T.C.

YEDITEPE UNIVERSITY

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DEPARTMENT OF PHYSIOLOGY

INVESTIGATION OF THE ELECTRICAL AND BEHAVIORAL EFFECTS OF CHRONIC HIGH-FAT DIET ON LEPTIN RECEPTOR NEURONS IN THE TUBEROMAMMILARY NUCLEUS IN TRANSGENIC MICE

MASTER THESIS

ZEHRA YAĞMUR EROL

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Istanbul, 2022

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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 ..... and numbered .....

Prof. Dr. Bayram YILMAZ

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.

Zehra Yağmur EROL

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LIST OF SYMBOLS AND ABBREVIATIONS

AAV: Adeno Associated Virus

aCSF: Artificial Cerebrospinal Fluid

AMPA: α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid

AGRP: Agouti-Related Peptide

BMI: Body Mass Index

$CaCl_2$: Calcium Chloride

CART: Cocaine and Amphetamine Regulated Transcript

CNO: Clozapine-N-Oxide

CNS: Central Nervous System

Cre: Causes of Recombination

CSF: Cerebrospinal Fluid

DAO: Diamine Oxidase

ddH2O: Double Distilled Water

DNA: Deoxyribonucleic Acid

DREAAD: Designer Receptors Exclusively Activated by Designer Drugs

EDTA: Ethylenediaminetetraacetic Acid

EPM: Elevated Plus Maze

FLEX: Flip-Excision

GABA: Gamma (γ)-Aminobutyric Acid

GFP: Green Fluorescent Protein

GLP1: Glucagon-Like Peptide 1

GPCRs: G-Protein-Couple Receptors

HCRT: Hypocretin

HDC: Histidine Decarboxylase

HF: High Fat

HNMT: Histamine N-methyltransferase

IHC: Immunohistochemistry

Ip: Intraperitoneal

JAK2: Jak Family Tyrosine Kinase

KCl: Potassium Chloride

LepR: Leptin Receptor

Lox: Locus of Crossing

$MgSO_4$: Magnesium Sulfate

mRNA: Messenger Ribonucleic Acid

MSH: Melanocyte-Stimulating Hormone

NaCl: Sodium Chloride

$NaHCO_3$: Sodium Bicarbonate

$NaHPO_4$: Sodium Phosphate

NAOH: Sodium Hydroxide

NMDA: N-Methyl-D-Aspartate

NMDG: N-methyl-D-Glucamine

NPY: Neuropeptide Y

NTS: Nucleus Tractus Solitarius

Ob: Obese

OFT: Open Field Test

OSA: Obstructive Sleep Apnea

PBS: Phosphate Buffered Saline

PCR: Polymerase Chain Reaction

PFA: Paraformaldehyde

PI3K: Phosphatidylinositol3-Kinase

PNS: Peripheral Nervous System

POMC: Pro-Opiomelanocortin

REM: Rapid-Eye-Movement

RNA: Ribonucleic Acid

RT-PCR: Real Time Polymerase Chain Reaction

siRNA: Small Interfering Ribonucleic Acid

STAT3: Signal Transducer and Activator of Transcription 3

SWS: Slow-Wave-Sleep

TBE: Tris Borate EDTA

TMN: Tuberomammillary Nucleus

TNF: Tumor Necrosis Factor

VMH: Ventromedial Nucleus of Hypothalamus

vTMN: Ventral Tuberomammillary Nucleus

WHO: World Health Organization

α-FMH: α-Fluromethylhistidine

ABSTRACT

Erol, Z. Y. (2023). Investigation of the Electrical and Behavioral Effects of Chronic High-Fat Diet on Leptin Receptor Neurons in the Tuberomammillary Nucleus in Transgenic Mice. Yeditepe University Institute of Health Sciences, Department of Physiology, Master Thesis. Istanbul.

Neuronal histamine generated by histaminergic neurons in tuberomammillary nucleus (TMN) in the posterior hypothalamus affects wakefulness by stimulating hypothalamic neurons. Whereas the underlying mechanisms are not yet fully understood, may be related to nutrition. Leptin has an important role in various obesity-related orexigenic and anorexigenic neural pathways. Thought that sleep and wakefulness problems experienced by obese people may be related to the leptin-histamine mechanism. Currently, no study demonstrating the relationship between leptin receptor neurons (LepR) and histaminergic neurons about food intake in TMN. In present study was intended to research the electrophysiological and behavioral effects of high-fat (HF) diet on LepR neurons in TMN region in LepR-Cre mice. Forty-eight LepR-Cre transgenic mice were used, some were fed a standard diet, and others on HF diet. For electrophysiological experiments, Cre-dependent GFP virus was intracranially injected into TMN, and electrical activity was recorded by patch-clamp technique. Behavioral effects were observed by open field and elevated plus maze tests. Chemogenetic activation/inhibition were performed by injection of Clozapine-N-Oxide intraperitoneally. For the statistical analysis, the GraphPad program was used. Firing frequency of LepR neurons was decreased significantly in mice fed with HF diet ($p<0.0001$). In behavioral tests, HF diet decreased the locomotor activity. Activation of LepR neurons decreases the locomotor activity and triggers the anxiety-like behaviors ($p<0.01$) in OFT. In this study, the electrophysiological and behavioral effects of LepR neurons in the TMN in mice fed with chronic HF diet were investigated for the first time. These findings have shown that HF diet alters behavioral characteristics and neuronal activity in LepR-Cre transgenic mice.

Key Words: Leptin, Histamine, Diet, Electrophysiology, Behavior

ÖZET

Erol, Z. Y. (2023). Kronik Yüksek Yağlı Diyetin Tuberomamiller Çekirdekteki Leptin Reseptör Nöronları Üzerine Elektriksel ve Davranışsal Etkilerinin Transgenik Farelerde Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü Fizyoloji ABD. Yüksek Lisans Tezi. İstanbul

Nöronal histamin, arka hipotalamus tuberomamilar çekirdekteki (TMN) histaminerjik nöronlar tarafından üretilir, hipotalamik nöronları uyararak uyanıklılığı etkilemektedir. Tam anlaşılamayan bu mekanizma, beslenme ile ilişkili olabilir. Leptinin obeziteyle ilgili oreksijenik, anoreksijenik yolaklarda önemli rolü vardır. Obez insanlardaki uyku-uyanıklık problemlerinin leptin-histamin mekanizmasıyla ilişkili olabileceği düşünülmektedir. Leptin reseptör (LepR) nöronları ve histaminerjik nöronlar arasındaki ilişkiyi, ve bu nöronların beslenme üzerine etkisini gösteren bir çalışma bulunmamaktadır. Bu çalışmada, LepR-Cre farelerde yüksek yağlı diyetin TMN bölgesindeki LepR nöronları üzerindeki elektrofizyolojik ve davranışsal etkilerinin incelemesi amaçlanmıştır. Çalışmada 48 adet LepR-Cre transgenik fare kullanıldı. Farelerin bir kısmı standart diyetle beslenirken bir kısmı kronik yüksek yağlı diyetle beslendi. Elektrofizyolojik çalışmalar için farelere Cre-bağımlı GFP virüsü TMN bölgesine intrakraniyal olarak enjekte edildi, nöronlardaki elektriksel aktivite değişiklikleri patch-clamp tekniği ile kaydedildi. Davranışsal etkiler açık alan ve artı labirent testleriyle gözlemlendi. Kemogenetik aktivasyon/inhibisyon, intraperitonel olarak N-Oksit Klozapin enjeksiyonuyla gerçekleştirildi. İstatistiksel analizler için GraphPad programı kullanıldı. Yüksek yağlı diyetle beslenen farelerde LepR nöronlarının ateşleme frekansının standart diyetle beslenenlere göre anlamlı derecede azaldığı görüldü ($p<0.0001$). Davranış testlerinde, yüksek yağlı diyet lokomotor aktiviteyi azalttı. OFT'nde bu nöronlarının aktivasyonu lokomotor aktiviteyi azalttı, anksiyete benzeri davranışları tetikledi ($p<0.01$). Bu çalışmada, kronik yüksek yağlı diyetle beslenen farelerde TMN alanındaki LepR nöronlarının davranışsal ve elektrofizyolojik etkileri ilk kez incelenmiştir. Bu bulgular, yüksek yağlı diyetin LepR-Cre farelerde davranış özelliklerini ve nöronal aktiviteyi değiştirdiğini göstermiştir.

Anahtar Kelimeler: Leptin, Histamin, Diyet, Elektrofizyoloji, Davranış

1. INTRODUCTION AND PURPOSE

Histamine is a neurotransmitter that has a modulatory role in the mammalian brain. Apart from being significantly associated with allergic conditions, it is also thought to play a fundamental role in wakefulness [1]. Histamine can be produced in two ways by mast cells to elicit an allergic response to pathogens in allergic conditions or it can be produced by the histamine neurons. Neuronal histamine is produced in the brain by histaminergic neurons which locate in the tuberomammillary nucleus (TMN) in the posterior hypothalamus, and these neurons project to almost all areas of the mammalian brain [2, 3]. Whole mechanism and function of histamine has not been fully understood but known that the hypothalamic neurons have excitatory wake-promoting effect and demonstrated that gamma-aminobutyric acid (GABA) suppress wakefulness [3] and glutamate increases the wakefulness by activating TMN [4]. In another study reveals that hypothalamic histamine neurons in the TMN have an effect on feeding behavior [5].

On the other hand, leptin which is a major peptide hormone regulates mainly food intake, energy expenditure, body mass and plays a crucial role in many physiological processes. This hormone is also a product of obese (ob) gene and it is produced from adipose tissue in the body. Leptin binds directly to the leptin receptors (LepRs) which are mostly found in hypothalamus [6, 7]. Thus, hunger is inhibited and satiety is mediated by activating orexigenic neural pathways to reduce food intake [8]. In the literature, there are various studies about leptin that have a key role on orexigenic and anorexigenic neural pathways also related with obesity [9, 10]. However, the relationship between histaminergic and LepR neurons in the TMN area and their effects on food intake is still unclear.

Food intake may be related to the wakefulness mechanism by LepR neurons in histaminergic area and this mechanism may have an effect on obesity which is one of the major problems of our age. It is known that; obese people experience sleep problems especially in deep sleep and they have low sleep quality and also in many obese people have obstructive sleep apnea (OSA) [11]. Short and low quality of night sleep is one of the common causes of daytime sleepiness and is considered also a risk factor for obesity. Short sleep duration has been shown to have a clear and direct effect on metabolism, promoting weight gain, increased appetite and insulin resistance [12]. In the present study was intended to research the electrophysiological and behavioral effects of high-fat (HF) diet on LepR neurons in TMN region in LepR-Cre mice.

