the mice and their behaviour. After each trial with an animal, the whole apparatus was cleaned with 70% ethanol again to begin in the same inert environment, unspoiled by interference with the previous animal of any sort.

The experiment lasted 5 days in total and comprised of three phases: habituation, familiarisation and the test itself.

Each animal was placed into the apparatus in the intersection of the two arms, securing its free will of which side of the L-maze to start exploring first.

For the habituation phase, each animal was placed in the apparatus for 10 min to get accustomed to the environment. This was done on 3 consecutive days at the same time of the day. This phase serves to reduce stress, avoid a potential neophobic response and to promote the exploratory activity in mice towards the objects used for testing the following days (Leger et al., 2013).

On the fourth day, for the familiarisation phase, two identical objects (A+A) were placed in each arm of the apparatus. The animals were free to explore both objects for

10 min to familiarise with them. This phase began at the same time as habituation phase on the previous days.

The first test was a test of short-term memory when after 2 h following familiarisation, one of the objects (A) was replaced with a new one – a novelty (B). It should be mentioned that to avoid object preference bias, objects A and B were counterbalanced so that one half of the animals in each experimental group were first exposed to object A and then to object B and vice versa (Griñán-Ferré et al., 2016). The animals were allowed to explore for 10 min.

The second test was a test of long-term memory. 24 h after familiarisation, i.e. on the fifth day, object B from short-term memory test was exchanged for a new object (C). The animals were left to explore the objects for 10 min.

Both short- and long-term memory tests were recorded by a camera that was situated above the apparatus. The videos were then analysed. For analysis, we used the discrimination index (DI):

$$DI = \frac{TN - TF}{TN + TF}$$

where TN = time exploring the novel object

TF = time exploring the familiar object

TN = total time exploring

DI serves to measure the difference between time spent exploring novel and familiar objects; this result can vary between +1 and -1. A positive score indicates more time spent with the novel object whereas a negative score indicates more time spent with the familiar object; zero score indicates a null preference (Antunes & Biala, 2012).

Importantly, exploration was defined as the orientation of the mouse's snout towards the object at a distance ≤ 2 cm, sniffing or touching with snout (Antunes & Biala, 2012).

5.3.2 Morris Water Maze (MWM)

The Morris water maze (MWM) is a test specially designed for rodents widely used to study and evaluate learning and spatial memory. The test is built on the principle of a rodent (mouse) trying to find a hidden submerged platform in a pool being navigated by distant cues around the perimeter of the area (Nunez, 2008). Spatial learning is assessed through repeated trials and reference memory by preference for the platform area when the platform is absent (Vorhees & Williams, 2006).

For this test, an open circular pool of 100 cm in diameter and 50 cm in height was used. It was filled half-way with water and maintained at constant temperature 25 ± 1°C. The pool was divided into four quadrants by two imaginary perpendicular axes. At each cardinal point of the axes, different objects were placed on the wall of the tank as visual clues allowing orientation. Five different starting position were determined (1, 2, 3, 4 and 5). A white escape platform was placed in the middle of one of the quadrants, in the one farthest from the starting points, submerged 1 cm below the water surface; to secure invisibility of the platform for tested mice, non-toxic latex was added to the water in order to provide opacity. The whole pool area was encircled by black curtains to disable mice from finding a place in the room that would serve as an orientation point. Moreover, the animals could not rely on scent to find the escape route.

MWM is a test that lasts six days in total. First five days are considered as learning phase. The mice were gently placed into the water at starting points, taking turns so one mouse would start in position 1 and finished with the last trial in position 5, and the next mouse started at 5 and finished at 1, etc. They were not allowed to rest during all the 5 trials. The following day, the mice would start in position 2 or 4 respectively, each day starting from a different location. This procedure took place, so the animal would not learn a specific route to find the platform. The mice were allowed to search for the platform for 60 s; if they did not find the platform after this time, they were guided to the platform by the investigator and remained in the platform for 15 s to memorise its location. On the 6th day, the platform was removed from the pool and the mice were placed in the pool in the most distant position from the platform – position 3 to perform the memory test. They were allowed to remain in the pool for 60 s for one trial only.

Their swimming paths were recorded by a video camera placed above the pool and the results were analysed with SMART® ver. 3.0 software.

5.3.3 Open Field (OF)

The open field test (OF) is a common test of exploratory behaviour, anxiety and general (locomotor) activity in mice, measuring both quality and quantity. In principle, the open field is an enclosed environment (box) surrounded by walls to prevent escape (Gould, 2009). OF is based on opposing drives of rodents, i.e. the urge to explore new environments and their fearful avoidance of bright or exposed areas (Sturman et al., 2018).

The apparatus used for OF had a character of a square box made of white plywood (50 × 50 × 25 cm) with red lines drawn on the floor dividing it into smaller 25-cm squares for the purpose of tracking the animals' position. The open field was placed behind black curtains encircling the area securing no visual interference with the testing room. Before the commencement of testing and after each trial, the apparatus was cleaned with 70% ethanol to minimise olfactory cues, as well as urine and faeces were removed.

To start the test, the mouse was placed in the centre of the open field. It was allowed to explore the environment for 5 minutes while the number of rears and grooming was counted by the experimenter.

Time spent in the centre area and total locomotor activity, as the sum of total distance, were analysed using SMART® ver. 3.0 software. Each trial was recorded using a video camera mounted to the ceiling at the height of 2,1 m situated above the apparatus.

5.3.4 Elevated Plus Maze (EPM)

The elevated plus maze (EPM) is a widely used behavioural assay for rodents validated to assess the anti-anxiety effects of pharmacological agents and steroid

hormones, as well as to define brain regions and mechanisms underlying anxiety-related behaviour (Walf & Frye, 2007). EPM is a test based on the natural aversion of mice for open and elevated areas. However, it is also based on their natural spontaneous exploratory behaviour in novel environments (Komada et al., 2008).

