T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

INVESTIGATION OF THE RELATIONSHIP BETWEEN COGNITIVE FUNCTION, SENSORY AND MOTOR BEHAVIOR AND EXTRACELLULAR MATRIX PROTEINS IN MICE ON THE HIGH-FAT DIET

MASTER THESIS

CİHAN CİVAN CIVAŞ, BSc.

İSTANBUL-2020

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APPROVAL

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The Jury judges that the candidate named above has satisfactorily completed his thesis.

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 01/09/2020 and numbered 2020/08-09

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

01/09/2020

Signature

Cihan Civan Cıvaş

DEDICATION

Dedicated to my lovely wife…

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ABSTRACT

Civas, C. C. (2020) Investigation of the Relationship Cognitive Functions, Sensory and Motor Behavior, and Extracellular Matrix Proteins in Mice on the High-Fat Diet, Yeditepe University, Institute of Health Sciences, Department of Physiology, MSc thesis, İstanbul.

Obesity is a worldwide health problem, and many people suffer from obesity and related diseases. Eating habits play an important role in developing obesity, and previous studies demonstrate that low-quality diets have adverse effects on the brain and behavior such as memory loss or impairment in the appetite and satiety mechanism. The effects of different diets have been investigated widely in the cellular mechanisms of the brain, and diets contained a high amount of fat damage to the cellular homeostasis and synaptic plasticity in the brain. However, how the extracellular matrix is modulated by a high-fat diet has not been evaluated enough. In this study, we assessed how lard based high-fat diet affects extracellular matrix proteins and the behavior of mice. For this purpose, male mice fed by the high-fat diet for 8 weeks, and then the behaviors of mice were assessed by the elevated plus-maze, light-dark box test, open field test, novel object recognition test, and rotarod. Then in different regions of the brain, the protein level of Reelin, tenascin C, and neuroligin 2 was evaluated with western blot, and the number of perineuronal nets positive somas was assessed by immunofluorescence staining. Our results reveal that 8 weeks cause the tendency of decrease in memory, and difficulties in learning of mice, and decrease performance in involuntary motor function. Also, changes in the protein amounts and perineuronal nets showed regional differences, and these differences are mostly related to synaptic plasticity and long-term potentiation. In conclusion, the high-fat diet may alter the behavior of mice by affecting the extracellular matrix structure that provides the proper function of the synaptic plasticity.

Keywords: Obesity, Extracellular Matrix, Reelin, Tenascin C, Neuroligin 2, Perineuronal Nets, Synaptic Plasticity

ÖZET

Civas, C. C. (2020). Yüksek Yağlı Farelerde Bilişsel İşlevler, Duyusal ve Motor Davranış ve Ekstrasellüler Matris Proteinlerinin İlişkisinin İncelenmesi, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi, İstanbul.

Dünyada obezite en önemli sağlık sorunlarından biridir ve birçok insan obezite ve obeziteye bağlı hastalıklardan muzdarip olmaktadır. Yeme alışkanlıkları obezitenin gelişmesinde önemli bir rol oynamaktadır ve önceki çalışmalar, düşük kaliteli diyetlerin beyin üzerinde hafıza kaybı veya iştah ve tokluk mekanizmasında bozulma gibi olumsuz etkilerinin olduğunu göstermektedir. Beyindeki hücresel mekanizmalarda farklı diyet türlerinin etkileri geniş çapta araştırılmıştır ve çalışmalar yüksek yağ içeren diyetlerin beyindeki hücresel homeostaz ve sinaptik plastisitede hasara yol açıtğını göstermiştir. Bununla birlikte, ekstrasellüler matrisin yüksek yağlı bir diyet tarafından nasıl değiştirildiği yeterince değerlendirilmemiştir. Bu çalışmada, domuz yağı bazlı yüksek yağlı diyetin ekstrasellüler matriks proteininlerini ve farelerin davranışını nasıl etkilediğini değerlendirdik. Bu amaçla erkek fareler 8 hafta boyunca yüksek yağlı diyetle beslendi ve ardından farelerin davranışları, yükseltilmiş artı labirent, aydınlık-karanlık kutu testi, açık alan testi, yeni nesne tanıma testi ve rotarod ile değerlendirildi. Daha sonra beynin farklı bölgelerinde Reelin, tenascin C ve neuroligin 2'nin protein seviyesi western blot ile değerlendirildi ve perinöronal ağ pozitif somaların sayısı immünofloresan boyama ile değerlendirildi. Sonuçlarımız, 8 haftanın hafızada azalma eğilimine, farelerin öğrenme konusunda zorluklara ve istemsiz motor fonksiyonda performansın düşmesine neden olduğunu ortaya koymaktadır. Ayrıca, protein miktarlarındaki ve perinöronal ağlardaki değişiklikler bölgesel farklılıklar göstermiştir ve bu farklılıklar çoğunlukla sinaptik plastisite ve uzun vadeli potansiyalizasyon ile ilgilidir. Sonuç olarak, yüksek yağlı diyet, sinaptik plastisitenin düzgün işlevini sağlayan hücre dışı matris yapısını etkileyerek farelerin davranışını değiştirebilir.

Anahtar kelimeler: Obezite, Ekstrasellüler Matris, Reelin, Tenascin C, Neuroligin 2, Perinöronal ağ, Sinaptik Plastisite

1. INTRODUCTION and PURPOSE

Obesity is one of the world-leading diseases and 2.1 billion people are classified as overweight or obese[1]. Besides other factors, dietary patterns have an essential role to develop obesity and western diets have adverse effects on energy metabolism. Especially, a diet contains high fat and high sugar content affects body energy and nutrient homeostasis. Thus, dysfunction in energy metabolism is the main reason for obesity and insulin resistance. In addition, recent studies reveal that a high-fat diet may contribute to the development of neurodegenerative disease[2]. One of the pioneer studies shows that a high-fat diet causes a decrease in cognitive abilities in rats[3]. Later, another study shows that decreasing cognitive ability with high-fat diets can depend on insulin resistance and glucose metabolism[4]. Alteration in the memory by the diets may also link with changes in the synaptic plasticity, but this mechanism is not well explained[5,6].

Besides insulin resistance, inflammation is observed in different brain areas sourced from a high-fat diet. In some brain areas like the hypothalamus, hippocampus, cerebellum, and amygdala, inflammatory markers such as interleukin-1β, interleukin-6, TNF-α, and NFκB are upregulated and these inflammatory markers are linked to cognitive dysfunctions and neurodegenerative diseases[7,8].

In a study, researchers show that high-fat diets cause decrease perineuronal nets (PNN) which are specialized extracellular matrix structure, without gaining weight, but they showed just in the prefrontal cortex[9]. Another recent study shows that perineuronal nets decrease in the medial prefrontal cortex when rats are fed by high fat-high sugar diets[10]. The other study investigated the effects of the high-fat diet on perineuronal nets in different genders and regions. They found that while the high-fat diet causes decrease PNNs in the prelimbic prefrontal cortex (PL-PFC), and ventral medial orbital frontal cortex (vmOFC), any significant changes were not observed in the infralimbic (IL-PFC) in male mice. In the females, increases in PNN number just were observed in the IL-PFC, but any significant changes were not observed in the PL-PFC and vmOFC[11].

Besides the perineuronal net's structure, the mRNAs of the extracellular matrix protein reelin protein, which plays a role in LTP and neuronal plasticity, are significantly reduced when animals are fed high-fat diets for 10 weeks[12]. Another study about reelin

shows that high-fat diets cause to decrease reelin positive cells in the medial prefrontal cortex, but the same effect is not observed in the dentate gyrus, CA1, and CA3 region in the hippocampus[13]. The other recent study about diet-induced obesity on reelin shows that amount of reelin was increased in the hypothalamus when animals fed by a high-fat diet[14].

Literature shows that extracellular matrix proteins play an essential role in the proper function of synapse and they regulate synaptic plasticity. We have hypothesized that alteration of synaptic plasticity by diet-induced obesity may be sourced from changes in the extracellular matrix proteins. For this purpose, we have investigated three different extracellular matrix proteins are reelin, tenascin C, and thrombospondin-1 play key roles in the synaptic plasticity and synaptogenesis in the hypothalamus, hippocampus, cortex, and cerebellum. Besides, PNN is another significant extracellular matrix structure to regulate synaptic plasticity, and we have investigated the number of PNN positive neuronal soma in the prefrontal cortex, cortex, hippocampus, and hypothalamus. Also, cell adhesion molecules regulate synaptic plasticity and recent studies show that neuroligin 2 has an important role in regulating synaptic plasticity and excitatory-inhibitory synaptic balance[15]. It may be associated with thrombospondin-1, and for this reason besides the extracellular matrix proteins, we also have investigated neuroligin 2 in the condition the diet-induced obesity. Besides post-mortem analysis, assessment of behavior changes was performed to compare with post-mortem data.

2. LITERATURE REVIEW

2.1. Obesity

2.1.1. Definition of Obesity

. Obesity is the 21st-century epidemic, and it can be defined as an excessive deposition of fat. It may lead to several diseases such as cardiovascular disease, some types of cancer, and neurodegenerative diseases, and it may relate to a decline of about 5-20 years in a lifetime[16]. In addition to medical problems, obesity is associated with socioeconomic issues with a reduced quality of life that may result in decreased work efficiency and unemployment[17]. Excessive fat deposition is a result of an imbalance between caloric intake and energy consumption. It is generally believed excessive eating is the cause of obesity rather than the consumption of energy. However, the etiology of obesity is more complex, and several factors may be involved. These factors regulate energy intake, deposition, and energy consumption[1].

2.1.2. Measurement

Body-Mass Index (BMI) is the most common and primary criterion to determine the obesity situation of people, and it is calculated weight divided by the square of height (kg/m2). For adults, if the BMI of a person is more than 30 kg/m2, he/she is accounted as obese. However, in children and teenagers, obesity criteria change with age and gender[18]. This value is not directly correlated with body fat amount, but most of the time, it gives an idea about that. In the athletes and older people, to determine the amount of fat in the body, BMI is not useful[19]. In addition to BMI, waist circumference, and waist to hip ratio have become other parameters to determine obese people recently, and they are important to measure for abdominal fat deposition[20]. Other widely used methods are CT scan, MRI, and bioelectrical impedance[1].

2.1.3. Epidemiology

Prevalence and tendency of obesity are heterogenic between people. Roughly, 40% of the world population is classified as obese or overweight, and %10.8 of adult men and %14.9 of adult women are obese. Thus, the tendency of women about obesity is more than men[21]. In addition to gender, ethnicity is another factor to affect the trend of obesity. One of the studies in the United States shows that Afro-American people have more lean

than other ethnicities[22]. Besides adults, obesity is becoming an essential problem between children, and approximately, %5 of children is suffering from obesity[21].

2.1.4. Factors

A variety of factors can cause obesity, and the most straightforward reason for obesity is overeating due to low-calorie consumption. However, in most cases, the pathology of obesity is more complex, and generally, it includes several systems such as the nervous or endocrine system. For understanding the causes of obesity, several factors should be taken into account, such as behavioral, economic or genetic differences.

2.1.4.1. Genetic Factors

Numerous studies have shown that many genes involve developing obesity and until now, researchers have revealed more than 60 genetic markers associated with developing obesity[23,24]. Also, studies on twins show that obesity might be an inherited disorder, and the inheritance of BMI can be up to %70[18]. Besides twin studies, studies about family histories show that childhood obesity can be related to genes inherited by parents, and childhood obesity is a vital sign for obesity in adulthood[25,26]. Also, adoption studies indicate that the BMI of adopted children is more similar to their biological family instead of the adopted family[27]. These studies show that genetic factors might induce obesity.

Genes that contribute to the development of obesity sometimes are inherited with Mendelian genetics, and these genes may be inherited with autosomal or X-linked chromosomes[28].

2.1.4.1.A Syndromic Monogenic Obesity

More than 30 syndromic monogenic obesities are reported, and these are rarely seen. In these syndromes, obesity is formed with mental retardation and developmental abnormalities[29].

One of the most studied neurodevelopmental monogenic obesity syndromes is Prader-Willi Syndrome (PWS), and the prevalence of this syndrome is 1 in 15000-30000[30]. In this syndrome, slowed development, hypotonia, and difficulties in feeding are characterized in the early stage of life. Then, with cognitive disabilities, childhood obesity, and hyperphagia develop. Most of the health problems develop with obesity[31]. %75 of PWS cases are- sourced from three kinds of genetic abnormalities in paternal

genes located in 15q.11.2-q13 whereas only %20-30 cases are sourced from maternal unilateral disomy[32]. Five different genes may be affected mutations in PWS areas, and these genes are makorin ring finger protein 3 (MKRN3), Mage family member 2 (MAGEL2), Necdin (NDN), nuclear pore associated protein 1 (NPAP1), SNRPN upstream reading frame (SNURF-SNRPN)[33].

Bardet-Bield Syndrome[34], Alström Syndrome[35], Albright hereditary osteodystrophy[36], Börjeson-Forssman-Lehmann Syndrome[37], Kabuki Syndrome[38], and Wilms tumor, aniridia, genitourinary abnormalities, and Mental Retardation Syndrome[39] are other important syndromes which cause early-onset obesity.

2.1.4.1.B Nonsyndromic Monogenic Obesity

Mutations in some genes cause early-onset obesity without any syndrome, and these genes are mostly related to the leptin-melanocortin pathway[40] (Figure 2.1). Most studied genes are responsible for the expression of Leptin (Lep), Leptin Receptor (Lepr), Melanocortin 4 Receptor (Mc4r), and Proopiomelanocortin (POMC) regulate food intake and energy balance[41].

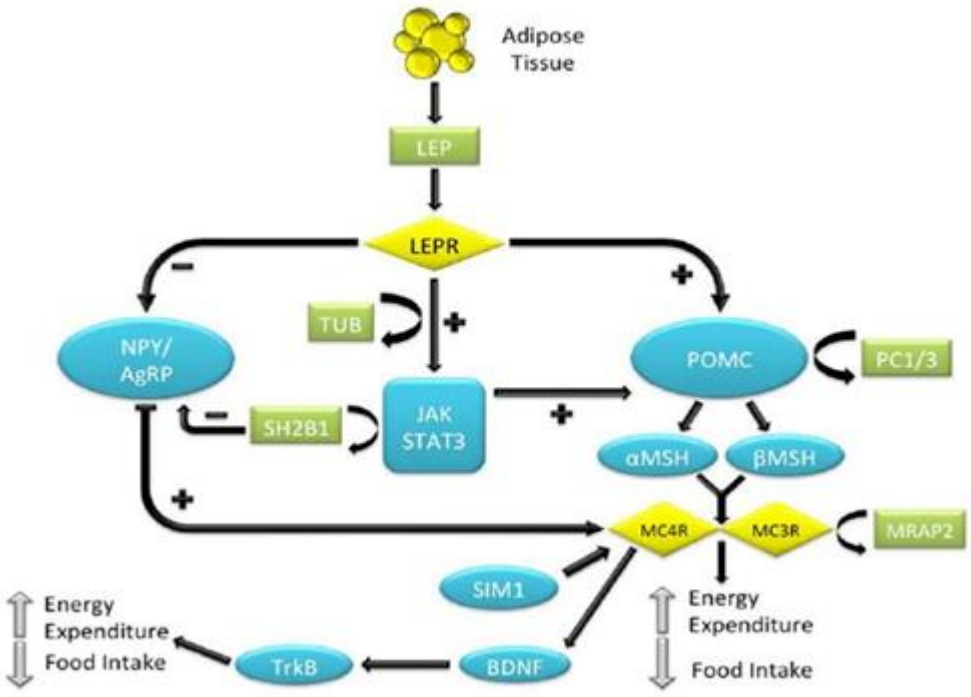

Figure 2-1 Leptin-melanocortin pathway and genes involved in the progression of obesity[41].

Mutations in the leptin or its receptor causes significant weight gain in the first year of life, and people who suffer from leptin mutations do not have any tolerance for food restriction[42]. Also, dysfunction in the leptin causes delayed puberty because of hypogonadism[43].

Besides leptin, mutations in the MC4R cause early rapid weight gain, hyperinsulinemia, and linear growth[44].

Another important protein to play a role in energy metabolism is POMC and mutations in the gene of this protein cause the recessive inheritance of obesity. Besides, the dysfunction of this protein causes hypopigmentation, hypocortisolism, and neonatal hypoglycemia[45,46].

Other genes responsible for nonsyndromic monogenic obesity are SH2B adaptor protein 1 (SH2B1)[47], neurotrophic tyrosine kinase receptor type 2 (NTRK2)[48], brain-derived neurotrophic factor (BDNF)[49], single-minded homologue 1 (SIM1)[50], kinase suppressor of Ras 2 (KSR2)[51], and Tubby bipartite transcription factor (TUB)[52].

2.1.4.2. Effects of Quality of Diet

One of the most major factors in the development of obesity is the quality of diets. Many researchers have conducted studies on these issues, and they showed how dietary patterns affect the development of obesity. The largest study has been run about obesity is the Nurses' Health Study (NHS), and they have produced valuable data about dietary effects on weight gain[53]. In this study, the relationship between dietary style and weight gain have been investigated. Their results reveal that diet contains potato chips, potatoes, sweetened beverages, processed meat, and red meats are mostly associated with weight gain. Besides, yogurt, nuts, fruits, vegetables, and whole grains are conversely related to weight gain (Figure 2.2).