2. GENERAL INFORMATION

2.1. Histamine

2.1.1. Histamine Metabolism (Synthesis and Inactivation)

Histamine (2-(4-imidazolyl)-ethylamine) is a bioactive amine and is formed as a result of the histidine amino acid decarboxylation with the histidine decarboxylase (HDC) enzyme [13]. Histamine can be produced in two ways in the body sources; by mast cells to elicit an allergic response to pathogens in allergic conditions or it can be produced by the histaminergic neurons in the brain.

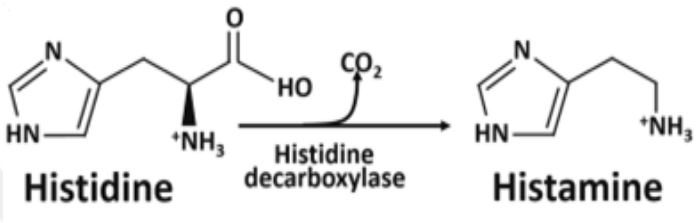

Fig1. Conversion of histidine to histamine by HDC enzyme [13].

Inactivation of histamine is provided by some enzymes. In the central nervous system (CNS), the neuronal histamine is inactivated by methylation via N-methyltransferase (HNMT). In the peripheral system; diamine oxidase (DAO) is one of the main degrading enzymes of histamine and it converts the histamine directly to the imidazoleacetic acid which is the $GABA_A$ receptor agonist. Under the basal conditions in the brain, the DAO activity is lower than the HNMT activity [14].

2.1.2. Histamine Function and Receptors

Histamine has four types receptors named as H_1R, H_2R, H_3R, H_4R and these histamine receptors belongs to the G protein coupled receptor (GPCR) family which are one of the largest groups of the membrane proteins that found on cell surface and they form the cellular response to many neurotransmitters and hormones [15, 16].

Each histamine receptor can locate different tissues and organs of both in the peripheral nervous system (PNS) and the central nervous system (CNS) and each receptor has different functions in the body. Therefore, histamine and its receptors can be associated with different diseases and physiological circumstances; both in the PNS and the CNS such as immunomodulation, gastric acid secretion, vasodilation, smooth muscle contraction.

In the gastrointestinal system, the vagus nerve is responsible for the regulation of histamine mobilization via enterochromaffin-like cells by controlling gastrin sensitivity in the stomach. Gastric acid secretion is controlled by histamine by activating the proton pumps via H_2R receptors [14].

In the immune system, histamine and its receptors have a significant role in both innate and acquired immunity. It can be produced by mast cells which is the main source of the histamine production to elicit an allergic response to pathogens in allergic conditions. For instance, H_1R is widely found in many cells especially in mast cells and this receptor is related to Type 1 hypersensitivity. Besides mast cell functions, histamine production and signalling are also controlled by various immune signals such as interferons and cytokines networks via H_2R and H_4R and these receptors are related to allergic diseases. H_3R is related to blood-brain-barrier function. [14, 17].

2.1.3. Histamine Receptors in the Brain

H_1R and H_2R are postsynaptic and these receptors have mostly excitatory effects on the neurons. H_3R is mainly found on histaminergic neurons, also on their somas, axons and dendrites of other neurons. In the lack of histamine, H_3R performs the spontaneous and constitutive activity. This constitutive activity in the brain has a potential regulatory role [1]. Recent studies have revealed that H_4R is also expressed in the brain and the highest levels of H_4R mRNA was detected in the spinal cord. The whole mechanisms and functions of histamine receptors have not been fully understood yet [1, 18].

2.1.4. Neuronal Histamine

In addition to being significantly associated with allergic conditions, histamine is a neurotransmitter that has a modulatory role in the mammalian brain, also thought to play a significant role in wakefulness in CNS. Neuronal histamine is generated by histaminergic neurons which locate in the TMN in the posterior hypothalamus additionally, these histaminergic neurons project to almost all areas of the mammalian brain including the olfactory bulb, caudate nucleus, hippocampus, spinal cord and cerebellum [2, 3].

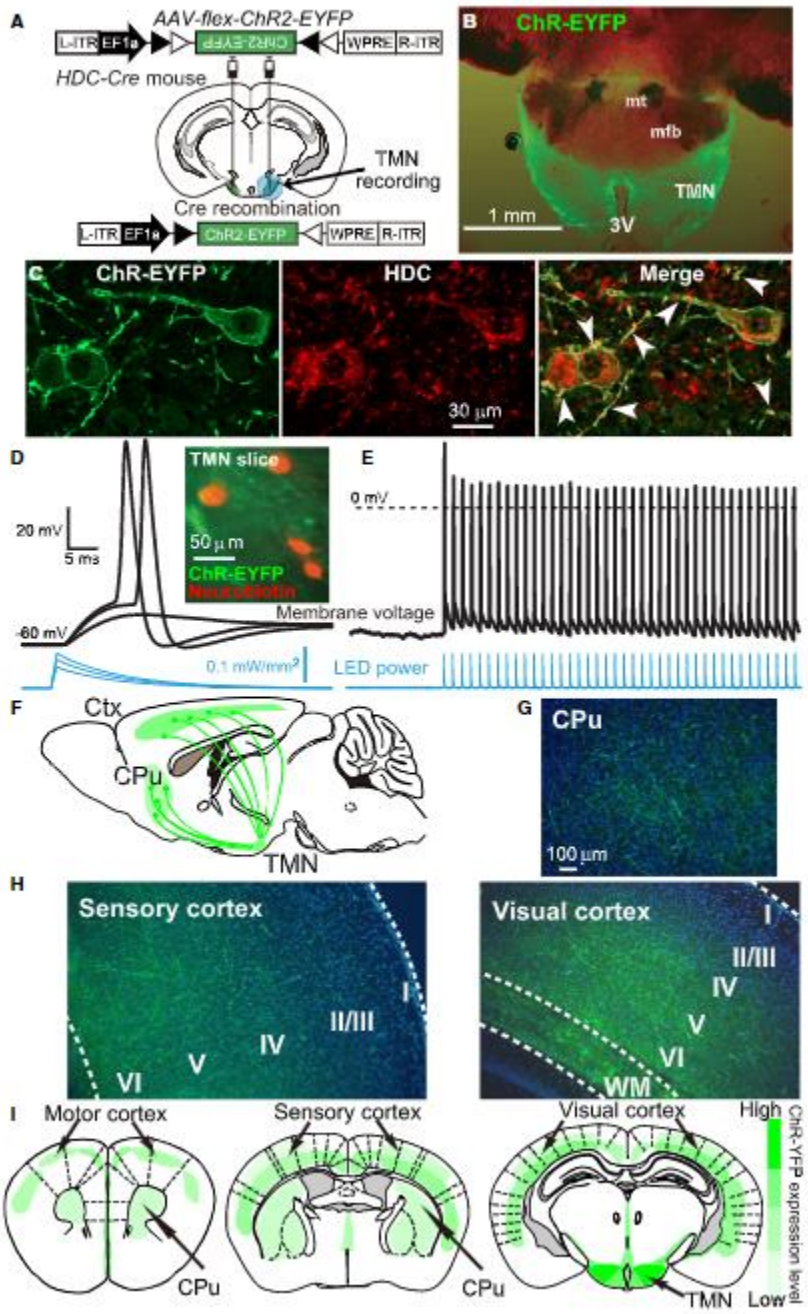

Fig2. The histaminergic neurons and their axonal projections from TMN to the neocortex, striatum and the other brain regions were shown in HDC-Cre mice after bilaterally ChR2-EYFP injection into TMN [3].

In human brain there are more than 64,000 histaminergic neurons and project their axons into almost all brain regions. The neuronal histamine also interacts with four histamine receptors. Each receptor locates different regions in the brain and have different functions. Therefore, histamine is related to many neurological diseases and physiological circumstances [2, 19].

Neuronal histamine is involved in the brain homeostasis and regulation of various neuroendocrine functions. It has a critical role in the regulation of behavioral state, energy metabolism, body weight, appetite, thermoregulation, sleep-wake cycle, circadian rhythms, locomotor activity, fluid balance, reproduction and stress. Additionally, it is also related to the higher brain functions for instance motor and sensory function, learning and memory, reward, emotional state [1, 20].

On the one hand, γ -aminobutyric acid (GABA) is one of the major neurotransmitters that shows inhibitory effects on the mammalian brain. The inhibitory action of GABA is performed by its two receptors; the $GABA_A$ the and $GABA_B$. These both receptors are commonly found in hippocampus, thalamus, basal ganglia, cerebral cortex, cerebellum, and brainstem. The $GABA_A$ receptors are the ligand-gated chloride (Cl^-) channels which are sensitive to the bicuculline. In contrast, the $GABA_B$ receptors which are insensitive to the bicuculline, also affect the calcium (Ca^{++}) and potassium (K^+) conductance [21].

Three groups of GABA containing neurons are found widely in the mammillary area of the posterior hypothalamus. These groups are the caudal, tuberal and postmammillary caudal magnocellular nuclei. TMN neurons also store GABA [2, 22]. TMN neurons can act as one of the most important sources of extrasynaptic GABA in the brain. These extrasynaptic GABA activate the $GABA_A$ receptors in various regions of brain such as the hippocampal dentate gyrus, cerebellar and cerebral cortex [2].

Until the discovery of nonbrain-penetrating options, scientists observed the drowsiness effect of antihistaminic anti-allergic drugs. This situation suggests that histamine has a significant role in some neuronal mechanisms related to wakefulness and sleep. Additionally, it is known that the hypothalamic neurons perform an excitatory wake-promoting effect. Several researches have revealed that the histaminergic network in TMN is a crucial element of the sleep–wake system and this system comprises histaminergic, serotonergic, cholinergic, noradrenergic, orexin and GABAergic signallings [2]. One study showed that, when injected intraperitoneally the α-fluoromethylhistamine (α-FMH) which is a specific HDC inhibitor decreased the wakefulness and increased the deep slow-wave sleep (SWS) in ventral posterolateral hypothalamus [2, 23]. Another study showed the GABA suppresses wakefulness; most histaminergic neurons store GABA and glutamic acid decarboxylase (GAD) enzyme, when the small interfering RNA (siRNA) knockdown vesicular GABA transporter of

histaminergic neurons, occurs the exceptional amount of continuous wakefulness in the hyperactive mice [3].