The apparatus for EPM consisted of two open arms and two closed arms. These were crossed in the middle perpendicularly to each other, forming a shape of a "+", creating a centre area. This apparatus was placed behind black curtains encircling the testing area to prevent any visual interference from the room. Before the test and after each trial, the apparatus was cleaned with 70% ethanol to minimise olfactory cues. Also, any present urine and faeces from the mice were removed.

At the beginning of the test, the mouse was placed at the junction of the open and closed arms, in the centre area. Then the investigator left the room and the mouse was allowed to explore the apparatus for 5 min. Each trial was recorded by a video camera and the number of rears, i.e. vertical standing of the mouse on two hindlegs, was counted. Time spent in open arms and closed arms were analysed with SMART® ver. 3.0 software. Moreover, locomotor activity, calculated as the sum of total distance, was observed.

5.3.5 Three-Chamber Social Test (TCT)

The three-chamber test (TCT), also known as the Crawley's sociability and preference for social novelty test, is a test of social behaviour and sociability in animals; sociability is defined as the subject mouse spending more time in the chamber containing the novel mouse than in the chamber that does not contain it/contains an inanimate object. (Yang et al., 2011) This test is applicable for assessing potential effects of pharmacological compounds on the sociability of animals (Kaidanovich-Beilin et al., 2011).

The apparatus used for this test was a rectangular box (15 × 15 × 20 cm) made of transparent plastic, divided into three equally sized compartments. The middle section of the walls connecting the chambers was open in order to allow the mouse free access

to each chamber. Two identical, metal wire cup-like containers, big enough to hold a mouse, were placed vertically in both side chambers, weighed down with a weight for balance scales so that in case of the animal leaning against the container, it would not move with its weight. Each container was comprised of metal wires to allow for air exchange between the interior and exterior but small enough to prevent direct physical interactions between an animal on the inside with one on the outside (Kaidanovich-Beilin et al., 2011).

Before testing and after each trial, the whole apparatus was properly cleaned with 70% ethanol in order to prevent olfactory cue bias.

The main principle of this test is based on the free choice of a subject mouse to spend time in any of the three box's compartments during two parts of the experiment. A mouse, previously unencountered by the tested animal, is placed under a wire cup and unable to move freely which prevents direct physical contact, eliminates aggressive behaviour or fighting; only sensory interactions, such as smell, sight, sound or taste, are allowed. Thus, it is the tested mouse who initiates and terminates any interactions, while having a choice between the unfamiliar control mouse and an empty container (Kaidanovich-Beilin et al., 2011).

Therefore, the subject mouse was taken from its home cage and transported in a box, placed in the middle chamber and was left to explore on its own for 10 min and thus habituate. After 10 minutes, another mouse, a SAMP8 control of the same sex and age, was also transported alone into the testing room and placed inside one of the containers. The subject mouse was again left to explore for 10 min.

Each session of the test was recorded by a video camera for later analysis. The fact that the investigator left the room for the duration of both exploration periods in order not to influence any behaviour of neither of the tested animals should be noted.

5.3.6 Glucose Tolerance Test and Triglycerides

Glucose tolerance test (GTT) evaluates the response of the body to exogenous glucose administration and determines its clearance. This test was performed 3 days before the mice were euthanised. After 12 h of overnight fasting, the mice were administered 2 g of glucose per kilogram of body weight intraperitoneally. A solution containing 0,4 g of glucose per ml in NaCl 0,9% (w/v) was used for administration.

Glucose blood concentrations were determined at 0, 5, 10, 15, 30, 60 and 120 min after glucose administration, using Accu-Chek® Aviva glucometer (Roche Pharma) and specific strips. Moreover, blood levels of triglycerides were determined using Accutrend® Plus meter (Roche Pharma) and specific strips. Blood was obtained from animals' tails after a small incision.

5.3.7 Data Analysis

Data are expressed as the mean ± Standard Error of the Mean (SEM) from at least 4-12 samples. Data analyses was conducted using GraphPad Prism ver. 6 statistical software. Means were compared with the Two-Way ANOVA test and Tukey's post-hoc test. Statistical significance was considered when p values were <0.05. Statistical outliers were carried out with Grubss' test and were removed from analysis.

6 RESULTS

6.1 Novel Object Recognition

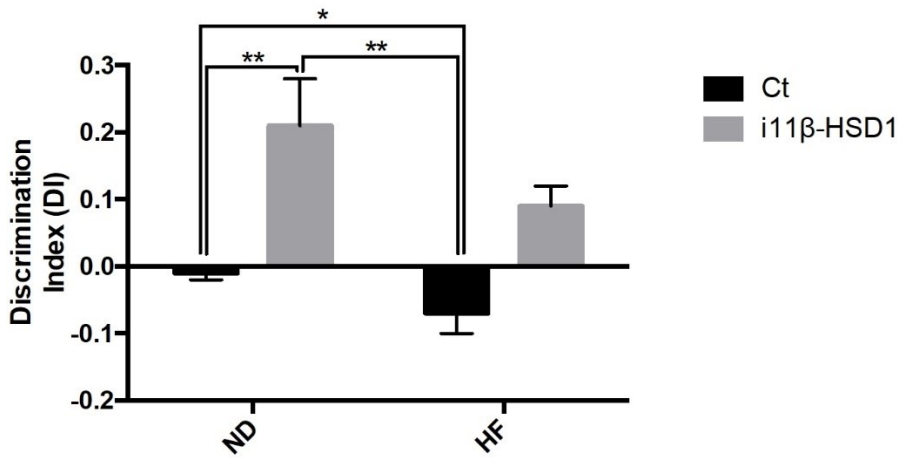

Figure 10 *Evaluation of short-term memory tested after 2 h after familiarisation in treated SAMP8 mice on normal (ND) and high-fat (HF) diet compared to controls. **p<0.01 and *p<0.05*

