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YAYIMLANMA VE FİKRİ MÜLKİYET HAKLARI BEYANI

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o Serbest Seçenek/Yazarın Seçimi

Dr. Barış Alten

22/06/2018

ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standarts. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor (Prof. Dr. M. Yıldırım SARA) and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

Barış Alten, MD

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ABSTRACT

Alten, B. The Effects of the GSK-3 Inhibitor TDZD-8 on Bipolar-like Behavioral Phenotype Induced by High Fructose Corn Syrup Consumption in Adolescent Rats, Hacettepe University Institute of Health Sciences Medical Pharmacology Doctor of Philosophy Thesis, Ankara, 2018. Children and adolescents are the top consumers of high fructose corn syrup (HFCS) sweetened beverages. Even though the cardiometabolic consequences of HFCS consumption in adolescents are well characterized, the neuropsychiatric consequences have yet to be determined. Adolescent rats were fed with 11% weight/volume carbohydrate containing HFCS solution, which is strikingly similar to the sugar-sweetened beverages of human consumption. HFCS-fed adolescent rats displayed bipolar-like behavioral phenotype along with hyperexcitability in hippocampal CA3-CA1 synapses. This synaptic hyperexcitability was associated with increased presynaptic release probability and increased pool of AMPA receptors to be incorporated to the postsynaptic membrane, possibly due to decreased expression of the neuron-specific α3-subunit of Na^+/K^+-ATPase (NKA) to 18±7% of control and an increased ser845-phosphorylation of GluR1 subunits of AMPA receptors to 4.6±1.25 times of control, respectively. TDZD-8 treatment was found to restore behavioral and electrophysiological disturbances associated with HFCS consumption by inhibition of Glycogen Synthase Kinase-3 (GSK-3), the most probable mechanism of action of lithium for its mood-stabilizing effects. Even though hippocampal neuroinflammation and local insulin resistance were suggested as other mechanistic links tying HFCS consumption to the emergence of bipolar-like behavioral phenotype, we failed to show such a link. This dissertation showed that HFCS consumption in adolescent rats led to bipolar-like behavioral phenotype with neuronal hyperexcitability, which is known to be one of the earliest endophenotypic manifestations of bipolar disorder.

Key Words: High fructose corn syrup, bipolar disorder, insulin resistance, GSK-3, hyperexcitability, inflammation, adolescent, Na^+/K^+-ATPase, AMPA

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ÖZET

Alten, B. Adölesan Sıçanlarda Yüksek Fruktozlu Mısır Şurubu Tüketimi ile İndüklenen Bipolar Benzeri Davranışsal Fenotipe GSK-3 İnhibitörü TDZD-8'in Etkileri. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Tıbbi Farmakoloji Doktora Tezi, Ankara, 2018. Çocuklar ve adölesanlar yüksek fruktozlu mısır şurubu (YFMŞ) ile tatlandırılmış içeceklerin baş tüketicileridirler. Adölesanlarda YFMŞ tüketiminin kardiyometabolik sonuçları iyi biliniyor olsa da nöropsikiyatrik sonuçları halen ortaya konamamıştır. Adölesan sıçanlar içeriği insan tüketimine sunulan tatlandırılmış içeceklere çok benzeyen %11 ağırlık/hacimce karbonhidrat içeren YFMŞ solüsyonuyla beslenmiştir. YFMŞ ile beslenmiş adölesan sıçanlar bipolar benzeri davranışsal fenotip ile beraber hipokampal CA3-CA1 sinapslarında hipereksitabilite göstermişlerdir. Bu sinaptik hipereksitabilite artmış presinaptik salıverilme ihtimali ile postsinaptik membrana yerleşebilecek AMPA reseptör havuzundaki artış ile ilişkilidir. Bu değişimlerden sırasıyla Na^{+}/K^{+}-ATPaz'ın (NKA) nöron spesifik α3 altbiriminin ifadesinin kontrolün %18±7'sine kadar azalması ve AMPA reseptörlerinin GluR1 altbiriminin ser845 fosforilasyonunun kontrolün 4.6±1.25 katı kadar artması sorumludur. TDZD-8 tedavisi YFMŞ tüketimi ile ilişkili hem davranışsal hem de elektrofizyolojik bozuklukları lityumun da duygudurum stabilleyici etkilerinin mekanizması olarak bilinen Glikojen Senteaz Kinaz-3 (GSK-3) inhibisyonuyla geri çevirmiştir. Hipokampal nöroinflamasyon ve local insülin direnci YFMŞ tüketimiyle bipolar benzeri davranışların ortaya çıkışını açıklayabilecek diğer mekanizmalar olarak öne sürülmüş olsa da bu tez böyle bir ilişkiyi ortaya koyamamıştır. Bu tez, adölesan sıçanlarda YFMŞ tüketiminin, bipolar bozukluğun en erken endofenotipik belirteçlerinden olan nöronal hipereksitabilite ve bipolar benzeri davranışsal fenotipe yol açtığını göstermiştir.

Anahtar Kelimeler: Yüksek Fruktozlu Mısır Şurubu, Bipolar Bozukluk, İnsülin Direnci, GSK-3, Hipereksitabilite, İnflamasyon, Adölesan, Na^{+}/K^{+}-ATPase, AMPA

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ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BD	Bipolar Disorder
CA1	Cornu Ammonis region 1
CA3	Cornu Ammonis region 3
CDK	Cyclin Dependent Kinase
DMSO	Dimethyl Sulfoxide
EPM	Elevated Plus Maze
FST	Forced Swim Test
FUST	Female Urine Sniffing Test
GluR1	Glutamate Receptor Subunit 1
GluR2	Glutamate Receptor Subunit 2
GSK-3	Glycogen Synthase Kinase 3
HFCS	High Fructose Corn Syrup
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IRS	Insulin Receptor Substrate
LDL	Low Density Lipoprotein
MWM	Morris Water Maze
NKA	Na^+/K^+-ATPase
NMDA	N-methyl-D-aspartate
OFA	Open Field Arena
OGTT	Oral Glucose Tolerance Test
PA	Passive Avoidance
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PKA	Protein Kinase A
PKC	Protein Kinase C
T2DM	Type 2 Diabetes Mellitus
TDZD-8	Thiadiazolidinones-8
TNF-α	Tumor necrosis factor alpha

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1. GENERAL INFORMATION

1.1. Western Diet and the Global Epidemic of Type 2 Diabetes Mellitus

Consumption of processed foods rich in saturated fats and refined sugars has become increasingly prevalent throughout the globe since the introduction of the industrial revolution (1). This diet style was named as *western diet* and shown to underlie many chronic disturbances of Western civilizations, such as obesity, dyslipidemia, hypertension, hyperglycemia and insulin resistance. Among other factors, the increased consumption of western diet has been contributing to the increased prevalence of diabetes globally, doubling it from 4.7% in 1980 to 8.5% in 2014 (2).

Insulin resistance and preceding type 2 diabetes mellitus (T2DM) is one of the most common causes of mortality both by itself and by its complications. According to the World Health Organization (WHO), the total burden of deaths directly from diabetes in 2012 has been estimated to amount to 1.5 million (2). However, an additional 2.2 million deaths are attributable to cardiovascular diseases and chronic kidney diseases related to hyperglycemia, which is higher-than-optimal but lower than the diagnostic threshold for T2DM (2). In 2014, about 422 million adults aged over 18 throughout the globe were living with diabetes, and it should be noted that this estimation must be much higher as the proportion of undiagnosed diabetes can be as high as 30-50%, even in the high-income countries (2, 3).

In parallel with the global trends, Turkey has also been threatened by the increased prevalence of obesity, insulin resistance, and preceding T2DM: According to the 2013 TURDEP-II study, about 6.5 million Turkish adults are suffering from diabetes. In the last 12 years, the increase in the prevalence of diabetes, glucose intolerance and obesity are 90%, 106% and 35%, respectively (4).

1.1.1. High Fructose Corn Syrup (HFCS) is the number one nutritional factor causing the increased prevalence of insulin resistance and T2DM.

Even though the definition of western diet includes both saturated fats and refined simple sugars, recent studies suggest that it is the increasing fructose consumption that disrupts glucose metabolism the most. New technologies brought out by the industrial revolution allowed the mass production of HFCS in the early 1970s and the consumption of HFCS has been increased approximately %1000 in the following 20 years (5, 6). When total energy intake was controlled for, aforementioned increased consumption of HFCS is the number one nutritional factor causing the increased prevalence of T2DM (7). In concordance with, the prevalence of T2DM is 20% higher in countries with higher availability of HFCS compared to countries with lower availability, independent of obesity (8).

The most commonly used form of HFCS, called HFCS-55, contains approximately 55% of fructose, 41% of glucose and 4% of other sugars. Even though it was initially thought to be a better replacement for sucrose, which is composed of bound glucose and fructose, HFCS was shown to have more detrimental effects on the overall well-being of consumers because of its free fructose content. Fructose in amounts close to usual daily consumption, apart from other simple sugars, can cause systemic insulin resistance quickly even in completely healthy young adults (9). There has been a debate whether the increased total caloric intake or fructose itself cause the insulin resistance. It was clearly demonstrated that fructose itself is the cause of fatty liver, impaired glucose tolerance and insulin resistance in three major insulin-responsive tissues, namely liver, fat, and muscle, independent of total caloric intake (10). As reviewed previously (11), fructose can cause each of the phenomena associated with the metabolic syndrome by distinct mechanisms. (I) Fructose is metabolized independently of insulin in liver and bypasses the rate-limiting step of glycolysis catalyzed by *phosphofructokinase*, rapidly increasing available hepatic acetyl-coA levels. Increased acetyl-coA is diverted to fatty acid synthesis, causing *fatty liver* and *hypertriglyceridemia*. (II) Excess circulating lipids are taken up by myocytes, which leads to *muscle insulin resistance*. (III) Byproducts of fructose metabolism in liver activate several protein kinases, which trigger serine phosphorylation and subsequent inactivation of IRS-1, leading to *hepatic insulin resistance*. (IV) Fructose metabolism by fructokinase depletes intracellular ATP in hepatocytes, generating the waste product of uric acid. Uric acid inhibits endothelial nitric oxide synthase, promoting *hypertension*. (V) Fructose is a gluconeogenic precursor and also induces the transcription of enzymes of gluconeogenesis, *increasing hepatic glucose output*.

1.1.2. Fructose has been consumed more and more for the last 30 years and adolescents are the top consumers.

Humans face this much of fructose consumption for the first time in their history of thousands of years. Before 1900’s, majority of fructose were consumed by means of fruits and vegetables (containing naturally occurring fructose). Both are less concentrated sources of fructose. In addition, they contain fibers, which is known to limit the absorption of fructose from the gut. As a result, the daily consumption of fructose approximates to 15 grams (4% of total calories) in 1900’s. Around 1970’s, when industrial revolution allowed mass and low-cost production of HFCS (added fructose), fructose intake had increased to 37 grams per day (7% of total calories). It was about 55 grams per day (10% of total calories), by 1994 (12). Today, fructose is mainly consumed within sugar-sweetened beverages, as HFCS is the sole caloric sweetener in soft drinks in majority of countries (12, 13). Different from fruits and vegetables, sugar-sweetened beverages are considered as one of the most concentrated sources of fructose available to human consumption. Since 1970’s, total and added fructose consumption have been increasing, whereas naturally occurring fructose intake has been decreasing or remaining constant (5). Nowadays, total of 74% of fructose ingested comes from foods and beverages other than whole fruits and vegetables (12). In addition, consuming fructose with soft drinks comes with several challenges apart from the fructose itself: (I) It has been shown that intake of beverages does not cause a corresponding reduction in intake of other food, suggesting that calories coming from beverages are *add-on* (6). (II) One study analyzing the sugar content and composition of several popular sugar-sweetened beverages found that many of the beverages contain more fructose than it is disclosed by the producers or otherwise assumed (14). (III) Sugar-sweetened beverages are most commonly consumed by adolescents (ages 12-18 years) (12). Biologically speaking, adolescent brain is much more vulnerable to reward-driven behavior, such as consumption of highly palatable HFCS. In addition, still developing prefrontal cortex causes diminished behavioral control on this highly sensitive reward-driven feeding behavior (15). These explain why adolescents consume almost 73 grams of fructose per day (%12 of total calories), while the mean daily fructose consumption for all age groups is around 55 grams (12). Moreover, adolescents consuming sugar-sweetened beverages are more likely to suffer from low intake of plain water (16).

1.1.3. HFCS consumption in adolescence period is associated with premature cardiometabolic diseases, but the neuropsychiatric consequences have yet to be determined.

Adolescence period is one of the developmental periods, which is known to determine the individual’s overall lifelong health (17). It has been shown that excess fructose consumption in adolescence period results in increased visceral adipose mass, impaired glucose tolerance, increased systolic blood pressure and dyslipidemia (18). Unfortunately, the majority of diseases caused by excess fructose consumption in adolescence do not become overtly apparent until adulthood and they develop cardiometabolic diseases at a younger age, resulting in premature deaths and disability in adulthood. Even though the metabolic consequences of HFCS consumption in the adolescent period have been relatively well known (6), the neuropsychiatric consequences have yet to be determined.

1.2. Western Diet, Central Insulin Resistance and Learning-Memory and Mood Disorders

Today, it is well known that insulin resistance in the key developmental periods, such as adolescence, results in cardiometabolic diseases of adulthood. However, recent studies suggest that patients with insulin resistance are also at risk for learning-memory and mood disturbances (19-25).

A review of the literature showed that patients with T2DM have an increased risk of developing any kind of dementia, including Alzheimer’s disease (26), which was given the name of *“Type 3 diabetes”* because of the close association between Alzheimer’s and T2DM (27). In addition, impaired glucose tolerance in humans was shown to be associated with poorer cognitive status and hippocampal atrophy (28, 29). Even though the exact mechanism of how insulin resistance influences learning-memory has not been fully elucidated yet (30), several studies have already shown the negative impact of western diet on hippocampal functions (31-36). Unfortunately, only a handful of studies investigated the neuropsychiatric consequences of exclusively high fructose consumption so far (37, 38). The most recent one showed that HFCS consumption in adolescent rats caused impairments in hippocampal spatial learning and memory while the adult rats were spared from the detrimental effects of HFCS (37). In a study conducted by Kanoski et al., it was shown that western diet disrupted hippocampus-dependent learning and memory tasks after the first 72 hours of the diet, whereas hippocampus-independent cognitive functions remained intact for almost a month (39). This clearly demonstrates that hippocampus-dependent functions are much more sensitive to western diet consumption and insulin resistance (39, 40). In addition, only a week of feeding with western diet was shown to be enough to cause structural alterations in the synaptic organization of hippocampus (41). If one considers that western diet can cause such detrimental changes in hippocampal structure and functions in the matter of days, one can speculate that these changes most likely occur due to more fast pacing changes instead of more late-developing consequences, like vascular complications of insulin resistance and T2DM in the brain.

Even though considerable attention has been given to learning and memory, less is known about mood disturbances seen in insulin resistance. Many studies reported increased prevalence of insulin resistance and T2DM in patients diagnosed with unipolar depression and bipolar disorder, and vice versa (25, 42-50). The literature up-to-date highly suggests that there is a mechanistic link in between insulin resistance/T2DM and mood disturbances, however several limitations do exist: (I) Almost all studies conducted so far are cross-sectional. This prevents to investigate the temporal relationship between the two, if there is any. (II) As both are chronic conditions, it is difficult to determine the temporal order. (III) Diagnosing mood disorders is relatively tentative, causing discrepancies between studies. (IV) Medications that are used to treat psychiatric conditions, such as antipsychotics and antidepressants, may cause weight gain and insulin resistance. However, a retrospective study of 243 patients with different psychiatric diagnoses, bipolar disorder was found to be a predictor of diabetes diagnosis, *independent* of the effects of medication use (20). This suggests that there should be an intrinsic relationship between diabetes and bipolar disorder apart from the risk of weight gain and insulin resistance due to medication use. A review of 7 large cohort studies conducted between 2009 and 2015, and found that age- and gender-adjusted risk for bipolar disorder was increased in patients with diabetes, and vice versa (50). To sum up, accumulating data strongly indicates that mood disorders, especially bipolar disorder, and insulin resistance have a link that has yet to be revealed.