In most of the cases, caloric restriction is applied in the clinics to weight loss[54]. Along with caloric restriction, various dietary patterns are studied how they affect weight loss[55,56]. Studies show the Mediterranean diet and low carbohydrate diets provide more weight loss than low-fat diets. Also, the PREDIMED study reveals that Mediterranean diets that are contained high unsaturated fat sourced from olive oil and nuts may provide weight loss because people consumption this diet shows the trend toward the loss of weight and lower waist circumferences[57,58].

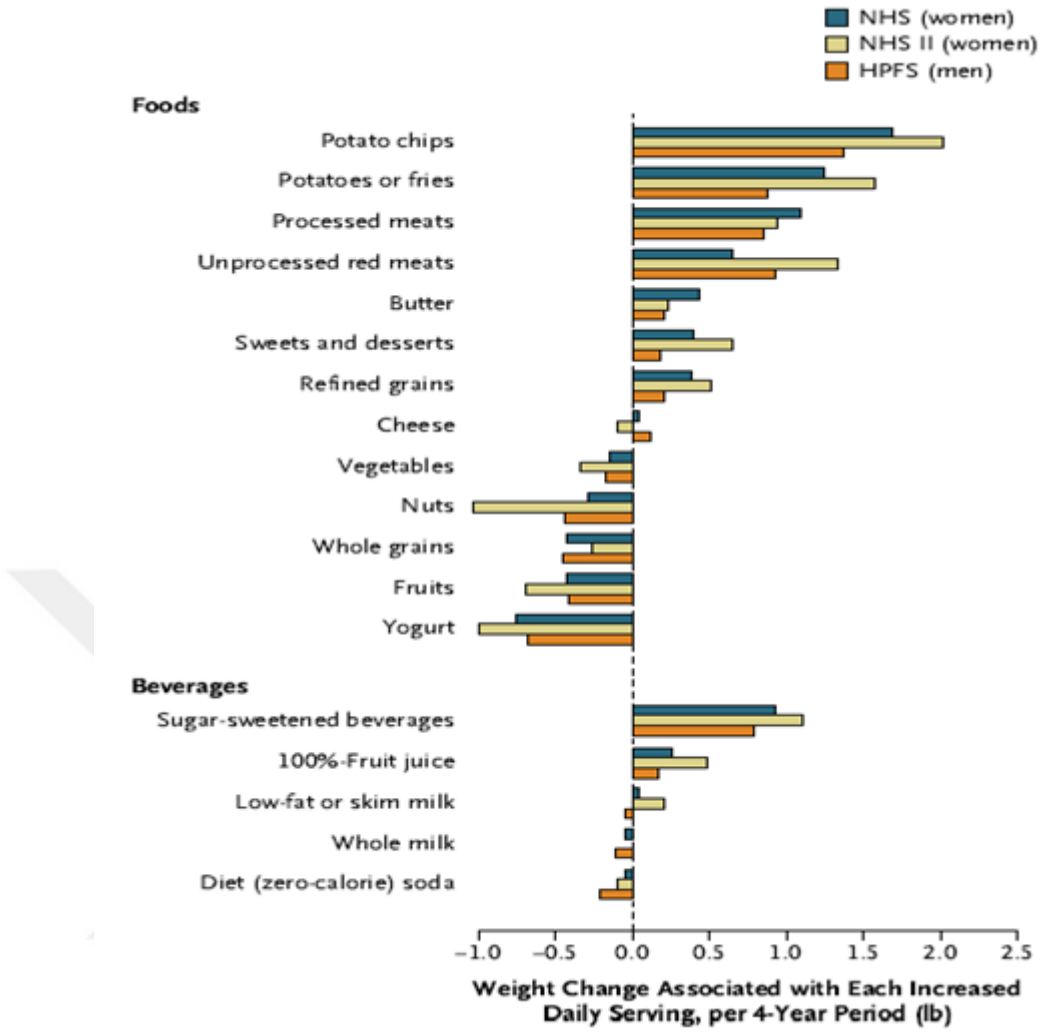

Figure 2-2 Association between food and weight change per 4-year period. (NHS (Nurses' Health Study from 1986 to 2006 involved 50422 women), NHS II (Nurses' Health Study from 1991 to 2003 involved 47898 women), HPFS (Health Professionals Follow-up Study from 1986 to to 2006 involved 22557 men)[53].

2.1.4.3. Effects of Lifestyle

In the NHS, the impact of physical exercise and sleep on weight gain were investigated. Their result reveals that increasing physical exercise has directly correlated with less weight gain. About the sleep, the ideal period of sleep is 6-8 h, and below 6h or above 8h, people tend to gain weight. Also, they show that increasing sedentary lifestyles such as watching television increases weight gain[53]. In addition, the American College of Sports Medicine recommended physical activity at least 150 minutes per week, but for long term weight loss, 250 minutes per week was recommended[59].

Other studies about sleep demonstrate that especially for young adults and adolescents, a shorter period of sleep than normal period causes more weight gain. The proposed mechanism about sleep deprivation is related to more calorie intake and less energy spent[60] (Figure 2.3).

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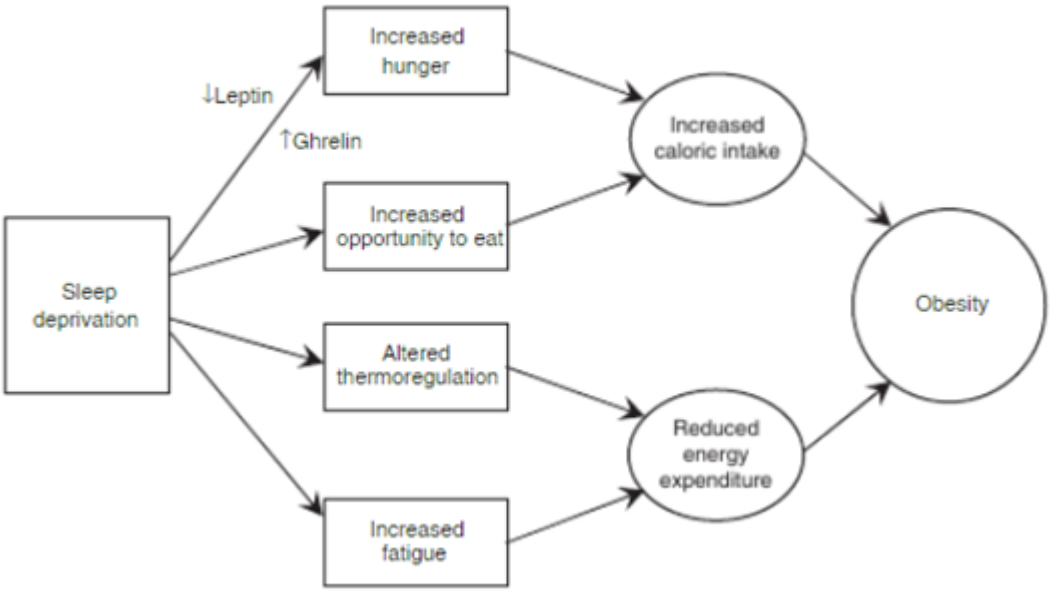


Figure 2-3 Relationship between sleep deprivation and obesity[60].

2.1.4.4. Socioeconomic Risk Factors

Worldwide, obesity is more prevalent in wealthier countries such as the USA. Besides, when countries have become richer, their prevalence of obesity has increased like in India and China. However, obesity is more common in poor people who live in the USA. This situation may be linked with access to fresh food and sedentary lifestyles[54,61,62].

2.1.4.5. Maternal Nutrition

Obesity may appear in developing fetus in the womb of a mother, and birth weight is one of the signs of obesity in childhood. Both high and low birth weight is a risk factor for obesity expenditure[63-65]. Another risk factor in infants is rapid weight gain[66]. Even before pregnancy, the feeding habits of both parents may affect the metabolic regulations

of infants[67]. Studies show that diet contains sugar-rich beverages and fast food in maternal consumption causes gestational diabetes and childhood obesity[68,69].

2.1.5. Role of Adipose Tissue

Two different types of adipose tissue are found in the body; brown adipose tissue (BAT) and white adipose tissue (WAT). These tissues play essential roles in energy homeostasis and regulate thermogenesis[70]. BAT mainly responsible for regulating body temperatures with consuming energy. Decreasing BAT is related to increasing BMI, and they are negatively correlated[71]. The other kind of adipose tissue is WAT is mostly related to the deposition of energy. Besides, in recent years, novel functions of adipocytes tissues discovered, and they release molecules called adipokines[72]. One of the well-known molecules released from adipocytes is leptin which controls satiety and appetite by acting on the hypothalamus[73].

Based on location, two distinct types of adipose tissue are found that are called subcutaneous and visceral adipose tissue. One of the located places of visceral tissue is the intra-abdominal area, and the accumulation of adipocytes in this area is mostly related to metabolic problems. Increasing visceral adipose tissue raises the risk of insulin resistance and cardiovascular diseases. In addition, visceral fat deposition is related to storing fat in the liver, heart, kidney, and pancreas. These depositions cause atherosclerosis, diabetes or cirrhosis[74].

2.2. Brain and Obesity

2.2.1. Appetite and Satiety Mechanism on Brain

The feeding regulation mechanism is tightly regulated, and it involves gastrointestinal, endocrine, and nervous systems. The hypothalamus is a critical site to regulate energy homeostasis in the brain, and two distinct neurons groups located in the arcuate nucleus (ARC) play an essential role. These are agouti-related peptide (AgRP)/ neuropeptide Y (NPY) neurons and POMC/cocaine and amphetamine regulated transcript (CART) neurons. These neuron groups work controversially, and AgRP/NPY neurons play an orexigenic role, and POMC/CART neurons play an anorexigenic role[75,76] (Figure 2.4). These neurons are affected by released hormones from adipose tissues and pancreas, which are leptin and insulin, respectively. These hormones enter the brain via passing blood-brain barrier (BBB), and they bind their receptor located in AgRP/NPY and POMC/CART neurons[77].

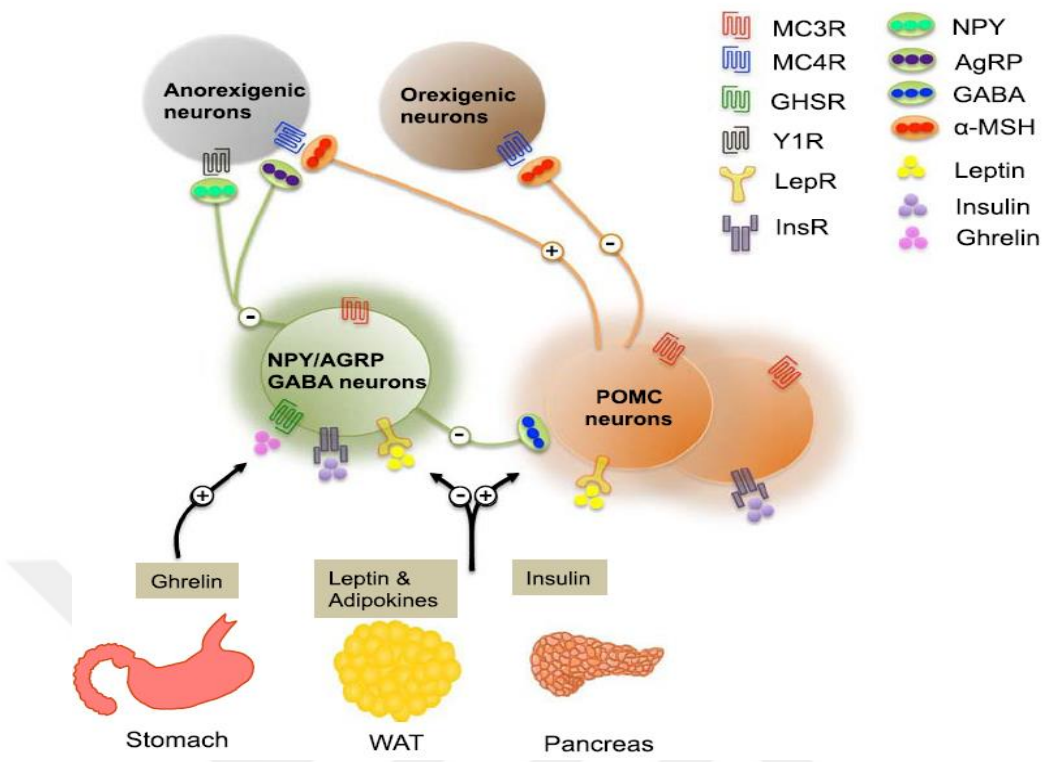


Figure 2-4 Mechanisms regulate appetite and satiety by the central melanocortin system[78].

The insulin hormone binds their receptor, and it activates phosphatidylinositol 3-kinase (PI3K). After the downstream of this pathway, forkhead box protein O1 (FoxO1) is inhibited. This protein is a transcription element, and it plays as an activator of the AgRP promoter, whereas it behaves inhibitory role for POMC promoter[78,80]. Like insulin, leptin has its receptor on POMC neurons, and when leptin binds leptin receptor, it activates Janus kinase (JAK)/ signal transducers and activators of transcription (STAT) pathway. Upon activation of STAT3, it induces gene expression of POMC[81]. AgRP and POMC neurons are projected to the paraventricular nucleus of the hypothalamus from the ARC, and these neurons release AgRP protein, and a-melanocyte stimulating hormone (a-MSH), respectively. These molecules stimulate melanocortin 3 and 4 receptors (MCR3/4) of neurons located in PVN, and these neurons have directed these signals to the nucleus tractus solitaries (NTS) located in the brain stem. These signals modulate feeding behaviors[82]. Another essential neuron group to maintain feeding behavior is steroidogenic factor-1 (SF-1) neurons located in the ventral medial nucleus of the hypothalamus (VMH), and they directly interact with POMC neurons to regulate

activation of anorexigenic response[83]. Besides POMC/CART neurons, PVN neurons release hormonal signals to regulate anorexigenic and thermogenesis responses that are thyrotropin-released hormones (TRH) and corticotropin-releasing hormones (CRH)[84,85].

Nevertheless, AgRP neurons secrete orexigenic response to stimulate food intake, and it is stimulated by ghrelin hormones released from the stomach[78,86]. These neurons release NPY, and it stimulates food intake by activating the NPY Y1/5 receptor. They mostly induce other neurons located in the lateral hypothalamus, and they release orexigenic and anti-thermogenic factors with melanin-concentrating hormone (MCH)[87]. This regulation system for appetite and satiety regulation is called the melanocortin system[85] (Figure 2.5).

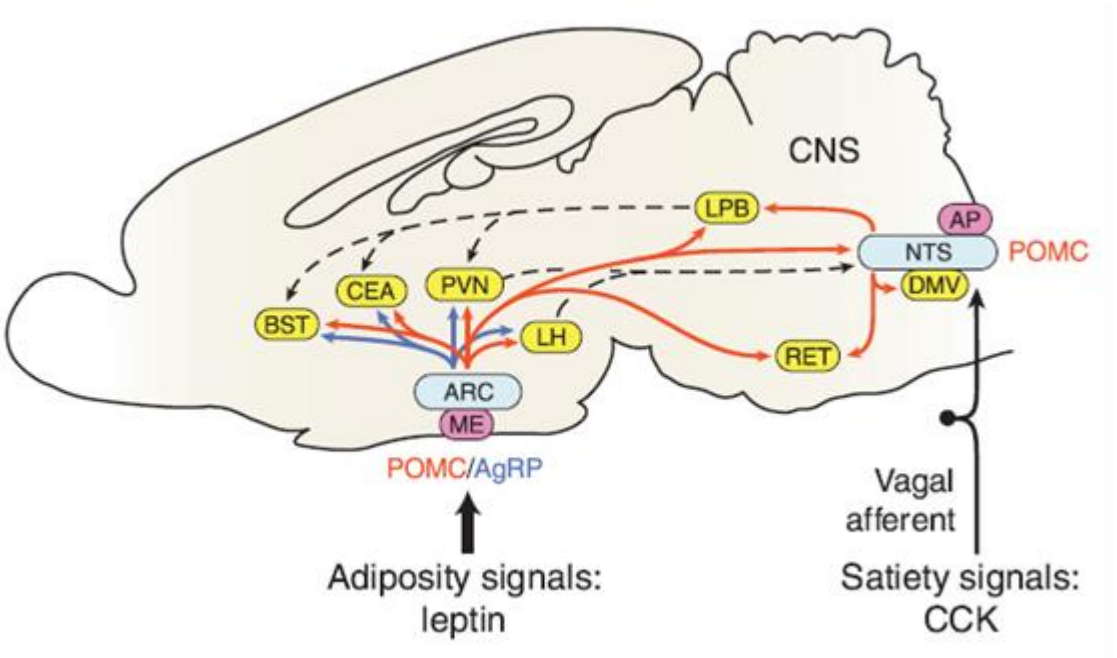


Figure 2-5 Central regulation of melanocortin system[85].

2.2.2. Inflammation Response of Brain to Obesity

Inflammation is a primary defense mechanism for tissue damage and foreign substances[88]. Previous studies show that overfeeding induces inflammatory signals in the hypothalamus[89]. The first study about the inflammatory effects of high-fat diet shows that high-fat diet induces c-Jun-N-terminal kinase (JNK) and nuclear-factor-κB (NFκB) pathway and these pathways enhance expression of interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) as proinflammatory factors. These molecules alter the

anorexigenic insulin signaling[90]. Subsequent studies demonstrate that astrocytes and microglia are vital components to induce inflammatory signals under the high-fat diet[91-94]. Inflammation response to HFD is observed in the 1 to 3 days consumption period in the ARC[95]. In the acute feeding of HFD, glial cells induce expression of neuroprotective molecules such as heat shock protein 70 (HSP70), and ciliary neurotrophic factor (CNTF) during gliosis. However, in chronic consumption, glial cell lost their neuroprotective ability, and they enhance the expression of TNF-α and promote positive energy regulation[96]. Studies show that inflammation signals in glial cells are activated by long-chain saturated fatty acid (SFAs) with inducing gliosis[97-99].