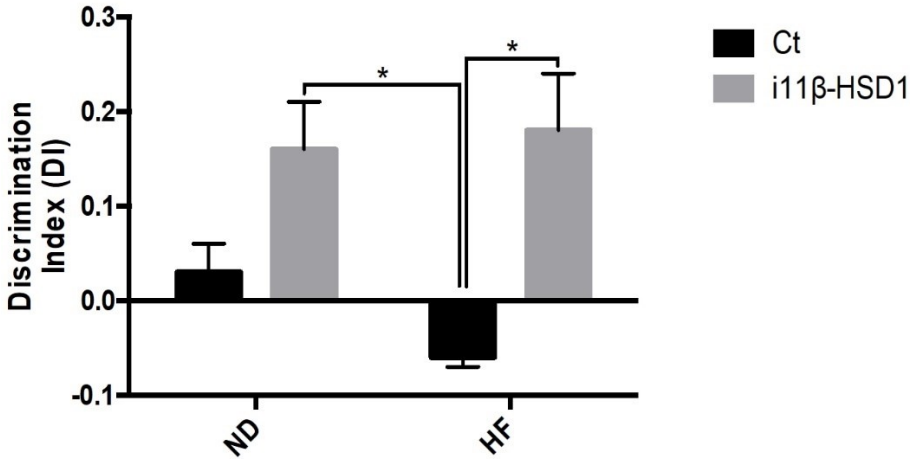

Figure 11 *Evaluation of long-term memory after 24 h after familiarisation in treated SAMP8 mice on normal (ND) and high-fat (HF) diet compared to controls. *p<0.05*

Novel object recognition test was used to evaluate short- and long-term recognition memory. Results of this test expressed as discrimination index are shown in figures 10 and 11. The discrimination index (DI) is higher in mice treated with RL-118 than non-treated control mice. This shows that treated groups spent more time

exploring the novel object and thus have a better recognition memory. According to the results of normal and HFD groups, high-fat diet worsens recognition memory in control normal-diet mice. We observe that treatment with RL-118 improved recognition memory even in HFD mice compared to non-treated controls.

6.2 Morris Water Maze

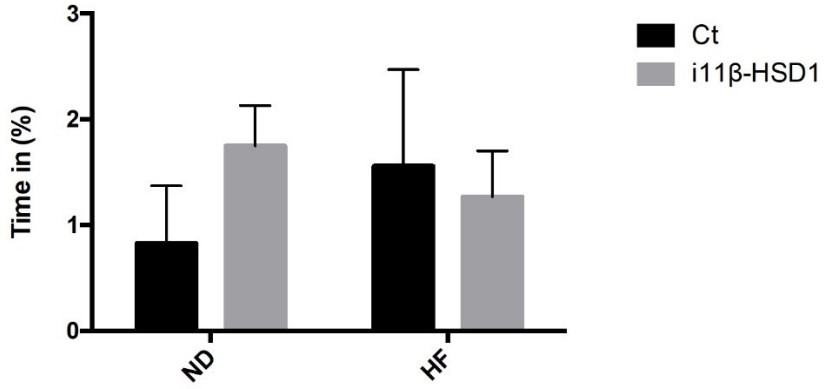

Figure 12 *Time spent in platform zone comparing treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls.*

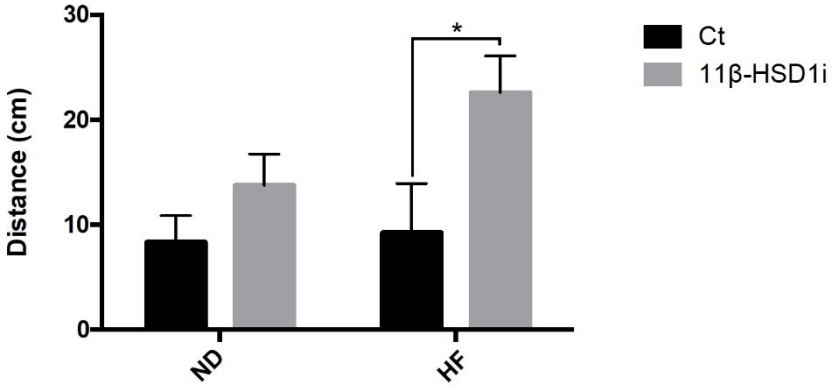

Figure 13 *Distance swum in platform zone comparing treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls. *p<0.05*

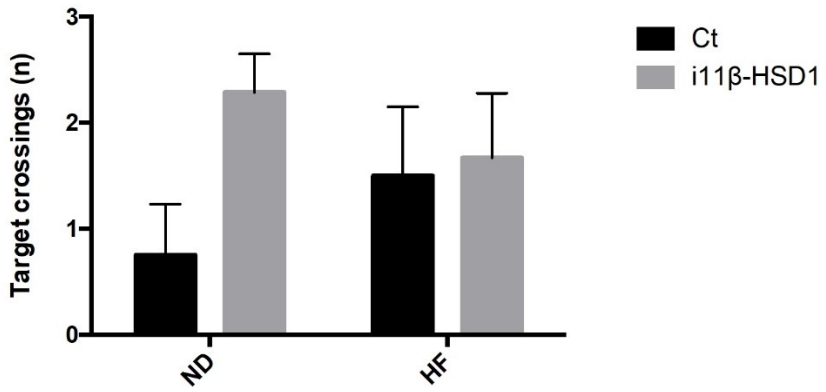

Figure 14 *Number of target crossings comparing treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls.*

Morris Water Maze was used to assess learning and spatial memory. Results of this test are shown in figures 12-14. When compared to controls, treated mice spent more time and swam longer distance in the platform zone, showing that treatment with RL-118 improves spatial memory.