Traditionally, the central nervous system had been known as insulin insensitive, however starting from the late 1970s, insulin receptors were found to be distributed in different parts of the brain. It should be noted that their distribution is quite heterogeneous: Insulin receptors are most abundant in limbic structures, mainly in olfactory bulb and hippocampus (51-54). Interestingly, the brain functions disrupted in the state of insulin resistance are the functions supported mainly by limbic structures, namely emotion, mood, behavior, learning, and memory.

The existence of insulin receptors in so-called insulin insensitive brain raises the first question that should be asked: What is/are the function(s) of insulin receptors in the central nervous system? Moreover, the overlapping features of the localization of insulin receptors in brain with the brain functions disrupted in insulin resistance cannot be explained as a simple coincidence. This raises the second one: What is the underlying mechanism that links the insulin resistance to the mood and learning-memory deficits seen in patients with T2DM? So far, no certain answer to either of the questions above has been revealed.

In order to understand the functions of insulin receptors in the brain, several studies introduced insulin to the central nervous system without altering plasma insulin and/or glucose concentrations. Majority of studies introduced insulin to both humans and animals intranasally and showed that insulin has direct effects on the central nervous system, improving learning and memory (55-60). The same results were reproduced when insulin was given intracerebroventricularly to mice (61). When the expression of insulin receptors and IRS in hippocampal neurons were decreased by genetic manipulations and viral vectors carrying siRNAs, hippocampal learning and memory were found to be impaired (62, 63). In addition, insulin receptor mRNA and protein levels have been shown to increase in hippocampus just an hour after animals were exposed to a hippocampus-dependent spatial learning task (64). These results showed that insulin and its receptors in the central nervous system have an undeniable role in normal functioning of hippocampal learning and memory.

1.2.1. It is still a mystery whether brain itself is insulin resistant, insulin deficient or is exposed to hyperinsulinemia in case of insulin resistance.

What could be the fast pacing change linking western diet consumption, insulin resistance and hippocampal dysfunction? It is still controversial whether neurons are insulin resistant, insulin deficient or are exposed to hyperinsulinemia in T2DM (30). There are several studies published so far, each suggesting a different mechanism.

Even though it is not still fully understood how insulin crosses the blood-brain barrier, several studies showed that it must cross by a saturable mechanism involving some kind of protein transporter (65-70). This saturation was found to occur at insulin concentrations maintaining euglycemia, so central nervous system should be protected from high circulating levels of insulin in physiological conditions (69). However, several factors have been shown to affect insulin transport through the blood-brain barrier. Diet-induced obesity decreased insulin transport through the blood-brain barrier (71, 72). The diabetic state itself increased insulin uptake into the central nervous system and this increase was thought to be due to long-lasting changes in the levels of glucose, insulin and other neuroendocrine agents (73, 74). One of these agents was shown to be triglycerides (72), which is known to increase abruptly upon feeding with high fructose.

1.2.2. Brain can be insulin resistant just like other insulin-responsive tissues.

Only a handful of studies so far showed disturbances in the neuronal insulin receptor signaling pathway, resulting in insulin resistance. Mielke et al. showed that lysates of whole brains obtained from rats fed with high fructose responded less to stimulation with insulin (75). Liu et al. showed that 12 weeks of high-fat feeding in mice caused insulin resistance in the whole brain, by showing decreased phosphorylation of secondary messengers of insulin signaling pathway (76). It is crucial to review the insulin signaling pathway and its effects on the central nervous system, in order to appreciate brain insulin resistance and its complications.

1.2.3. Brain insulin receptors respond to insulin as peripheral insulin receptors do.

As reviewed by Plum et. al. (77), insulin exerts its biological effects by binding to membrane-bound insulin receptors, which belong to the receptor tyrosine kinase family. When insulin binds to extracellular α_2 domains of insulin receptors, β_2 domains bearing kinase activity start autophosphorylating each other on tyrosine residues. Subsequently, intracellular insulin receptor substrates (IRS) become phosphorylated by IR, allowing it to interact with adapter proteins, namely PI3K, Grb2, and Syp. Afterward, adapter proteins successfully activate downstream secondary messengers. Two main different cascades are activated by insulin signaling system as a result: Ras-Raf-MEK-ERK cascade and PI3K-Akt cascade. Even though the cellular responses evoked by either of the cascades have been investigated separately in the central nervous system, the crosstalk between the two complicates the interpretation.

1.3. Possible Mechanisms Linking Insulin Resistance to Learning-Memory and Mood Disturbances

1.3.1. AMPA Receptors and Synaptic Strength

Growing evidence suggests that glutamatergic neurotransmission and neuroplasticity plays a central role in the pathophysiology and treatment of several mood disorders, specifically bipolar disorder (78, 79). As glutamate is the major excitatory neurotransmitter in the mammalian brain and has an undeniable role in synaptic plasticity, glutamate and its receptors gained significant attention.

Glutamate is synthesized as a byproduct of Krebs cycle or transamination of oxoglutarate in neurons or regenerated from recycled Glutamine given by glia. Then, it is transported into synaptic vesicles, in which they reside until Ca^{2+} induced presynaptic release machinery is triggered. When released, glutamate rapidly binds and activates its receptors, which can be divided into two: ionotropic and metabotropic. Ionotropic glutamate receptors are further divided into three: AMPA receptors, NMDA receptors, and Kainate receptors. Enzymatic cleavage is not used as a mechanism to terminate glutamate action in the synapse instead, glutamate is taken up by excitatory amino acid transporters (EAATs) into glia. Glia synthesizes glutamine from glutamate and delivers it to neurons as a substrate for glutamate synthesis.

AMPA receptors are responsible for the fast excitation at the majority of synapses by the Na^+ influx and rapidly depolarizes the membrane when bound with glutamate. However, glutamate binding is not enough to activate NMDA receptors by itself. Postsynaptic membrane depolarization repels Mg^{2+} in the pore of NMDA receptors, allowing both Na^+ and Ca^{2+} influx, which further depolarizes the membrane and activates secondary signaling pathways related to synaptic plasticity.

Specifically, the modulation of AMPA receptor trafficking has gained attention because of being a target for the two most common mood stabilizing agents: lithium and valproate (80). Chronic treatments with therapeutically relevant concentrations of lithium or valproate were shown to decrease the synaptic expression of GluR1 subunit of AMPA receptors in the hippocampus by attenuating ser845-phosphorylation of GluR1 (81-83). Normally, increased ser845-phosphorylation of GluR1 creates a readily available pool of AMPA receptors to be incorporated into the synapses. This increased AMPA receptor density in synapses is necessary for the emergence of bipolar-like behavioral phenotype, as AMPA antagonists attenuate amphetamine-induced hyperactivity, one of the most valid animal models of mania (81). In addition to postsynaptic alterations, presynaptic changes were also linked to BD. One study reported increased glutamate levels is in postmortem human bipolar brains (84). In addition, both lithium and valproate were shown to decrease the synaptic glutamate levels by increasing its reuptake (85, 86). In addition, long-lasting treatment with lithium was shown to inhibit NMDA receptor-mediated Ca^{2+} influx, protecting from excitotoxicity (87, 88). Glutamate levels were found to be increased in all mood states in brains of patients with bipolar disorder (89). Last but not least, a recent study showed that hippocampal neurons of patients with BD had hyperexcitability, which was reversed by lithium only in neurons derived from patients who also clinically responded to lithium (90). Thus, neuronal hyperexcitability is considered one of the earliest endophenotypic manifestations of BD. Interestingly, the neuronal excitability was shown to be modulated by diet. Ketogenic diet, a low carbohydrate diet, has been used for the treatment of intractable seizure disorders for many decades and shown to counter neuronal hyperexcitability both in vivo and in vitro (91, 92). A recent case report demonstrated the efficacy of ketogenic diet in the treatment of BD (93).

The principal cascade regulating synaptic strength, plasticity, and neuronal survival seems to be the PI3K-Akt cascade (94, 95). In addition, learning, which involves synaptic plasticity, itself both triggers and requires activation of Akt (96, 97). PI3K-Akt cascade can be activated by various ligands, including but not limited to BDNF, NGF, NMDA, and insulin. Insulin has been shown to cause endocytosis of AMPA receptors in GluR2 dependent manner. Then these AMPA receptors become eliminated from the recycling machinery (98-101). As AMPA receptors are implicated in the majority of the excitatory synaptic transmission in hippocampus, insulin, by activating PI3K, results in a depression of excitatory synaptic transmission. Interestingly, insulin, just like NMDA receptor activation, also causes insertion of AMPA receptors to the neuronal membrane, however the subunit involved is GluR1 (102). When stimulated by insulin, GluR1 containing AMPA receptors accumulate in extrasynaptic sites (102). However, GluR2 containing AMPA receptors are directly endocytosed from synaptic sites (102). This difference in insulin-induced spatiotemporal pattern of AMPA receptor distribution may explain the overall decrease in synaptic strength when insulin is introduced to hippocampal slices (103).

This is in concordance with the hypothesis of central insulin resistance giving rise to neuronal hyperexcitability, which was shown to be closely associated with bipolar disorder. Insulin also modulates NMDA receptor activity, which is known to be a key factor in learning and memory (104-106).

1.3.2. Na^+/K^+-ATPase

Apart from the changes in AMPA receptor trafficking, alterations in Na^+/K^+-ATPase (NKA) levels and/or activity may also cause neuronal hyperexcitability. One of the prominent hypotheses for the mechanism of BD is that a primary or secondary dysfunction of NKA predisposes or even directly causes the clinical manifestations. This hypothesis was first proposed almost half a century ago (107), and later supported and expanded by further evidence (108). NKA is a vital membrane-bound transporter, which maintains the electrochemical gradient of excitable cells to function properly. NKA consists of a catalytic α-subunit, which determines all the major properties of the NKA, and a regulatory β-subunit. In the central nervous system (CNS), α-subunit exists as three isoforms: α1 and α2 are found in both neurons and glia, whereas α3 is exclusively expressed in neurons (109). Several single nucleotide polymorphisms across all three α isoforms in CNS have been associated with the BD (110). Furthermore, intracerebroventricular administration of ouabain, an inhibitor of NKA, is widely recognized as a valid animal model of mania (111). Mice carrying an inactivating mutation in neuron-specific α3-subunit of NKA showed a behavioral phenotype resembling those of patients with BD in a recent study (112). It is still not known how this decrease in NKA amount and/or activity translates to the behavioral changes evident in patients with BD. Nevertheless, several mechanisms were proposed (108): (I) less negative resting membrane potentials causing hyperexcitability, (II) secondary impairments of Na^+/Ca^{2+} exchangers causing intracellular Ca^{2+} overload, (III) activation of signal transduction pathways directly by NKA or indirectly by increased intracellular $[Ca^{2+}]$. The last proposal was linked to the PI3K-Akt pathway, modulating the activity of glycogen synthase kinase 3 (GSK-3).

Interestingly, diabetes has been shown to decrease the amount and/or activity of NKA in skeletal muscles, heart ventricular muscle and peripheral nerves (113), as well as in different brain regions (114). The region with the greatest and most significant decrease in NKA activity in the brain was the hippocampus (114), which was known to be involved in the pathophysiology of bipolar disorder.

1.3.3. Glycogen Synthase Kinase 3 (GSK-3)

GSK-3 is a serine/threonine kinase, which phosphorylates more than a hundred substrates (115). Thus, it is a critical secondary messenger involved in many signaling pathways, linking insulin receptor signaling pathway to learning, memory and mood disturbances. T2DM, Alzheimer's disease and several other neurodegenerative diseases, bipolar disease, unipolar depression, chronic inflammatory conditions, and several cancers were found to be directly related to GSK-3 and its activities.

As reviewed by Hur and Zhou (116), GSK-3 was initially identified as a key regulatory enzyme in glycogen metabolism (117). Soon after, its significant role in the central nervous system was recognized. GSK-3 has two different isoforms, namely GSK-3α and GSK-3β, that are encoded by separate genes, but have similar homology in structure over 85%. The ratio of homology increases further in their kinase domains, approximating to 97%. This extreme homology in between two provides both the capability of perpetuating similar biological processes, and little is known about the isoform-specific functions, if there are any. However, the major one found in the central nervous system is GSK-3β.

Apart from the majority of kinases, GSK-3 has a constitutive activity in resting conditions and becomes phosphorylated on serine residues and inhibited in response to several upstream signals. The major family that is responsible to regulate GSK-3 is Akt, whose activity is induced by insulin/growth factor receptor tyrosine kinase signaling (118). In addition, many of the substrates of GSK-3 should be phosphorylated first, also known as *primed*, so that GSK-3 can phosphorylate those more efficiently (119). This substrate-specific control provides another layer of regulation, which aids GSK-3 to coordinate a lot of cellular functions with a great specificity, even though it can phosphorylate dozens of substrates. Additional mechanisms to regulate GSK-3 is to regulate its localization in subcellular levels and its interactions with GSK-3 binding proteins (120).

Diabetes is associated with hyperactivity of GSK-3.

When insulin binds IR and triggers insulin receptor signaling, activated Akt phosphorylates constitutively active GSK-3β on Ser9, decreasing its activity. Thus, in state of insulin resistance, GSK-3 remains highly active (121). Increased GSK-3 activity has been reported in patients with T2DM and obesity-prone animal models of T2DM (122, 123). Even though GSK-3 hyperactivity is the result of upstream unresponsiveness to insulin, it also aggravates insulin resistance by phosphorylating IRS-1, making it more and more unresponsive to actions of IR (124, 125). As expected, GSK-3 inhibitors have been shown to alleviate insulin resistance in both patients with T2DM and animal models of T2DM (126-131).

GSK-3 is the single most important target for mood stabilizers, and increased GSK-3 activity is associated with both manic- and depressive-like behaviors.

Lithium has been the first-line treatment for prevention of mood swings of bipolar disorder for almost 50 years. The primary effect of lithium is its strong antimanic effect. In addition, lithium has a weak antidepressant activity and an ability to augment the effects of conventional antidepressants when used in combination (132). More importantly, lithium has a specific antisuicide effect independent from mood-stabilization (133), as lithium reduces the risk of suicide also in patients who are not responders to lithium treatment (133). Since mortality due to suicide approximates to almost 15% in patients with bipolar disorder, lithium has been and will be the mainstay treatment for these patients.

Even though lithium is just a simple cation and has been extensively used for many years, its mechanism of action was relatively recently revealed: It has been shown that lithium exerts its mood-stabilizing effects by inhibiting GSK-3 at concentrations relevant for the treatment of bipolar disorder (134, 135). Lithium inhibits GSK-3 by both directly and indirectly (Figure 1.1). First, lithium competes with Mg^{2+}, which causes a *direct inhibition* of GSK-3 (136). Second, lithium was shown to augment the inhibitory phosphorylation of GSK-3, causing an *indirect inhibition* of it (137, 138). This indirect inhibition appears to be mediated by a feedback loop involving regulation of protein phosphatase-1 or increased Akt activity following lithium treatment (138, 139). As indirect inhibition tends to occur more slowly and longer lasting, it may explain why lithium should be administrated chronically to exert its therapeutic effect in bipolar disorder. This also supports the ability of lithium to inhibit GSK-3 to a greater extent than would be possible based on only the direct inhibitory action (K_i of 2 mM) at very low therapeutic concentrations approximating 1 mM (140). Even with both mechanisms of inhibition, lithium can only moderately inhibit GSK-3 at this low concentration. This may be a therapeutic advantage, as complete inhibition of GSK-3 would have widespread consequences, yielding a wide variety of side effects. Nowadays, several specific GSK-3 inhibitors were shown to have mood-stabilizing effects on animal models, suggesting that the primary mode of action of lithium is, indeed, inhibition of GSK-3 (141-144). Haploinsufficiency of GSK-3 mimics the behavioral actions of lithium (145). In addition, many of the drugs used in the treatment of bipolar disease, such as valproate, lamotrigine, antipsychotics, and antidepressants, have been shown to inhibit GSK-3 by increasing inhibitory serine phosphorylation (146).