Histamine neurons commonly exhibit a spontaneous firing with low frequency approximately 1-4 Hz. but when these neurons are waking up, their firing rate is increased. During SWS the firing is also very low and also no firing may be observed during rapid-eye-movement (REM) sleep. From the ventrolateral preoptic area during sleep, the GABAergic control mediates the histaminergic neurons inhibition [24].

On the other hand, another study reveals that, the glutamate which is one of the main excitatory neurotransmitters in the brain increases wakefulness by activating TMN area. According to the study when the glutamate and glutamatergic receptor agonists (N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)) injected dose dependently into the TMN region, it increased wakefulness and the firing rate of action potentials of histaminergic neurons by activating TMN area. Additionally, when the NMDA receptor antagonist was injected, the firing rate of these neurons decreased and non-rapid eye movement sleep increased in rats. These findings reveal that glutamate increased wakefulness via activating the histaminergic neurons in TMN in rats results from a NMDA action and H_1Rs [4].

2.1.5. Histaminergic System and Feeding

Additionally, some study reveals that hypothalamic histamine neurons in the TMN have an effect on feeding behavior. Appetite and satiety are the key factors of eating behaviors.

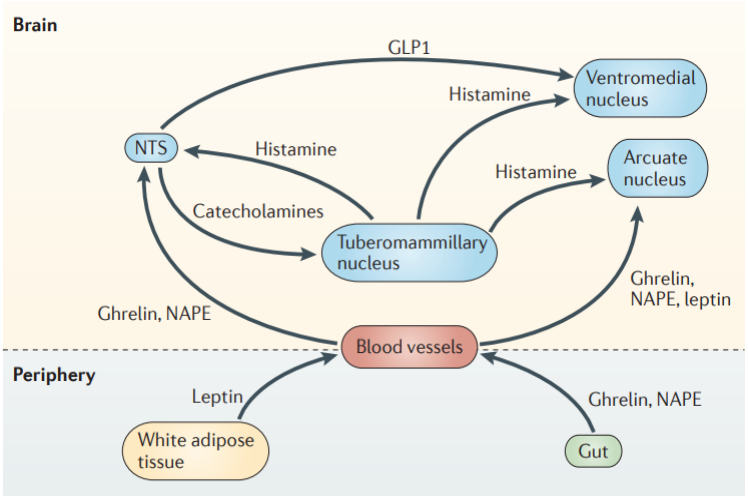

Fig3. Some brain regions that regulate feeding and their innervation via histaminergic fibers are shown [2].

Histamine affects food intake by increasing the arousal states of the animals. Histaminergic system is important for arousal during motivated behaviors. H_1R and H_3R have a fundamental role in food intake and control of the body weight because it is also an autoregulator in synthesis and release of histamine. These receptors may have an anti-obesity effect [2, 25].

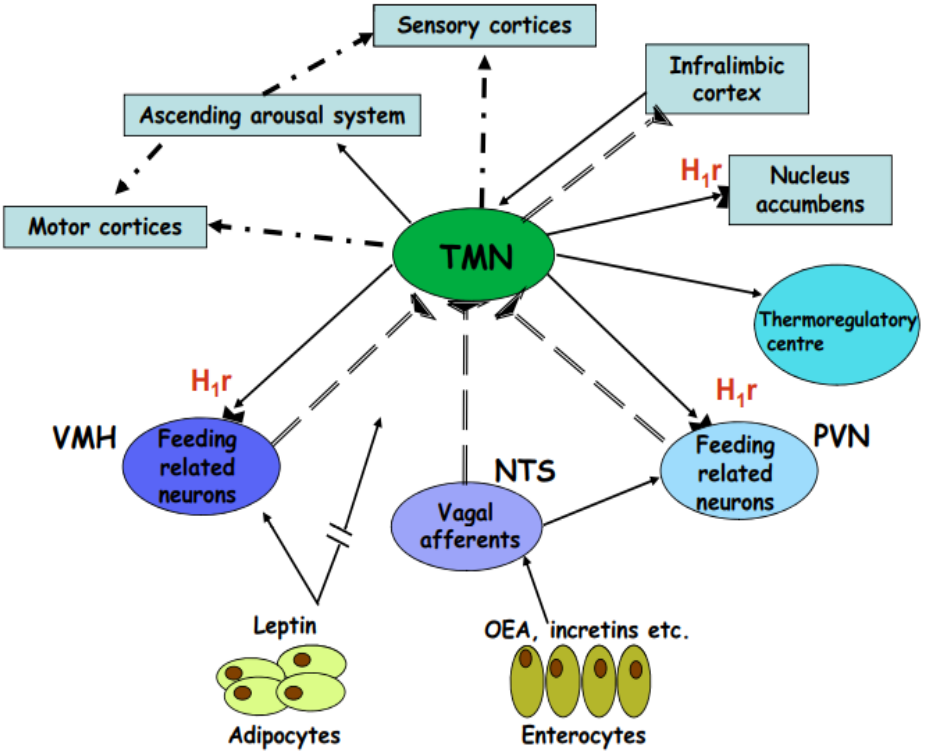

Fig4. Histaminergic neurons in TMN and their several hypothesized actions in feeding behavior [25].

Above figure double lines refer to the presumed connections between feeding behavior related regions and TMN. The dot-dash lines refer to the presumed connections between cortical regions related to appetitive behavior and TMN.

The vagal complex, arcuate nucleus, the nucleus tractus solitarius (NTS) and ventromedial nucleus of hypothalamus (VMH) are highly innervated by the histamine containing fibers from TMN, and there is high amount of H1R binding is in these areas. Histamine decreases food intake via H1R in the VMH. The NTS also innervates the histaminergic neurons in TMN [2]. TMN also provides the cortical arousal and supports the thermal responses [2, 25].

2.2. Sleep Disorders and Obesity

2.2.1. Sleep Disorders

Proper sleep is vital to health. Sleep disorders have been associated with various health problems. For instance, heart diseases, obesity and diabetes. According to the International Classification of Sleep Disorders lists, sleep disorders are categorized into 7; insomnia, parasomnia, sleep-wake disorders about circadian rhythm, central disorders of hypersomnolence, sleep related breathing disorders, movement disorders and others [26] In addition, the reduced sleep quality and sleep durations are related to increase in adiposity also body weight. It is known that obese people experience sleep problems especially in deep sleep and they have low sleep quality and also in many obese people have OSA [11]. Short and low quality of night sleep is a common cause of daytime sleepiness and is considered also a risk factor for obesity. Short sleep duration has been shown to have a clear and direct effect on metabolism, promoting weight gain, increased appetite and insulin resistance [12].

Moreover, rodent and human studies that containing healthy individuals and patients have narcolepsy showed the histaminergic network in TMN is a significant factor of the sleep-wake system. Narcolepsy which is a rare sleep disorder and is also defined as excessive daytime sleepiness. According to the pathophysiological studies, in the early state the orexinergic neurons loss in the hypothalamus lead to narcolepsy [1]. The sleep-wake system includes cholinergic, noradrenergic, hypocretin (HCRT or orexin), serotonergic, histaminergic and GABAergic signalling and provides rapid transition from wakefulness to the sleep [1, 2]. Neuronal histamine promotes wakefulness by activating raphe serotonergic and basal forebrain cholinergic neurons. Other studies reveal that histamine levels in cerebrospinal fluid (CSF) are decreased in people with narcolepsy and supports the histamine promotes the wakefulness and alertness. While the absence of HCRT can lead to narcolepsy in contrast, H3R antagonist increased the sleep quality in patients have narcolepsy and increased wakefulness in the narcoleptic animals. These results have shown that low amounts of histamine correlate with the low amount of wakefulness and increased sleepiness [2].

2.2.2. Obesity

Nowadays, obesity is one of the major problems of our age. According to the World Health Organization (WHO), obesity is identified as a disproportionate increase in body weight as a consequence of excessive amounts of adipose tissue accumulation. It is associated with many diseases; type-2 diabetes, cardiovascular diseases and some cancer types. Globally, obesity and also obesity related disorders are increasing worldwide. Body mass index (BMI) is commonly utilized to indicate obesity in adults. Obesity is a BMI greater than or equal to 30 [27, 28].

2.3. Leptin and Feeding Behavior

Feeding behavior has complex regulatory mechanisms and several factors have crucial roles to regulate satiety and food intake. These factors consist of central peptidergic factors (neuropeptide Y, melanocortin, pro-opiomelanocortin, glucagon-like peptide 1 (GLP1), cocaine and amphetamine regulated transcript (CART)), the peripheral petidergic factors which are leptin and ghrelin, and the nutrient-derived factors (glucose and lipids) and they are involved in regulation of satiety food intake. However, the precise roles of these factors in feeding are not understood well. In addition, the cortical and sensory areas, hypothalamus, brainstem nuclei are all involved in food intake regulation. In these several regions, histamine also regulates some factors that participates in food intake [2].

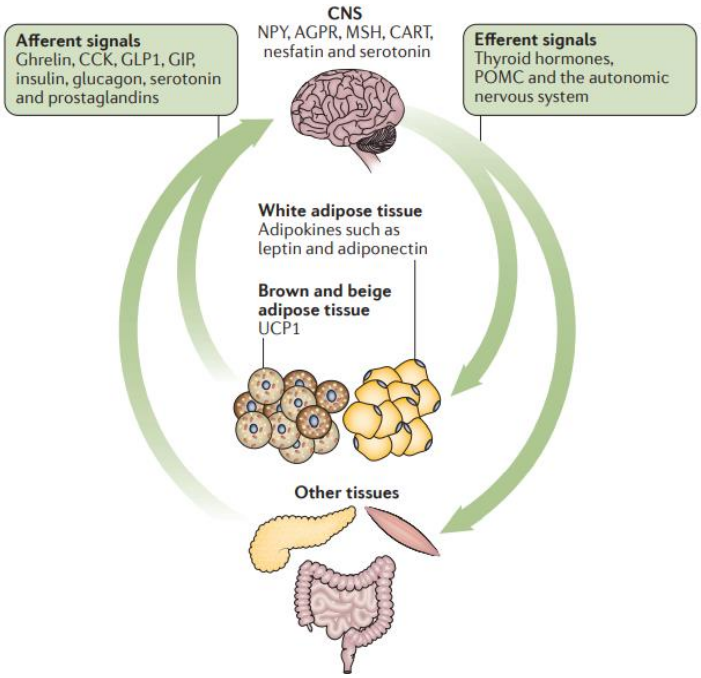

Fig5. The control of the hunger and the satiety [28].