6.3 Open Field

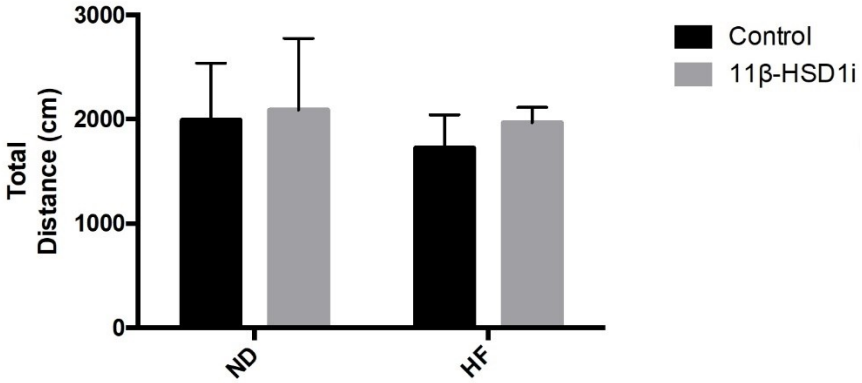

Figure 15 *Evaluation of locomotor activity as the total distance travelled comparing locomotor activity in treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls.*

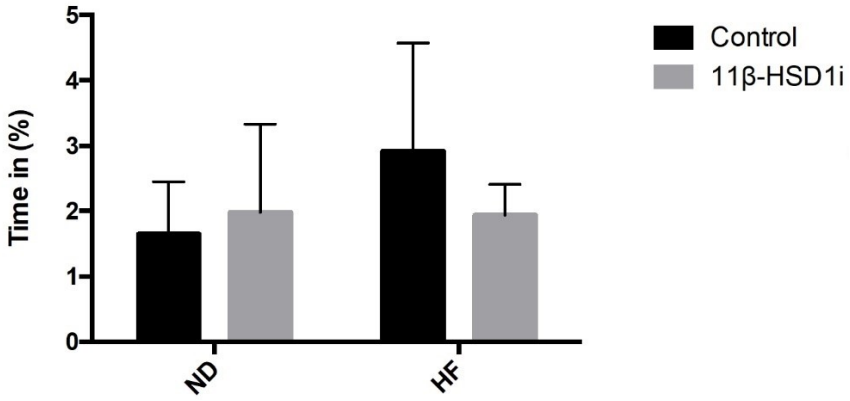

Figure 16 *Time spent in the centre zone comparing treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls.*

Open field test was used to measure general locomotor activity and anxiety levels. Results of this test are shown in figures 15 and 16. Treated mice show slightly higher distance travelled and time spent in the centre zone when compared to non-treated controls. Diet does not seem to play a role as the results of both dietary groups differ minimally. Treated groups show increased exploratory behaviour and therefore decreased levels of anxiety and thigmotaxis (preference to stay close to the walls of the field).

6.4 Elevated Plus Maze

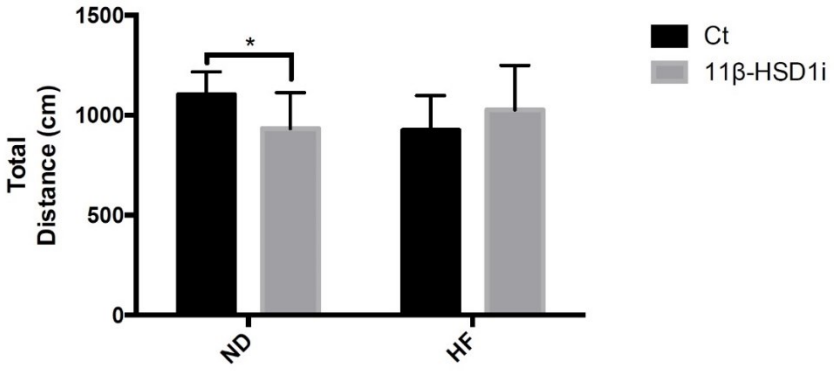

Figure 17 *Total distance travelled as evaluation of locomotor activity in treated SAMP8 mice on normal (ND) and high-fat (HF) diet compared to controls. *p<0.05*

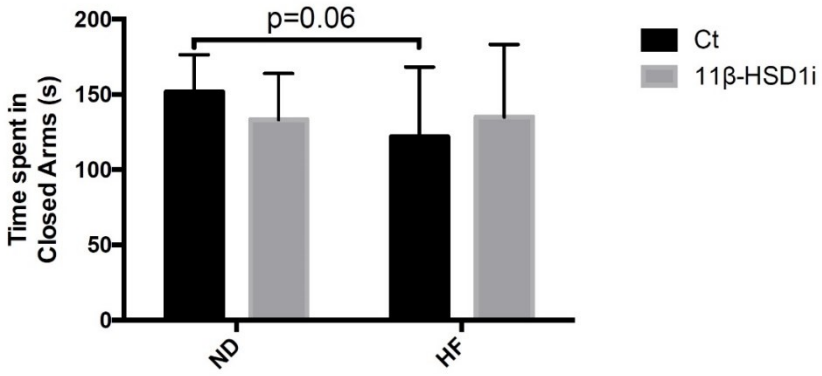

Figure 18 *Time spent in closed arms comparing treated SAMP8 mice on normal (ND) and high-fat (HF) diet to controls.*

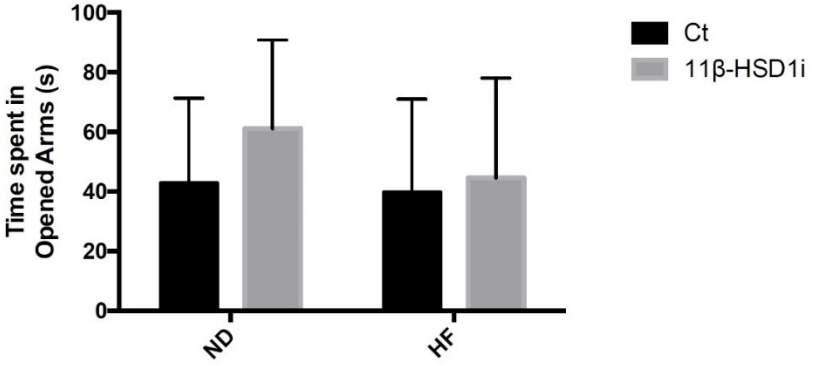

Figure 19 *Time spent in opened arms comparing treated SAMP8 on normal (ND) and high-fat (HF) diet to controls.*