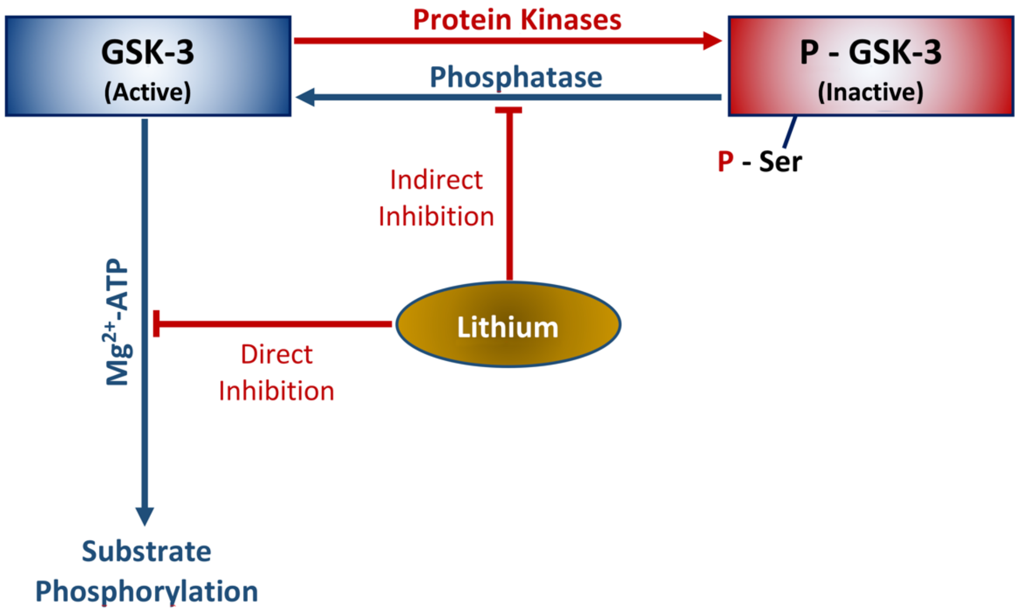

Figure 1.1. Mechanisms of inhibition of GSK-3 by lithium. Lithium inhibits GSK-3 by both competing with Mg^{+2} (direct inhibition) and enhancing inhibitory serine phosphorylation of GSK-3 (indirect inhibition).

Apart from the clues obtained from lithium therapy, transgenic animal studies also implicate the involvement of GSK-3 in bipolar disorder. For instance, transgenic mice overexpressing GSK-3 was validated as an animal model of hyperactivity and mania (147). GSK-3 hyperactivity is not only implicated in mania-like behavior, but also important in unipolar depression. Beaulieu et al. showed that 5-HT deficiency is associated with overactive GSK-3, which causes depressive behaviors. Pharmacological and genetic approaches reducing the activity of GSK-3 alleviated the depressive behaviors caused by 5-HT deficiency (148). In addition, GSK-3 is also implicated in social behaviors (149). Interestingly, Polter et al., changed serine to alanine at 9th position, preventing the inhibitory Ser9 phosphorylation of GSK-3β. These modified animals displayed susceptibility to amphetamine-induced hyperactivity and to stress-induced depressive-like behaviors (150). Even though it seems confusing that overactivity of GSK-3 causes both manic- and depressive-like behaviors, the mood-stabilizing action (strong antimanic and weak antidepressant effects) of lithium can be explained by its inhibitory actions on GSK-3. However, studies in transgenic animals overexpressing GSK-3 suggest that GSK-3 overexpression should be considered as not producing a resistance to depressive-like behaviors, but rather as inducing behavioral changes suggestive of mania-like effects (147, 151).

GSK-3 is also implicated in learning-memory and several GSK-3 inhibitors are in clinical trials to treat Alzheimer's disease.

Studies conducted with transgenic animals overexpressing GSK-3 in the brain led to the discovery that GSK-3 is crucial for normal learning and memory processes. GSK-3 overexpressing transgenic mice were found to have spatial learning and memory deficits (152-154). Same studies showed that GSK-3 is tightly associated with LTP and LTD, which are known to be the best cellular representations of learning and memory. GSK-3 overactivity caused impairments in LTP. In addition, it was shown to decrease some of the key proteins involved in presynaptic release machinery, postsynaptic NMDA and AMPA receptor subunits and related structural scaffolding proteins (155). Inhibition of GSK-3 restored LTP and synapse-associated impairments. Another study showed that induction of LTP itself caused GSK-3 inhibition, allowing the expression of short- and long-lasting changes associated with LTP induction and maintenance (156). In addition, LTP induction can block LTD expression via GSK-3 inhibition, which is thought to permit the initial consolidation of learned information (157).

As mentioned before, insulin was found to enhance endocytosis of AMPA receptors, thus decreasing AMPA receptor-mediated excitatory synaptic transmission. A recent study showed that GSK-3 inhibitors caused a similar reduction in AMPA receptor-mediated excitatory synaptic transmission by increasing internalization of AMPA receptors, which was occluded by insulin (158). This suggests that insulin may facilitate the endocytosis of AMPA receptors via GSK-3 inhibition.

GSK-3 is vital in inflammation and GSK-3 inhibitors provide a strong anti-inflammatory action.

Interestingly, all the diseases mentioned previously share a common feature: chronic inflammation. It may or may not be a major contributing factor, nonetheless, inflammation accompanies T2DM, mood disorders (mainly bipolar disorder), and neurodegenerative diseases (with a focus on Alzheimer's disease). By promoting inflammation (159), GSK-3 may also link these disorders to each other, apart from proposed hypotheses of insulin resistance causing GSK-3 overactivity.

GSK-3 is required to produce the most important pro-inflammatory cytokines, namely Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α), whereas it decreases the production of the major anti-inflammatory cytokine, Interleukin-10 (IL-10) (159). This elucidates the anti-inflammatory effects of GSK-3 inhibitors, allowing animals to survive from otherwise lethal dose of lipopolysaccharide (159). Several other studies showed the anti-inflammatory effects of GSK-3 inhibition in animal models of arthritis, colitis, peritonitis, and endotoxemia (160-163).

The main rationale to use GSK-3 inhibitor in order to treat T2DM is its beneficial effects on glucose intolerance mentioned previously. However, inflammation is commonly associated with T2DM, and inhibition of GSK-3 may have additional therapeutic potential by alleviating inflammation. As reviewed by Pickup (164), patients with T2DM were found to have increased circulating levels of acute phase reactants, IL-6 and TNF-α. Moreover, IL-6, which is known to orchestrate this acute phase reactant response, was found to increase gradually, as T2DM gets more severe (165, 166). Interestingly, circulating TNF-α is increased only in established overt T2DM, but not in impaired glucose tolerance (167). There are several studies suggesting different mechanisms that may explain how TNF-α and IL-6 augment insulin resistance: Interestingly, just like GSK-3 (125), TNF-α can phosphorylate and reduce the activity of IRS-1, halting insulin receptor signaling and causing insulin resistance (168). On the other hand, IL-6 was shown to modulate gene expression to decrease insulin sensitivity (169-171). Even though it is still not fully understood whether this inflammatory response is a primary event causing insulin resistance or a secondary event caused by many biochemical alterations evident in T2DM, it is certain that inflammation plays an important role in T2DM. If one considers the boosting effect of GSK-3 on IL-6 and TNF-α, one can appreciate the added benefit that GSK-3 inhibitors would yield by decreasing inflammatory state associated with T2DM.

Similar to T2DM, many neuropsychiatric diseases are also associated with inflammation. This association was first suggested when Smith claimed that cytokines released from macrophages as a cause of depression, also known as *macrophage theory of depression*, more than 20 years ago (172). Today, it is known that inflammation is relevant to many neuropsychiatric diseases, including but not limited to Alzheimer's disease, major depressive disorder and bipolar disorder (173-175). Even though it will be discussed in greater depth in the following chapters, it should be noted that inflammation must be considered as one of the possible mechanisms linking T2DM to neuropsychiatric diseases. GSK-3 may also be a favorable drug target to reverse this disruptive inflammatory link in between these diseases mentioned.

1.4. Treatment with GSK-3 Inhibitors

After the discovery of lithium providing mood stabilizing effects by inhibition of GSK-3, much effort has been directed to develop novel GSK-3 inhibitors for a wide variety of indications with an improved side-effect profile.

Basically, GSK-3 inhibitors developed up-to-date can be divided into three major categories according to their mechanism of inhibition (Table 1.1.) (176): (I) The first category includes cations, such as lithium, zinc, and tungstate, which were shown to inhibit GSK-3 by competing with Mg^{2+}. An indirect inhibitory pathway for lithium was also revealed and is discussed above. (II) The second category includes natural and synthetic inhibitors of GSK-3, which compete with ATP for ATP binding domain of GSK-3. However, a typical human cell has more than 500 different protein kinases, all of which share a similar ATP-binding domain. Targeting this mostly homologous pocket comes with an expense: These inhibitors are not very selective for GSK-3 and resulting side-effects from inhibition of other kinases bring out serious drawbacks for them to reach clinics. (III) The third and the last category includes inhibitors targeting GSK-3 from domains other than the ATP-binding pocket. These non-ATP-competitive inhibitors provide more selectivity than those targeting the ATP-binding pocket, since they bind to regions unique to GSK-3, but not to other kinases. This is of utmost importance for treatment with GSK-3 inhibitors, for having the therapeutic effect while avoiding side-effects from non-specific kinase inhibition.

Small heterocyclic thiadiazolidinone (TDZD) family of inhibitors was the first developed family as a non-ATP-competitive inhibitor of GSK-3 (177). Even though their exact mechanism of action has yet to be confirmed, a possible interaction with cysteine 199, a key residue located in the active site of GSK-3, was suggested (178). As expected, TDZD was not shown to have any inhibitory action on various kinases, including PKA, PKC, and CDK1/cyclin B (177). TDZD-8 is one of the most well-characterized inhibitors among this family (Figure 1.2.). Even though it has not reached clinical trials yet, it has been used in many animal models with success.

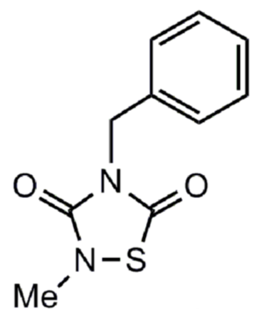

Figure 1.2. Structure of TDZD-8.

Type	Source	Inhibitor	Inhibition potency (IC_{50})	Biological activity in CNS	Drug development state
Cations	Inorganic atom	Lithium	2 mM	Mood stabilizer, neuroprotection, therapeutic benefits (tau and amyloid) in AD models (1-9).	Drug of reference for bipolar dis-orders. Conflicting results in the clinic for AD.
		Zinc	15 μM	Antidepressant (10, 11).	----
		Tungstate	Indirect inhibition	Decrease tau phosphorylation (12)	Phase I as antiobesity agent
ATP-competitives	Marine organism	**Indirubins** 6-BIO	5-50 nM 1.5 μM	Neuroprotection, axon formation, maintain pluripotency, reduced tau phosphorylation (13-16).	Pre-clinical
		Hymenialdisine Dibromocantharelline	10 nM 3 μM	Decrease tau phosphorylation, neuroprotection (17).	Pre-clinical
		Meridianins		Decrease tau phosphorylation (18).	-----
	Organic synthesis	**Aminopyrimidines** CT98014 CT98023 CT99021 TWS119	0.6-7 nM	Block LTD, maintain pluripotency, enhance neurite outgrowth, supports cell renewal of ESC, reduce tau phosphorylation (5, 19-22). Induces neuronal differentiation. Support self renewal of ESC (23).	----- -----
		Arylindolemaleimide SB-216763 SB-41528	 34 nM 77 nM	Neuroprotection, induce axon formation, shows therapeutic benefits in AD and schizophrenic models(5, 9, 24-32).	Pre-clinical
		Thiazoles AR-A014418 AZD-1080	 104 nM n.r.	Neuroprotection, shows antidepressive and anti-manic activity, shows therapeutic benefits in AD and ALS model (33-39).	----- Withdraw from clinical phase I
		Paullones Kenpaullone Alsterpaullone Cazpaullone	4-80 nM	Neuroprotection, blocks LTD, reduces Aβ production, reduces tau phosphorylation (19, 26, 40-45)	Pre-clinical
		Aloisines	0.5-1.5 μM	Blocks cell proliferation (46).	-----
Type	**Source**	**Inhibitor**	**Inhibition potency (IC_{50})**	**Biological activity in CNS**	**Drug development state**
Non-ATP competitives	Marine organism	**Manzamines** Manzamine A	 1.5 μM	Decrease tau phosphorylation (47).	-----
		Furanosesquiterpenes Palinurine Tricantine	 4.5 μM 7.5 μM	Decrease tau phosphorylation	-----
	Organic synthesis	**Thiadiazolidindiones** TDZD-8 NP00111 NP031115 NP031112(tideglusib)	 2 μM 2 μM 4 μM n.r.	Neuroprotection, decrease tau phosphorylation, beta-amyloid and gliosis in vivo, antidepressants, affects locomotor activity and reverse schizophrenic like behavior, promotes tissue recovery after spinal cord injury (48-55).	----- ----- ----- Phase II as orphan drug for PSP Phase IIb for AD
		Halomethylketones HMK-32	 1.5 μM	Neuroprotection, decrease tau phosphorylation (56).	-----
	Peptides	L803-mts	40 μM	Neuroprotection, antidepressive activity, axon morphogenesis (14, 57-59).	Pre-clinical

Table 1.1. Classification of GSK-3 inhibitors. Adapted from the review by Eldar-Finkelman and Martinez (176).

Side effects are the major challenge with GSK-3 inhibitors. First, it is obvious that inhibitors targeting domains other than ATP-binding pocket should be used to achieve selectivity of inhibition. Even though the selective inhibition of GSK-3 with non-ATP-competitive inhibitors can be achieved, there is still a huge concern raised related to the wide variety of cellular processes that GSK-3 regulate. Many think that inhibition of GSK-3 would prevent cells from functioning normally. However, one should consider that in many disease states, GSK-3 activity has already been increased. Inhibition would reduce this overactivity to normal levels, but not below normal. Another theoretical side effect associated with GSK-3 inhibition is to have increased risk of developing cancer due to disruption of β-catenin/Wnt pathway. However, more than 50 years-experience with lithium did not show any increased risk of developing any type of malignancy. In fact, recent studies suggested a decreased cancer risk with lithium treatment in patients with bipolar disorder (179, 180).

2. INTRODUCTION

2.1. Metabolic Characterization of HFCS Consumption in Adolescent Rats

As previously mentioned in the first chapter, HFCS consumption has been considered as the most important nutritional cause of T2DM worldwide (7). Unfortunately, adolescents are the top consumers of HFCS through consumption of sugar-sweetened beverages (12). The widespread use of HFCS as an additive coincides with the emergence of articles reporting the increased incidence of T2DM in children and adolescents (181). There has been a debate regarding the causality of increased consumption of HFCS sweetened beverages and increased prevalence of obesity (182-189). Studies showed that decreasing sugar-sweetened beverage consumption reduces obesity among children and adolescents (190, 191). Some argued that HFCS and sucrose were essentially the same and there was no reason to believe that HFCS poses a metabolic risk (182). However, HFCS was shown to cause a higher fructose exposure due to more rapid absorption kinetics with significantly different acute metabolic effects (192). Some studies showed that children consuming sugar-sweetened beverages had an increased risk of developing obesity once they reach into adulthood (193-195). Regardless of the obesogenic effect of HFCS sweetened beverages, it has been very well shown that HFCS consumption caused insulin resistance with associated hyperinsulinemia and dyslipidemia (196).