Satiety and hunger are regulated by some complex interactions between the CNS, circadian rhythms, mechanical sensing, nutrients and hormones. In the hypothalamus many neurotransmitters and many neuropeptides participate in food intake regulation. Neuropeptide Y (NPY) and agouti-related peptide (AGRP) stimulate food intake in contrast, melanocyte-stimulating hormone (MSH), CART and pro-opiomelanocortin (POMC) inhibit the food intake.

Adipocytes are the significant functional unit of the adipose tissue that secretes the adipokines for instance, adiponectin, leptin, visfatin, interleukin-6, apelin, tumor necrosis factor (TNF) generated by adipose tissues and they are related with appetite, inflammation, thermogenesis, and fat deposition [28, 29]. Thermogenin participates in heat generation. Pancreatic and gastrointestinal hormones such as GLP1, insulin, glucagon, ghrelin, gastric inhibitory polypeptide, and serotonin are involved in food intake, and control the hunger. Some hormones such as ghrelin increase the food intake in contrast, others such as GLP1, serotonin, insulin decrease food intake [28]. Here, leptin and ghrelin are the significant hormones. Ghrelin is also known as hunger hormone oppositely; leptin is known as the satiety hormone and it inhibits hunger by regulating energy balance [30].

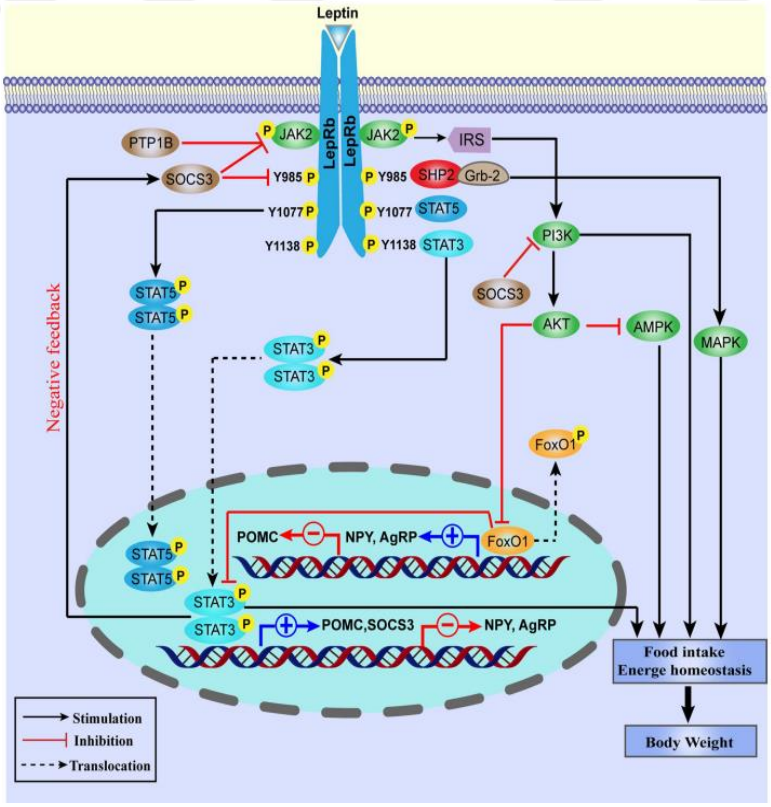

Fig6. Leptin signalling pathway [31].

Leptin is a 16-kDa protein and encoded by Ob gene and has important roles mainly in hypothalamus; inhibit appetite, and control food intake and body weight. There are six isoforms of the leptin receptor and they are named as ObRa, ObRb, ObRc, ObRd, ObRe and ObRf. The ObRb is most abundant in hypothalamus. These receptors are expressed widely in peripheral tissues and all physiological actions are still not fully understood. However, leptin deficiency is a major cause of the obesity, in humans. This situation may be explained with the leptin resistance because a high concentration level of leptin does not allow the reduction in food intake. Leptin resistance can result from a mutation of the LepR [30, 32].

LepR (mostly ObRb) is involved in intracellular signalling and it is vital for leptin action. Binding of leptin to Ob-Rb initiates the signals with the activation of the Jak family tyrosine kinase (JAK2) on some tyrosine residues during leptin stimulation. Then, tyrosine is phosphorylated and JAK2 phosphorylates of tyrosine residues (Tyr985, Tyr1077, Tyr1138) on ObRb.

The phosphatidylinositol3-kinase (PI3K) signalling promotes the energy balance regulation by leptin because inhibiting the PI3K, the feeding suppression is blocked. Deletion of signal transducer and activator of transcription 3 (STAT3) which is the transcription factor that encoded by the STAT3 gene, activates the transcription of the neuropeptide and POMC, causing overfeeding and obesity. STAT3 signal is necessary for the regulation of energy expenditure and feeding by leptin. After STAT3 phosphorylation, it is translocated from cytoplasm to the nucleus. JAK2/ STAT3 signaling pathway has a crutial role in energy homeostasis and also neuroendocrine function [32].

3. MATERIALS AND METHODS

All experiments were carried out at Yeditepe University Faculty of Medicine Department of Physiology Brain Research Laboratories. The experimental protocol was approved by Yeditepe University Faculty of Medicine Animal Experiments Ethics Committee with the decision numbered 022/01-3 on 28.01.2022. All statistical analyzes were performed by using GraphPad Prism v.9.0 program. $P<0.05$ was considered as statistically significant. For the scientific figure illustration BioRender program was used.

3.1. Experimental Animal Used in Study

In this study, 48 female and male (3-4 months age) transgenic LepR ObRb-Cre transgenic mice (Jackson Lab Code: #008320) were used. Mice were kept at 21±1 ˚C and 12 hours light-dark environment.

Food and tap water were given ad libitum to all mice and mice were divided into behavioral (n=36) and electrophysiology groups (n=12) and each group, two different diets were applied to these mice. In each group, the half of mice were fed with a standard diet (CHOW) and the other half were fed with chronic high-fat (HF) diet (consist of 60 kJ% fat)

3.2. Mice Production Strategy

In this study, LepR-Cre transgenic mouse line was imported from Jackson Laboratory as 1 male and 1 female mouse and produced by applying Homozygous x Homozygous mating strategy. In accordance with this strategy, a mating cage consisting of two females and one male was created. Production and maintenance of mice were carried out at Yeditepe University Faculty of Medicine Experimental Research Centre.

3.3. Genotyping

The newborns were separated according to their gender. After the lactation period, the tissue samples were taken from their ears from mice were collected into DNase free eppendorf tubes for DNA isolation and the standard PCR. Tubes were stored at -21°C.

3.3.1. DNA Isolation and Polymerase Chain Reaction (PCR)

For DNA isolation of samples 100 µl alkalizing buffer 25 mM NaOH and 0.2 mM EDTA were added in each sample respectively. Then, all samples were incubated at 98°C for 1hour. Then, 100 µl neuralization buffer (40 mM Tris-HCl) was added in each sample and centrifuged at 4000 rpm for 4 min. (Microcentrifuge, Sigma 1-14). Then, supernatants

were separated into new tubes and they were used in PCR, and PCR mix solution was prepared.

Table 1. Control and transgenic PCR contains and their concentration were shown.

Chemicals	Concentration
10X Reaction Buffer	1X
Forward & Reverse Primers	0.5 µM
dNTP Solution Mix	200 µM
Taq Polymerase	1,25 unit/50 PCR
dH2O	
Isolated DNA	

Table2. The used Cre primers in the study.

Primers	Sequence 5´→ 3´
Cre Forward	GCC AGC TAA ACA TGC TTC ATC
Cre Reverse	ATT GCC CCT GTT TCA CTA TCC

Table3. PCR protocol for thermal cycler.

Number of Cycle	Temperature (°C)	Time (s)
1	95	30
38	95	30
38	60	30
38	68	60
1	68	300

According to the above Table 1 ingredients, a PCR mix was prepared as 12,5 µl for each sample. Then, samples were run at thermal cycler (Multigene Optimax, TC9610). After PCR protocol was used, 1 µl dye (DNA gel loading dye) was added on all samples and samples were loaded in 2% agarose gel. Before loaded samples, the ladder which refers to the different size of DNA fragments as bands was loaded in the first well,

negative and positive controls were loaded in the next wells. Samples were loaded into other wells respectively. Then, gel electrophoresis was performed at 120V, 300 mA for 18 min. After that, the 2% agarose gel was monitorized by using Biorad Chemidoc device.

Fig7. PCR result of LepR-Cre mice.

3.4. Experimental Groups

Mice were divided into behavioral (n=36) and electrophysiology groups (n=12) and each group, two different diets were applied to these mice.

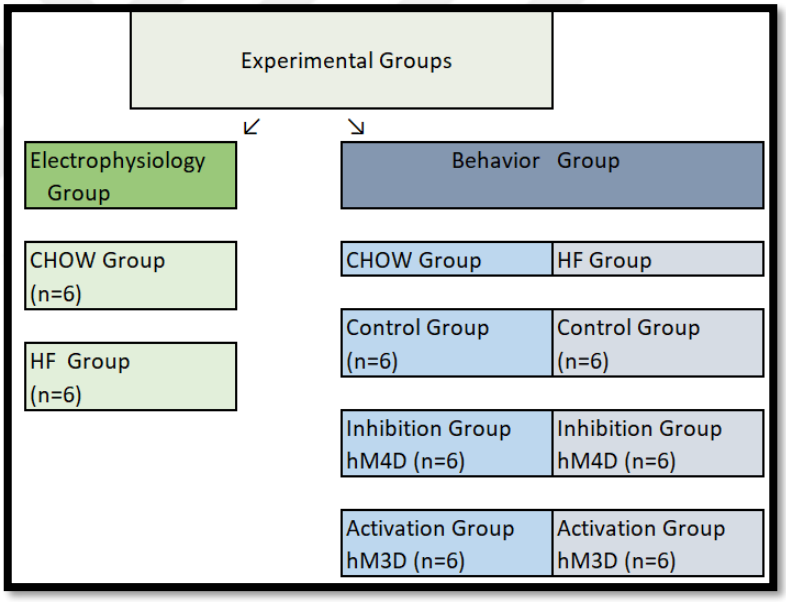

Fig8. Behavior and electrophysiology experimental groups.