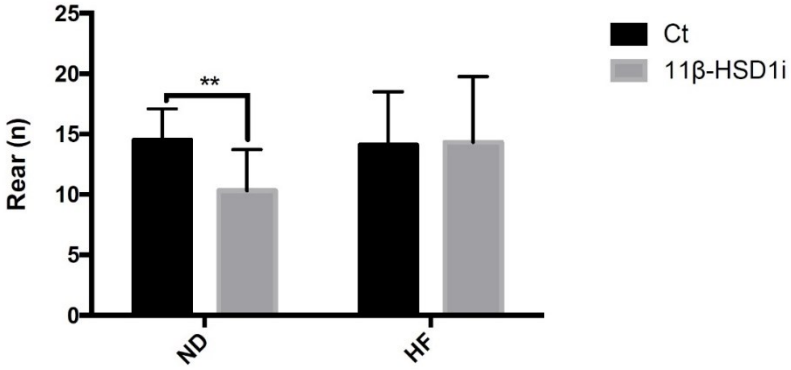

Figure 20 *Number of rears counted comparing treated SAMP8 on normal (ND) and high-fat (HF) diet to controls. **p<0.01*

Elevated plus maze was used as a test of anxiety and locomotor activity. Results are shown in figures 17-20. Locomotor activity observed was more significant in untreated normal-diet mice compared to treated mice. Comparison of all groups as for the time spent in closed and open arms showed differences within the groups. HFD fed mice spent more time in closed arms compared to normal-diet mice which suggests higher anxiety levels. Treated mice in both dietary groups showed decreased levels of anxiety spending longer in opened arms. Count of rears as another parameter of anxiety suggested the control the treated group to have lower levels of anxiety than the untreated control group.

6.5 Three-Chamber Test

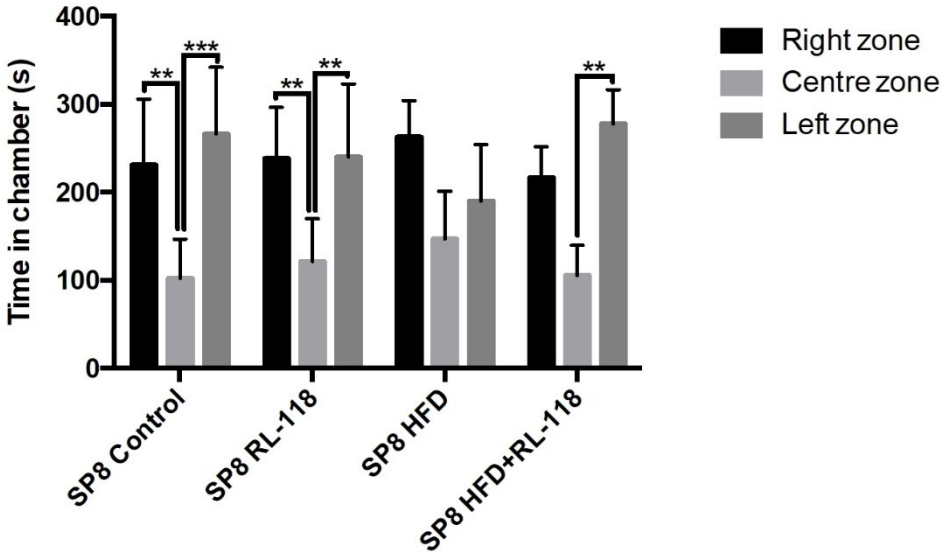

Figure 21 *Time spent exploring in each of the three chambers (zones) comparing treated SAMP8 mice on normal and high-fat diet to controls. Habituation phase. ***p<0.001 and **p<0.01*

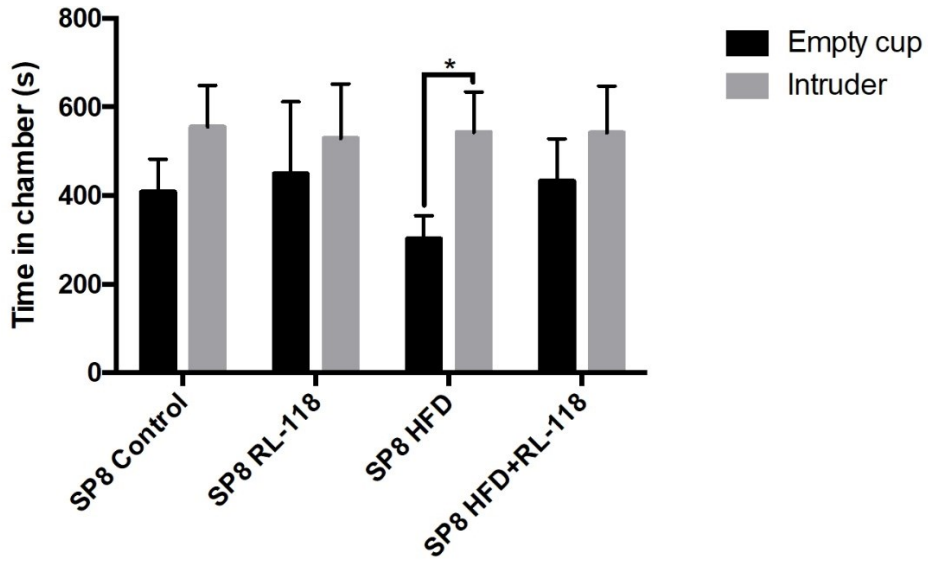

Figure 22 *Time spent exploring the empty cup vs time spent exploring the cup with intruder comparing treated SAMP8 mice on normal and high-fat diet to controls. Sociability testing. *p<0.05*