2.2.3. The mechanism behind Inflammation in Brain

Previous studies established that the most well-explained mechanism about the inflammatory effects of HFD is through toll-like receptor 4 (TLR4). This receptor is a kind of pattern recognition receptors (PPRs), which is activated by pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS)[100-102]. Triggering TLR4 by LPS enhances the expression of pro-inflammatory cytokines such as TNF-α with stimulating the NFκB pathway[103]. Because of structural similarity, SFAs activate the TLR4 by mimicking LPS[104]. Many researchers mention that some SFAs play as a ligand for TLR4, such as palmitic acid or lauric acid, and they activate the inflammatory cascades[94,100,102,104,105]. Contrary, one the recent study propose that SFAs do not trigger the inflammatory pathway via TLR4, but they induce the JNK pathway in a different way. Besides, TLR4 contributes to the inflammatory response of SFAs indirectly[106].

Another proposed mechanism about the modulation of insulin resistance is related to ceramide, which is a kind of sphingolipid synthesized from palmitate. Researchers show that ceramides act as inhibitors of insulin signaling, and their inhibition rescues glucose metabolism in obesity and diabetes models[107]. Ceramides manipulate insulin action via inhibiting Akt/PTB pathway. Previous studies demonstrate that ceramides directly activate protein kinase Cζ and protein phosphatase 2A (PP2A) that inhibit Akt/PTB cascades[108]. Further suggesting mechanism that enlightens the adverse effect of palmitic acid is linked to the PKCθ translocation to the cell membrane of AgRP/NPY neurons, and it is associated with decreasing the activity of insulin signaling[109]. An *in vitro* study with NG2 hypothalamic cell line supports the hypothesis about the deleterious effects of palmitic acid through ceramide partially, but it is not related to TLR4[110].

Endoplasmic reticulum (ER) stress is another proposed player to have a role in insulin resistance. ER is the main site responsible for protein synthesis, folding, and trafficking. Functional abnormalities in the ER cause the misfolding proteins, and it is called unfolded protein response (UPR). Improper function of ER causes the activation of inflammatory pathways and leads to insulin resistance and obesity[111]. Researchers show that inducing ER stress in the hypothalamus via injection of thapsigargin (TG) promotes the insulin and leptin resistance. Besides, this induced stress causes an increase in food intake and body weight. They also show that DIO causes the ER stress in the hypothalamus and enhances the activation of the spliced form of X-box binding protein 1 (Xbp1s), C/EBP homology protein (CHOP), glucose-regulated/binding immunoglobin protein-78, and inositol-requiring kinase-1(IRE1) that are ER stress markers[112]. Another group investigated the effects of palmitate on a hypothalamic neuronal cell line. Their results indicate that palmitate induces ER stress molecules eukaryotic translation initiation factor 2A (elF2A) and Xbp1 via JNK cascades[113]. However, the role of Xbp1s is not clear in the hypothalamus, and Williams et al. show that the constitutive expression of Xbp1s in POMC neurons restores insulin sensitivity[114]. As stated in previous studies, it is evident that ER stress is contributed to the inflammation pathway with feeding HFD[89].

2.2.4. Effects of Obesity on Cognition and Behaviors

Besides genetic models, diet-induced obesity models are commonly used to study the effects of obesity. In this model, animals are fed by special diets that contain a high amount of fat or sugar.

The first study about the effects of saturated fatty acid on cognition was published in 1990, and in this study, Long-Evans rat, fed by 20% fat diets that contain a high amount of saturated fatty acid (lard-based) or polyunsaturated fatty acids (soybean oil-based) or standard chow diets. The performance of rats was assessed three different tests that are Olton's radial arm maze, a variable-interval delayed alternation task, and Hebb-Willams maze and results show that while animals fed by polyunsaturated fatty acids showed relatively less memory impairment when compared to chow-fed animals. However, animals fed by saturated fatty acids had serious impairment effects on learning and memory performance[3]. After this study, mechanisms behind memory impairment that is caused by fatty acid are investigated, and the same research group proposed that insulin

resistance might be one of the mechanisms to play a role in difficulties in learning mechanism and memory retardation. They investigated both epidemiological studies and rodent studies[115].

Another affected behavior from dietary content is anxiety and depression behavior. One of the studies shows lard based high-fat diet-fed mice performed more anxiety-like behavior than mice fed by fish oil-based high fat diet both 3 days and 4 weeks period[116]. Besides, another study shows that 4 months feeding by a high-fat diet caused anxiety-like behavior and increased corticosterone levels by inducing the innate immune system[117]. There are other studies to show the effects of dietary types on anxiety-like behaviors in rodents[118-122].

Dietary types affect also motor behavior and one of the studies illustrated that western diets reduced locomotor activity in both acute and chronic consumption[123]. The other study showed that despite the corn oil-based high-fat diet reduced locomotor activity after 6 weeks feeding, olive oil-based high fat diet did not have a significant effect on locomotor activity[124].

2.3. Extracellular Matrix Structure

Extracellular matrix (ECM) encircles the cells inside the brain and they fill up to 20% of the brain[125]. These structures play essential in the development of the brain and it modulates synaptogenesis, axonal guidance, and cell migration. In adults, ECM is involved in tissue repairing and synaptic plasticity. Basement membrane (BM), perineuronal nets (PNN), and perisynaptic nets are three different compartments of the brain extracellular matrix and their matrix is mostly composed of a kind of glycosaminoglycan (GAG) that is hyaluronan (HA)[126-128].

Besides HA, other main components of ECM are sulfated proteoglycans, chondroitin sulfate proteoglycans (CSPGs), tenascin R, and link proteins, and the basement membrane is composed of collagen type IV, laminin, fibronectin, and heparin sulfate proteoglycans (HPSGs) surround blood vessel in CNS to join blood-brain barrier (BBB) structure. Also, there are several glycoproteins, growth factors, membrane receptors, and cell adhesion molecules to interact with these main components[126,129] (Figure 2.6).

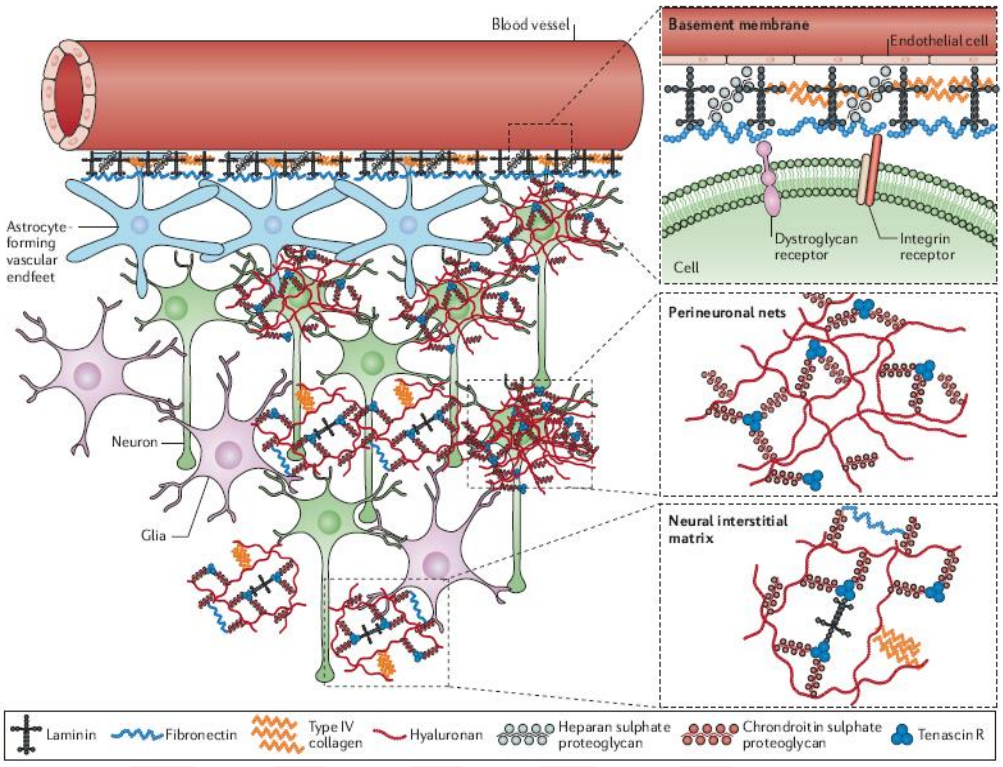


Figure 2-6 Composition of Extracellular Matrix that are Perisynaptic Nets, Perineuronal Nets, and Basement Membrane[127].

2.3.1. Hyaluronan

HA is formed by repeating a long chain of N-acetylglucosamine and glucuronic acid. These polymer chains are extruded by hyaluronan synthase (HAS) that is located at plasma membranes of neurons. There are isoforms of HAS is found in the mammalian body that are HAS1, HAS2, and HAS3, and their expression changes in different parts of the brain[130,131]. All three are located in the plasma membrane of the neuronal soma, but just HAS1 is also located in the axonal membrane[132]. The polymer structure of hyaluronan forms a backbone for ECM structure and several proteins bind this structure. Their structure can vary in size and biophysical properties. Besides, HA acts signaling molecules and it binds several receptors that are CD44, the receptor for HA-mediated motility (RHAMM), Stabilin-2, lymphatic vessel endothelial hyaluronan receptor-1 (LYVE), and HA-binding Toll-like receptors (TLR-2 and TLR-4) [129,133].

2.3.2. Proteoglycans

In the CNS, two fundamental types of proteoglycans are found and these are CSPGs located in mostly PNN, and HSPGs located in the basement membrane. The core protein of proteoglycans is linked to multiple GAGs chains, and the GAG is formed by polymers of disaccharides that are amine derived from galactose (N-acetylgalactosamine, GalNAc) or glucose (N-acetylglucosamine, GlcNAc) bonded to galactose (Gal) or uronic acid (glucuronic acid, GlcA, or iduronic acid, IdoA)[134]. GAG is started to synthesize from the tetrasaccharide structure (xylose-galactose-galactose-glucuronic acid) by Golgi Apparatus with xylosyl transferase 1. Amino sugar or uronic acid groups are sulfated by sulfotransferases[135] (Figure 2.7). Besides hyaluronan, four distinct types of GAG are found in the brain their repeated units are different from each other, and they are attached to a core protein. Repeated unit of chondroitin sulfate (CS) is formed by GlcA and GalNAc, heparin sulfate (HS) is formed by glucuronic acid and GlcNAc, dermatan sulfate (DS) is formed by IdoA and GlcNAc, and keratin sulfate (KS) is formed by Gal and GlcNAc[136].

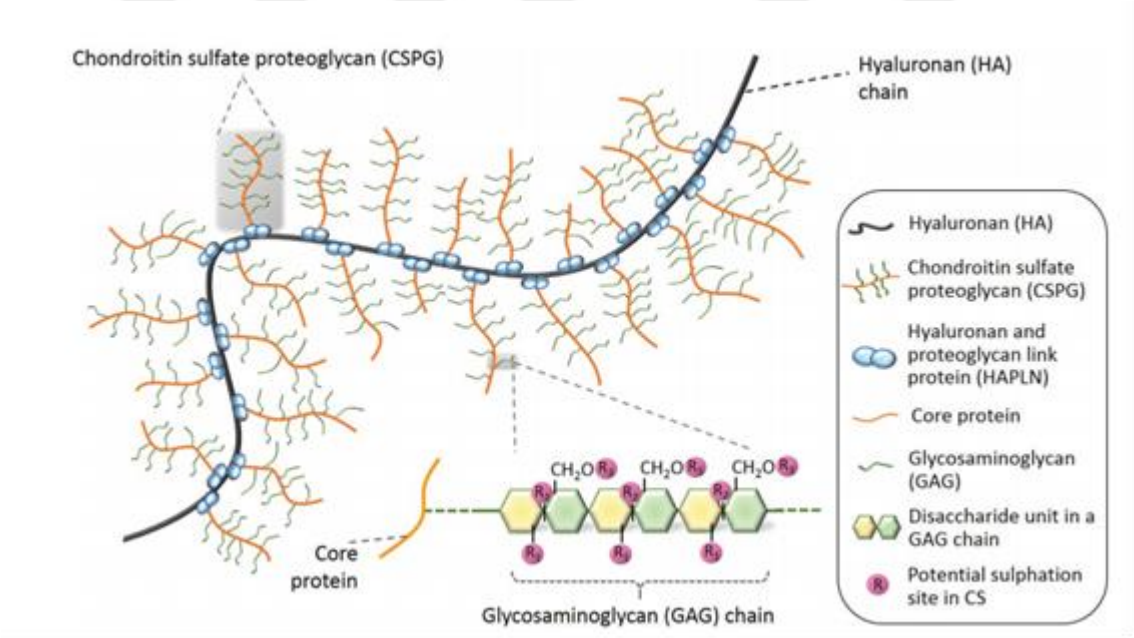
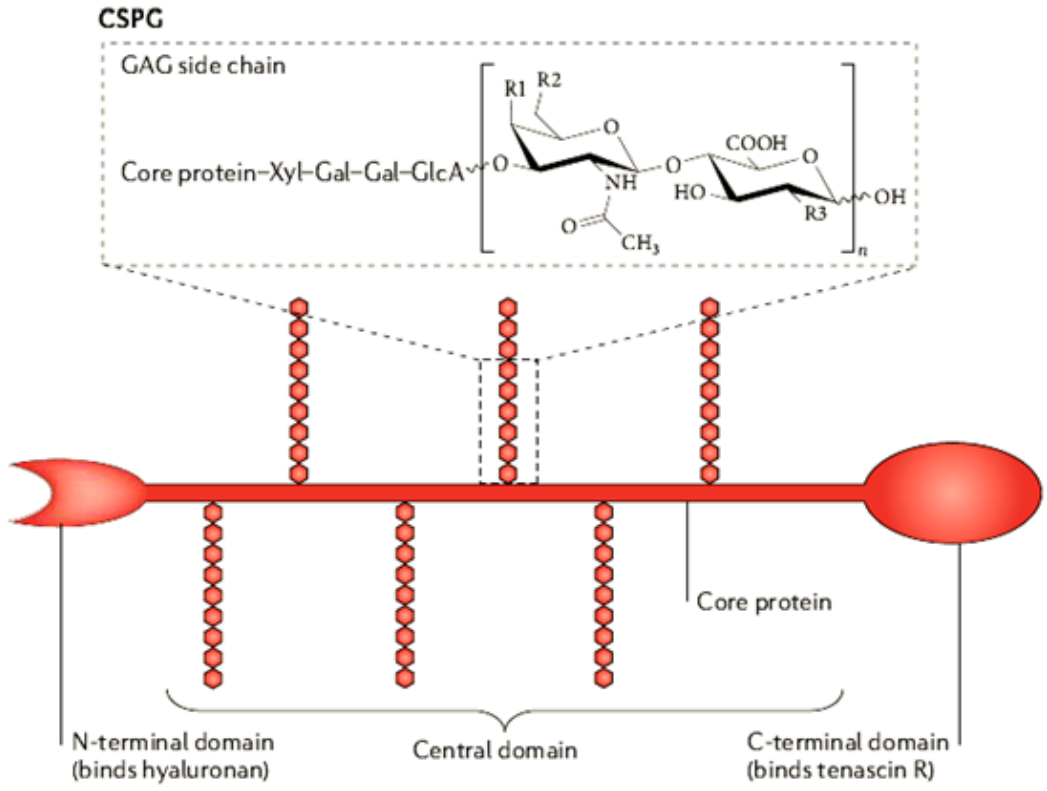


Figure 2-7 Structure and Aggregation of Chondroitin Sulphate Proteoglycans (CSPGs)[137].

The major population of proteoglycans of the central nervous system is CSPG and at least 16 different core proteins may bind CS to form CSPG. These proteins are mostly synthesized by astrocytes[138-140]. There are several CSPGs groups are found and the most common group is lecticans which are also called hyalectans[141]. Four main types of lecticans are found in the CNS and these are aggrecan, versican, neurocan, and brevican. All of them are found in the ECM, but brevican may also link glycosylphosphatidylinositol on the cell membrane[142,143]. These lecticans have two main regions that are an N-terminal globular G1 region and a C-terminal globular G3 region. N-terminal globular G1 region contains two important domains that are immunoglobulin(Ig)-like loop and a hyaluronan-binding domain, and the C-terminal globular G3 region contains three important domains that are C-type lectin domain, epidermal growth factor (EGF)-repeats, and a complement regulatory protein-like motif[144,145]. G1 region binds the HA with link proteins and the G3 region mostly interacts with Tenascin-R[126] (Figure 2.8).



Gal, galactose; R, sulphate attachment site; Xyl, xylose.

Figure 2-8 Structure of proteoglycans and their domains[127].

2.3.3. Link Proteins

Link proteins provide stabilization between the G1 region of proteoglycans and the HA chain. These proteins are a member of the hyaluronan- and proteoglycan-binding link protein gene family (HAPLN), and this family has four different members[143,146]. These proteins are called HAPLN1, HAPLN2, HAPLN3, and HAPLN4 and except for HAPLN3, the other three proteins are expressed in the nervous system[147]. Their structures are similar to each other and all of them contain an N-terminal signal sequence, an Ig domain, and two consecutive link modules (or proteoglycan tandem repeat) which are necessary to stabilize CSPGs and HAs[148]. HAPLN2 is mostly related to the myelinated axon and it expresses together with versican at the nodes of Ranvier which is called perinodal nets[149]. HAPLN1 and HAPLN4 are mostly located in the perineuronal nets and colocalized with versican and neurocan respectively[143]. Both link proteins are necessary for the proper function of perineuronal nets and perinodal nets[150,151].