Many human studies have already shown the detrimental effects of fructose consumption. For instance, one study examined the effects of either glucose or fructose-sweetened beverages providing 25% of daily energy intake for 10 weeks. Even though both groups completed the study with the similar amount of weight gain, those consumed fructose suffered from insulin resistance, increased fasting plasma glucose levels and increased hepatic de novo lipogenesis, hypertriglyceridemia, increased LDL and apoB levels (197). When healthy normal-weight male volunteers consumed sugar-sweetened liquids in a double-blind, randomized, cross-over trial, high fructose consumption was associated with impaired insulin sensitivity, higher LDL, total cholesterol and free fatty acids when compared with high glucose consumption (9). In another human study, sugar-sweetened beverage consumption was associated with increased liver fat, increased visceral fat and increased triglyceride levels (198). A meta-analysis of prospective cohort studies of sugar-sweetened beverage intake demonstrated that individuals in the highest quantile of sugar-sweetened beverage intake (most often 1-2 servings/day) had a 26% greater risk of developing T2DM (199). In a subcohort of EPIC (European Prospective Investigation into Cancer and Nutrition) study, one serving of sugar-sweetened beverage consumption was significantly associated with an increased risk of developing T2DM, even after adjustments were made for total energy intake and body mass index (200). When these detrimental effects of increased fructose consumption through sugar-sweetened beverages were considered, one should not be surprised to see a 35% higher risk of coronary artery disease and all-cause mortality in individuals who are frequent consumers of sugar-sweetened beverages (201, 202).

Rodents studies showed that HFCS consumption resulted in increased body fat, notably in the abdominal region and triglyceride levels when rats were allowed to consume HFCS in both short-term (8 weeks) and long-term (6-7 months) (203). One interesting study demonstrated that consumption of 15% fructose-containing solution increased visceral adiposity, even though the total energy intake remained constant as mice decreased their chow intake (204). Furthermore, the pattern of lipid accumulation in case of fructose feeding was quite similar to that is observed in humans with metabolic syndrome (205, 206).

To begin with, we performed the metabolic characterization of HFCS consumption in adolescent rats. Adolescent rats of HFCS group (of age 21 days) were fed with 11% weight/volume carbohydrate containing HFCS solution which was designed to mimic the sugar-sweetened beverages available to human consumption.

This approach was thought to be a better representation of real world compared to exaggerated diets containing excessive amounts of carbohydrates and saturated fats incompatible with human consumption. In addition, the duration of HFCS consumption was somewhat shorter compared to previous studies. Here, rats were fed with HFCS solution for 4 weeks and an additional 2 weeks during their behavioral tests were performed. This 4-week time period covers the entire adolescence period of rats, starting from the postnatal day 21 to day 64. Metabolic characterization was performed with oral glucose tolerance test. OGTT was chosen as it is one of the most physiologic representations of the handling of glucose in response to insulin. As high fructose feeding as short as 7 days was already shown to disrupt glucose tolerance and induce insulin resistance (207-209), no further tests were performed.

2.2. Behavioral Characterization of HFCS Consumption in Adolescent Rats

Western diet was associated with learning and memory disturbances in both rodents and humans. However, very little is known about the specific effects of exclusive HFCS consumption on mood disorders.

Majority of studies investigated the eating habits in patients with learning-memory and mood disturbances, however the effect of diet on learning-memory and mood disturbances gained attention just recently. As cardiometabolic diseases are the number one killer in patients with bipolar disorder, the manipulation of diet and other cardiovascular risk factors is highly crucial (210). Today, diet was shown to be as crucial to psychiatry as it is to endocrinology, cardiology, and gastroenterology (211). A recent systematic review found an evidence of unhealthy dietary patterns and poorer mental health in children and adolescents (212). The pattern of diet consumed during early developmental periods was shown to determine the behavioral and emotional impairments later in life (213, 214).

Even though many studies showed how diet alters mood in general and specifically in depression, evidence to the association between diet and bipolar disorder is poor. For instance, a couple of meta-analyses demonstrated that consumption of western diet increases the odds of having depression whereas healthy Mediterranean style diet is protective from depression (215-217). Furthermore, positive diet interventions were shown to improve the outcomes of depression and anxiety (218, 219). Comorbid insulin resistance causes a more chronic course with rapid cycling of bipolar disorder (220, 221). After carefully reviewing the literature regarding the association between diet and bipolar disorder, Lopresti and Jacka discussed that whether “diet has a causative role in bipolar disorder or is simply a lifestyle based factor associated with this disease” (222). Beyer and Payne further reviewed the most recent literature and suggested that more studies are needed to explore the possibility that diet actually causes and/or worsens bipolar disorder (223). As there are only a handful of studies focusing on this subject, more and more data must be obtained before ending the discussion for good.

As clinical studies investigating the relationship between diet and bipolar disorder are already scant, the preclinical studies investigating the mechanism tying the two are almost none existent. A few showed how diet may affect hippocampal formation in general. A recent study demonstrated that hippocampal spatial learning and memory tasks were impaired upon short-term feeding with 11 w/v carbohydrate containing HFCS solution in adolescent rats. However, adult rats were spared from the detrimental effects of HFCS consumption in the same study (37). Similarly, high fructose feeding for 7 weeks in mice resulted in impairments in LTP and LTD, reduction in hippocampal neurogenesis and hippocampal dysfunction, leading to a decrease in hippocampal learning and memory (224). Unfortunately, the complete behavioral characterization of adolescent rats fed with HFCS has not been performed before. That’s why we performed behavioral characterization of rats fed with HFCS and try to categorize the observed findings as discrete learning-memory and mood disorders.

2.3. Electrophysiologic and Molecular Characterization of HFCS Consumption in Adolescent Rats

We demonstrated that HFCS consumption in adolescent rats led to development of bipolar-like behavioral phenotype, including spontaneous hyperlocomotion, decreased anxiety, increased risk-taking behavior, increased social/sexual reward-seeking behavior and increased sensitivity to behavioral despair paradigm with subtle learning deficits and preserved long-term memory which were readily reversible with the inhibition of GSK-3, which is known as the most probable mechanism of action of lithium for its mood-stabilizing effects. BD is a genetically heterogeneous, highly heritable and devastating condition with a mean prevalence of 1.8% in children and adolescents (225). It is the fourth leading cause of disability-adjusted life years among the adolescents (226). Even though several plausible mechanisms have already been suggested, the etiology and pathophysiology of BD have yet to be elucidated.

As introduced in the first chapter, alterations in the synaptic structure, transmission, and plasticity, reduced levels and/or activity of NKA and inflammation have been suggested as the underlying pathophysiology of BD. Neuronal hyperexcitability is one of the most commonly reported alterations associated with bipolar disorder. A recent high impact study demonstrated that hippocampal-like neurons obtained from patients with bipolar disorder with the aid of induced pluripotent stem cell technology showed neuronal hyperexcitability. In addition, this observed hyperexcitability was particularly reversed by lithium only in neurons obtained from patients who also responded to lithium (90). This study clearly demonstrates that hyperexcitability is not only a coincidental feature seen with bipolar disorder, but also a feature contributing to the pathophysiology of it. Several other studies reported bipolar disorder associated with neuronal hyperexcitability and mood-stabilizing agents attenuates this hyperexcitability by various mechanisms of action. For instance, increased glutamate levels were found in brains of patients with bipolar disorder in postmortem examinations (84, 227). In addition, a meta-analysis showed increased glutamate levels in brains of bipolar patients measured by magnetic resonance spectroscopy (228, 229). In concordance, mood stabilizing agents were shown to decrease glutamate levels. For instance, the first-line mood stabilizer lithium was shown to decrease synaptic glutamate levels by increasing its reuptake (85, 230, 231). In addition, chronic lithium treatment was shown to be protective from excitotoxicity of excess glutamate (87, 88). Similar to lithium, anticonvulsants having mood-stabilizing activity, most notably valproate, was also shown to decrease synaptic glutamate levels (86).

Apart from the regulation of amount of glutamate released into the synaptic cleft, AMPA receptor trafficking has also gained attention as both lithium and valproate target it (83). Both lithium and valproate decrease synaptic expression of AMPA receptors by attenuating the ser845-phosphorylation of GluR1 subunit, dissipating the readily available pool of AMPA receptors to be incorporated into the postsynaptic membrane (80-83). This postsynaptic AMPA receptor insertion is crucial for the emergence of bipolar-like behavioral phenotype, as AMPA receptor antagonists attenuate amphetamine-induced hyperactivity (81). Interestingly, the neuronal excitability was shown to be modulated by diet. Ketogenic diet, a low carbohydrate diet, has been used for the treatment of intractable seizure disorders for many decades and shown to counter neuronal hyperexcitability both *in vivo* and *in vitro* (91, 92). A recent case report demonstrated the efficacy of ketogenic diet in the treatment of BD (93).

We further aimed to characterize the HFCS-fed rats in respect to aforementioned aspects. Our last effort was to find out whether observed bipolar-like behavioral changes were associated with alterations in either synaptic strength, levels of NKA or inflammation, that can be reversed by inhibition of GSK-3.

3. MATERIALS AND METHODS

3.1. Animals. Male Wistar rats of 21 days of age (Kobay, Ankara, Turkey), weighing approximately 45-55 grams at the time of arrival, were housed as triplets on a 12:12h light:dark schedule in a temperature (21°C) and humidity (40%) controlled room. All animals had access to tap water or 11% w/v carbohydrate containing HFCS solution and chow *ad libitum*, unless stated otherwise. For the behavioral tests and electrophysiology experiments, 27 male Wistar rats used. Additional 15 rats were used for intracerebroventricular insulin injections and immunoblotting. Rats were given at least a week for acclimation prior to any procedure and handled extensively during this period. All experiments and tissue harvesting were done in the light cycle. All procedures were approved by the Hacettepe University Animal Experimentations Local Ethics Board.

3.2. Experimental Groups. The diets and treatments allocated to groups are shown in the table (Table 3.1.).

Group	Diet	Treatment
Control	Tap water + Chow	Vehicle (1% DMSO – 99% saline)
HFCS	HFCS solution containing %11 w/v carbohydrate + Chow	
HFCS + TDZD-8		TDZD-8 dissolved in 1% DMSO – 99% saline

Table 3.1. Experimental groups and their corresponding diets and treatments.

3.3. Diets. Control group had *ad libitum* access to tap water and chow, whereas HFCS group had *ad libitum* access to HFCS solution containing %11 w/v carbohydrate and chow. Chow and liquid consumption were tracked weekly. Diets were started when rats were on postnatal day 21 and continued for 6 non-interrupted weeks (Figure 3.1.).

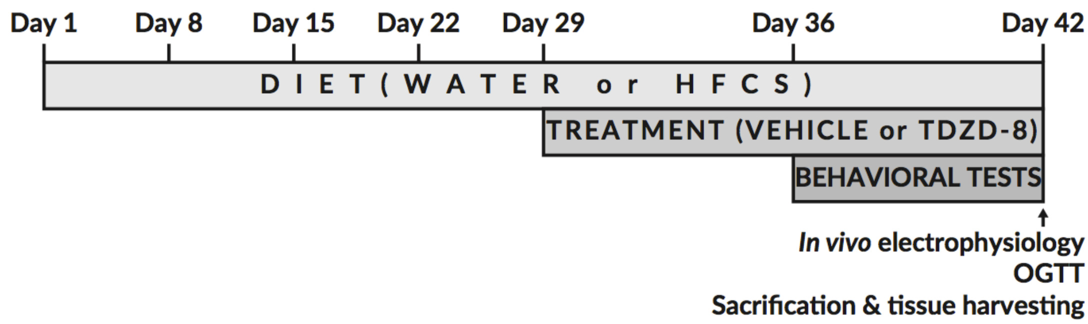

Figure 3.1. Experimental timeline.

3.4. Treatment Preparation and Administration. TDZD-8 (supplied by Dr. Ana Martinez, Spain) was dissolved in anhydrous DMSO (40 mg/ml). Afterward, the TDZD-8 solution in DMSO was aliquoted and stored at -80°C. When needed, the aliquoted stock solution was left in room temperature to thaw. Normal saline was added to reconstitute the anhydrous DMSO stock solution of TDZD-8, reaching a final concentration of 0,4 mg/ml in 1% DMSO – 99% saline. This solution was briefly vortexed to obtain the final solution used to administer TDZD-8 intraperitoneally. 2 mg/kg of TDZD-8 was administered once daily for 14 days from day 29 to 42. The TDZD-8 dose was determined from previous literature (232). Control group and HFCS group received equivalent volume (5 ml/kg) of vehicle treatment, which was composed of 1% DMSO – 99% saline.

3.5. Oral Glucose Tolerance Test. In order to assess the glucose tolerance, rats were undergone OGTT after receiving 14 days of vehicle or TDZD-8 treatment. In order to provide an easy access to tail vein blood, their tail tips were cut (2-3 mm in length) using sterile technique a day prior to OGTT. Rats were fasted for 6 hours prior to the experiment. Blood samples were obtained at the following time points by gently removing the clot from the tail tip: 15 minutes prior to glucose administration (-15 min), just before the glucose administration (0 min), and 15, 30, 45, 60, 75, 90, and 120 minutes after the glucose administration. 2 mg/kg of glucose was administered by oral gavage at 0 min. After removal of the blood clot, the first drop of blood was removed by sterile gauze, and the second drop obtained by gently milking the tail was used to assess blood glucose levels. Blood glucose readings were done by calibrated Accu-Chek Performa Nano (Roche, Switzerland).

3.6. Sacrification and Dissection of Fat Pads. After experiments were completed, rats were sacrificed by rapid decapitation. Total trunk blood was collected and centrifuged at 7.000g for 10 minutes. Serum was separated and stored at -80°C until further use. Before exposing the abdominal cavity, the inguinal fat pad was dissected by a midline cut lifting the skin but preserving the remaining layers of the abdominal wall. Afterward, the abdominal cavity was exposed further advancing the midline cut. Testicles were pulled into the abdominal cavity in order to better visualize the epididymal fat pats and dissect them. Later on, the mesenteric fat pad was scraped from the first part of the duodenum to cecum. The liver was dissected. Kidneys were removed and prerenal fat pads dissected. Retroperitoneal fat pads were dissected starting from the sacral region towards the diaphragm. In order to dissect the subscapular fat pads, a horizontal cut was made starting from the thoracic region, extending from the axilla, reaching to the subscapular region. The skin was scraped off towards the back of the neck to visualize and dissect the subscapular fat pad. All the fat pads dissected kept on a cold plate and weighted immediately by a precision scale.

3.7. Behavioral Tests. After a week of treatment with either vehicle or TDZD-8, rats were tested in a series of behavioral experiments. Rats were given an hour to acclimate to the testing room prior to the intraperitoneal injections. All rats received either vehicle or TDZD-8 treatment half an hour before the behavioral experiments. The order of experiments was determined from the least stressful one to the most stressful one. All behavioral tests were conducted in a specialized room, which was composed of two different chambers connected to each other by a sound and lightproof sliding door. The first chamber consisted of a computer system equipped with the behavioral tracking software, was connected to the cameras in the second chamber in order to visualize and track the rats during behavioral experiments. All apparatuses were black, in order to create a contrast difference between the background and white rats to have a more sensitive tracking of the animals.

3.8. Open Field Arena. Open field arena is extensively used to primarily assess the locomotion of animals. It is also secondarily helpful to assess anxiety-like and exploratory behaviors. The test apparatus was a square glass chamber (45x45x45 cm) with a black disposable base plate. A camera was placed a meter above the base plate. Four distinct visual cues (red circle, blue cross, green triangle and magenta square) were attached on each wall of the glass chamber. In between experiments, the black base plate was changed and the glass chamber was wiped with 70% EtOH to remove any olfactory cues left by the previous rat. Briefly, rats were placed and allowed to explore the arena for 1 hour. The total distance traveled was recorded as a measure of locomotion (Figure 3.2.).