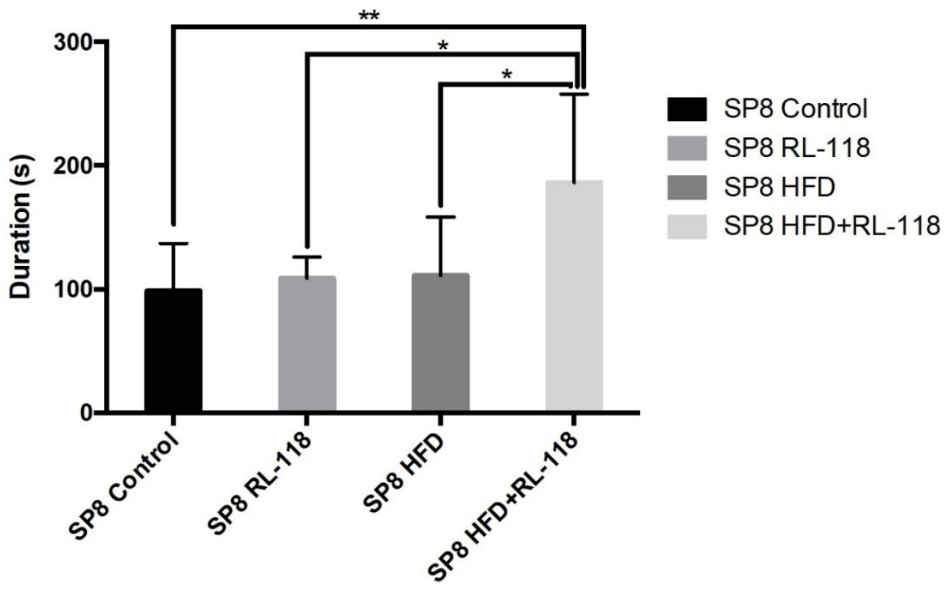

Figure 23 *Time exploring and sociability comparing treated SAMP8 mice on normal and high-fat diet to controls. **p<0.01 and *p<0.05*

The three-chamber test was used to test sociability and social behaviour in our subjects. Results of this test are shown in figures 21-23. There were no markable differences found among the groups as far as exploration of either empty cup or cup with the intruder is concerned. However, there was a slightly increased sociability in treated control and HFD mice, and significantly increased sociability in treated HFD mice.

6.6 Glucose Tolerance Test and Triglycerides

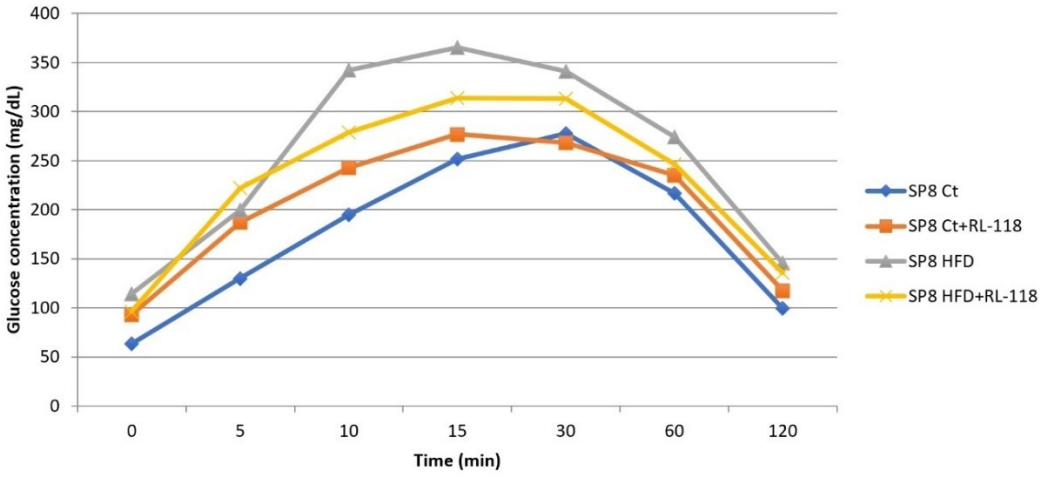

Figure 24 *Results of the glucose tolerance test comparing treated SAMP8 mice on normal and high-fat diet to controls.*

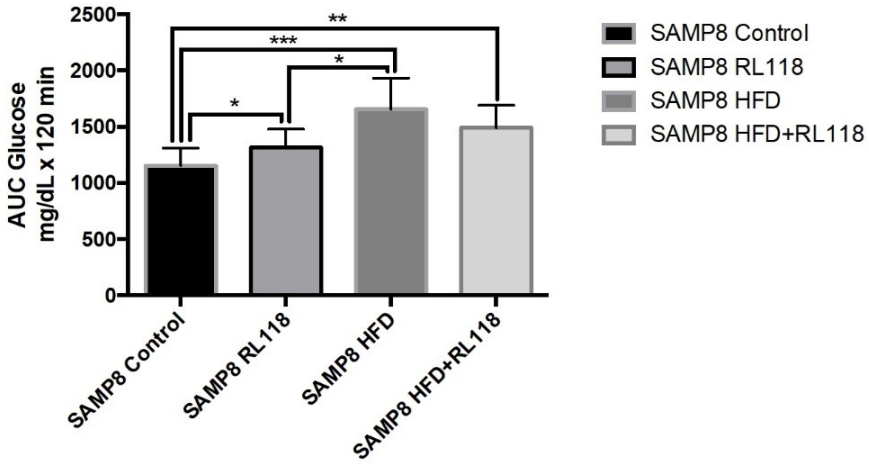

Figure 25 *Area under the curve of glucose – blood concentration (mg/dl) at 120 min after administration comparing treated SAMP8 on normal and high-fat diet to controls. ***p<0.001, **p<0.01 and *p<0.05*