2.3.4. Tenascins

In the mammalian body, four distinct types of tenascins are found, but only Tenascin R (TnR) and Tenascin C (TnC) are expressed in the CNS. These molecules are multimeric glycoproteins that cross-linked with proteoglycans and are mostly expressed in the neurogenic niche[152]. Tenascin R is the most commonly expressed tenascin in the CNS. Two different isoforms are found which are 160kDa and 180kDa and they probably form dimers and trimers structure together. After the translational period, post-translational modifications are done with sulfated oligosaccharides[153]. These modifications contribute to the function of TnR such as adhesion, migration, and motility[153,154]. Tenascin R binds lecticans in the fibronectin III repeats at the G3 domain with a calcium-dependent manner[155] (Figure 2.9). Tenascin R plays various roles depend on target cells and receptors such as promotes growth or adhesion and may show opposite effects of these functions like inhibits growth[156].

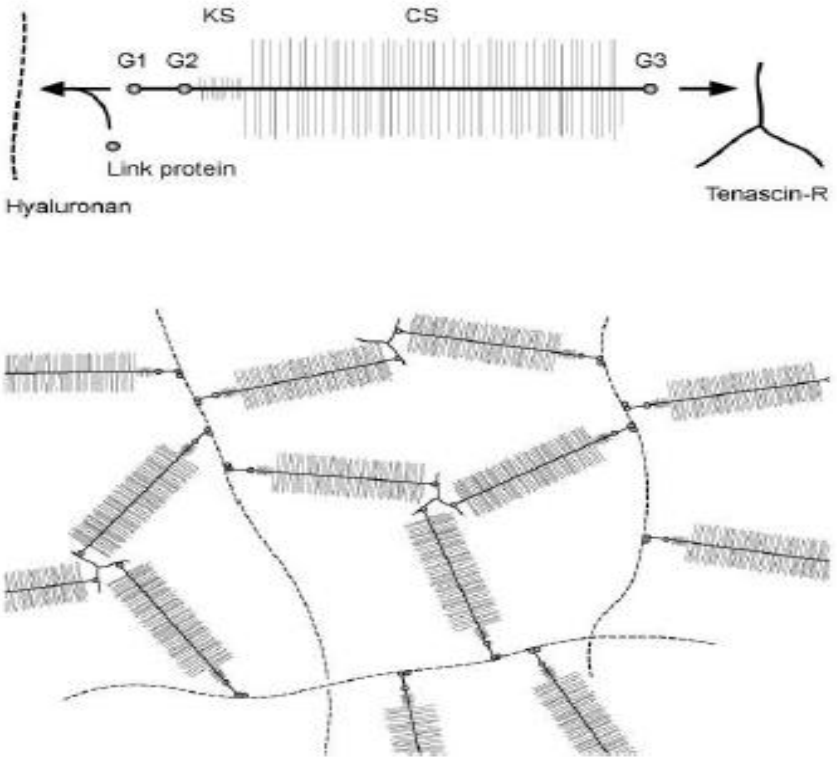


Figure 2-9 Binding of Tenascin R to proteoglycans and assembly of this structure[155].

2.3.4.1 Tenascin C

Tenascin C is another kind of tenascins expressed in the central nervous system, and it is the assembly as hexamer called hexabrachion. Mouse tenascin C is formed by 14.5 EGF-like domains, 8 main and 6 alternative spliced FNIII-like domains. In the mouse CNS, 27 alternatives of Tenascin-C were identified and splicing of this isoforms is regulated by homeobox transcription factor Pax6 in mouse neural stems[157,158]. Besides EGF-like and FNIII-like domains, it contains a tenascin assembly (TA) domain, cysteine residues, and four α-helical heptad repeats[159,160] (Figure 2.10).

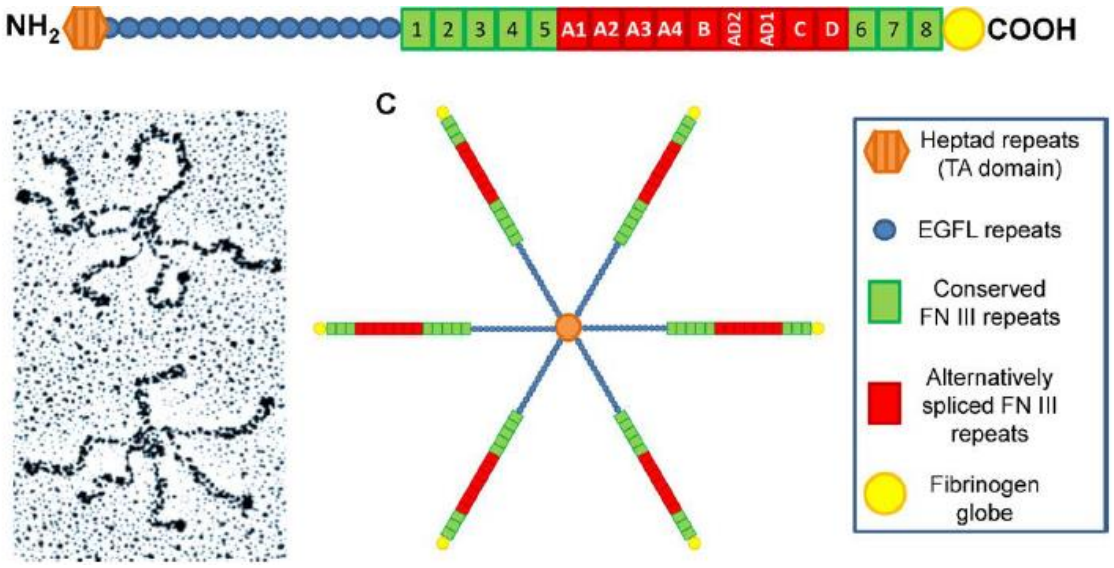


Figure 2-10 Structure of peptides of Tenascin C and their assembly as a hexabrachion[161].

TnC may interact with several surface receptor and its receptors are integrins, cell adhesion molecules(CAMs), phosphacan/receptor protein tyrosine phosphatase beta/zeta (RPTP z/b), and annexin II[162-164]. It also interacts with proteoglycans and fibronectin[165-167].

In the central nervous system development, tenascin C is expressed by radial glial cells, then, it is mostly expressed by astrocytes[168,169]. TnC modulates stem cells like neural stem cells precursor cells or oligodendrocytes precursor cells at embryonic day E14-15 such as maturation and proliferation[158,170,171]. In the adult brain, expression of TnC is downregulated, but it continues to express canonic neurogenic zones in the hypothalamus and cell types like Golgi epithelial cells in the cerebellum[172-175].

Due to their diverse isoforms, TnC can play various roles in both developing and adult brains. For example, it contributes to the regulation of cell migration by both its adhesive and anti-adhesive properties[176-179]. Besides, both these features provide directing axonal growth, guidance, and growth cone deflection[180-182]. Studies show that TnC both induces, and inhibits cell proliferation[183,184]. Another dual function of TnC is about apoptosis and it both suppresses and induces apoptosis[170,185]. Also, several researchers reveal that TnC contributes to the synaptic plasticity in the hippocampus and cerebellum[161,186,187]. Studies show that TnC plays a significant role in the formation of long term potentiation (LTP) in the CA1 region of the hippocampus and this mechanism

is a dependent L-type voltage-dependent Ca2+ channel (L-VDCC)[188]. Two different mechanisms are proposed for the action of TnC. One of them is TnC interacts with their integrin receptor and it activates L-VDCC, another mechanism is L-VDCC directly activated by TnC[161,189-191] (Figure 2.11).

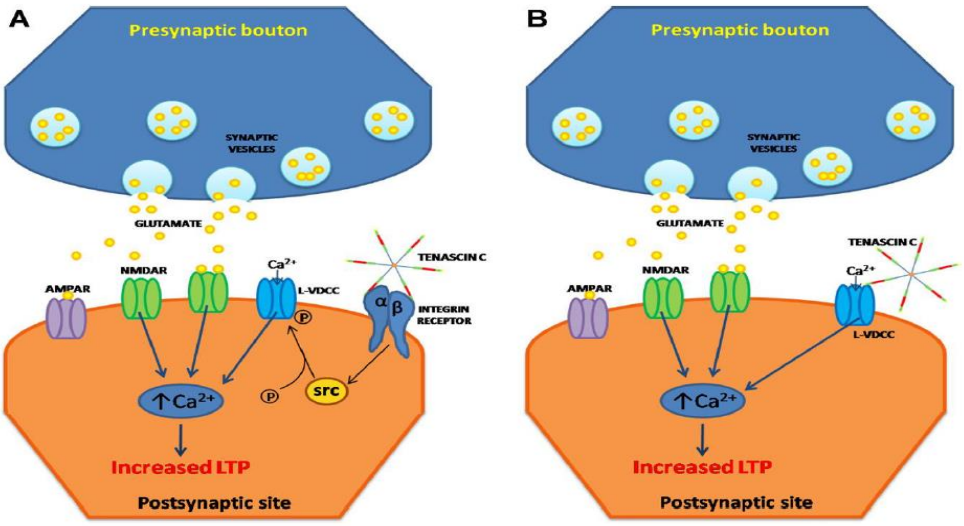


Figure 2-11 Proposed mechanism of TnC action on LTP via L-VDCC A) indirect action of TnC on L-VDCC with activating integrin receptors B) direct action of TnC on L-VDCC[161].

2.3.5. Reelin

Reelin is a glycoprotein that has 440 kDa molecular mass, and it has been widely studied. Studies show that reelin is necessary for normal brain development and it plays a significant role in neural migration. Besides neuronal migration, it plays a role in dendritic growth, dendritic spine development, and synaptic plasticity[192,193].

During the embryonic development, reelin is secreted by Cajal-Retzius cells in the marginal zone of the hippocampus and cerebral cortex[194-196]. Besides, it is expressed by granule cell precursor in the granular layer of the embryonic cerebellum[194,197]. Reelin regulates neuronal migration in migrating neurons and radial glial cells with two different lipoprotein receptors that are ApoER2 and VLDLR[198,199]. After the binding of reelin to lipoprotein receptors, these receptors form oligomer and activates adaptor protein disabled-1 (Dab-1) with phosphorylated by Src kinases[200]. This phosphorylation is mediated by Fyn kinases and it initiates further key steps of Reelin signaling[201]. It recruits

phosphoinositide 3-kinase (PI3K), Crk/CrkL, Lis1 to further actions[202-205] (Figure 2.12). This signaling cascade mediates neuronal migration and formation of the cerebral cortex layer[206].

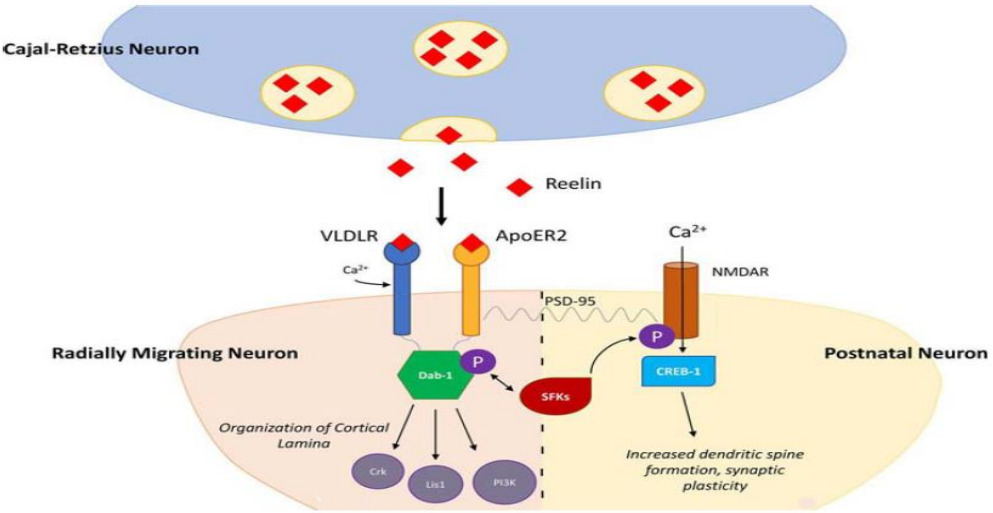


Figure 2-12 Signaling cascades of Reelin action in neural migration[207].

Besides neuronal migration, synapse formation, and synaptic plasticity are the main roles of reelin[208]. The presence of reelin in the hippocampus induces long term potentiation (LTP) significantly by binding lipoprotein receptors VLDLR and ApoER2[209]. Further studies reveal that Reelin regulates LTP via activating the NMDA receptor[210,211].

2.3.6. Thrombospondins

Thrombospondins (TSP) are glycoproteins has five different members (TSP-1, TSP-2, TSP-3, TSP-4, TSP-5), and they all contain conserved C-terminal region, calcium-binding type 3 repeats, and epidermal growth-(EGF)-like repeats[212]. Thrombospondins interact with various kinds of structures and receptors such as ECM, growth factors, and cytokines[213]. As a receptor, they bind integrins, neuroligins, lipoprotein receptors, and growth factor receptors[214].

TSP1 and TSP2 are expressed by astrocytes during development and adult life[212,215]. Studies reveal that TSP1 and TSP2 are a key factor in the formation of excitatory synapses[215,216]. They control synaptogenesis via neuronal gabapentin α2δ-1 and EGF-like domains of TSP bind the von Willebrand Factor A domains of the

receptor[216]. However, induced synapses by TSP1 are postsynaptically silent, so other factor needs to activate this synapses[214].

2.3.7. Perineuronal Nets Structure

Perineuronal nets is a condensed ECM structure that surrounds soma of determined neurons. In the cortex, the structure of perineuronal nets is found mostly around the parvalbumin-expressing (PV+) GABAergic interneurons, but it is also found pyramidal neurons in deep cortical layers[217-219]. In the CA2 region of the hippocampus and the amygdala, some excitatory neurons are encircled by PNNs[220]. Most of the PNNs are stained by Wisteria floribunda agglutin (WFA), but recent studies reveal that there are also WFA negative PNNs in the CNS[217].

In the PNN, the main structure is formed by hyaluronan and lecticans family of CSPGs, but mostly aggrecan joins the structure of PNN. The other three members of lecticans are also found in the PNNs[144,221,222]. In this structure, CSPGs bind the hyaluronan backbone with link proteins (HAPLN1, HAPLN4), and other sides of the lecticans link with tenascin R[223]. Especially, brevican makes strong interaction with TnR and HAPLN4, and it has different actions from other members of the lecticans family[224-227]. Other molecules that join the structure of PNN are semaphorin 3A, neurotrophins, and OTX2[219] (Figure 2.13).

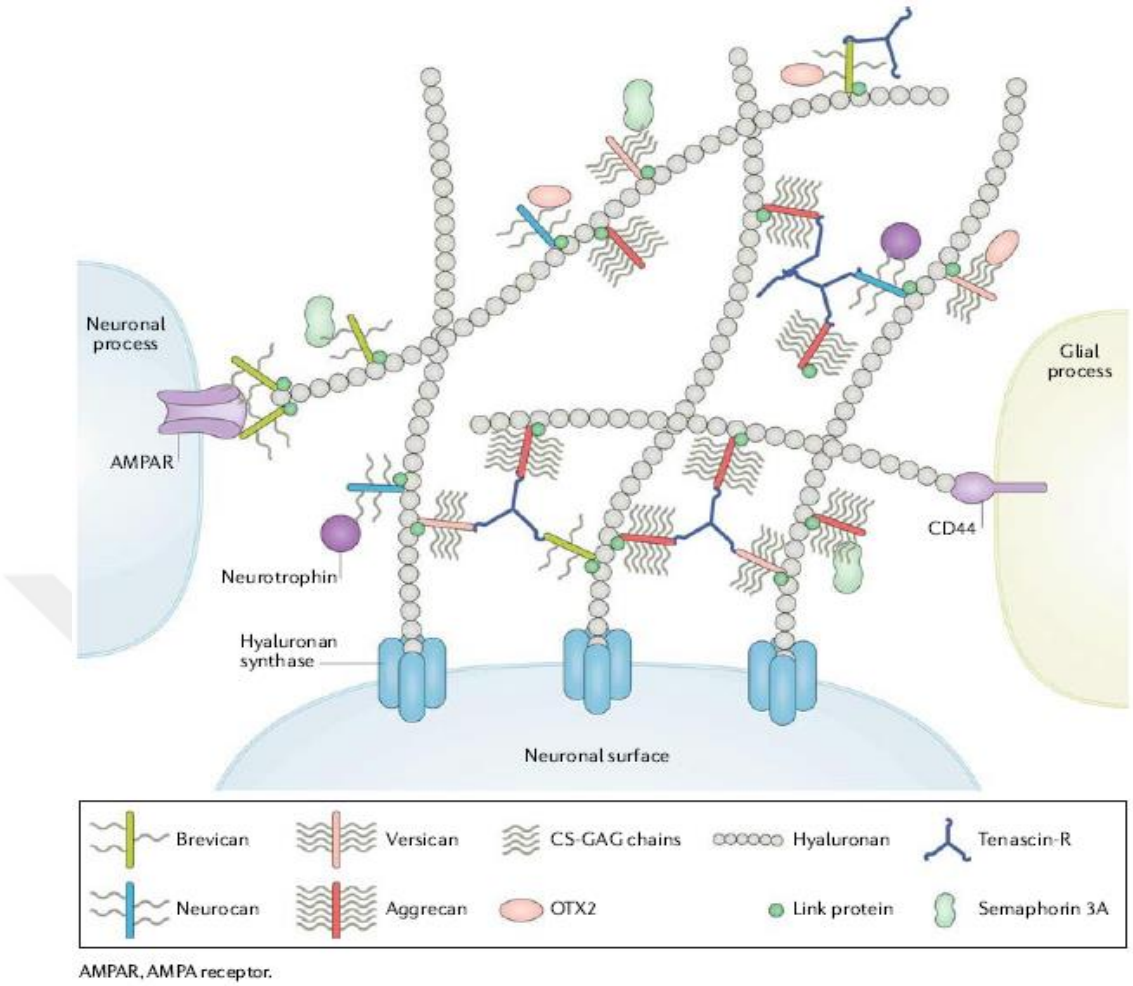


Figure 2-13 Components and assembly of PNN structure[219].