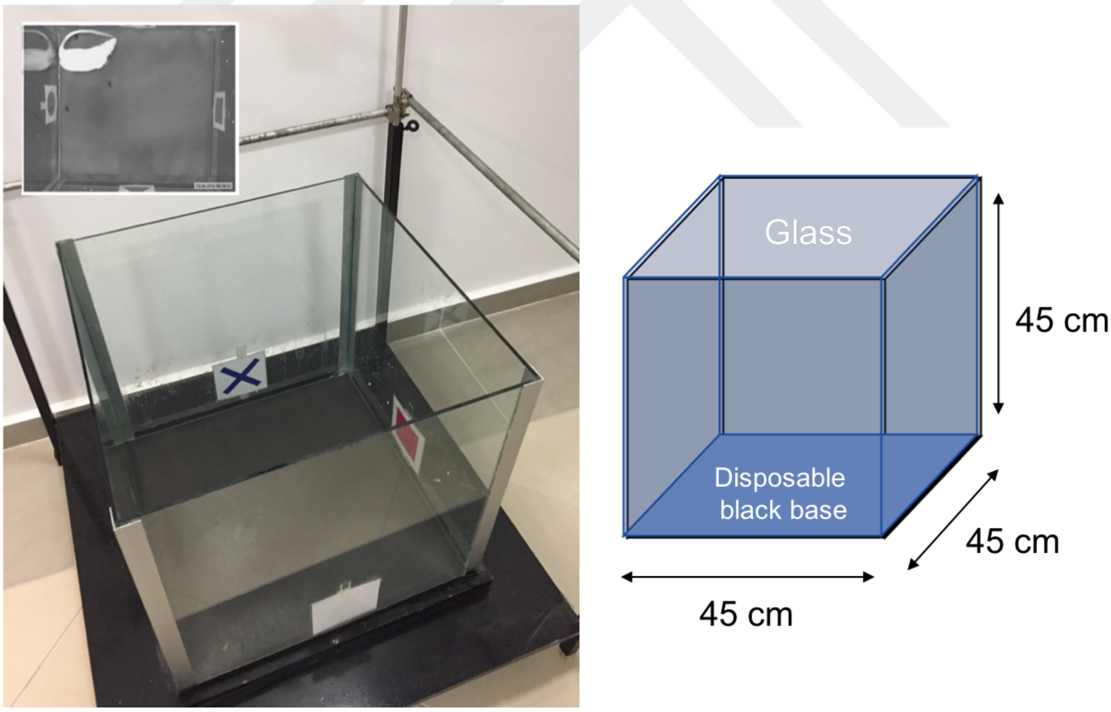

Figure 3.2. Apparatus of open field arena. Inlet: Image captured by the camera above the apparatus during the behavioral testing.

3.9. Elevated Plus Maze. Elevated plus maze is used to assess the anxiety-like behaviors and is known to be superior to the open field arena for this particular purpose. The apparatus is an elevated maze (1 meter above the ground) which is composed of four perpendicular arms (40 cm long and 15 cm wide), forming a plus shape. Two of the arms, facing each other are open (open arms). The remaining two are enclosed with 45 cm high walls (closed arms). A camera was placed 1,5 meters above the maze. Briefly, rats were placed at the junction of these four arms, facing towards an open arm. They were allowed to explore the apparatus for 5 minutes. The apparatus was wiped with 70% EtOH in between experiments. This test relies on the rats' propensity toward the dark, enclosed spaces (closed arms), and the fear of heights/open spaces (open arms). The time spent and distance traveled in arms were recorded. Increased time spent and/or distance traveled in open arms reflects of anti-anxiety behavior (Figure 3.3.).

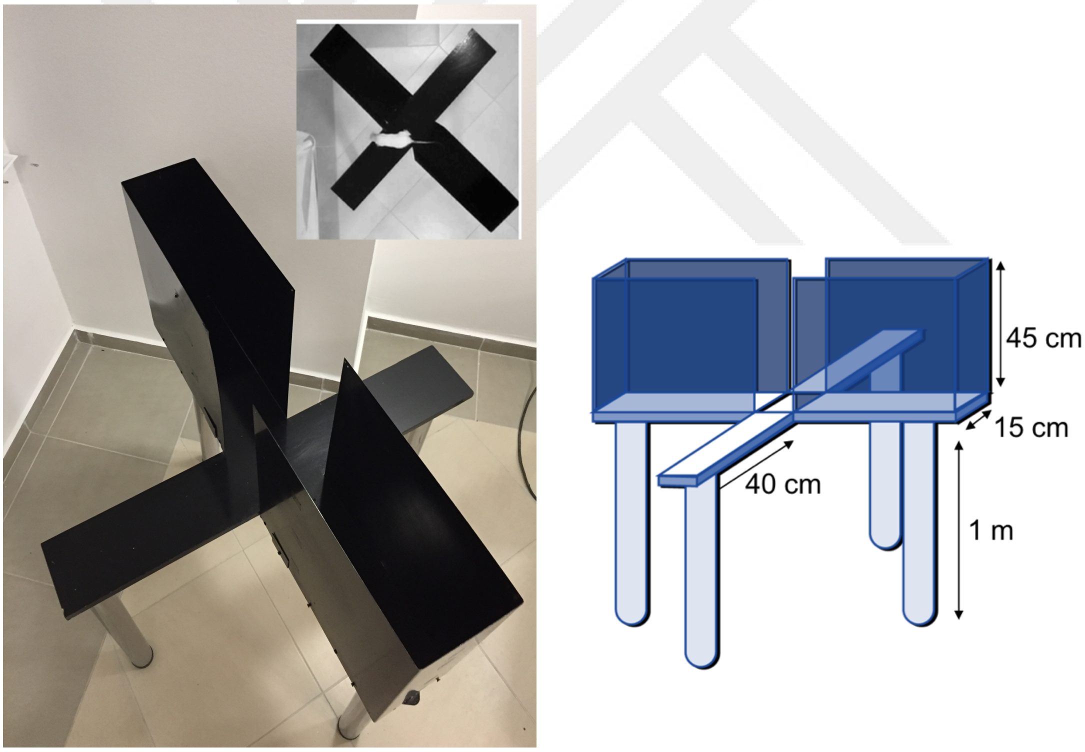

Figure 3.3. Apparatus of elevated plus maze. Inlet: Image captured by the camera above the apparatus during the behavioral testing.

3.10. Passive Avoidance Task. Passive avoidance task is a measure of ability to learn and remember to suppress a specific response (get into the dark compartment) to avoid from a noxious stimulus (inescapable foot shock). This test requires an intact hippocampus, as well as an intact amygdala. The apparatus consisted of two neighboring compartments (26x30x30 cm) divided by an upward-sliding door. One of the compartments (light compartment) was enclosed with transparent acrylic and illuminated by a halogen lamp (2800 K 1980 lm). The other compartment (dark compartment) was enclosed with black opaque acrylic with no obvious light inside. The base of the apparatus consisted of steel rods placed intermittently with gaps of 1,5 cm in between. The steel rods were connected to a control unit, which enabled the experimenter to apply predetermined current. This type of passive avoidance task relies on the innate preference of rats for the dark compartment, and the suppression of this innate preference after an inescapable foot shock (Figure 3.4.).

Passive avoidance task was conducted at three different stages: (I) Habituation, (II) Training, and (III) Test. (I) In the habituation stage, a rat was placed in the middle of the light compartment and the door in between the compartments was opened 5 seconds later. The latency of the rat to get into the dark compartment was recorded. The door was closed immediately after, and the rat was returned to its cage. (II) 5 minutes after, the rat was placed into the light compartment for the training stage. In this stage, when the rat entered the dark compartment, the door closed and an inescapable foot shock (square waves of 3 s of duration, 50 Hz, 1 mA) was applied through the steel rods. The rat was returned to its cage. (III) 24 hours after the training stage, the rat was placed in the light compartment for test stage. In this phase, the rat was subjected to the same procedure as in the habituation phase. The latency of the rat to enter the dark compartment was recorded. If the rat did not enter the dark compartment in 300 s, the experiment was terminated and the rat was returned to its cage.

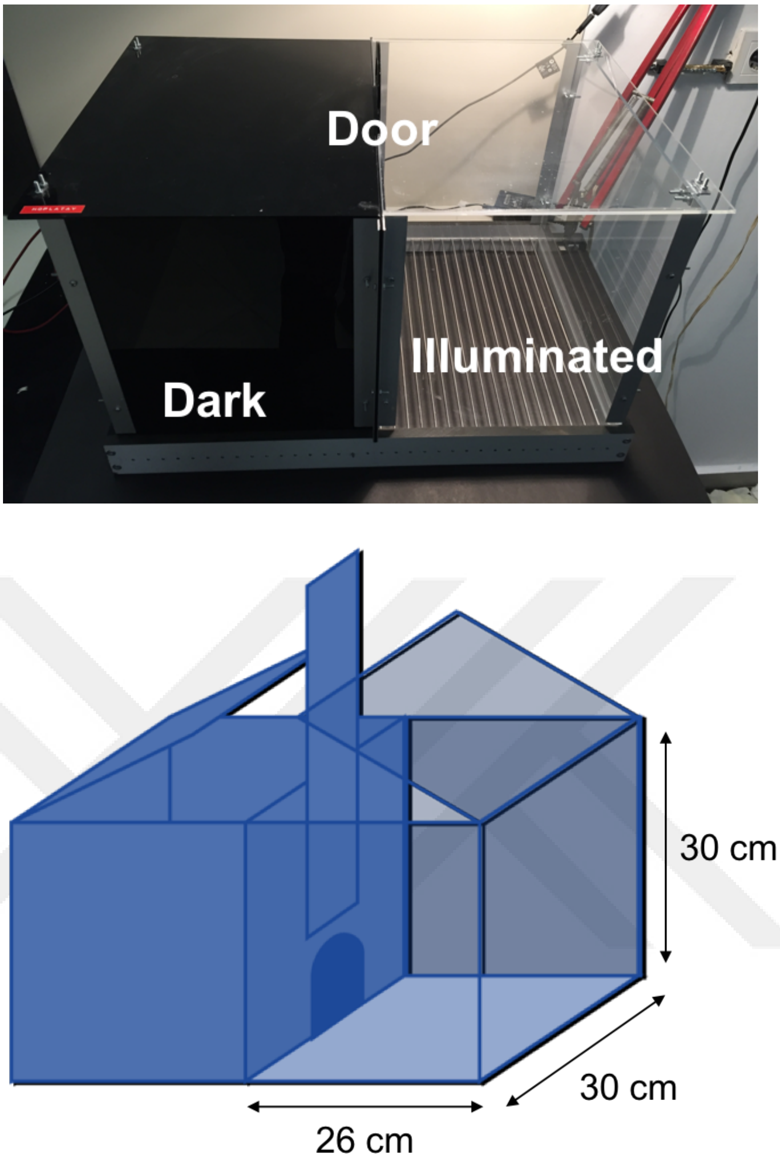

Figure 3.4. Apparatus of passive avoidance.

3.11. Morris Water Maze. Morris water maze is widely used to study hippocampus-dependent spatial learning and memory. However, this test is very sensitive to manipulations of normal brain functions also related to brain areas other than hippocampus. This is why water maze can be used almost like a screening test to confirm the “normality” of the brain function. The water maze apparatus was a black circular pool with the diameter of 180 cm. The pool was filled approximately half-way with warm water (22-23°C). The interior of the pool was featureless as possible, except for 4 visual cues (red circle, blue cross, green triangle and magenta square) attached (Figure 3.5.). The pool was divided into 4 equal quadrants by two imaginary axes intersecting each other perpendicularly. The intersecting axes defined the east, west, south, and north points of the pool. The quadrants were named according to the axes limiting them: northeast, northwest, southeast, and southwest (Figure 3.6.). A black cylindrical platform (height of 40 cm, diameter of 12 cm) was placed in the middle of the northwest quadrant. The platform was hidden as it was submerged 1-2 cm below the water surface. A camera was placed above the center of the pool. The test was composed of two different stages: (I) Acquisition, and (II) Probe. (I) The acquisition stage was composed of 4 subsequent days. In each acquisition day, each rat was undergone 4 different trials with intervals of 10 minutes in between. The rats were placed into the pool, facing towards the pool wall. The starting position in each trial was different in order to prevent the rat to memorize a path to platform instead of spatially learning where the platform was. In addition, the order of this different starting positions was also different in different acquisition days (Table 3.2.). After the rat was placed into the water, it was given 2 minutes to find the platform. If the animal fails to find it within the allotted time, it was gently guided by the experimenter towards the platform. When the rat found the platform, it was kept there for 15 seconds in order to appreciate and learn where the platform was located compared to visual cues. The latency to find the platform was measured and recorded by the behavior tracking software. Rats who are able to learn where the platform was, should have decreasing latencies with increasing number of trials. (II) 48 hours after the last trial of acquisition stage, a probe trial was given in order to assess the long-term memory. In probe trial, the hidden platform was removed. The rat was placed into the water from a novel starting point, that it had not been used before. This ensured that its quadrant preference was an indication of true spatial memory, rather than a memorized specific swim path. The rat was allowed to swim for 60 seconds. Time spent in target quadrant (the quadrant where the platform was located in the acquisition stage) was measured by the behavior tracking software.

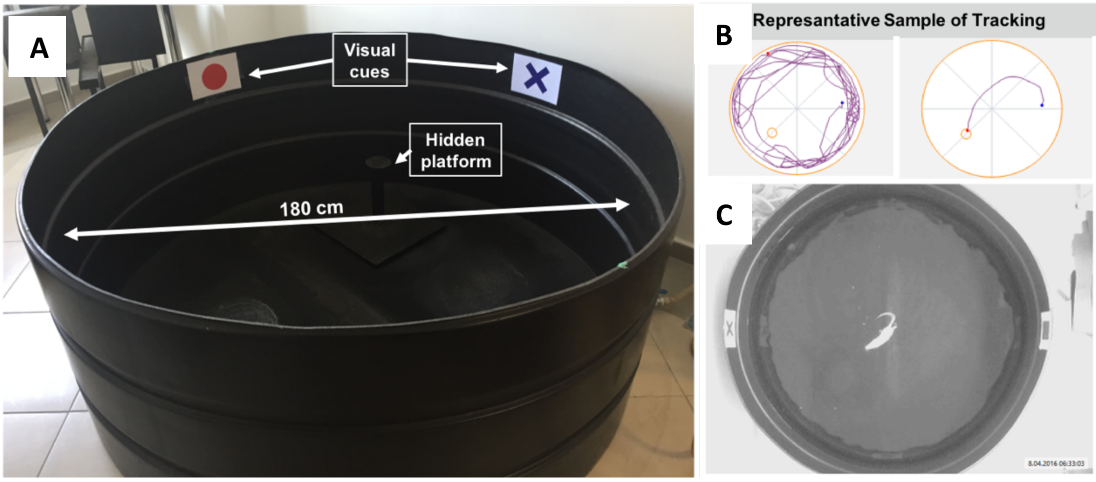

Figure 3.5. Morris Water Maze. (A) Apparatus of Morris water maze. (B) Representative sample of tracking. Left first acquisition day, Right last acquisition day. (C) Image captured by the camera above the apparatus during the behavioral testing.

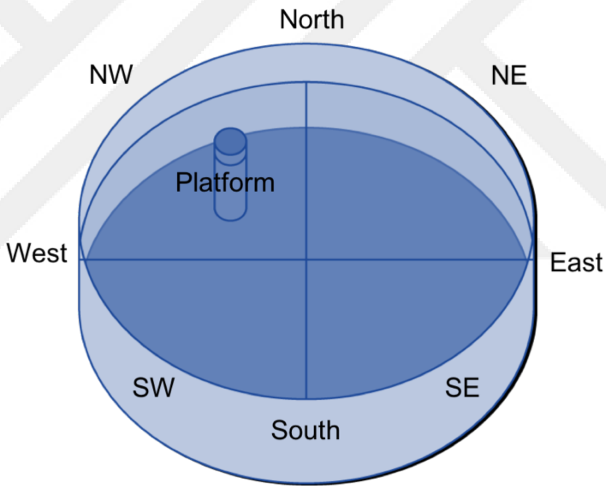

Figure 3.6. Virtual quadrants of Morris water maze. Hidden platform is located at the middle of the NW quadrant.