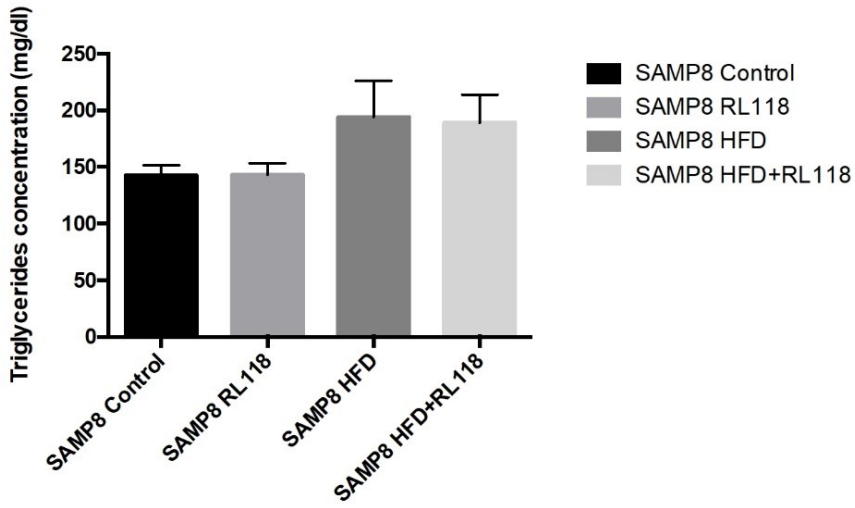

Figure 26 *Concentration of triglycerides (mg/dl) in the blood of treated SAMP8 mice on normal and high-fat diet in comparison with the control groups.*

At the end of the study, glucose tolerance test was performed to evaluate the response of the body to exogenous glucose. Results of this test are shown in figures 24 and 25. HFD mice had a worsened profile of glucose clearance compared to normal-diet control group. Treated HFD mice had a slightly improved glucose clearance profile. Also, the area under the curve of blood glucose shows significant differences between normal and HFD groups echoing the previous results.

Together with GTT, blood levels of triglycerides were determined. These levels were also significantly different comparing normal and high-fat diet groups. High-fat diet increases levels of blood TAG. Treatment with RL-118 slightly improves TAG profile in HFD mice.

7 DISCUSSION

The population is ageing and the physical and mental body changes that accompany this inevitable process are nowadays a more significant factor influencing outbreak and onset of many diseases that occur in the global population, many of which manifest as non-communicable diseases affecting global health everywhere. Cognitive ageing is closely affiliated with ageing of the whole organism. Ageing, if pathological, can lead to cognitive and other mental impairments that are more severe than in the case of normal physiological ageing, and manifest in the form of dementia, AD being the most common type of this cognition-affecting group of diseases. As the aetiology and pathophysiology that lies behind this disease are multifactorial and yet to be fully understood, different research teams all over the world are currently trying to study the bases of AD. Another problem of AD is a lack of tests that could be used for very early AD diagnostics. (Zvěřová, 2017) As there are many hypotheses reasoning the onset of this disease, there are also many research studies, each one of which focuses on different aspects in the complexity of AD pathophysiology, resulting in various different drug studies. At the moment, there are only a few approaches in the treatment of AD and none of them really answers the question of why. The currently available AD treatment merely slows down the progression and delays the inevitable – loss of memory, personality and in the final stage, death.

One of the current research approaches in the potential treatment of AD is with no doubt the inhibition of 11β-hydroxysteroid dehydrogenase type 1, an enzyme important in the metabolism of endogenous glucocorticoids. According to the existing hypotheses, lowering cortisol/corticosterone concentrations leads to improved cognition, reduced anxiety, increased locomotor activity and, as described in studies on rodents, neuroprotective effects (Puigoriol-Illamola et al., 2018).

In this master's thesis, the goal was to study a novel potent pyrrolidine-based 11β-HSD1 inhibitor RL-118, its effects on cognition, behaviour, memory and possible neuroprotection in female SAMP8 mice, a widely used model of the sporadic AD. It is now known that lifestyle plays an important role in the onset of the sporadic AD. In line with this, we tested the compound in groups which were divided according to the

treatment and normal chew or HFD. A series of behavioural and social tests was performed and at the end of the study, a test of glucose tolerance and triglycerides to assess the relationship between treatment with RL-118 and type of diet.

Based on the acquired results, it was observed that SAMP8 mice treated with RL-118 showed better results in behavioural tests of novel object recognition and MWM implying that treatment with the compound improved their recognition memory, spatial memory and learning. These results are in line with findings of Sooy et al., 2010 and Wheelan et al., 2015, whose spatial learning studies on C57BL/6J mice both confirmed, that in this strain 11β-HSD1 inhibition improved spatial learning. Moreover, they confirm the results of Puigoriol-Illamola et al., 2018 that in SAMP8, inhibition of 11β-HSD1 ameliorates spatial and recognition memory. Treated mice showed decreased anxiety in EPM test which echoes Puigoriol-Illamola et al., 2018 suggesting that RL-118 reduces stress level and anxiety. Moreover, increased sociability was observed within the treated groups.

As far as HFD and its relation to cognition are concerned, we have observed that in NORT and partially MWM, recognition and spatial memory of HFD mice were worse than in mice on normal chew, and 11β-HSD1 inhibition improved memory in HFD mice. Anxiety levels seemed rather intact by HFD and we observed slightly increased sociability. Lastly, as expected, glucose clearance was decreased, and TAG levels increased in HFD mice. However, we confirmed that treatment with RL-118 improved glucose clearance in HFD mice but left the TAG levels intact.