PNNs play several important roles in the CNS, and one of the important roles of PNNs are ion buffering. Components of PNNs have negative charges and around the neurons, they build up a polyanionic environment[228]. PNNs act as a reservoir consisting of positive ions, and they provide ion sorting to regulate high neuron spike frequencies[229]. Besides, PNN reduces the effects of oxidative stress[230]. Because of the hydrophilic properties of the HA and aggrecan, PNNs play a role in the water homeostasis[231]. Another significant function of PNNs is regulating synaptic plasticity. For example, in the hippocampus, PNNs affect the lateral diffusion of the AMPA receptor[232]. Studies reveal that type of sulphation of CSPGs influences synaptic plasticity, and while 4S GAGs have an inhibitory role, 6S GAGs have a permissive role[233]. Also, PNNs has a key role between excitatory and inhibitory neurons balancing[234,235]. Lastly, in the long term memory

formation, PNNs are required in the hippocampus, and for fear learning, PNNs are needed in the amygdala and medial frontal cortex[236-238].

2.3.8. Basement Membrane

BM is a specialized extracellular matrix structure located between endothelial cells and parenchymal tissue[239]. Two distinct types of BM are found and these are called endothelial basement membrane and parenchymal basement membrane that are disassociated by pericytes[240]. BM is a sheet-like structure which is composed of four main components; laminin, collagen IV, nidogen, and perlecan[241]. These proteins are produced by brain microvessel endothelial cells, pericytes, astrocytes that are joining the blood-brain barrier (BBB) structure.

2.4. Neuroligin 2

Neuroligins are cell adhesion molecules that have four distinct class NLGN 1, 2, 3, 4 located mostly post-synapses of neurons, and they mostly have a role in synaptogenesis and synaptic function[242,243]. Neuroligins mostly interact with neurexins located at pre-synapses of neurons[244]. Previous studies demonstrate that neuroligin 2 is mostly located at the inhibitory (GABAergic and glycinergic) synapses[245,246]. It provides recruitment of GABAA receptors interacting with Gephyrin[247,248]. Some studies suggest that neuroligin 2 binds PSD95 to regulate excitatory neurons, but it mostly prefers binding gephyrins[249,250]. Another study also shows that knockdown of neuroligin 2 causes a reduction in the glutamatergic event with regulating KCC2 a chloride transporter[251]. This channel plays a significant role in excitatory synapse development and dendritic spine maturation[252]. To these data, neuroligin 2 plays a role in the balancing of excitatory and inhibitory synapses[252]. Besides neurons, glial cells also express neuroligins[250]. Interestingly, one of the recent studies shows that the deletion of neuroligin 2 in astrocytes causes a decrease in the excitatory synapse in the visual cortex. Electrophysiology results reveal that loss of the neuroligin 2 induces reduction in miniature excitatory postsynaptic currents (mEPSC), whereas causes a rise in miniature excitatory postsynaptic currents (mIPSCs) in the layer V cortical pyramidal neurons. Thus, astrocytic neuroligin 2 is a significant molecule to regulate the balance between excitatory and inhibitory synapse. In the same study, researchers reveal that neuroligins also take place in the regulation of the morphology of the astrocytes[15].

3. MATERIALS and METHODS

3.1. Experimental Setup and Animal Husbandry

Male C57BL/6 animals were used, and all animals were eight weeks old at the beginning of the experiment. They were divided into two different groups. The first group was fed by a standard chow diet for two months; the second group was fed by a High Fat Diet (HFD) (Sniff E15742-34 %60 KJ Lard-based Fat) for two months.

3.2. Behavioral Tests

3.2.1. Elevated Plus Maze

Elevated Plus Maze is a commonly used behavioral method to determine the anxiety levels of rodents and the test just lasts 5 minutes. To assess the behavior, total arm entries, the ratio of the number of open arm entries to total arm entries, the ratio of time spent in the open arm to the total spent time are used as parameters[253].

Equipment: In this test, a plus-shaped Plexiglas platform is used. In this platform, opposite two arms are enclosed by high walls (45cm), and the other opposite arms do not have walls.

Protocol: During the test, the temperature (21 ˚C) and lux (100) of the room are optimum. Animals were placed in the center of the arena toward the open arms. They were freely moving for 5 minutes and after each animal, the platform was cleaned with %70 ethanol to remove odor. Their movement was recorded and analyzed with EthoVision XT6 – Noldus.

3.2.2. Light Dark Box

Light Dark Box test is a specially designed platform to assess anxiety behavior to aversive light stimuli. This platform contains two different sections. One of them is dark and the other area is illuminated with aversive light. Animals can be moving freely between these areas and the test lasts 10 minutes. Anxiety level is determined by the ratio of time spent in the dark are to the total spent time, and total entry number to the dark area[254].

Equipment: The Plexiglas area has two different sections which are the same size (30x45x30) and they are connected by a door (7x7). One of these sections is darkened and prevented the entering of light (0-5 lux) and the other area is enlightened by the aversive light source (300-330 lux).

Protocol: Mice were placed to the enlightened area that their face is directed on the opposite side of the dark area. They were moving freely in these two sections for 10 minutes. After each animal, the arena was cleaned with 70% ethanol to remove odor. Videos of the test were recorded and analyses were done as double-blind.

3.2.3. Open Field Test

Open Field test is commonly used to evaluate the locomotor activity and anxiety behavior of rodents. In this test, animals freely move in a square or circular arena. Locomotor activity is determined by measuring the total distance moved in this area. To assess anxiety behavior, the arena is divided equally into three pieces. These areas are found in the center, near the outer wall, and between these two areas. Anxiety level is determined by the ratio of spending time in the outer area and center area to total time[255].

Equipment: A plexiglass square arena is used and its size is 80x80x45 cm.

Protocol: Temperature of the room (21˚C) and lux of the room (100 lux) is at optimum condition. Mice were placed to the center of the arena and they were moving freely during the 10 minutes. After each mouse, the arena was cleaned by 70% ethanol to eliminate stress behavior sourced from the odor of the previous animal. Videos were recorded and analyzed by EthoVision XT6 –Noldus.

3.2.4. Novel Object Recognition Test

Novel object recognition test is widely used to evaluate the learning and memory performance of mice. This test has three sessions and these sessions are habituation session, training session, and test session. In the habituation session, rodents freely explore the arena without any objects and they have become familiar to the environment. In the training session, two identical objects are found in the opposite corner of the arena, and animals recognize these objects during this session. In the test session, one of the objects should replace with another novel object. In the test session, animals should remember previous objects and should explore the novel object. This test is based on the visual exploratory behavior of mice and healthy rodents should identify similar and different objects to each other. Learning and memory performance of mice is determined by comparing the ratio of time spent around the novel object to total time and the ratio of time spent around the old object to total time[256].

Equipment: A plexiglass square arena is used and its size is 45x45x20 cm.

Protocol: This method was composed of three different sessions and they lasted three days. The first session was a habituation session and in this session, mice were placed into the center of the arena. Mice explored the arena for 5 minutes. On the second day, the training session was performed and for this session, two identical objects were placed in two opposite corners of the arena. Animals were put in the same location and position as the previous day. This session lasted 10 minutes for each animal. The third day was a test day and on this day one of the objects was replaced with a novel object. Mice were put into the same place as before the day and mice spent 10 minutes in this arena. After each animal, the arena, and objects were cleaned by 70% of ethanol. Videos were recorded and analyzed by EthoVision XT6 –Noldus.

3.2.5. Rotarod test

Rotarod test is a widely used method to assess the motor skills of rodents and in this platform, animals running and try to stay in balance on the rotating rod. The speed and acceleration of the rod can be vary depending mouse model and study. The performance of mice is evaluated by latency time and acceleration of dropping points [257].

Equipment: Harvard apparatus (Panlab) Rotarod system is used.

Protocol: This behavioral assessment lasted four days and on the first day, results were accounted as a habituation day of animals. For the habituation to the platform, animals were placed to constant 10 rpm rotating rod and this process was repeated until the stay at least 30 seconds on the rod. After the habituation, animals were placed to the rod again and they were running in an accelerating rod from 4 rpm to 40 rpm in 5 minutes. This protocol was repeated following three days and results were recorded. After each animal, the platform was cleaned by 70% Ethanol.

3.3. Cardiac Perfusion

Mice were anesthetized by using inhaling anesthetic isoflurane and their chest cavity was opened. Then, a small incision was made into the right atrium while 0.9% isotonic sodium chloride solution was circulated through the left ventricle to remove blood from the body. Five (5) animals of each group were separated for western blot and just 0.9% sodium chloride solution was circulated and the other three animals were separated for immunofluorescence. In these three animals, 4% paraformaldehyde were circulated to fixation of tissue after the isotonic saline solution. For the western blot, brains were dissected into the cerebellum, hypothalamus, hippocampus, and cortex, and

tissues were stored in -80 C. For the immunofluorescence, brain tissues were saved in 4% paraformaldehyde overnight and after then they stored in a 30% sucrose solution.

3.4. Imaging

3.4.1. Brain Slicing

Firstly, brains were embedded into the 4% agarose solution, and they were frozen in the 4˚C in the refrigerator. After that brain was slicing with vibratome into the 50µm coronal sections.

3.4.2. Immunofluorescence Staining

Slices contain the prefrontal cortex, hypothalamus, and hippocampus were selected. Slides were washed by 1X-PBS (Invitrogen, 00-3002) three times for 15 minutes. Tissues were blocked by 3% bovine serum albumin (BSA) (Bioshop, ALB001) for 1 hour and then they were incubated with Wisteria floribunda agglutinin (WFA) (1:500) (Vector Laboratories, FL-1351) overnight. Then, they were washed by 1X-PBS three times for 15 minutes. They were dried and mounted by mounting medium.

3.4.3. Microscopy

Imaging of slices was performed by Zeiss Confocal Microscope (LSM 700). For visualizing PNN, 488nm laser was used and images were obtained 20x objective with using tile scan and z-stack options.

3.5. Western Blot

3.5.1. Protein Isolation

The weight of tissues varied from 0.01 mg to 0.2 mg and they were thawed with pellet pestle (Sigma-Aldrich, Z359971-1EA) in the radioimmunoprecipitation assay (RIPA) (Santa Cruz, sc-24948A) at 4˚C. Approximately, 100µl RIPA buffer was used for 10mg tissue. After this process, tissues were incubated for 1 hour on the ice, and then they were centrifuged at 13000 rpm for 20 minutes at 4˚C. Supernatants of tissues were collected and they were centrifuged at 13000 rpm for 20 minutes at 4˚C. After that supernatant was collected and stored at 80˚C for further analysis.

3.5.2. Protein Determination

Protein determination was done using the BCA assay kit (Thermo Fisher Scientific, 23225). In this assay, albumin standards were prepared from 2mg/ml stock solution (Table 3.1).

Table 3-1 Final dilutions of standards for BSA assay

Vial	Volume of Diluent(µL)	Volume and Source of BSA (µl)	Final Concentration of BSA (µg/ml)
A	0	300 of Stock Solution	2000
B	125	375 of Stock Solution	1500
C	325	325 of Stock Solution	1000
D	175	175 of Vial B dilution	750
E	325	325 of Vial C dilution	500
F	325	325 of Vial E dilution	250
G	325	325 of Vial F dilution	125
H	400	100 of Vial G dilution	25
I	400	0	0

After the preparation of standards, for each sample and standard, a working solution was prepared 1:50 respectively solution A and solution B. 200 µl working solution was added for each 25 µl standard and sample, and they were incubated at 37˚C for 30 minutes. After that absorbance of samples and standards was measured using spectrophotometry at 562nm.

3.5.3. SDS Gel Electrophoresis

Samples were diluted as 60 µg final protein amount in final 35µl solution and it contained 3.5 µl reducing agent (Invitrogen, B0009) and 8.75 µl LDS sample buffer (Invitrogen, B0008). After preparing the samples, they were denaturated at 78˚C for 10 minutes. Then, samples were loaded to Bolt™ 4 to 12%, Bis-Tris gel (Invitrogen, NP04122BOX), and they were run 1 hour 30 minutes with NuPAGE™ MOPS SDS Running Buffer (Invitrogen, NP000102). As a marker, 5µl PageRuler™ Prestained

Protein Ladder (Thermo Scientific, 26617) and 5µl Spectra™ Multicolor High Range Protein Ladder (Thermo Scientific, 26625) were mixed and used.

3.5.4. Transfer of Protein from Gel to Membrane

Separated proteins in NuPAGE™ 4 to 12%, Bis-Tris gel were transferred to the PVDF membrane (Thermo Scientific, 88518). Before transfer, the PVDF membrane was washed with absolute Methanol for 5 minutes (J.T. Baker™, 8405), and then it was washed with 1X Transfer Buffer (25mM Tris Base (Sigma-Aldrich, T1503), 192mM Glycine (VWR, M103), 20% Methanol). Besides, two pieces of filter paper were wet with 1X Transfer Buffer. Membrane and Gel were put inside the two filter papers in the transfer cassette and they were smoothened with a cylindrical roll-on. They were locked with the upper part of the transfer cassette to form the sandwich model. The transfer was done in 22V for 45 minutes with a semidry transfer method.

Table 3-2 List and dilution of antibodies

Antibodies	Dilution	Brand	Catalog No
Reelin Polyclonal Antibody	1:2000	Invitrogen	PA5-78413
Tenascin C Monoclonal Antibody (MTn-12)	1:1000	Invitrogen	MA1-26778
Neuroligin 2 Polyclonal Antibody	1:1000	Invitrogen	PA5-72822
Thrombospondin 1 Monoclonal Antibody (A6.1)	1:250	Invitrogen	39-9300
Anti-GAPDH Rabbit Monoclonal Antibody	1:5000	BosterBio	M00227
Anti-rabbit IgG, HRP-linked Antibody	1:1000	Cell Signaling	7074S
Goat anti-Rat IgG (H+L) Secondary Antibody, HRP	1:10000	Invitrogen	31470

3.5.5. Detection of Desired Protein

After the transfer of protein from gel to the PVDF membrane, the membrane was blocked by 5% dry fat milk for 1 hour on the shaker (BioFrox, 1172GR500) which was diluted on 1X TBST buffer (10x TBS (24 g Tris-HCl (BioBasic, TB0103), 5.6 g Tris base (Sigma-Aldrich, T1503) 88gr NaCl (Sigma-Aldrich, 31434) in 1L final solution), distilled water, 0.1% Tween 20 (Sigma, P1379)). Primary antibodies were diluted with 5% of dry fat milk and after the blocking, the membrane was incubated by primary antibodies

overnight at 4˚C under the shaking condition (Table 2). At the end of the incubation time, antibodies were gathered and the membrane was washed 6x10 minutes with 1x TBST. Subsequently, it was incubated with secondary antibody for 1 hour at room temperature. After that membrane was washed 6x10 minutes with 1x TBST. Following to washing step, the bands were visualized with chemiluminescence method using SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Scientific, 34096) on ChemiDoc XRS+ (Biorad), and quantification of bands was done with Image Lab™ software (version 6.0.0, Bio-Rad).

3.6. Statistical Analysis

All statistical analyses were gathered by GraphPad Prism® 8.4.3. For the normality test, the Shapiro-Wilk normality test was used. Data were analyzed using Student's t-test, two-way ANOVA, or nonparametric test. For significance, lower than 0.05 was accepted for P-value.

4. RESULTS

4.1. Measurement of Metabolic Parameters

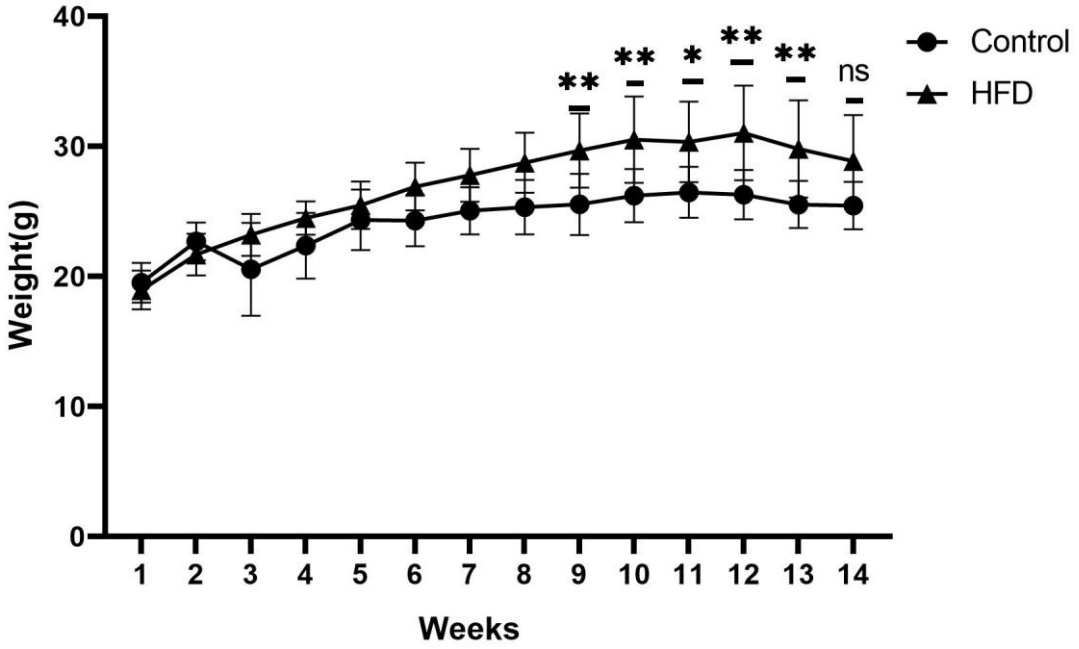


Figure 4-1 Weekly body weight changes of animals who consume chow diet and high-fat diet (n=8, $p<0.05$, Two-way ANOVA).