3.5. Intracranial Injection of Cre-Recombinase Enzyme-Dependent AAV Viruses

LepR mice will be injected with AAV-CAG-Flex-GFP virus. The genetic map of this virus includes GFP gene and LoxP sites which the Cre-recombinase enzyme binds. Thus, the GFP expression of specific neurons is ensured by marking these neurons that contain Cre-recombinase enzyme in Cre-dependent transgenic mouse line. In this study, these target neurons will be LepR neurons and the intracranial injection was applied into

vTMN region in the posterior hypothalamus bilaterally by using stereotaxic device (David Kopf) under the anesthetic isoflurane gas.

After mice were deeply anesthetized the hair was cut on the scalp. Then, an antiseptic was applied to this area and the surgical incision was made. Cold saline was applied. Subsequently, the length between the Bregma-Lambda points on the skull was measured by the electrode holder.

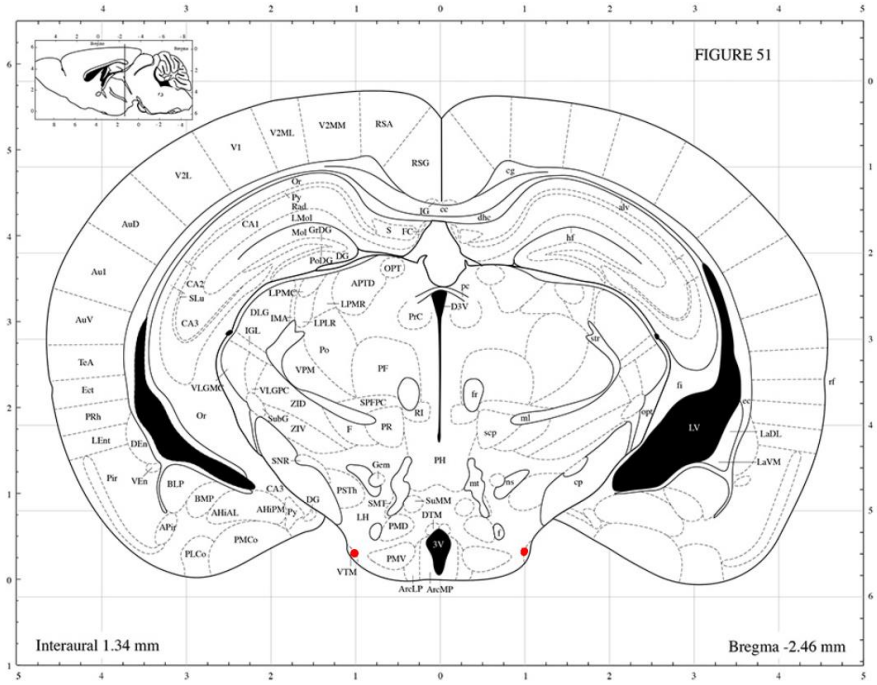

Fig9. Coronal slice of the mouse brain that shows the vTMN area with the red dots [33].

The coordinates of the vTMN region determined according to the Paxinos Mouse Brain Atlas are 2.46 mm posterior, ± 1.0 mm lateral and 5.5 mm vertical [33]. Measuring the distance between the Bregma and Lambda points was done for all mice and new coordinates were calculated by proportioning when necessary. Then, the small holes were made using a surgical drill in these coordinates as bilaterally. For the virus injections, the glass micropipettes (Drummond Scientific, Wiretrol, Broomall-PA) were used and these micropipettes tips were further thinned by using pipette puller (Narishige, Japan) to reduce the brain tissue damage. Then, mineral oil was added into micropipette and placed into the micromanipulator. A low amount of mineral oil was taken out and 1.2 µl of virus was drawn. Then 600 nl virus was injected bilaterally into the vTMN region into the hypothalamus. After the injection of virus, waited for 8 minutes. After all processes completed the scalp was sutured with 4.0 surgical suture and cleaned again with an antiseptic.

Fig10. Intracranial virus injection with glass pipette in mouse brain in stereotaxic device.

Same protocol was used for the hM3D and hM4D virus injections into the vTMN. Activation of hM3D which is a cation channel, and inhibition of hM4D which is an anion channel will be achieved with Designer Receptors Exclusively Activated by Designer Drugs (DREAAD) technology which is based on the sensitization of muscarinic receptors to Clozapine-N-oxide (CNO) instead of their own antigen. With these channel proteins inserted into the virus DNA, only the targeted LepR neurons are infected. At the end of 2 weeks, LepR neurons have channel proteins that can be activated/inhibited with CNO.

3.6. Preparation of Brain Slices and Electrophysiologic Recordings (Patch Clamp)

For in vitro electrophysiological recordings, the mice will be anesthetized deeply with isoflurane. Then, they will be perfused transcardially by using ice-cold N-methyl-D-glucamine (NMDG)-based cutting solution (92 mM NMDG, 1.25 mM $NaHPO_4$, 2.5 mM KCl, 30 mM $NaHCO_3$, 25 mM glucose, 20 mM HEPES, 2 mM thiourea, 3 mM sodium pyruvate, 5 mM sodium ascorbate, 10 mM $MgSO_4.7H_2O$ and 0.5 mM $CaCl_2.2H_2O$) and mice were decapitated and brains were removed.

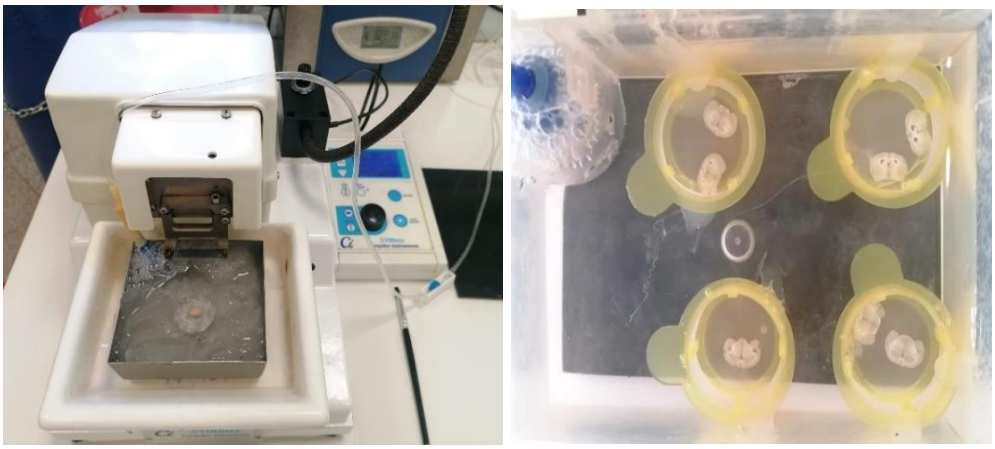

Fig11. (Left) Obtaining coronal brain slices in vibratome and (Right) obtained 250 μm-thick slices.

The 250 μm-thick coronal slices were obtained by using the vibratome device as seen in the above figure and they were incubated with 95% O_2 - 5% CO_2 gas mixture continuously. After incubation of vTMN-containing slices were placed into aCSF solution (92 mM NaCl, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 2 mM thiourea, 30 mM $NaHCO_3$, 25 mM glucose, 20 Mm HEPES, 3 mM sodium pyruvate, 5 mM sodium ascorbate, 2 mM $CaCl_2.2H_2O$ and 2 mM $MgSO_4.7H_2O$) and incubated for 1 hour. Then slices were put into a recording chamber that perfused with recording aCSF solution (124 mM NaCl, 1.25 mM $NaHPO_4$, 2.5 mM KCl, 5 mM HEPES, 12.5 mM glucose, 24 mM $NaHCO_3$, 2 mM $CaCl_2.2H_2O$ and 2 mM $MgSO_4.7H_2O$) by in patch clamp device (Multi Clamp 700B Axon CNS). Then, LepR neurons in vTMN were identified and recorded as cell-attached. As a result of this, the firing frequencies of LepR neurons were analyzed.

3.7. Feeding Behavior

After waiting for the virus injection period of injected LepR mice, the mice were taken into behavioral cages. Then, in their feeding behaviors between control that fed with CHOW and the experimental groups that fed a chronic high-fat diet for 6 weeks and their daily food intake were calculated every day and their body weight were weighed once in three days then, analyzed.

3.8. Locomotor Activity (Home Cage Activity)

In order to obtain the locomotor activity recordings, a camera system was attached to the behavior cages and 24h recordings were obtained. It was applied twice as the first day and the last day of the chronic diet in the cage. Thus, locomotor activity of mice was recorded for 24h with these cameras.

3.9. Behavioral Tests

All behavioral tests were performed at the same time at 10 am. Two days before the test day, mice were handled to avoid stress. On the test day, mice were housed into the new cages and transferred to the behavior testing room and waited for 1 hour to get used to the room conditions. 30 minutes before testing in the arena, each mouse was injected CNO intraperitonially. With this way, the LepR neurons activated and inhibited with CNO. The hM3D injection group was activation group, the hM4D injection group was inhibition group and GFP injection group was the control group in these tests.

3.9.1. Open Field Test (OFT)

OFT was performed to observe locomotor activity and the anxiety-like behaviors. Mice were placed into the Plexiglass chamber (80cm*80cm*40cm) and mice were kept for 10 min. Total spent time in center, the frequency in center and the velocity of mice in the chamber were observed and analyzed.

3.9.2. Elevated Plus Maze (EPM)

This test was used to analyze anxiety-like behaviors of LepR mice. Mice were placed into the Plexiglass chamber (110cm*110cm*40cm) that consisting of plus-shaped arms and their movements were observed and analyzed according to their preferences of arm choices (as opened or closed arm).