Trials	Acqusition Days			
	1	2	3	4
1	S	SW	NE	E
2	E	S	SW	NE
3	SW	NE	E	S
4	NE	E	S	SW

Table 3.2. Starting positions according to the acquisition days and the trials of Morris Water Maze.

3.12. Forced Swim Test. Forced swim test is the experimental paradigm of learned helplessness. It has been traditionally used to assess the depressive-like behavior and is a useful measure to assess antidepressant-like activity in rats. The apparatus was a 50 cm tall glass cylinder (diameter of 20 cm) which was filled to a depth of 30 cm with warm water (23-25°C). 20 cm space was left at the top so that the rat cannot escape. A camera was placed to the side of the cylinder (Figure 3.7.). The test was composed of 2 stages: (I) Pretest and (II) Swim test. (I) In pretest stage, a rat was gently placed into the cylinder and kept in there for 15 minutes. At the end of the session, the rat was removed from the water, gently dried with towels and returned to its cage. In this session, the rat was supposed to learn that there is no way for him to escape. 24 hours after the pretest, the swim test was performed. (II) In swim test, the rat was subjected to the same procedure, except that the session lasted 5 minutes. The time spent immobile was assessed by the behavior tracking software. The immobile behavior in the swim test is thought to reflect the failure to keep performing escape-directed behavior due to the behavioral despair learned in the pretest.

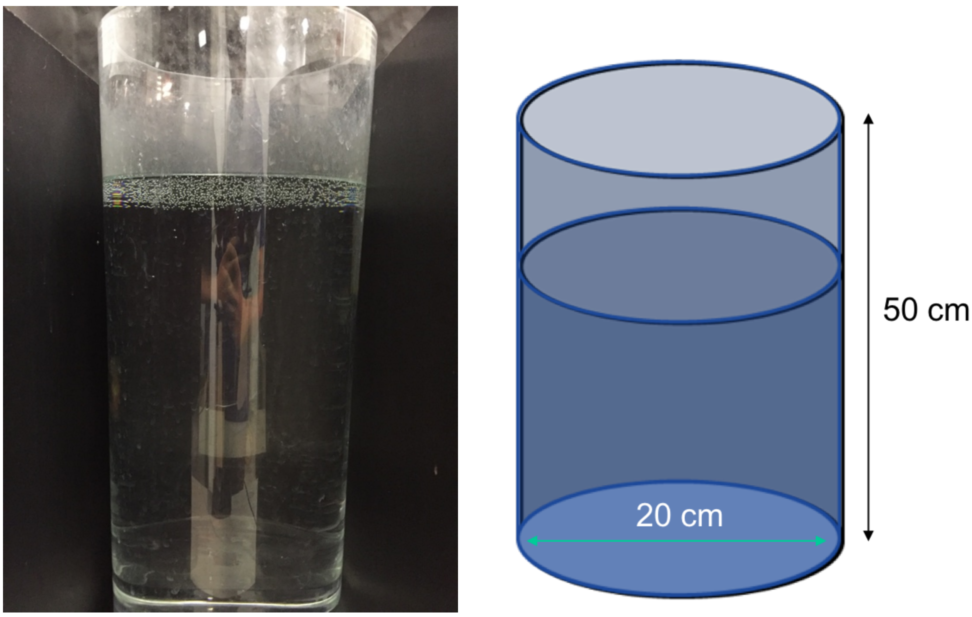

Figure 3.7. Apparatus of forced swim test.

3.13. Female Urine Sniffing Test. Female urine sniffing test is a non-operant test used to assess the sexual reward-seeking behavior (hedonic behavior). This test depends on the appetitive response of male rats towards the pheromonal odors found in the urine of female rats. In order to maximize the attractiveness of the urine, it was collected from the female rats, who were found to be in the estrus phase of their cycle.

3.14. Determination of the Phase of Estrous Cycle of Female Rats. Rodent estrous cycle is divided into 4 stages: proestrus, estrus, metestrus, and diestrus. In order to determine the stage of which the female rat is going through, rapid identification of cells collected via vaginal lavage was performed. Briefly, 100 μl of normal saline was drawn to a sterile pipette. The tip of the pipette was gently introduced from the opening of the vagina. Quarter to half (25-50 μl) of the total volume of saline was expelled and drawn back quickly. This was repeated 4 to 5 times, which was enough to collect a sufficient number of cells. The fluid of vaginal lavage was placed on a glass slide and kept in room temperature, allowing it to completely dry. The dry slide was stained with crystal violet for 1 minute and subsequently washed with dH_2O for another minute. Approximately 15 μl of glycerol was placed on top of the smear and covered with a coverslip. The slides were examined under light microscopy to identify the cell types, making possible to determine the phase of estrous cycle. Estrus cycle was characterized by the presence of cornified squamous epithelial cells, which were packed as dense clusters.

Urine from female rats was collected freshly using a metabolic cage before every experiment. The apparatus used for FUST is an acrylic chamber (26x30x30 cm), in which the cotton swab was placed. One hour before the test, the rats were habituated to the testing environment, including the cotton swab. The test was composed of 2 subsequent stages. (I) For the first stage, the tip of the cotton swab was dipped in sterile water and placed into the apparatus. The rat was placed into the apparatus and allowed to explore for 3 minutes, during which the sniffing duration was measured. The rat was returned to its cage and allowed to rest for 45 minutes. (II) In the second stage, the tip of the cotton swab was dipped in the freshly collected urine sample of female rats in estrus phase. The rat was subjected to the same procedure afterward. In both stages, the behavior of rats was recorded by a camera, in order to assess the time sniffing afterward. The time male rats spent sniffing the cotton tip dipped in female estrus urine was used to assess the hedonic behavior.

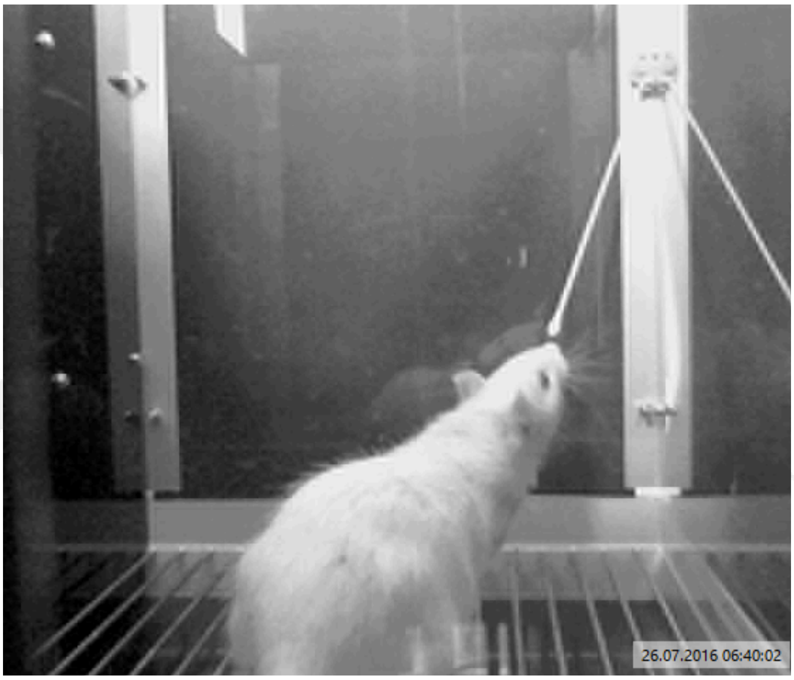

Figure 3.8. Sniffing behavior of a rat in female urine sniffing test. Image captured by camera next to the apparatus during the female urine sniffing behavioral testing.

3.15. *In vivo* Electrophysiology. Rats were anesthetized by intraperitoneal administration of 1,4 g/kg urethane (Sigma Aldrich). The depth of anesthesia was confirmed by the absence of paw withdrawal reflex. Afterward, the rat was placed to the rat stereotaxic surgical frame (Stoelting Co., IL, USA). The body temperature of the rat was kept constant at 36.8°C throughout the experiment with the help of homoethermic blanket and its control unit (Harvard Apparatus, MA, USA). A midline incision exposing both bregma and lambda was made. The horizontal and vertical alignment of the rat in the stereotaxic frame was performed according to the bregma and lambda before further proceeding with the experiment. The coordinates that were used to open burr holes and target specific brain areas were obtained from Paxinos Rat Brain Atlas. 3 individual burr holes were opened for a ground electrode, a stimulating electrode, and a recording electrode (Figure 3.9.). The coordinates according to the bregma was given in the table (Table 3.3.). A gold-plated ground electrode was placed caudal to lambdoid suture, in contact with dura mater.

Targeted Structure	Electrode	Anteroposterior (mm)	Mediolateral (mm)	Depth (mm)
VHC	Stimulating	-1,2	0,1	4,5
CA1	Recording	-3,9	2,2	2,5

Table 3.3. Coordinates targeting VHC and CA1 of the hippocampus.

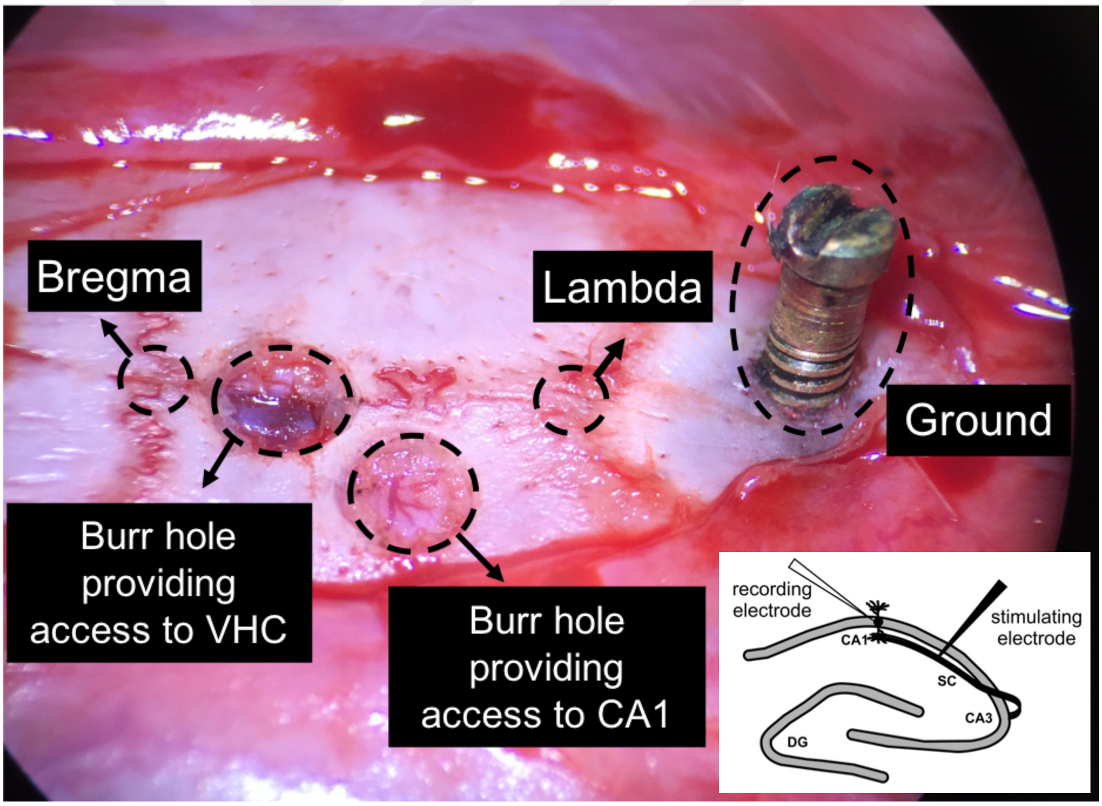

Figure 3.9. Image of rat skull captured from dissection microscope showing bregma, lambda, burr holes providing access to VHC and CA1, and ground. Inlet. Schematic representation of recording and stimulating electrodes and their corresponding targets in the hippocampus. (SC Schaffer collaterals, DG dentate gyrus)

The Schaffer collaterals passing through the VHC were stimulated every 20s with 0.1ms pulses using a stimulator (S44, Grass Instruments, RI, USA) isolated from the recording system with a stimulus isolation unit (SIU5, Grass Instruments) to evoke CA1 field excitatory postsynaptic potentials (fEPSPs) and field population spikes (pSpikes). The locations of both electrodes were optimized in order to obtain the maximal response possible. The evoked fEPSPs and pSpikes from *stratum radiatum* and *stratum pyramidale* were recorded and amplified with a headstage (Batiray, YSED, Turkey) and an amplifier (Kaldiray EX-2C, YSED) and digitized by a data acquisition system (PowerLab 8/SP, ADInstruments, Australia). Data recordings and analysis were done by using LabChart software (AD Instruments, Australia) and MiniAnalysis (Synaptosoft Inc., GA, USA), respectively. Input-output curves were constructed by applying stimuli with increasing intensities, ranging from 1V to 15V. The slopes of fEPSPs and the amplitudes of pSpikes were normalized according to the maximal response obtained from that particular recording. Paired-pulse facilitation and inhibition phenomena were assessed by applying paired-pulses with varying interpulse intervals at a stimulus intensity evoking 50% of the maximum response. The rats were sacrificed afterward and hippocampi were extracted as described below for qPCR and ELISA experiments.

3.16. Sacrification and Extraction of Hippocampi. Rats were rapidly sacrificed by decapitation. Whole brains were isolated and put in ice-cold PBS (Sigma Aldrich, Germany) solution. Afterward brains were put on an ice-cold plate for further dissection. First, the cerebellum was gently removed. Then, a midline cut was made to separate two cerebral hemispheres from each other. One kept on the plate for dissection, whereas the other was put into the ice-cold PBS solution. Frontal cortex and midbrain were removed using a scalpel. Afterward, two blunt plastic instruments were used to dissect the hippocampus without harming it. The second hippocampus was isolated likewise. The whole dissection, beginning from decapitation to extraction of both hippocampi, lasted less than a minute. The hippocampi were put into either ice-cold RNAlater (Qiagen, Germany) for qRT-PCR or homogenizing buffer containing protease and phosphatase inhibitor cocktails (see below) for immunoblotting.

3.17. Protein Isolation. A commercially available plasma membrane protein extraction kit (ab65400, Abcam, UK) was used to specifically isolate plasma membrane proteins. The isolation was performed according to the manufacturer's protocol. Briefly, the isolated tissues were immersed in 2-3 volume of an ice-cold 1X Homogenizing Buffer that includes both protease inhibitor (included in ab65400 kit) and phosphatase inhibitor (ROCHE PhosSTOP, Roche Diagnostics, Germany) cocktails. The samples were sonicated until completed lysed. Then, the homogenate was centrifuged at 700xg for 10 minutes at +4°C, by which, the total protein fraction was acquired. The cytosolic protein fraction was acquired by further centrifuging at 10,000xg for 30 min at +4°C. Then, the pellet was further used for the purification of plasma membrane proteins following the manufacturer's protocol. Both isolated cytosolic and plasma membrane protein fractions were stored at -80°C in aliquots.