It is worth mentioning that hypotheses that AD in fact is type 3 diabetes mellitus have been raised in the past years (de la Monte, 2008). Also, 11β-HSD1 inhibitors are being tested for their potential in the treatment of metabolic syndrome and obesity, diabetes mellitus type 2 or cardiovascular diseases. Therefore, results of this study, particularly of the glucose tolerance test and TAG levels in HFD mice, correlate with the hypothesis that 11β-HSD1 inhibition is a potential approach in treatment of these diseases (Stimson & Walker, 2013; Zou et al., 2017). This is because 11β-HSD1 has been suggested to be an important risk factor for diabetes, as diabetes is associated with tissue-specific alterations in the metabolism of glucocorticoids. Activation of the enzyme followed by excess glucocorticoid production results in enhanced GR-mediated insulin

resistance and glucose intolerance (Zou et al., 2017). Referring to this fact, our compound RL-118 showed a promise in lowering glucose levels and improving the overall glucose clearance and tolerance profile.

During evaluation and analysis of the results, some discrepancies between tests and even groups of mice that lead to the fact that some of the results did not correlate could be observed. These discrepancies might have been a result of the age of the mice. The previous study that was performed with SAMP8 mice and RL-118 by the Neuropharmacology in Aging and Neurodegeneration research group used mice of 12 months of age for behavioural and cognitive testing (Puigoriol-Illamola et al., 2018). As the testing started at the age of 6 months, the factor of senescence might be considered to play a role. Also, the different results of OF test and EPM can be explained by the fact, that these tests are based on different paradigms of anxiety. Moreover, these opposite results can be explained by decreased movement of HFD mice who, after being placed in the middle of the box in the OF, moved less and thus stayed in the centre zone for a longer period of time.

The previous study that was performed at the University of Barcelona shows that administration of RL-118, when performed in female SAMP8 mice of 12 months of age, resulted in improvement in cognition induced by 11β-HSD1 inhibition. Biochemical tests also showed significant neuroprotection induced by RL-118 inhibition of 11β-HSD1. It was discovered, that inhibition by RL-118 leads to autophagy which is a possible mechanism of neuroprotection induced by this compound (Puigoriol-Illamola et al., 2018). As this is only the beginning of testing of this group of compounds, there is not a vast pool of information about all their effects in SAMP8 mice, but also on cognition in general. However, the positive results acquired in this study and the results of the previous study of RL-118 (Puigoriol-Illamola et al., 2018) determine that there is a big potential within pyrrolidine-based 11β-HSD1 inhibitors in cognitive decline treatment.

Studies of 11β-HSD1 inhibition and its impact on cognition and memory processes have also been performed in the previous years with inhibitors A-918446, A-801195 (Mohler et al., 2011), UE1961 (Sooy et al., 2010) and UE2316 (Wheelan et al., 2015). The presented study confirmed that although the compounds used were synthesised on different bases and different subjects were used, all the compounds in

all the models improved cognitive performance by 11β-HSD1 inhibition. Interestingly, in contrast to currently available AD treatments, the study by Mohler et al., 2011 11β-HSD1 inhibition increased neither acetylcholine, nor dopamine levels. This was not investigated in the presented behavioural study; however, it might be interesting for further studies of the effects of RL-118.

To further study the effects of RL-118 in line with our hypotheses, biochemical, immunohistochemical and other tests will be performed *in vitro*.

To conclude, RL-118 is a pioneer of the new group of 11β-HSD1 inhibitors and as such holds a great potential for further research. As this compound shows signs of neuroprotection and also cognitive improvements in female SAMP8 mice (Puigoriol-Illamola et al., 2018), it is implicated that this approach sheds some light on the treatment of age-related cognitive disorders or neurodegenerative diseases, such as AD.

8 CONCLUSIONS

In this study, the effects of 11β-HSD1 inhibition by RL-118 on cognition and behaviour in 6-month-old female SAMP8 mice, as well as influence of HFD were evaluated. Our results support the hypothesis that 11β-HSD1 inhibition improves cognition and memory in a mouse model of accelerated ageing and sporadic AD.

It was discovered that:

- normal and high-fat diet mice treated with 11β-HSD1 inhibitor showed changes in anxiety and recklessness. Moreover, high-fat diet SAMP8 mice treated with 11β-HSD1 inhibitor improved their motivation for social behaviour;
- 11β-HSD1 inhibitor-treated mice ameliorate their cognitive performance in both memory types, recognition and spatial. On the contrary, high-fat diet impaired recognition memory;
- high-fat diet impairs the glucose metabolism, as well as increases TAG blood levels. In addition, 11β-HSD1 inhibitor improves glucose metabolism profile and decreases TAG blood levels in treated mice.

In conclusion, 11β-HSD1 inhibition is a promising therapeutic strategy for cognitive decline associated with high-fat diet in female SAMP8 mice.

ABBREVIATIONS

11β-HSD1	11β-hydroxysteroid dehydrogenase type 1
AACD	age-associated cognitive decline
AD	Alzheimer's disease
ADI	Alzheimer Disease International
ApoE4	apolipoprotein E4
APP	amyloid precursor protein
Aβ	β-amyloid
CBG	corticosteroid-binding globulin
CNS	central nervous system
eFAD	early-onset familial Alzheimer's disease
EPM	elevated plus maze
GABA	gamma-aminobutyric acid
GC	glucocorticoidl
GR	glucocorticoid receptor
GTT	glucose tolerance test
HF	high-fat
HFD	high-fat diet
HPA	hypothalamus-pituitary-adrenal axis
ICD	International Classification of Diseases
LOAD	late onset Alzheimer's disease
MR	mineralocorticoid receptor

MWM	Morris water maze
NAD^+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NCDs	non-communicable diseases
ND	normal diet
NFT	neurofibrillary tangles
OF	open field
NORT	novel object recognition test
SAMP8	Senescence-Accelerated Mouse Prone 8
SP	senile plaques
TAG	triglycerides
TCT	three-chamber test
WHO	World Health Organization
WT	wild-type

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