3.10. Immunohistochemistry (IHC) and Confocal Imaging

IHC is the tool to observe and analyze the localization and distribution of the biological molecules in the biological tissues. In this study, IHC technique was used to observe the colocalization of the histaminergic and the LepR neurons in the vTMN region of hypothalamus in mCherry-labelled hM3D and hM4D injected mice. mCherry makes the LepR neurons glow in red color. Firstly, the coronal slices were washed with 1M glycine 3 times for 15 min. Then, washed with 1X PBS for 15 min. Then 0.1% TritonX-100 based permeabilization solution was added onto slices and incubated at 4ºC for 1 hour on the shaker. After that, the blocking solution (0.3% BSA in 1X PBS, 0.05% TritonX-100) was added and incubated at 20ºC for 1hour on the shaker. Then, anti-histamine primary antibody (Thermo Scientific, H7403) 1:2000 was added and incubated at 4ºC overnight. After that, the solution was removed and the primary antibody dilution buffer (1% BSA in 1X PBS, 0.05% TritonX-100) was added on slices and washed for 15 min. Washed again with 1X PBS 3 times for 15 min on shaker. Then, secondary antibody (Cell Signaling, #44125) was added and incubated at dark and at room temperature for 1 hour on the shaker. Then, washed with dilution buffer for 15 min and washed again with 1X PBS 3 times for 15 min on shaker. After IHC was completed, the slices were closed using a slide and a coverslip by adding mounting medium on slices. These processes were carried out in the dark. After all, the slices were viewed under a confocal microscope.

4. RESULTS

4.1. Feeding Behavior

According to the results of 6 weeks of chronic diets observed that, as seen in **Figure 12 A-B,** mice fed HF diet had significantly increased in their body weight ($p<0.05$) although they consumed less feed than mice fed with CHOW ($p<0.001$). **Figure 12 C,** analysis of the body weights alterations as on the first and last days of their diets showed there was no significant increase in body weight in CHOW, while a significant increase in body weight was observed in HF fed mice ($p<0.001$). **Figure 12 D-E,** when body weights were analyzed according to gender, observed significant increase in body weight in females fed with HF compared to males (n=26 $p<0.0001$, n=19 $p<0.05$).

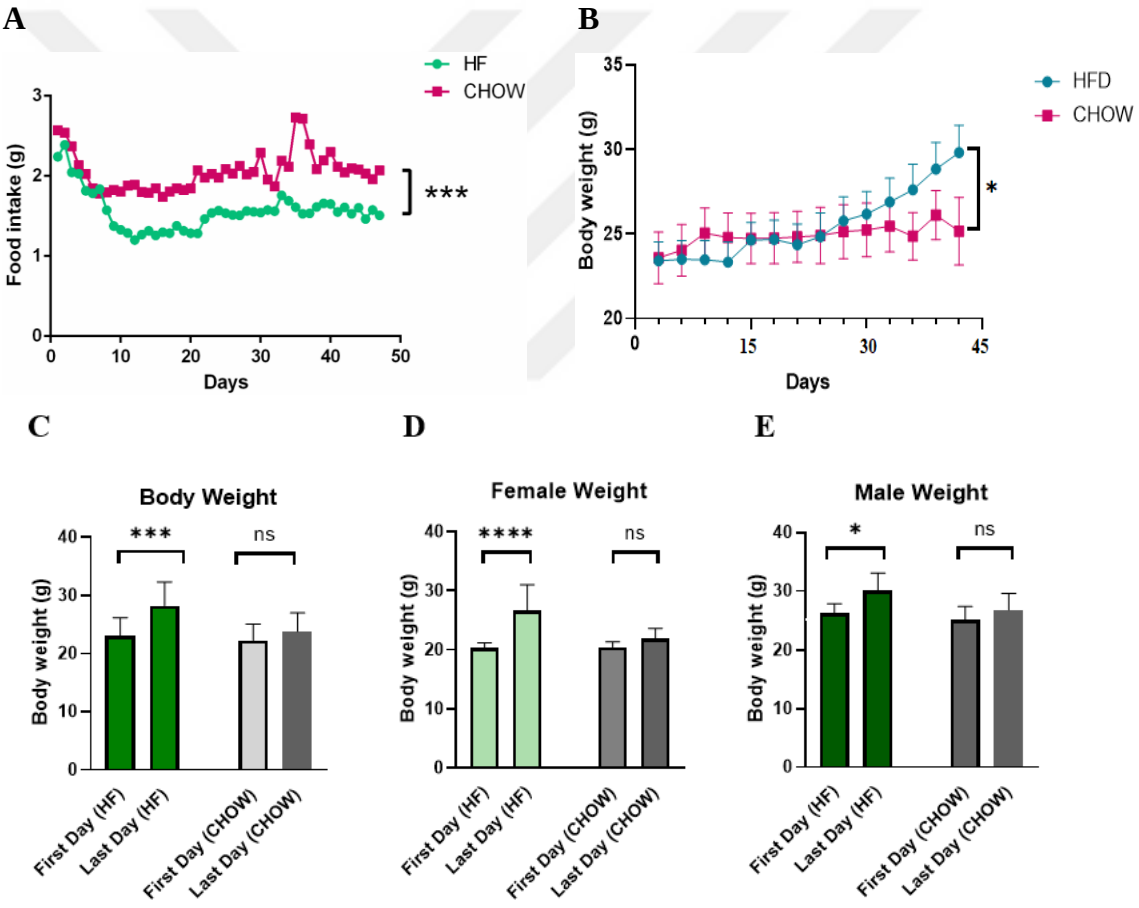

Fig12. Feeding behavior results of LepR-Cre. A; Daily food intake of mice that were fed with chronic HF diet and fed with CHOW diet (n=45). B; Body weights of mice that fed with chronic HF diet and fed with CHOW diet (n=45) C, D, E; the body weights were also shown according to gender as all mice, female, and male mice respectively (n=45, n=26, n=19). Student's t-test and Kruskal-Wallis's test were used for statistical analyses.

4.2. Electrophysiological Recordings (Patch Clamp)

Patch-clamp technique was used to measure electrical activity alterations and to examine the firing frequency of LepR neurons in the vTMN region. As seen in **Figure 13,** the cell-attached recordings were obtained from the specific GFP virus infected LepR neurons in the vTMN region by using that the recording pipette. The effect of HF diet on LepR neurons was investigated and as seen in **Figure 14 A-B,** the firing frequency of LepR neurons was significantly decreased in mice fed with HF diet.

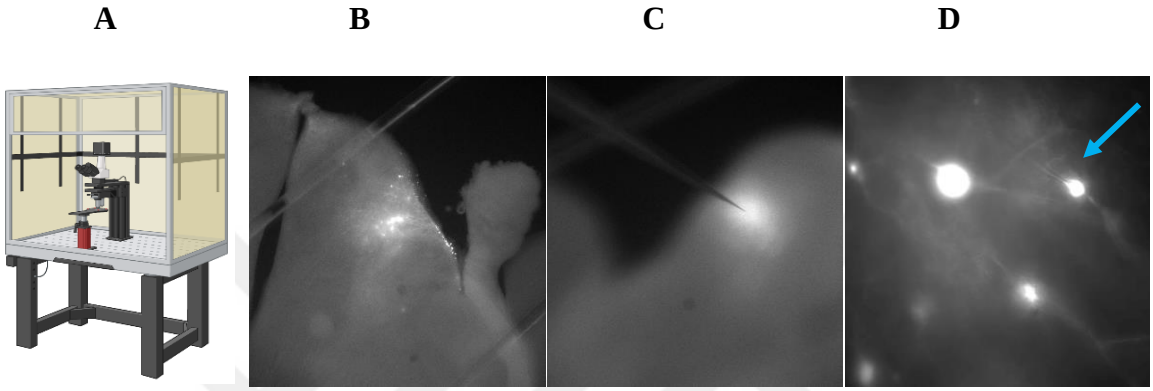

Fig13. A; Illustration of the patch clamp system. B; GFP virus infected LepR neurons in the vTMN region. C; The recording pipette at the injection region. D; GFP expressing LepR neurons in the vTMN region and for the cell-attached recording, a specific LepR neuron that the recording pipette is attached shown with the blue arrow.

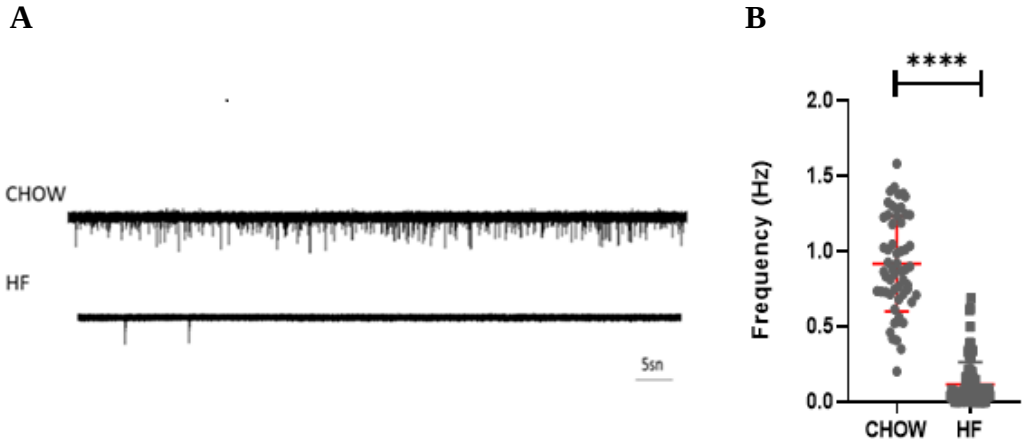

Fig14. A; Electrophysiological recordings obtained from LepR neurons in the vTMN region of CHOW and HF fed mice by patch clamp (n=6 for each diet group). B; Firing frequency of LepR neurons (HF: n=88 neurons/ n=6 mice, CHOW: n=57 neurons/ n=6 mice) Statistical analyzes were performed with Student's t-test and Mann-Whitney U test ($p<0.0001$).

4.3. Effects of High-Fat (HF) Diet on Behavior of LepR-Cre Mice

4.3.1. Open Field Test

OFT was performed to observe locomotor activity and the anxiety-like behaviors. According to the behavioral results, as seen in **Figure 15B,** HF fed mice spent less time in the center zone than the CHOW fed mice group but the activation of LepR neurons cause to significantly decreased the spent time in the center ($p<0.01$). In **Figure 15 C-D**; However, the activation and inhibition of LepR neurons did not change the velocity of mice but the activation of these neurons significantly decreased the frequency in the center ($p<0.05$).