3.18. Determination of Protein Levels of Lysates. For western blot analysis, protein concentrations were determined using commercially available RC DC Protein Assay Kit (BIO-RAD, CA, USA) using bovine serum albumin (BSA) as the standard. Briefly, BSA standards were prepared in different concentrations ranging from 0,2 to 1.4 mg/ml. The reagent A' was prepared by adding 20 μl reagent S to 1 ml reagent A. 5 μl of the standards and unknown samples were put into designated wells of 96 well plate. Afterwards, 25 μl of reagent A' and 200 μl reagent B were added to each well. The plate was mixed for 5 seconds and incubated at room temperature for 15 minutes. Finally, absorbance of each well at 750 nm was measured by using a spectrophotometer (ThermoFisher Scientific, USA). Protein concentrations of unknown samples were determined according to the standard curve constructed by the BSA standards.

3.19. Immunoblotting. Extracted protein samples (10 µg) from rat hippocampus were loaded and ran on a 10% acrylamide gel (BIO-RAD TGX Stain-Free™ FastCast™ Acrylamide Solution, BIO-RAD) at 90 V for 90 minutes. Protein was transferred to PVDF membranes using BIO-RAD Trans-Blot® Turbo™ Transfer System at 25 V for 7 minutes. Blots were blocked for 1 hour at room temperature with 5% nonfat dry milk in TBS-T Blotting-Grade Blocker (BIO-RAD), and subsequently probed with their relevant antibodies at 1∶1000 dilution overnight at 4°C. Anti-Rabbit-ECL secondary (ABCAM) at a concentration of 1∶100000 was applied for 1 hour at room temperature, blots were briefly washed in TBS-T and then TBS, then incubated with ECL substrate (Immobilon™, Millipore, MA, USA) for 5 minutes in the dark. Exposures were performed using Kodak GEL Logic 1500 Transilluminator Integrated Imaging System (Kodak, NY, USA). The intensities of the target bands were normalized according to the intensities of the corresponding beta-actin bands. Relative change of protein levels was reported as fold changes of the control group.

3.20. RNA Isolation. Hippocampus was taken from RNAlater solution and washed with PBS to remove excess RNAlater. Afterwards, it was put into a new DEPC-treated microcentrifuge tube. 1 ml of TRI reagent was added and the tissue was homogenized using a sonicator. It was incubated at room temperature for 10 minutes. Obtained solution was centrifuged at 12,000 x g at 4°C for 10 minutes. The supernatant was taken into a new microcentrifuge tube and the pellet was discarded. 200 µl of chloroform was added to supernatant and vortexed for 30 seconds to completely dissolve the supernatant. The clear solution was incubated at room temperature for 3 minutes. Then, it was centrifuged at 12,000 x g at 4°C for 15 minutes. This centrifugation yields a three-phasic suspension. The lower phase contained proteins and the middle phase contained DNA. The upper phase containing RNA was taken to a new microcentrifuge tube. The last 2 steps (solubilization in chloroform and centrifugation) were repeated to remove any DNA and/or protein contamination in the upper phase. 200 µl chloroform was added and centrifuged one more time to avoid any DNA and protein contamination. Upper phase was solubilized with 500 μl isopropanol. It was vortexed until the turbidity fades. 2 μl glycogen was added and incubated at -20°C for 40 minutes. Following the incubation, the sample was centrifuged at 12,000 x g at 4°C for 25 minutes. The supernatant was discarded and the pellet was washed with 1 ml of 75% ethanol. The sample was centrifuged at 7,500 x g for 5 minutes at 4°C. The ethanol was discarded and the microcentrifuge tube left open to allow air dry of any ethanol left. Finally, 35 μl of nuclease-free water was added to dissolve isolated total RNA in the pellet. The final RNA containing solution was incubated at 65°C for 10 minutes in order to eliminate any possible clusters of DNA and/or protein due to contamination or avoid any interference of qPCR by RNAlater solution. The concentrations of RNA samples, A_{260}/A_{280} and A_{260}/A_{230} ratios were determined by NanoDrop 2000 (ThermoFisher Scientific, USA).

3.21. Primer Synthesis. The primers were designed according to the NCBI gene database. The primers were designed to encompass at least one intronic sequence, binding to the end of the previous exon and the beginning of the next one. Thus, the possibility of primers to bind to intronic sequences, which can be found in potentially contaminating DNA, was reduced. This provides an additional increased specificity towards primer-specific mRNAs. The selected forward and reverse primers are listed in the table (Table 3.4.). The primers were synthesized by Sentegen Biotechnology (Ankara, Turkey). These primers were both used for cDNA synthesis and the quantitative real time polymerase chain reaction. The amount of nuclease free dH_2O that should be added to obtain a 100 μM main stock was given by the data sheet provided by the manufacturer. A 25 μM intermediatery stock was prepared from the 100 μM main stock. Finally, 5 μM working solution was prepared by further diluting the intermediatery stock. This working solution of 5 μM was used for all experiments.

Gene	Type of Primer	Primer Sequence (5'-3')	Melting Temperature (°C)	dH_2O (µl) for 100 µM stock
ATP1A1	Forward Primer	TCCTTAAGCGTGCAGTAGCG	54	2098
	Reverse Primer	CTCATCTCCATCACGGAGCC	56	1864,7
ATP1A2	Forward Primer	TAGCATACGAAGCGGCTGAG	54	1647,7
	Reverse Primer	GATCATGCCGATCTGTCCGT	54	1790,8
ATP1A3	Forward Primer	GCCAAGATGGGGGACAAAAA	52	1874,5
	Reverse Primer	TGCACGCAGTCGGTATTGTA	52	1762,6
GAPDH	Forward Primer	TCCCATTCTTCCACCTTT	46	990,2
	Reverse Primer	TAGCCATATTCATTGTCATACC	49	1740,6

Table 3.4. Primers used in cDNA synthesis and qPCR experiments.

3.22. cDNA Synthesis. A commercially available RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Germany) was used to convert RNAs to cDNA for qPCR studies. The volumes of the RNA samples for cDNA synthesis was adjusted according to the concentration of each sample. The cDNA synthesis was performed according to the protocol provided by the manufacturer. Gene-specific primers were used in cDNA synthesis to increase the specificity. The reagent volumes used in cDNA synthesis reaction are shown in the table (Table 3.5.).

Reagent	**Volume**
Template RNA	According to RNA concentration of the lysate
Gene specific Forward Primer (5 µM)	2 µl
Gene specific Reverse Primer (5 µM)	2 µl
5X Reaction Buffer	4 µl
RiboLock RNase Inhibitor (20 U/µl)	1 µl
10 mM dNTP Mix	2 µl
Reverse Transcriptase Enzyme (M-MulV) (200 U/µl)	1 µl
Nuclease-free dH_2O	Complete to 20 µl
Total Volume	**20 µl**

Table 3.5. Reagents and their corresponding volumes for cDNA synthesis.

3.23. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR). SybrGreen Jump Start qRT-PCR kit (Sigma Aldrich, Germany) was used for the qRT-PCR reactions and the reactions were performed according to the manufacturer's instructions. The reactions were run by using the Corbett Rotor-Gene 6000 (Qiagen, Germany) and the results were reported with the instrument's software. To make sure that the reactions were specific for each gene, melting curves were obtained and analyzed for each reaction. Moreover, all reactions were performed as duplicates. Each run also contained a non-template control to make sure that there was no amplification of primers or formation of primer-dimers that may interfere with the results. The optimized qRT-PCR reaction conditions were given in the following table (Table 3.6.).

Reagent	**Volume**
SybrGreen Mix	10 µl
Forward Primer (5 µM)	1,6 µl
Reverse Primer (5 µM)	1,6 µl
$MgCl_2$	2,4 µl
cDNA	2 µl
dH_2O	2,4 µl
Total Volume	**20 µl**

94°C	2 min	
94°C	15 sec	30 cycles
60°C	60 sec	
Melt	60°C - 90°C	

Table 3.6. Reagents, their corresponding volumes and reaction conditions for qRT-PCR experiments.

The threshold cycles for each sample were determined. The expression levels of target genes relative to housekeeping gene were calculated using the following formula. This formula allows the calculation of expression levels even if the reaction efficiencies are not equal.

$$Ratio = \frac{(E_{target})^{\Delta Ct\ Gene\ of\ Interest\ (control-sample)}}{(E_{housekeeping})^{\Delta Ct\ Housekeeping\ gene\ (control-sample)}}$$

where E_{target} is the reaction efficiency for gene of interest; $E_{housekeeping}$ is the reaction efficiency of housekeeping gene (GAPDH); ΔCt is the difference between the Ct values of control and treated samples.

3.24. ELISA. Commercially available ELISA kits (Elabscience, MD, USA) for rat TNF-α, IL-1β, and IL-6 were performed according to the manufacturer's instructions. The total protein levels of hippocampal lysates were determined by using RC DC Protein Assay (Bio-Rad, CA, USA). Absorbance measurements were carried out by Multiskan GO spectrophotometer (Thermo Fisher Scientific).

3.25. Statistical Analysis. Data were presented as means ± standard errors. Statistical analyses were performed by GraphPad Prism (GraphPad Software Inc., CA, USA). Student's t-test or analysis of variance (ANOVA, one-way or two-way) followed by Tukey's post-hoc test was used when there were 2 or 3 groups to compare, respectively. $p<0.05$ was considered statistically significant. *, †, and ‡ were used to demonstrate statistical significance between the values of control vs. HFCS, HFCS vs. TDZD-8, and control vs. TDZD-8, respectively.

4. RESULTS

4.1. Chow and Liquid Consumption. In the first week, the average liquid (tap water) consumption of control group was 678±24.4 mL/cage, whereas the average liquid (11% w/v carbohydrate containing HFCS solution) consumption of HFCS group was 775±47.3 mL/cage. Even though HFCS was consumed more than tap water by their corresponding groups, the difference in between was not statistically significant in the first week. Beginning from the second week, HFCS group consumed more liquid compared to control group, which was also statistically significant (Figure 4.1. A). Even though HFCS group consumed more liquid compared to control group, HFCS-fed rats significantly reduced their mean chow intake starting from the first week (Figure 4.1. B).

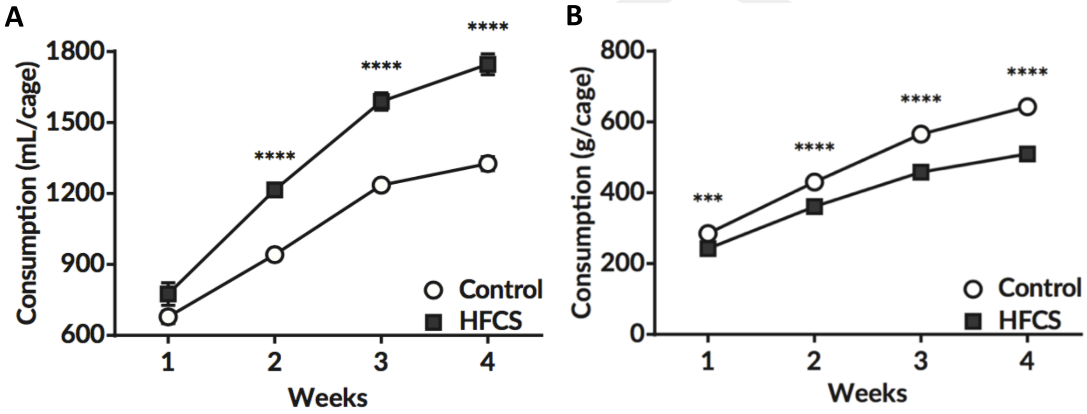

Figure 4.1. Weekly liquid and chow consumption. (A) Weekly liquid consumption. HFCS group had a significantly higher mean consumption of liquid compared to the control group, starting from the second week. (B) Weekly chow consumption. HFCS consumption caused a significant reduction in weekly chow consumption throughout the tracking period of a month. Some error bars are not clearly visible due to very small standard errors of mean. ***p<0.001, ****p<0.0001 between control vs. HFCS. (n=9/group).

4.2. Weight. There was no difference in mean body weights of control and HFCS group rats initially. Even though there was a considerable trend of control group having greater mean body weights, this difference was not statistically significant in the first 2 weeks. Starting from day 17, the control group had significantly higher mean body weight compared to that of HFCS group. It should be noted that the rats of both groups had appropriate weight gains according to their growth charts; however, the amount of weight gain was different in control and HFCS groups (Figure 4.2.)

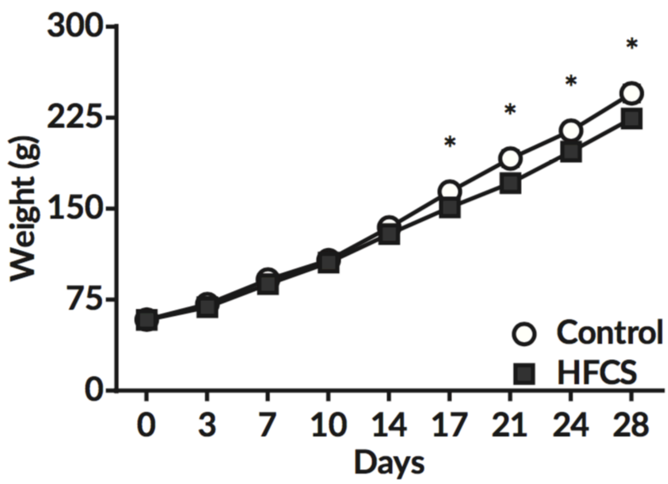

Figure 4.2. Body weights. The mean body weight of the HFCS group was slightly less than that of the control group, starting from the day 17. Some error bars are not clearly visible due to very small standard errors of mean. *p<0.05 between control vs. HFCS. (n=9/group)

4.3. Oral Glucose Torelance Test. Rats were challenged with oral glucose tolerance test (Figure 4.3. A) to measure glucose intolerance as a sign of insulin resistance and T2DM. Initially, the mean fasting blood glucose levels (at -15 min) of control, HFCS and TDZD-8 groups were 81.2±3.21 mg/dL, 130±3.41 mg/dL and 109±3.92 mg/dL, respectively. HFCS group had significantly higher mean fasting blood glucose level compared to both control and TDZD-8 groups (Figure 4.3. B) (Tukey's multiple comparisons test, Control vs. HFCS, HFCS vs. TDZD-8 and Control vs. TDZD-8, p<0.0001, p<0.01 and p<0.001, respectively). Just before glucose administration through gastric lavage, the fasting blood glucose measurements were repeated to test whether stress changed the initial measurement. However, the mean fasting glucose level remained significantly higher in HFCS group compared to both control and TDZD-8 groups. HFCS consumption and TDZD-8 treatment were shown to have a significant effect on how rats handle glucose in OGTT (two-way ANOVA, group effect, $F_{(2,22)}$=11.48, p=0.0004). After the glucose administration, all 3 groups reached to peak at 15 min, and the mean blood glucose levels at peak of control, HFCS and TDZD-8 groups were 186±6.28 mg/dL, 195±7.97 mg/dL and 197±6.77 mg/dL, respectively. Even though there was no statistical significance in between groups at the peak, HFCS group had a higher mean blood glucose level. After the peak, the mean blood glucose levels of HFCS and TDZD-8 groups dropped suddenly whereas that of control group declined smoothly. Thereafter, the mean blood glucose levels of both control and TDZD-8 groups continued to decline until they reached to similar levels prior to glucose administration, whereas the mean blood glucose level of HFCS group remained constant and resulted in significantly higher 2-hour postprandial blood glucose levels than control and TDZD-8 groups (Figure 4.3. B): The blood glucose levels of HFCS remained high compared to those of control and TDZD-8 rats (Control 101±1.4 mg/dL, HFCS 133±5.93 mg/dL, TDZD-8 106±4.08 mg/dL, $F_{(2,22)}$=16.6, p<0.0001). Previous studies showed that area under the curve (AUC) of OGTT may be a better index for glucose intolerance, insulin resistance and diabetes than fasting plasma glucose and 2-hour postprandial glucose levels. Both HFCS consumption and TDZD-8 treatment had a significant effect on the area under the curve of OGTT (ANOVA, $F_{(2,22)}$=8.46, p=0.0019). Here, HFCS group had significantly higher AUC compared to control group and TDZD-8 treatment reversed HFCS-induced increase in the AUC back to control levels (Tukey's multiple comparisons test, Control vs. HFCS and HFCS vs. TDZD-8, p<0.05 and p<0.01, respectively) (Figure 4.3. C).