To assess the metabolic effect of High Fat Diet (HFD), the bodyweight of animals was measured weekly for 14 weeks, and the weight of mice in both groups increased until the 11th week continuously (Figure 4.1). However, the raise rate of High-fat diet-fed groups was higher than the chow diet-fed group, and differences between HFD fed groups and control groups became statistically significant. To this result, in the last two weeks, HFD fed animals lost weight and although the mean weight of the HFD group was higher than control groups, differences were not significant.

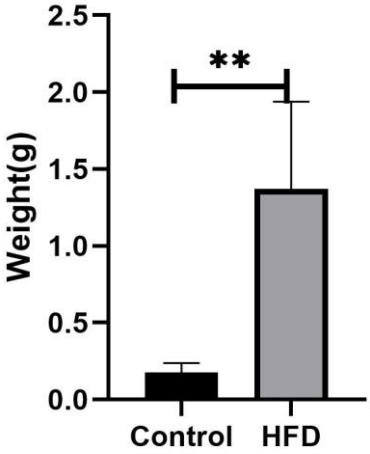


Figure 4-2 Total white adipose tissue of animals (n=5, $p<0.05$, Student's t-test).

Besides total weight measuring, the amount of total white adipose tissue is an important metabolic parameter to assess obesity. During dissection, white adipose tissues were gathered from only saline perfused animals. Data shows that the weight of white adipose tissue is much higher in HFD fed mice than the control group (Figure 4.2). Despite the bodyweight, if we compare white adipose tissue weight with bodyweight, differences between the control group and HFD groups increased (Figure 16). Thus, HFD led to higher fat deposition.

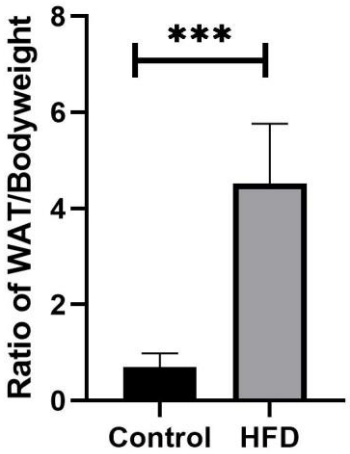


Figure 4-3 Percentages of total white adipose tissue to bodyweight (n=5, $p<0.05$, Student's t-test).

Besides, the daily food intake of mice was measured for 15 days after 6 weeks starting the diet. To this measurement, the daily food intake of control mice was significantly higher than animals fed by HFD (Figure 4.4). Differences in food intake between the control group and HFD were nearly two grams.

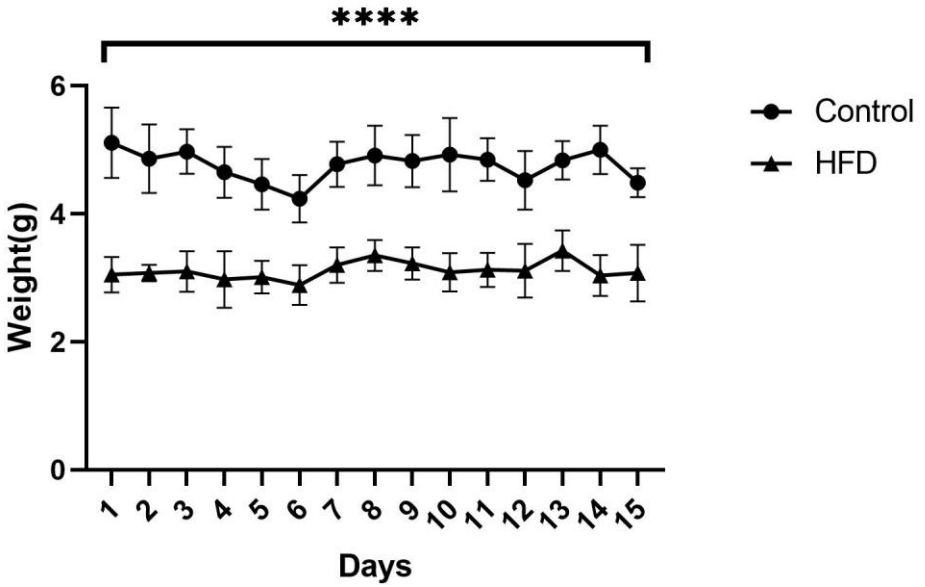


Figure 4-4 Daily of intake of mice for the succession of 15 days (Significance is valid for all separate days) (n=8, $p<0.05$, Two way ANOVA).

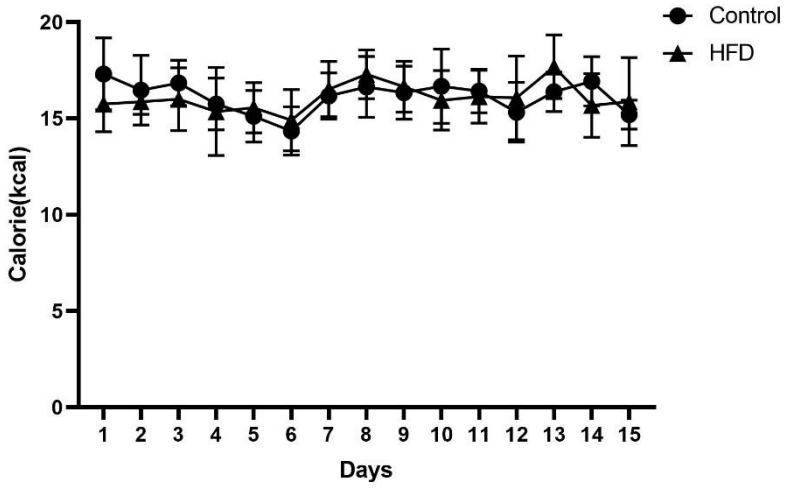


Figure 4-5 Daily calorie intake succession of 15 days for both groups (n=8, $p<0.05$, Two-way ANOVA).

However, if we compare daily calorie intake instead of the weight of food, both groups consumed nearly the same amount of calories (Figure 4.5). Thus, feeding by a high-fat diet did not affect daily calorie intake.

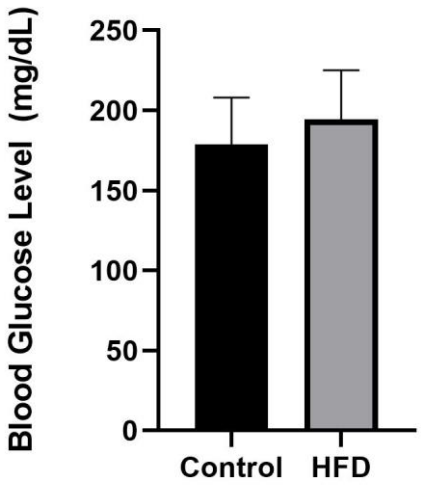


Figure 4-6 Blood glucose level of Control group and high-fed diet consumed group (n=8, $p<0.05$, Student's t-test).

Another important obtained parameter was blood glucose level and when mice were sacrificed, the blood sugar level of animals was measured with a blood glucose meter. Results showed that there were no significant differences between the control group and the HFD group (Figure 4.6).

4.2. Results of Behavioral Experiments

4.2.1. Elevated Plus Maze

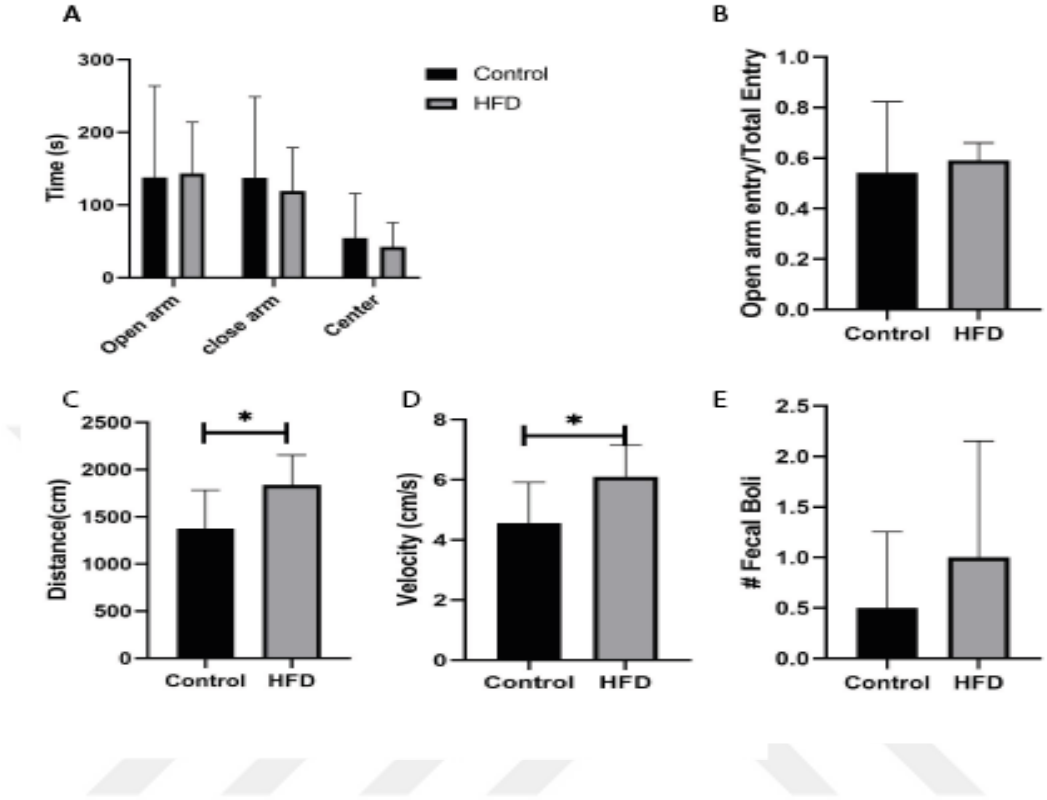


Figure 4-7 Behavioural results of Elevated Plus Maze test for both group A) Spent time on the open arm, close arm, and the center point of elevated plus-maze B) Ratio of open arm entry number to total entry arm entry number of mice C) Distance walking of mice on the maze D) Velocity of mice E) Number of fecal boli (n=8, $p<0.05$, Two-way ANOVA, Student's t-test).

To determine the anxiety level of mice, one of the used test is elevated plus maze test, and after 8 weeks starting the diet, the anxiety levels of mice were measured by the elevated plus-maze. Data demonstrate that both groups spent a similar time on the open arm, close arm, and center point (Figure 4.7 A). Besides, in each group, their spending time in open arms and close arms was nearly the same, and also their ratio of open arm entry to total entry number was not significantly different (Figure 4.7 B). Another stress marker in the elevated plus-maze is the number of fecal boli. Differences between the number of fecal boli were not significant (Figure 4.7 E).

On the other hand, significant differences were measured in the distance move of mice and HFD fed mice moved more distance than the control group (Figure 4.7 C). Also, the velocity of mice was higher in the HFD group than the control group (Figure 4.7 D).

4.2.2. Open Field Test

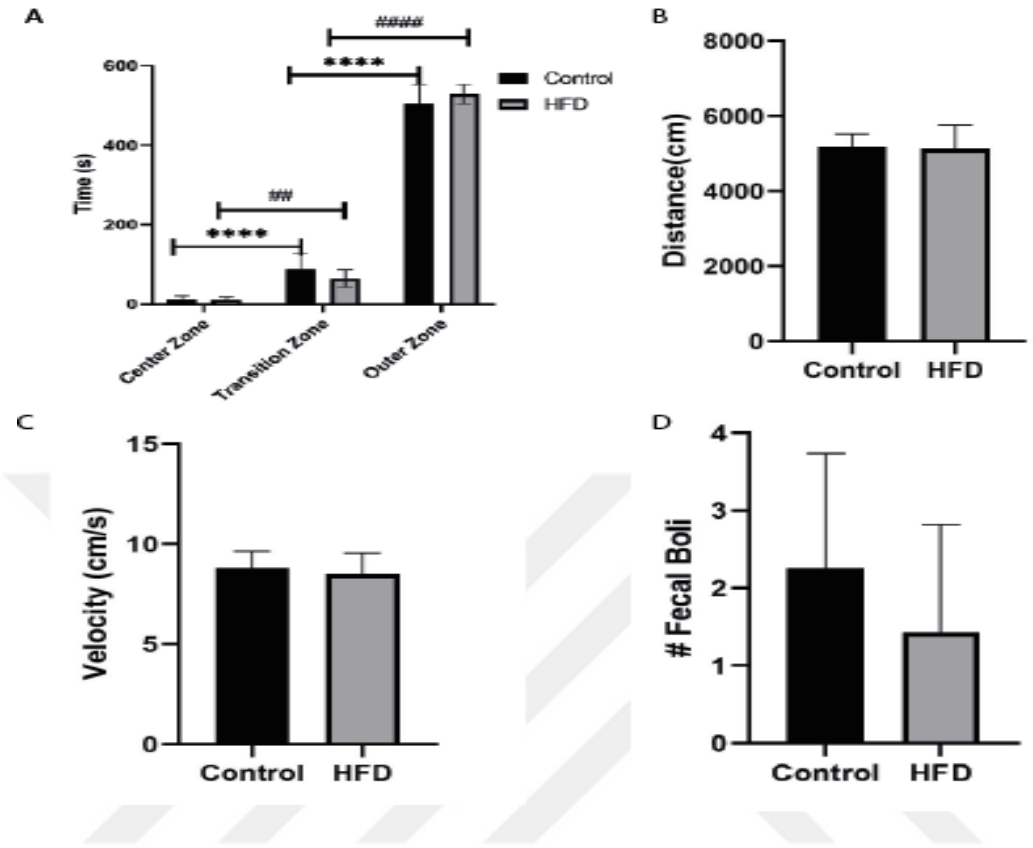


Figure 4-8 Behavioral result of open field test A) Spending time in different zones of arena B) Total distance walking by both groups C) Velocity of mice D) Number of fecal boli (n=8, $p<0.05$, Two-way ANOVA, Student's t-test).

Another commonly used test to assess anxiety behavior is an open field test. The arena was divided into three different parts that are called an outer zone, transition zone, and center zone. As a result of the open field test, the control group, and HFD group stay a similar amount of time in all zones, and both groups spent more time in the outer zone and less time in the center zone (Figure 4.8 A). In addition, meaningful differences were not measured in the number of fecal boli between both groups (Figure 4.8 D).

In addition to anxiety behavior, the open field test is used to evaluate locomotor behavior. Despite the differences were observed in the elevated plus-maze, there were no differences in distance moved by mice and the velocity of mice between the chow group and the high-fat diet group (Figure 4.8 B, Figure 4.8 C).

4.2.3. Light-Dark Box Test

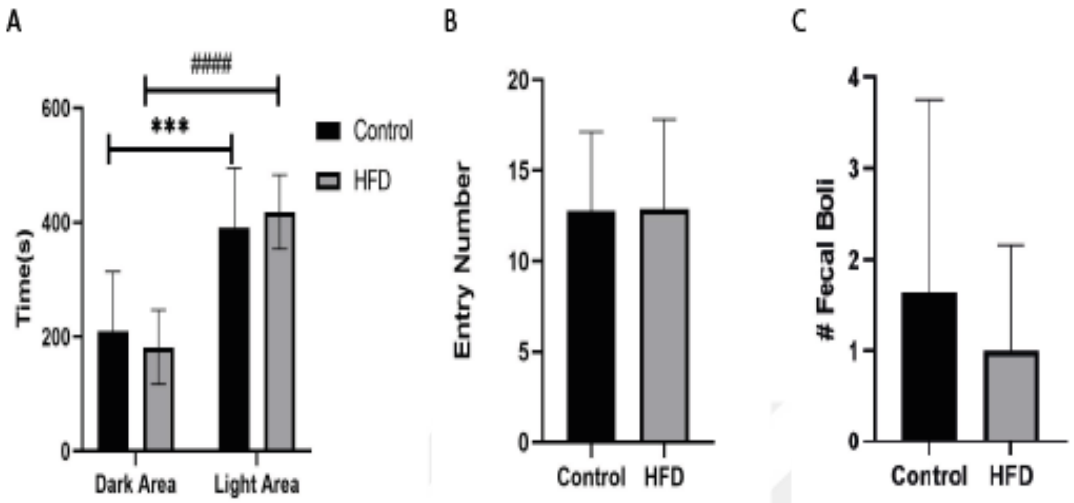


Figure 4-9 Behavioral results of the light-dark box test. A) Time spent in the dark area and light area by both groups B) Total entry number of Dark area C) Number of Fecal boli (n=8, $p<0.05$, Two-way ANOVA, Student's t-test).