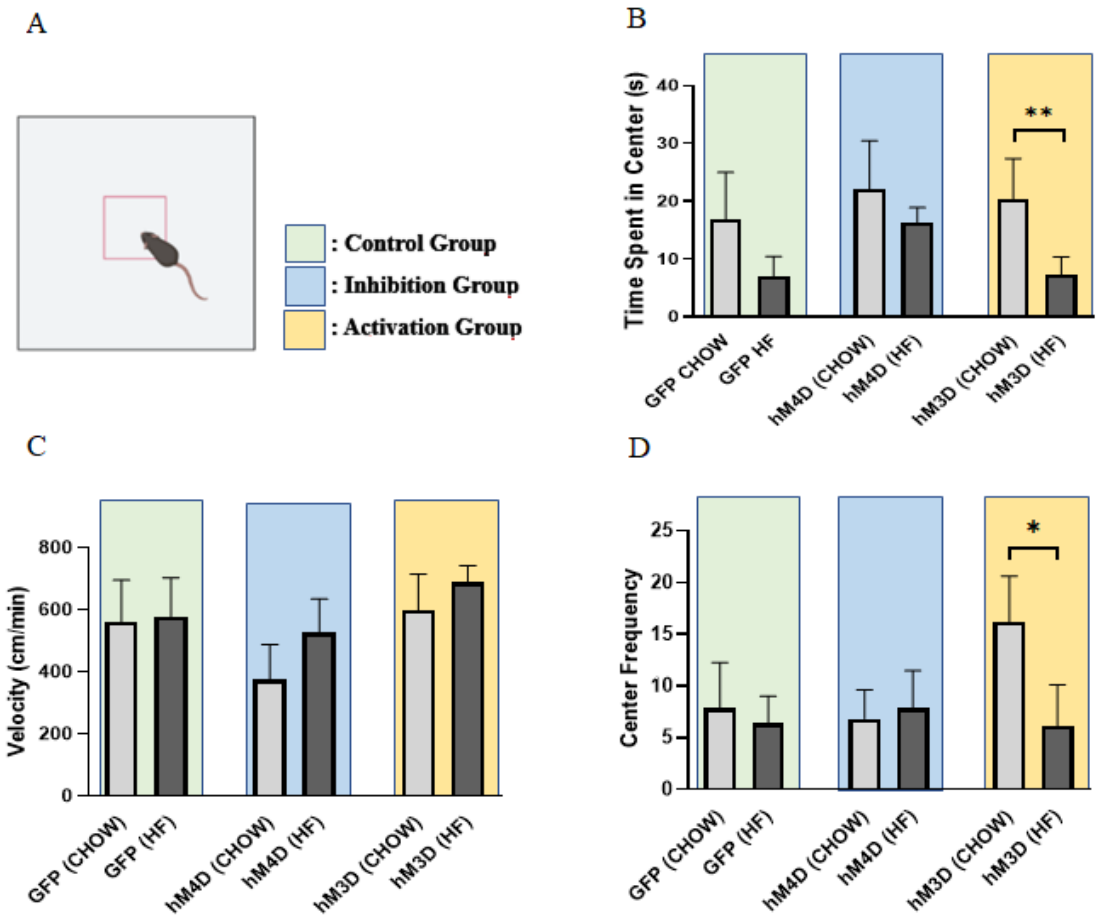

Fig15. OFT test results. A; Illustration of OFT test. B, C, D; Total spent time in center, the velocity and the frequency in center between control, inhibition and activation groups of LepR-Cre mice in the OFT chamber, respectively. Student's t-test and Kruskal-Wallis's test were used to analyze statistically (n=6 for each group, $p<0.01$, $p<0.05$).

4.3.2. Elevated Plus Maze Test

EPM test was preferred to observe anxiety-like behaviors of LepR mice. According to the behavioral results, as seen in **Figure 16 B-C**, in both diet groups mice preferred to spend time mostly in closed arms. However, the activation of LepR neurons significantly increased the closed arm preferences in CHOW diet group but it did not occur any alterations in HF group ($p<0.05$). On the contrary, the inhibition of LepR neurons significantly decreased the opened arm preferences in the HF diet group but significant alterations were not observed in the CHOW diet group ($p<0.05$). In **Figure 16 D-E**, when observed the spent time in the opened arm in both diet groups, the activation/inhibition did not make a significant difference but the activation of LepR increased the velocity of mice ($p<0.01$).

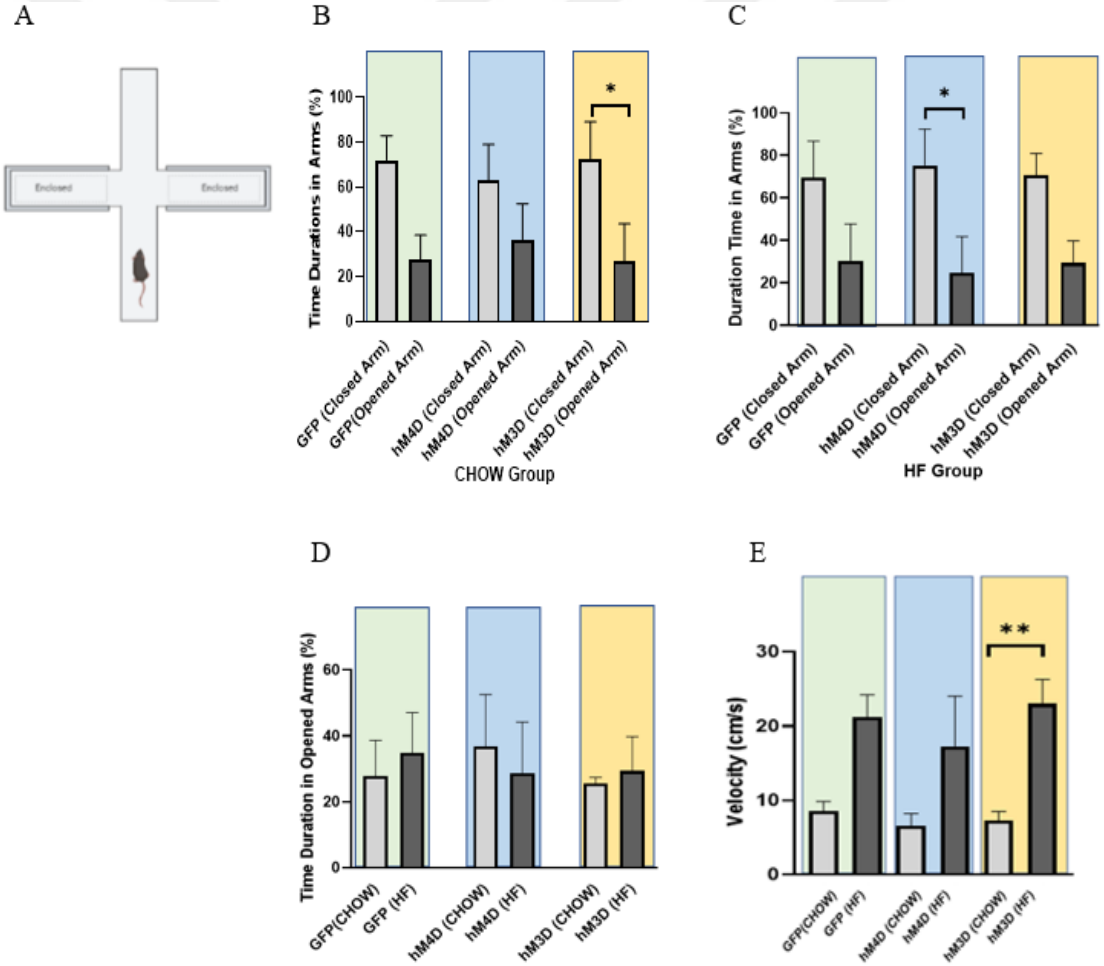

Fig16. EPM test results of control, inhibition and activation groups of LepR-Cre mice. A; Illustration of EPM test. B, C; Spent time (%) in the arms of EPM in Chow diet fed mice and HF fed diet mice respectively. D; Spent time (%) in opened arms of the diet

groups. E; Velocity of LepR-Cre mice in the EPM chamber. Student's t-test and Kruskal-Wallis's test were used for statistical analyses (n=6 for each group).

4.4. Locomotor Activity (Home Cage Activity)

Home cage activity test was used to observe locomotor activity of LepR-Cre mice. As seen in **Figure 17 B;** according to the heat map results, after 6 weeks chronic standard diet, the locomotor activities of the LepR-Cre mice in the cage increased oppositely, the HF diet decreases the locomotor activity. However, these increasing and decreasing locomotor activities (movements and velocity) did not create a statistically significant difference as seen in **Figure 17 C-D**.

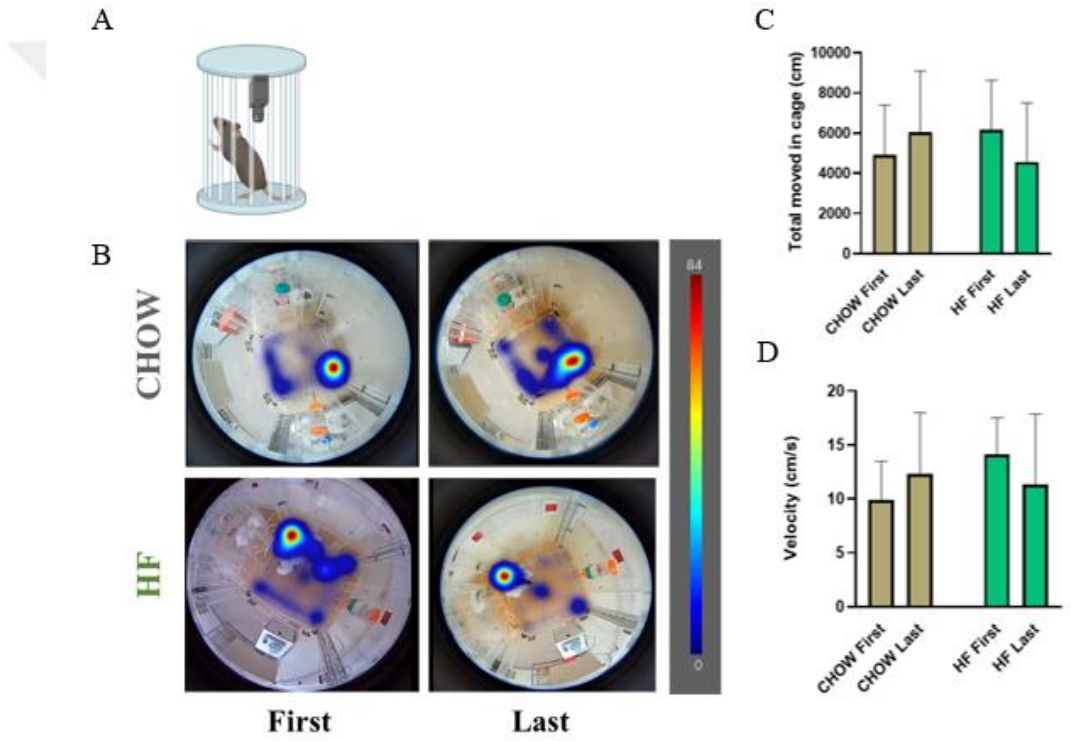

Fig17. Home cage activity results of LepR-Cre mice. A; Illustration of the using camera that placed into the cage of mice. B; The heat map of LepR-Cre mice in CHOW and HF diet fed groups respectively and obtained from the first day and the last day of diets of mice. C and D; Total movement in the cage and the velocity of mice fed with CHOW and HF diet respectively. Student's t-test and Kruskal-Wallis's test were used to analyze statistically (n=11 for each diet group).

4.5. Confocal Imaging

IHC technique was used to observe the colocalization of the histaminergic and the LepR neurons in the vTMN region of hypothalamus. As seen in the below figures there is colocalization of the histaminergic neurons (seen green in below figures) and the LepR neurons (seen red in below figures) in the TMN area.

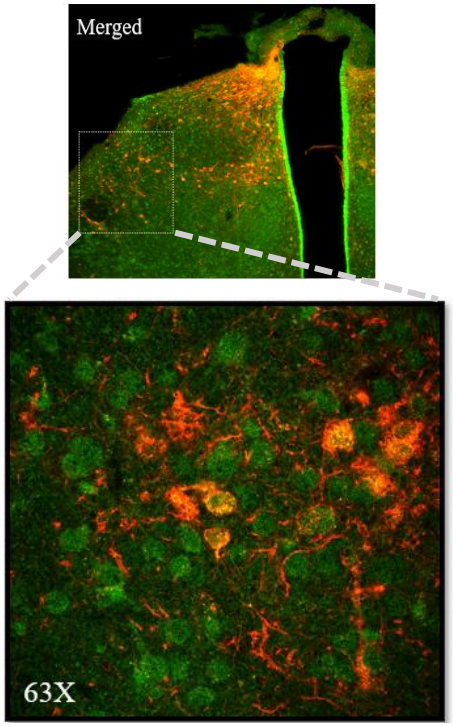

Fig18. In CHOW diet group mCherry-labelled LepR neurons (red) in vTMN were seen at 20X and 63X. By IHC staining technique histaminergic neurons were seen in green.

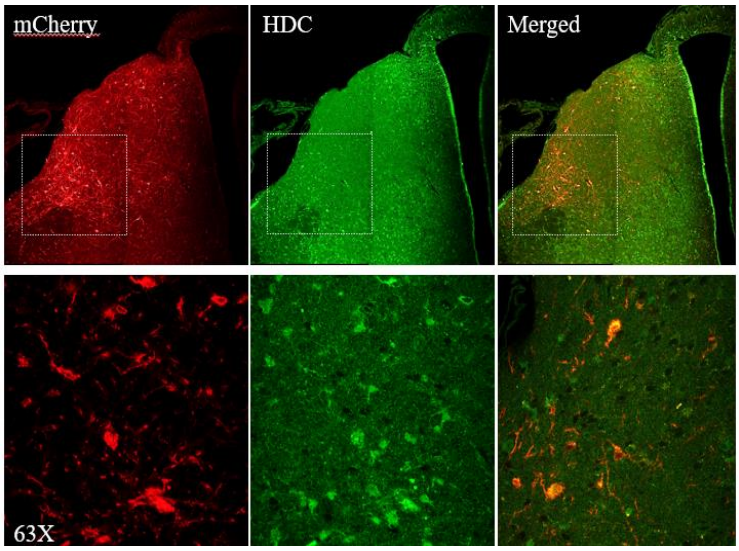

Fig19. In HF diet group mCherry-labelled LepR neurons (red) in vTMN were seen at 20X and 63X. By IHC staining technique histaminergic neurons were seen in green.

5. DISCUSSION AND CONCLUSION

Neuronal histamine is generated by histaminergic neurons in TMN in the posterior hypothalamus and affects wakefulness directly by stimulating hypothalamic neurons. However, the underlying mechanism of neuronal histamine is not yet fully understood. This situation may be related to feeding. Leptin has a crucial role in various obesity-related orexigenic and anorexigenic neural pathways. Moreover, it is known that sleep and wakefulness problems experienced by obese people and these wakefulness problems in obese people may be related to the leptin-histamine mechanism and this mechanism can affect the feeding behaviors. Currently, no study demonstrating the relationship between histaminergic neurons and leptin receptor neurons (LepR) about food intake in TMN. In this study, in order to observe histamine-leptin mechanism in obesity, we investigated the electrophysiological and behavioral effects of HF diet on LepR neurons in TMN region in LepR-Cre transgenic female and male mice.

Feeding behaviors between control group that fed with standard diet and the experimental groups that fed a chronic HF diet for 6 weeks of LepR-Cre mice were performed by measuring their daily food intake and their body weight. According to the results of feeding behavior, mice fed with HF diet had significantly increased in their body weight although they consumed less than mice fed standard diet. When we analyzed the body weights, there was no significant increase in body weight in CHOW but a significant increase was observed in body weight of HF fed mice. In addition, when we compare this result according to gender our data showed that, the reason of this significant increase in body weights is due to the fact that female mice gain more weight than males. This data correlates with the literature. According to a study was revealed by Adele Kennedy and her colleagues in 1997, the serum leptin increases in progressive obesity in both women and men but these leptin levels were observed higher in women than in men also, this situation is relative to leptin resistance [34].

In order to observe the electrophysiological effects of the HF diet on LepR neurons, the patch clamp technique was used. Then, with the obtained cell attached recordings from specific LepR neurons and their firing frequencies in the vTMN area were identified. Our data showed that, HF diet severely suppressed the firing frequency of LepR neurons in the TMN. On the other hand, as I mentioned before the TMN is the region where the histaminergic neurons are also located which means the electrophysiological data obtained from LepR neurons may include also histaminergic

neurons, our confocal imaging data that shows the colocalization of LepR and histaminergic neurons also supports this situation. Besides it is known that; histaminergic neurons have a wake-promoting effect and these neurons are more active in the wake state [24]. Therefore, the chronic HF diet may change this situation inversely and it can suppress these neurons firing.

In order to observe locomotor activity and the anxious behaviors of LepR-Cre mice, OFT, EPM test were performed and also home cage activity of mice was examined. According to obtained results from OFT, HF fed mice spent less time in the center zone than CHOW group but the activation of LepR neurons cause to significantly decreased the spent time and frequency in the center. Activation of LepR neurons in the HF group, it decreases the locomotor activity and triggers the anxious behaviors.

According to the EPM test results, the mice in both diet groups were preferred to spent time mostly in closed arms. However, the activation of LepR neurons in the CHOW group and the inhibition of these neurons in the HF groups significantly increased the closed arm preferences in LepR-Cre mice. Interestingly, activation of the LepR neurons increased the velocity of mice in the HF group.

In addition, home cage activity was used to observe locomotor activity of LepR-Cre mice in their cage. According to the heat map results, the locomotor activity of the LepR-Cre mice fed with standard diet increased oppositely, the HF diet decreases the locomotor activity. However, these increasing and decreasing locomotor activities (movements and velocity) in the cage were not statistically significant.

Moreover, one study was revealed by Ming Guo and his colleagues in 2013, leptin deficient or LepR deficient mice show anxiogenic behavior [35]. Taken together, this situation can support our OFT and home cage activity data because we showed that activation of LepR neurons in the HF group, it decreases the locomotor activity and triggers the anxiety-like behaviors. In normal situations, activation of these neurons may increase the locomotor activity but here, the HF diet may reverse this situation.

In conclusion, the behavioral and electrophysiological effects of LepR neurons in the TMN in LepR-Cre mice fed with chronic HF diet were investigated for the first time within this thesis study. Our findings have shown that HF diet alters the behavioral characteristics and the neuronal activity in transgenic LepR-Cre mice.

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7. APPENDICES

7.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2022-003	28.01.2022	2022/01	2022/01-3	Prof. Dr. Bayram Yılmaz
'Kronik Yüksek Yağlı Diyetin Tuberomamiller Çekirdekteki Leptin Reseptör Nöronları Üzerine Elektriksel ve Davranışsal Etkilerinin Transgenik Farelerde Araştırılması' isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				

Hayvan Türü / Irkı	Toplam Hayvan Sayısı	Hayvanın Cinsiyeti
Fare	57	Erkek ve Dişi

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Dr. Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Prof. Dr. Özlem MALKONDU	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMİCİ BAŞOL	
Üye	Atakan Mücahit YAVUZ	
Üye	Ahmet ŞENKARDEŞLER	

7.2. Curriculum Vitae

Personal Informations

Name	ZEHRA YAĞMUR	**Surname**	EROL
Place of Birth		**Date of Birth**	
Nationality		**TR ID Number**	
E-mail		**Phone number**	

Education

Degree	**Department**	**The Name of the Institution Graduated From**	**Graduation year**
Master	Physiology	Yeditepe University	2022
University	Genetics and Bioengineering	Yeditepe University	2020
High school	-	Izmir Anatolian High School	2013

Languages	**Grades (#))**
English	70

Work Experience (Sort from present to past)

Position	**Institute**	**Duration (Year - Year)**
		-
		-

Computer Skills

Program	**Level**
MS Office Programs	Excellent

Scientific works

The articles published in the journals indexed by SCI, SSCI, AHCI

Articles published in other journals

Proceedings presented in international scientific meetings and published in proceedings book.

Journals in the proceedings book of the refereed conference / symposium

Others (Projects / Certificates / Rewards)

219S554, TUBITAK 1001 Research Project Investigation of Central Regulation and Mechanisms of Ovarian Functions by Optogenetic and Pharmacogenetic Methods in Transgenic Kisspeptin-Cre Mice with Polycystic Over Syndrome. Participation/Leaving Dates of Project: 01.11.2020-continues, Start/End Dates of Project: 01.07.2020-01.07.2023
2209-A TUBITAK Research Project: 'Proteomic Analysis of HeLa Cells Upon 3-Bromopyruvate Treatment' (Project No: 1919B011703070) Dates: 15.06.2018-15.06.2019