Besides the elevated plus maze and open field test, the light-dark box test is used to evaluate the anxiety level of mice. Spending time of light area and the dark area was the main parameter for anxiety and if we compare high-fat diet-fed mice by chow-fed mice, significant differences were not measured(Figure 4.9 A). Both groups spent more time in the light area than the dark area. In addition to the time spent in different areas, the entry number of the dark area was almost the same in all groups (Figure 4.9 B). There were no significant differences in the number of fecal boli (Figure 4.9 C).

4.2.4. Novel Object Recognition Test

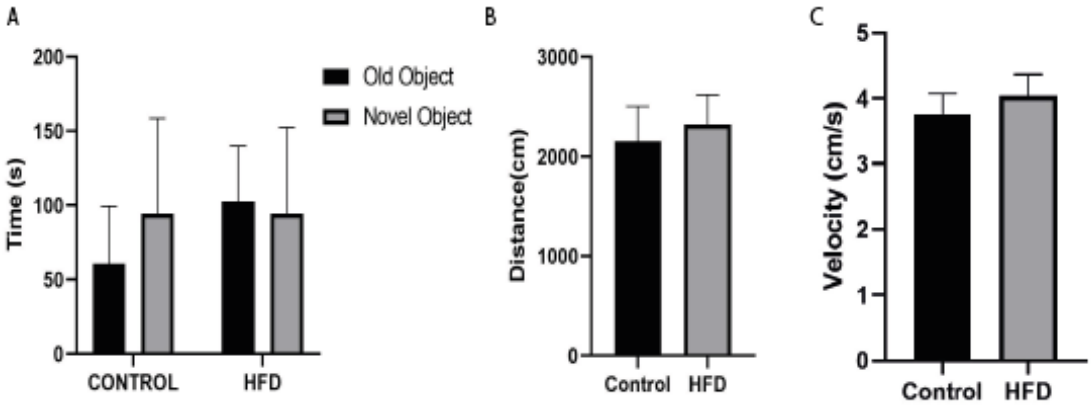


Figure 4-10 Behavioral results of novel object recognition test A) Spending time around the novel object and old object in both groups B) Total distance moved by animals C) Walking velocity of mice (n=8 $p<0.05$).

Novel object recognition test is a commonly used method to evaluate the learning ability and memory of rodents. Our results show that although the control group spent more time on the novel object, the HFD group spent nearly equal time around objects (Figure 4.10 A). However, these values were not statistically significant. About the locomotor activity, no significant differences were not measured in distance moved and velocity of mice in both groups (Figure 4.10 B, C).

4.2.5. Rotarod Test

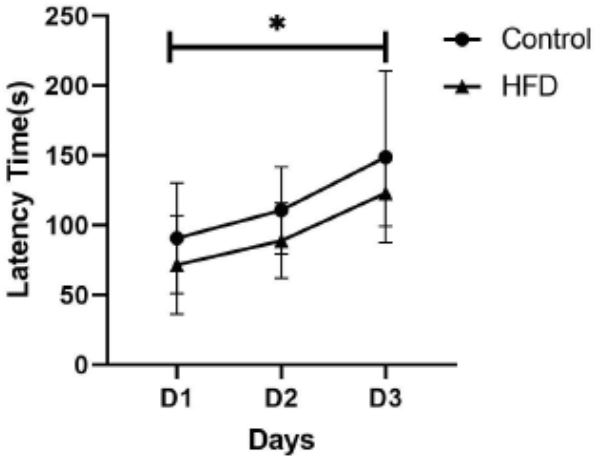


Figure 4-11 Latency time of rotarod test for three days (n=8, $p<0.05$, Two-way ANOVA).

The locomotor activity of mice was also measured with the rotarod test. Both groups improved their locomotor performance day by day and they significantly showed better performance on the last day by comparing the first one (Figure 4.11). In addition, despite the insignificant data, the chow diet-fed group was more durable on the rod than the high-fat diet-fed group.

4.3. Post-mortem Analysis

4.3.1. Imaging of Perineuronal Nets

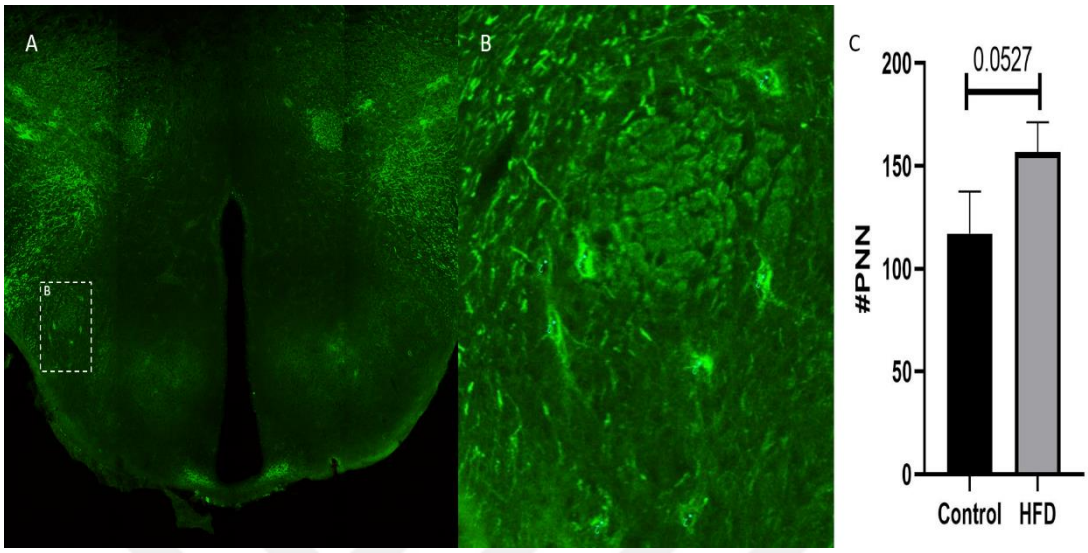


Figure 4-12 Results obtained from immunofluorescence staining of hypothalamus A) Confocal image of PNN in the hypothalamus section (20x, 488nm) B) Zoom in labeled area C) Number of PNN in both control group and HFD group (n=3, $p<0.05$, Student's t-test).

For illustrating PNN, slices were taken from the hypothalamus, hippocampus, prefrontal cortex, and other parts of the cortex. PNN (green, 488nm) was stained by fluorescein-labeled WFA, and it was visualized with confocal microscopy (Figure 4.12 A, B). To determine the number of PNN, PNN positive soma was counted. In the hypothalamus, the number of PNN was higher in HFD fed mice than chow-fed mice (Figure 4.12 C). The p-value of differences obtained by Student's t-test was 0.0527 and differences between HFD and control groups were almost significant.

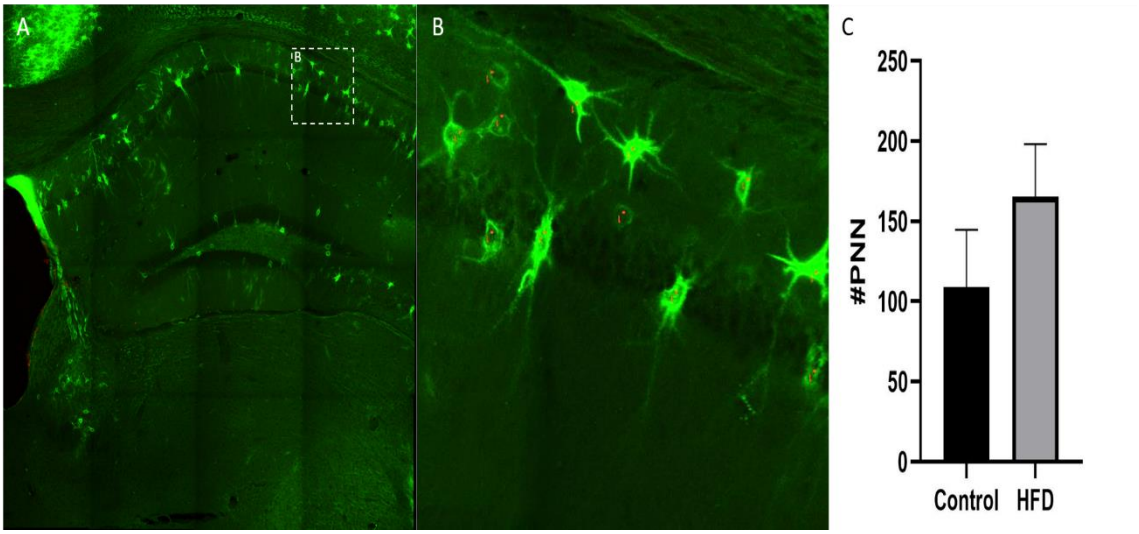


Figure 4-13 Results obtained from immunofluorescence staining of hippocampus A) Confocal imaging of PNN in the hippocampus section (20x, 488nm) B) Zoom in labeled area C) Number of PNN in both control group and HFD group (n=3, $p<0.05$, Student's t-test)

Another visualized part of the brain was the hippocampus (Figure 4.13). Our data shows that there were no statistically significant differences, but it was seen that number of PNN is higher in HFD-fed mice (Figure 4.13 C).

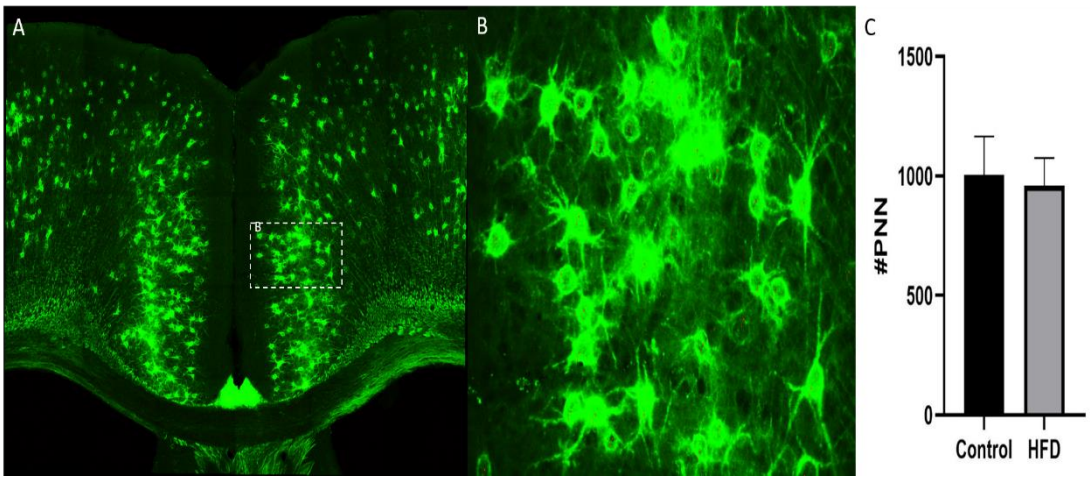


Figure 4-14 Results obtained from immunofluorescence staining of cortex A) Confocal imaging of PNN in the cortex (20x, 488nm) B) Zoom in labeled area C) Number of PNN in both control group and HFD group (n=3, $p<0.05$, Student's t-test)

However, the situation was different in the cortex. Our data reveal that the density of PNN was higher in the cortex than the hypothalamus and hippocampus (Figure 4.14

A, B), but any differences were not measured between the control group and the high-fat diet group (Figure 4.14 C).

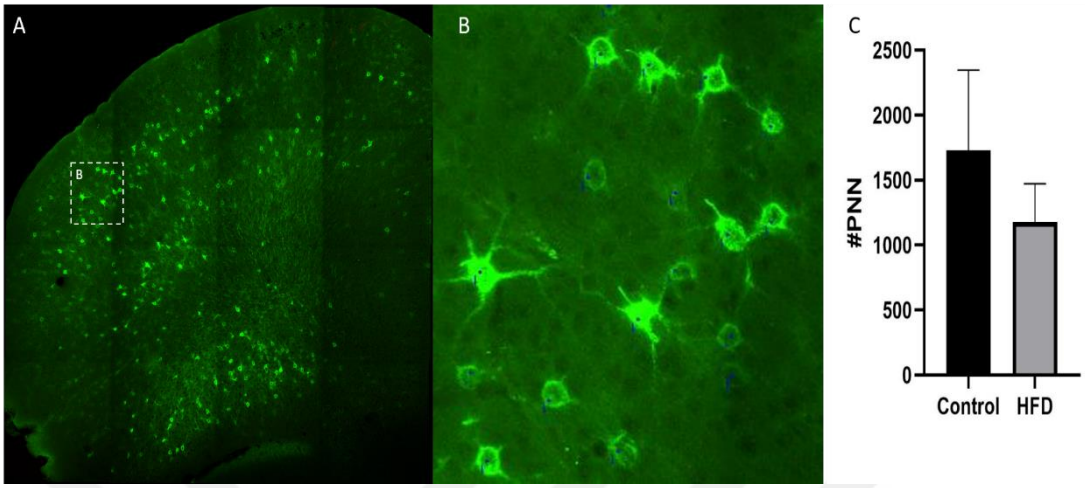


Figure 4-15 Results obtained from immunofluorescence staining of prefrontal cortex A) Confocal imaging of PNN in the prefrontal cortex (20x, 488nm) B) Zoom in labeled area C) Number of PNN in both control group and HFD group (n=3, $p<0.05$, Student's t-test).

In the prefrontal cortex, although there were no significant differences between both groups, the number of perineuronal nets was higher in the control group than the HFD group (Figure 4.15 C).

4.3.2.Western Blot

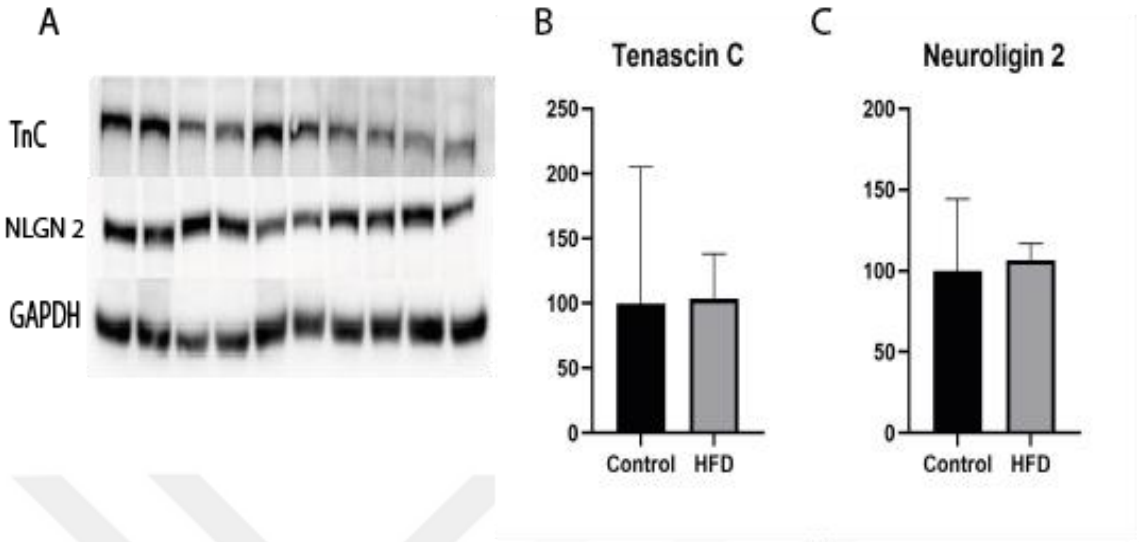


Figure 4-16 Western results obtained from hypothalamus A) Western bands of Tenascin C (TnC), Neuroligin 2 (NLGN 2) and Gliseraldehid 3-Fosfat Dehidrogenaz (GAPDH) B) Quantification analysis of Tenascin C with comparing GAPDH C) Quantification analysis of Neuroligin 2 with comparing GAPDH (n=5, $p<0.05$, Student's t-test).

The expression level of proteins was evaluated by the western blot method. In the hypothalamus, tenascin C and neuroligin 2 protein amount was determined and GAPDH is used as a control protein. Our results indicate that the protein amount of tenascin C and neuroligin 2 in high-fat diet and control was nearly equal to each other (Figure 4.16 B, Figure 4.16 C).

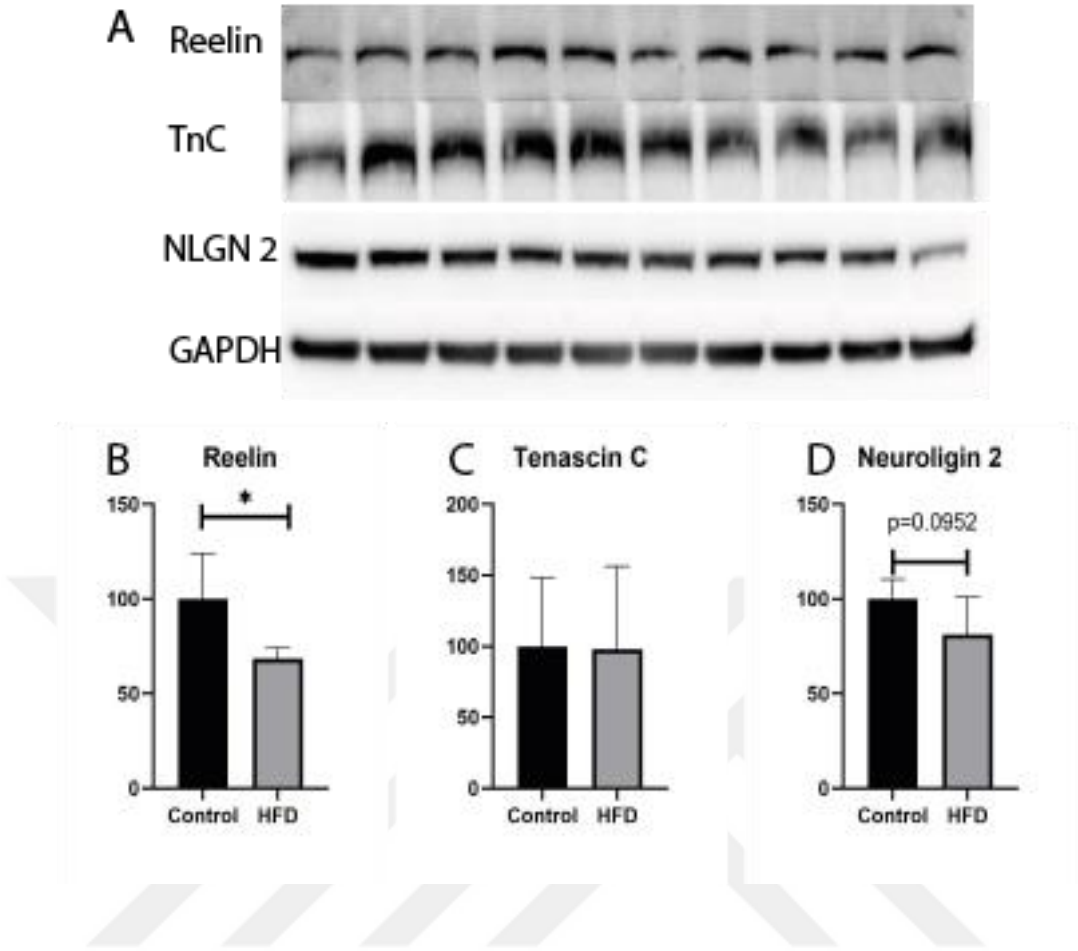


Figure 4-17 Western results obtained from hippocampus A) Western bands of Reelin, TnC, NLGN 2, and GAPDH B) Quantification analysis of Reelin with comparing GAPDH C) Quantification analysis of Tenascin C with comparing GAPDH D) Quantification analysis of Neuroligin 2 with comparing GAPDH (n=5, $p<0.05$, Student's t-test).

Another dissected tissue to determine the protein amount was the hippocampus and protein amount of Reelin, Tenascin C, and Neuroligin 2 were detected (Figure 4.17 A). Our results reveal that the high-fat diet significantly affected Reelin and it caused a decrease in its amount (Figure 4.17 B). About Neuroligin 2, our findings show that there were no significant differences between the two groups significantly, but the expression in control groups seems higher than HFD groups (Figure 4.17 D). However, for Tenascin C, the protein amount was almost equal for both groups (Figure 4.17 C).

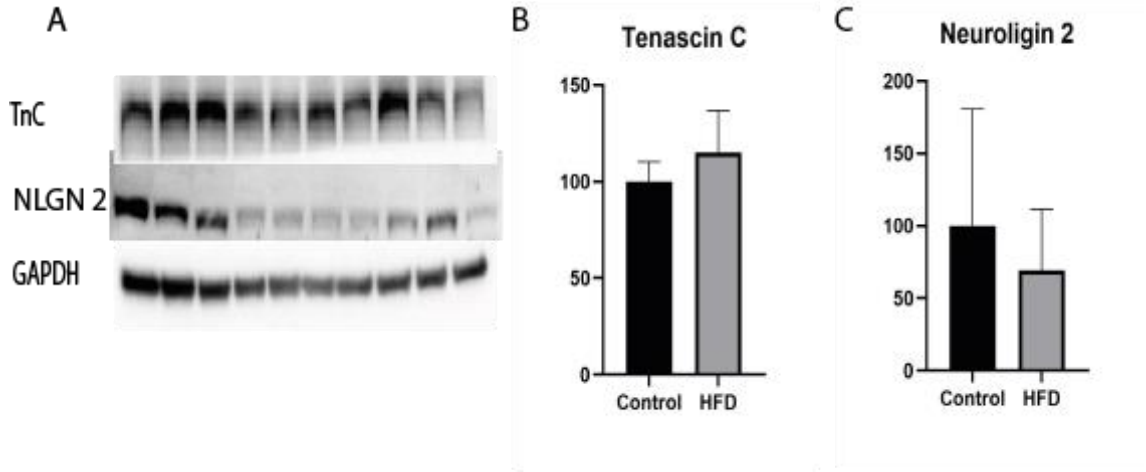


Figure 4-18 Western results obtained from cortex A) Western bands of Tenascin C(TnC), Neuroligin 2 (NG2) and Gliseraldehid 3-Fosfat Dehidrogenaz (GAPDH) B) Quantification analysis of Tenascin C with comparing GAPDH C) Quantification analysis of Neuroligin 2 with comparing GAPDH (n=5, $p<0.05$, Student's t-test).

In the cortex, protein levels of tenascin C and neuroligin 2 were assessed (Figure 4.18 A). Our results reveal that HFD did not affect the amount of Tenascin C and Neuroligin 2 (Figure 4.18 B, Figure 4.18 C).

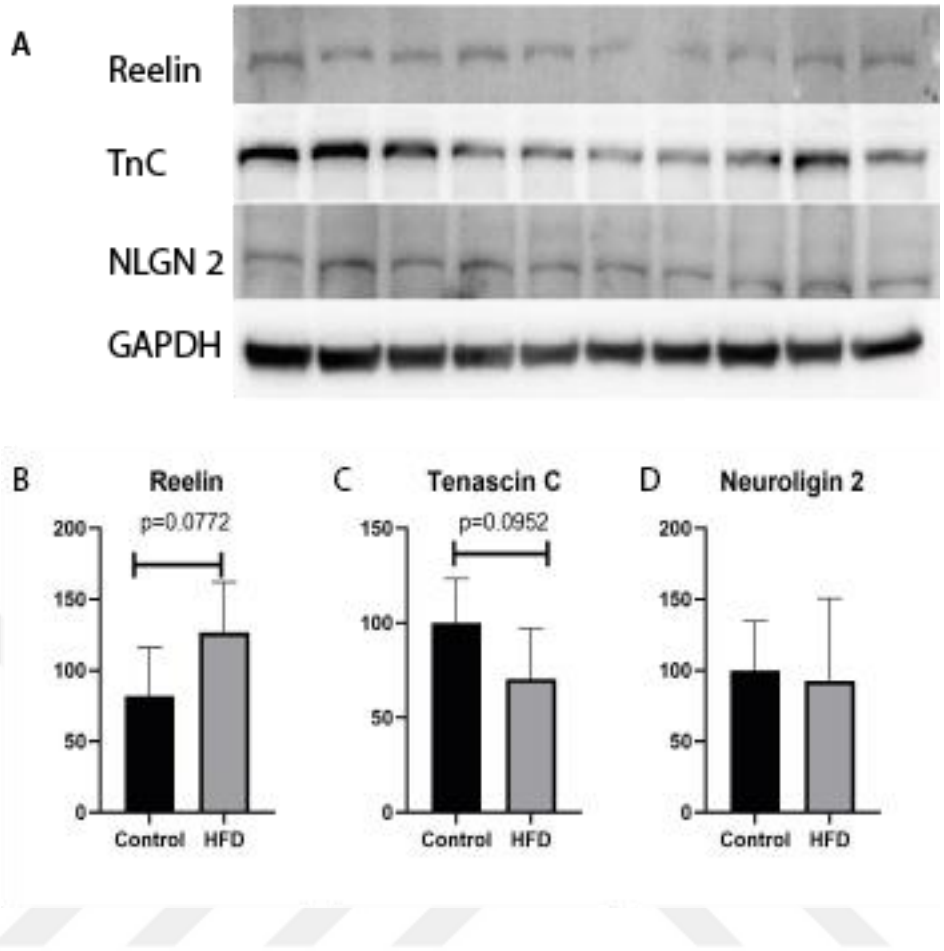


Figure 4-19 Western results obtained from cerebellum A) Western bands of Reelin, TnC, NG2, and GAPDH B) Quantification analysis of Reelin with comparing GAPDH C) Quantification analysis of Tenascin C with comparing GAPDH D) Quantification analysis of Neuroligin 2 with comparing GAPDH (n=5, $p<0.05$, Student's t-test).

The amount of Reelin, Tenascin C, and Neuroligin 2 was evaluated in the cerebellum. Despite the nonsignificant data statistically, it was seen that HFD affected Reelin, and it was increased in the cerebellum when animals fed by HFD (Figure 4.19 B). Another protein was Tenascin C that was decreased in high-fat diet-fed mice comparing with control groups, but it was not significant (Figure 4.19 C). No significant differences were measured in the Neuroligin 2 amount (Figure 4.19 D).

5. DISCUSSION and CONCLUSION

In this study, we have investigated how a high-fat diet affects extracellular matrix proteins with detailed behavioral analysis. For this purpose, animals fed by a 60% high fat diet, and then were assessed using five different behavioral tests. After behavior tests, animals were sacrificed, and then post-mortem analyses were done. For post-mortem analysis, protein expression levels of Tenascin C, Reelin, and Neuroligin 2 were measured. Probably because of the antibody, thrombospondin-1 could not be visualized and quantified. In addition, reelin was just visualized in the hippocampus and cerebellum. For perineuronal nets, the immunofluorescence staining method was applied, and PNN positive cell somas were counted for assessment.

First of all, we evaluated the effects of a high-fat diet on mice. Weekly measurement of bodyweight data shows that the HFD leads to an increase in the bodyweight significantly. After the 9^{th} week, differences in bodyweight between control and HFD became significant and it continued until the end of the experiments. The total white adipose tissue of animals was measured and in the HFD group, the amount of total WAT was higher than the control group. Also, the ratio of WAT to bodyweight showed that animals fed by the high-fat diet accumulated more fat animals fed by the control diet. Thus, mice fed by the high-fat diet became obese. However, this situation was not associated with the amount of caloric intake, because our measurements demonstrated that total calorie intake was almost the same for both groups. Consequently, the accumulation of WAT tissue was sourced from the adverse effects of the fat. Besides, the blood glucose level of both groups was at a similar level, so there was no disruption on the regulation of glucose level by insulin. As a result, HFD caused obesity without alteration of blood sugar level in the same amount of caloric intake.

For the behavioral assessment of animals, five different behavioral tests were applied, and anxiety level, learning and memory, and motor functions were assessed. For the evaluation of anxiety, elevated plus maze, light-dark box, and open field test was applied. All test results demonstrate that between the control group and the high-fat group any differences were not observed about the anxiety. Besides the anxiety, learning, and memory of mice were tested by a novel object recognition test. Significant differences were not observed between groups. However, while the control group spent more time around the novel object, the HFD groups spent nearly equal time around the object. Thus,

learning and memory in the mice fed with the HFD were disrupted. The last tested behavior of mice was the motor function, and for this purpose, open field test and rotarod were used. In the open field test, any differences were not measured in the total distance moved and movement velocity. In the rotarod, both groups showed better performance on the third day compared with the first day significantly. However, it seemed that the performance of the control groups was more than the HFD group without significance. In conclusion, although animals did not show a significant difference, they tended to underperform in animals fed a high-fat diet than control animals in the involuntary motor function and learning function. However, we did not observe any significant difference in anxiety behavior.

For the post-mortem analysis, immunofluorescence imaging and western blot were performed in the different tissues. In the hypothalamus, firstly, the number of PNN positive soma was evaluated, and nearly, significant increases were seen in the HFD group. However, we didn't measure any differences in the tenascin C, and neuroligin 2 levels. Unfortunately, we couldn't measure reelin and thrombospondin 1 level because of the low quality of antibodies. Previous studies demonstrated that increasing hypothalamic PNN is mostly related to restriction in the plasticity, but their function depends on the subregion of the hypothalamus[258]. Thus, feeding by a high-fat diet may be related to decreasing neural plasticity in the hypothalamus. However, to determine which behavior of mice is affecting the high-fat diet, subregional analysis is needed. Another tissue of interest was the hippocampus and our post mortem results showed that a significant decrease of reelin was measured in the high-fat diet group, and a noticeable decreasing of neuroligin 2 was measured in the hippocampus. Researches about the role of reelin on the adult brain demonstrate that Reelin has a role in controlling synaptic plasticity[209]. Our data appear to be consistent with previous studies about the effects of the high-fat diet on reelin expression[12]. Besides, the noticeable decrease in neuroligin 2 may be related to disruption in the excitatory and inhibitory synaptic balance in the hippocampus[259]. In immunostaining results, although there were no significant differences between the control group and the HFD group, there was a discernible increase in the number of PNN positive soma in the HFD groups. An increasing number of PNN is related to the restriction of synaptic plasticity and they support synaptic stability[260]. All of these results reveals that HFD may alter the synaptic plasticity and synaptic function in the hippocampus. Another dissected tissue was the cortex, and in the cortex, we did not

measure any differences in the amount of the tenascin C, and neuroligin 2. To our results, differences were not observed in the PNN positive soma in the cortex. However, in the prefrontal cortex, there were distinct differences between the control and the HFD group, and the number of PNN positive soma was higher in the HFD group. This result is correlated with the previous studies, and it may be associated with food-seeking behavior [11]. The last tissue that we investigated was the cerebellum and in the cerebellum, we just analyzed protein levels of the reelin, tenascin C, and neuroligin 2. Our results showed that there was an obvious decrease in the amount of the tenascin C level and an increase in the reelin level. In the cerebellum, tenascin C is associated with the functional synaptic plasticity, decreasing of tenascin C level with the HFD may cause the improper plasticity in the synapse[261]. Unfortunately, there are relatively very few studies about the increasing reelin level in the cerebellum, but in adulthood, reelin is mostly related to the regulation of synaptic plasticity. Increasing the reelin amount in the cerebellum may be linked with impairments in the function of synapse and synaptic plasticity.

In conclusion, our data demonstrated that besides the accumulation of fat and obesity, the high-fat diet may alter the synaptic plasticity in the different regions of the brain. In all regions, this regulation may link to different extracellular matrix proteins in the different regions and their mechanism may change. Also, it can be argued that altering the expression of the extracellular matrix protein by HFD can trigger changes in the behavior of mice.

To further studies, besides the male mice, the effects of the HFD on the extracellular matrix should be investigated on the female mice. Also, changes in other extracellular matrix proteins should be assessed. To the better understanding role of extracellular matrix changes, their levels can be manipulated in the HFD condition and their effects should be evaluated.

6. REFERENCES

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7. Appendices

7.1 Ethical Approval

YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU (YÜDHEK)

ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
28.05.2019	755	06.05.2019	Prof. Dr. Bayram YILMAZ

'Yüksek yağlı diyet ile beslenen farelerde beyin ekstraselüler matriks proteinlerindeki değişiklikler ile kognitif fonksiyon, duysal ve motor davranışlar arasındaki ilişkinin araştırılması' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 3 Yıl	**Hayvan Türü ve cinsiyeti:** **Fare** ♀♂	**Hayvan Sayısı:** 48

GÖREVİ	ADI SOYADI
Başkan	Prof. Dr. Bayram YILMAZ
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA
Raportör	Vet. Hekim Engin SÜMER
Üye	Prof. Dr. M. Ece GENÇ
Üye	Prof. Dr. Rukset ATTAR
Üye	Doç. Dr. Soner DOĞAN
Üye	Doç. Dr. Ediz DENİZ
Üye	Prof. Dr. Gamze TORUN KÖSE
Üye	Doç. Dr. Aylin YABA UÇAR
Üye	Hakan GÖKSEL
Üye	Ahmet ŞENKARDEŞLER

7.2 Curriculum Vitae

Kişisel Bilgiler

Adı	Cihan Civan	**Soyadı**	Cıvaş

Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora			
Yüksek Lisans	Fizyoloji	**Yeditepe Üniversitesi**	2020
Lisans	Moleküler Biyoloji ve Genetik	**İzmir İleri teknoloji Ensititüsü**	2017
Lise	Sayısal	**Kastamonu Fen Lisesi**	2012

[#] Başarılmış birden fazla sınav varsa (KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Bildiği Yabancı Dilleri	Yabancı Dil Sınav Notu ([#])
IELTS	6.5

İş Deneyimi (Sondan geçmişe doğru sıralayın)

Görevi	Kurum	Süre (Yıl - Yıl)

Bilgisayar Bilgisi

Program	Kullanma becerisi
Microsof Office	İyi
MATLAB	zayıf

*****Çok iyi, iyi, orta, zayıf olarak değerlendirin

Bilimsel Çalışmaları

SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

Inactivation of Magel2 suppresses oxytocin neurons through synaptic excitation-inhibition imbalance

Diğer dergilerde yayınlanan makaleler

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler

Effects of Optogenetic and Chemogenetic Manipulations of Hypothalamic Kisspeptin Neurons on Hippocampal Astrocytes in Kiss-Cre Mice

Hakemli konferans/sempozyumların bildiri kitaplarında yer alan yayınlar

Investigation of the effect of kisspeptin neurons in the arcuate nucleus on the spatial learning and memory in Alzheimer's disease by pharmacogenetic methods.
The effect of different fat containing diet exposure in maternal-maturation periods on oily taste perception and fat preference in male Sprague-Dawley offspring rats.

Diğer (Görev Aldığı Projeler/Sertifikaları/Ödülleri)