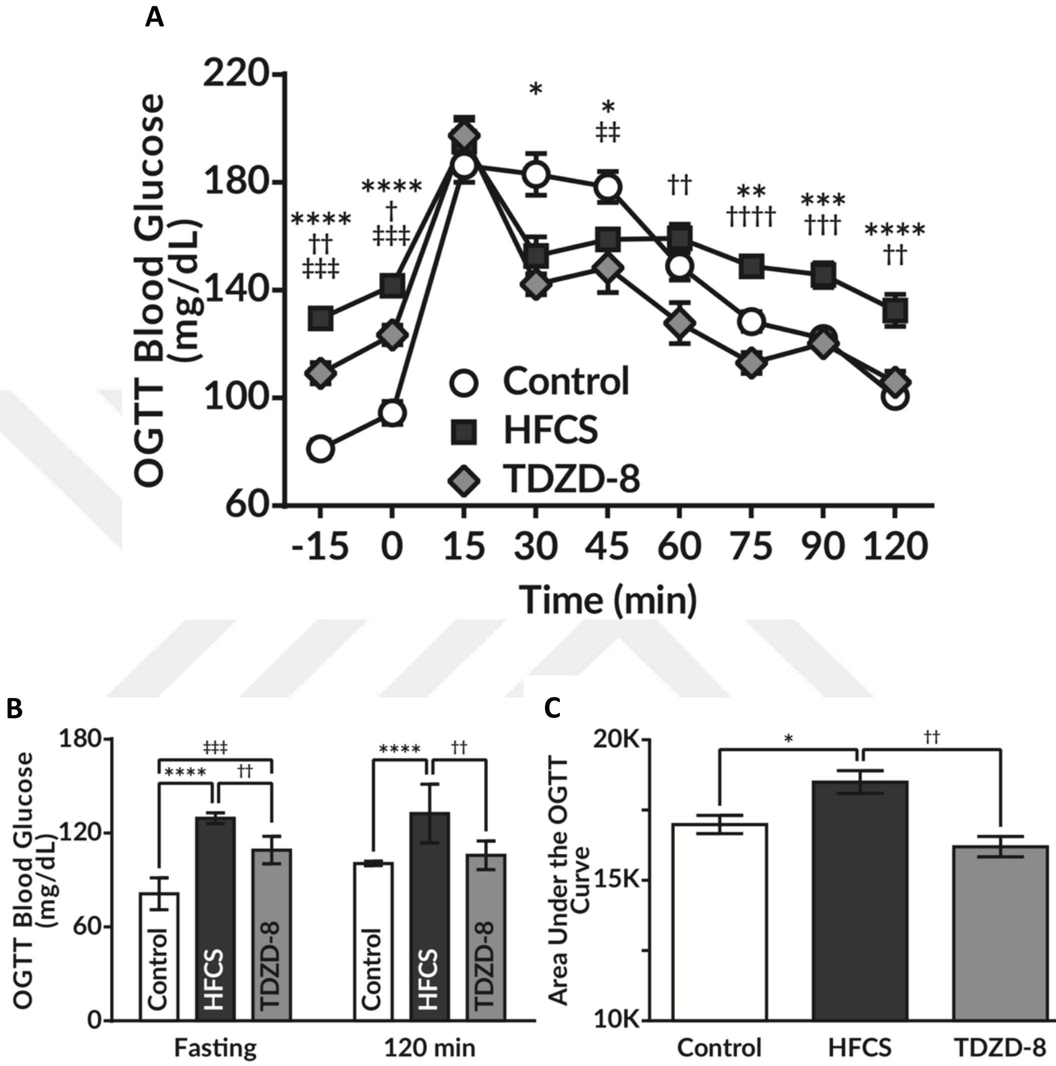

Figure 4.3. Oral Glucose Tolerance Test. (A) Oral Glucose Tolerance Test. (B) Fasting and 2-hour postprandial blood glucose levels. (C) Area under the OGTT curve. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 between control vs. HFCS. †p<0.05, ††p<0.01, †††p<0.001, ††††p<0.0001 between HFCS vs. TDZD-8. ‡p<0.05, ‡‡p<0.01, ‡‡‡p<0.001 between control vs. TDZD-8. (n=10/group for control and HFCS, n=5/group for TDZD-8).

4.4. Fat Pads. HFCS consumption and TDZD-8 treatment had a significant effect on the fatty body mass of rats (ANOVA, F(2,26)=7.00, p=0.0037). Even though HFCS group had a lower total body mass compared to control group at the end of a month of tracking period, the mean ratio of fatty mass to total body mass was significantly higher (Control 3.76±0.2%, HFCS 4.54±0.2%, Tukey's multiple comparisons test, Control vs. HFCS, p<0.05), suggesting an increase in fatty mass with a reduction in lean body mass. Furthermore, TDZD-8 treatment failed to revert HFCS-induced increase in fatty mass (TDZD-8 4.79±0,2%, Tukey's multiple comparisons test, HFCS vs. TDZD-8, not significant) (Figure 4.4.).

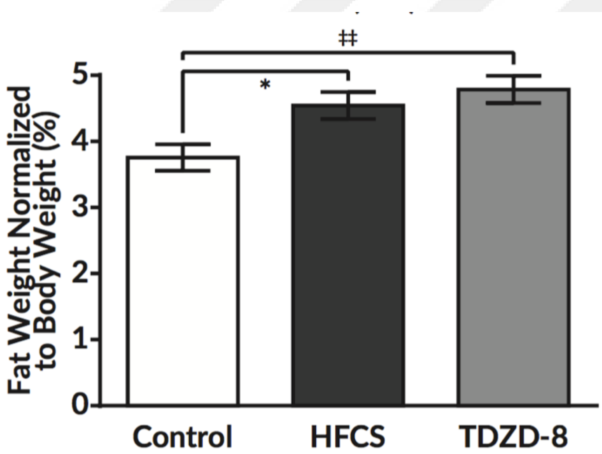

Figure 4.4. Ratio of weight of fat pads to total body weight.

4.5. Open Field Arena. To measure the spontaneous locomotion of rats, the open field arena test was utilized (Figure 4.5.). There was a significant effect of HFCS consumption and TDZD-8 treatment on the distance traveled in open field arena (ANOVA, F(2,21)=7.39, p=0.0037). In the 60 min of tracking period, control group traveled 18.5±4.47 m, whereas HFCS group traveled 34.6±3.78 m, suggesting spontaneous hyperlocomotion associated with HFCS consumption (Tukey's multiple comparisons test, Control vs. HFCS, p<0.05). Furthermore, TDZD-8 group traveled 15.3±2.98 m, which shows TDZD-8 treatment restored HFCS-induced spontaneous hyperlocomotion (Tukey's multiple comparisons test, HFCS vs. TDZD-8, p<0.01).

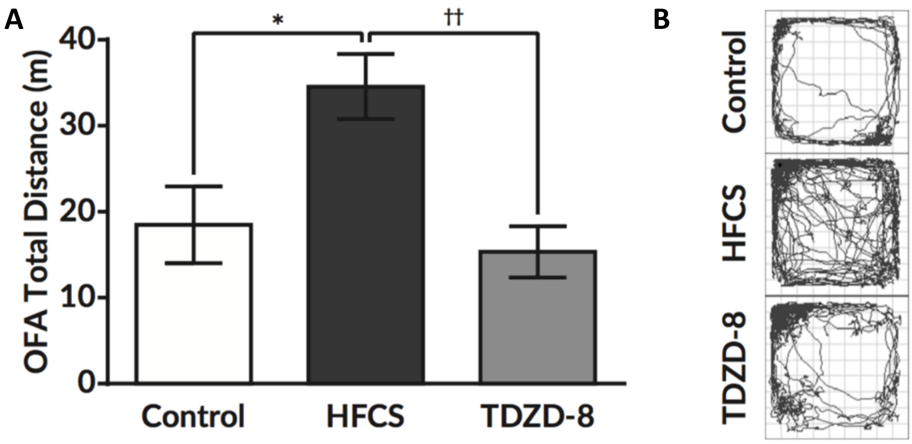

Figure 4.5. Open Field Arena. (A) Distance traveled in open field arena. (B) Representative track plots of rats from their corresponding groups. *p<0.05, between control vs. HFCS. ††p<0.01 between HFCS vs. TDZD-8. (n=8/group).

4.6. Elevated Plus Maze. To measure anxiety-related behaviors, elevated plus maze was utilized (Figure 4.6.). There was a significant effect of HFCS consumption and TDZD-8 treatment on the time spent (ANOVA, $F(2,24)=6.21$, $p=0.0067$) and distance traveled (ANOVA, $F(2,24)=8.01$, $p=0.0022$) on the open arms. Control group spent a mean of 6.01 ± 2.46 s on the open arms, whereas HFCS group spent a mean of 42.7 ± 13.7 s. Furthermore, TDZD-8 group showed a similar response to that of the control group, spending 7.62 ± 3.82 s. Tukey's multiple comparisons tests further revealed that HFCS group spent significantly more time and traveled more distance on the open arms of elevated plus maze compared to both control and TDZD-8 groups (Tukey's multiple comparisons tests, Control vs. HFCS and HFCS vs. TDZD-8, $p<0.05$).

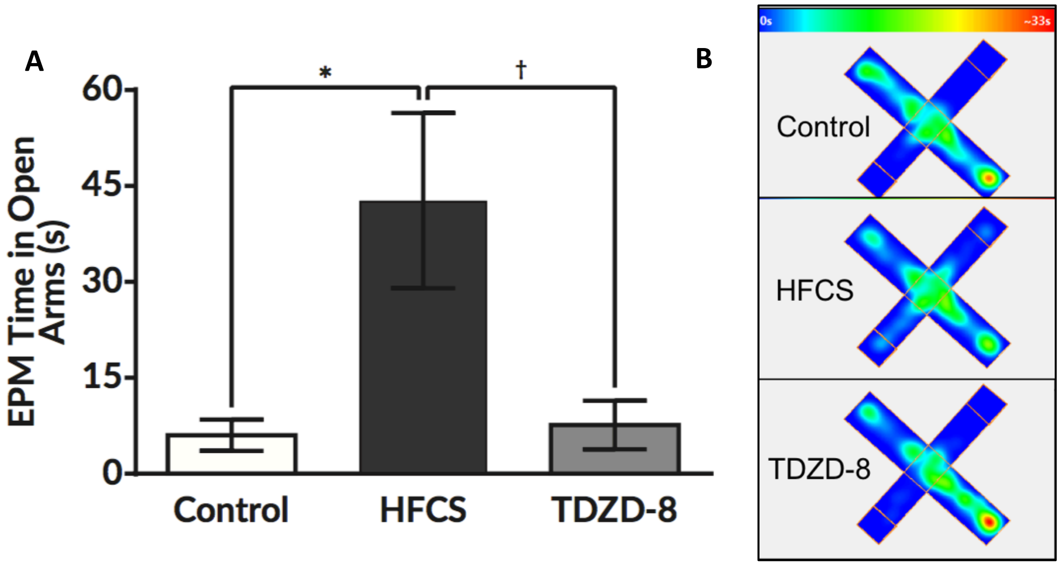

Figure 4.6. Elevated Plus Maze. (A) Time spent in open arms of elevated plus maze. (B) Averaged heat maps of groups. *p<0.05, between control vs. HFCS. †p<0.05 between HFCS vs. TDZD-8. (n=9/group).

4.7. Female Urine Sniffing Test. To measure both sexual and social reward-seeking hedonic behaviors, the female urine sniffing test was utilized (Figure 4.7.). All three groups had similar mean duration of sniffing when presented with distilled water (Control 4.01±1.01 s, HFCS 4.88±0.96 s, TDZD-8 3.79±0.99 s) and all three groups sniffed significantly more after presented with female urine (Control 8.08±1.4 s, HFCS 14.04±1.47 s, TDZD-8 6.24±2.12 s, two-way ANOVA, stage effect $F(1,21)=21.8$, $p=0.0001$). When presented with female urine, HFCS consumption and TDZD-8 treatment had a significant group effect on the mean time of duration of sniffing (two-way ANOVA, group effect $F(2,21)=6.24$, $p=0.0075$). Post-hoc tests revealed that HFCS group had a greater mean duration of sniffing compared to both control and TDZD-8 groups (Tukey's multiple comparisons test, Control vs. HFCS and HFCS vs. TDZD-8, $p<0.01$ and $p<0.001$, respectively).

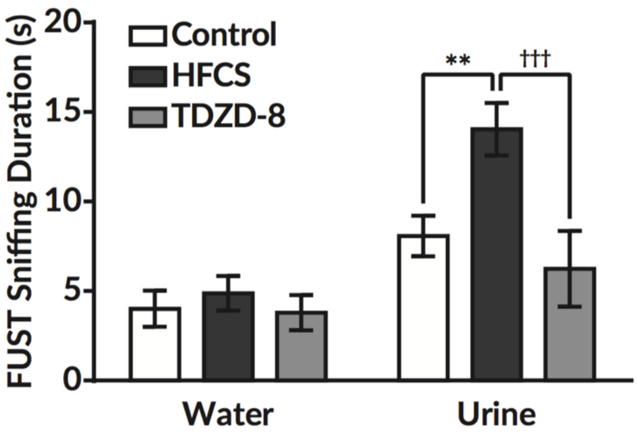

Figure 4.7. Female Urine Sniffing Test. **p<0.01 between control vs. HFCS. †††p<0.001 between HFCS vs. TDZD-8. (n=9/group).

4.8. Forced Swim Test. To measure the behavioral despair in response to learned helplessness, forced swim test was utilized. There was a significant effect of HFCS consumption and TDZD-8 treatment on the immobility time at the forced swim test (ANOVA, $F(2,24)=4.84$, $p=0.0171$). Control group had a mean immobility duration of 28.9±4.12 s, whereas HFCS group had a greater mean immobility duration of 45.9±4.61 s (Tukey's multiple comparisons test, Control vs. HFCS, $p<0.05$). TDZD-8 treatment successfully reverted the HFCS-induced greater immobility back to control levels with a mean immobility duration of 29.9±4.30 s (Figure 4.8.) (Tukey's multiple comparisons test, HFCS vs. TDZD-8, $p<0.05$)

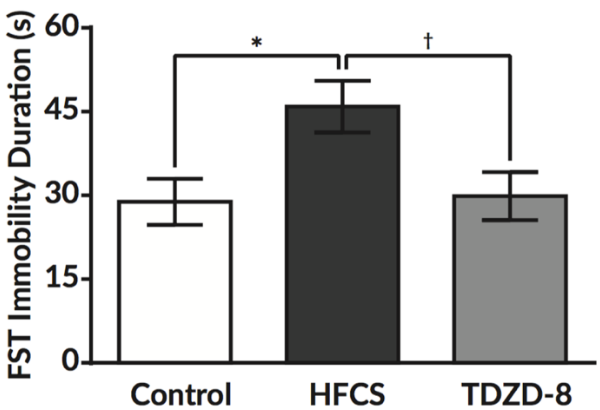

Figure 4.8. Forced Swim Test. *p<0.05 between control vs. HFCS. †p<0.05 between HFCS vs. TDZD-8. (n=9/group).

4.9. Passive Avoidance Task. To assess spatial learning and memory, which relies on intact hippocampal functions, both passive avoidance task and Morris water maze were utilized. Passive avoidance task also requires an intact amygdala as well as an intact hippocampus. In the shock phase, all three groups showed a similar latency to enter into the dark compartment, where rats received an inescapable foot shock. 24 hours the foot shock, the same experiment was repeated, called the test phase. In the test phase, none of the rats entered the dark compartment in order to avoid the foot shock (Figure 4.9.). Thus, neither HFCS consumption nor TDZD-8 treatment was unable to affect the behavior in the passive avoidance task compared to control group.

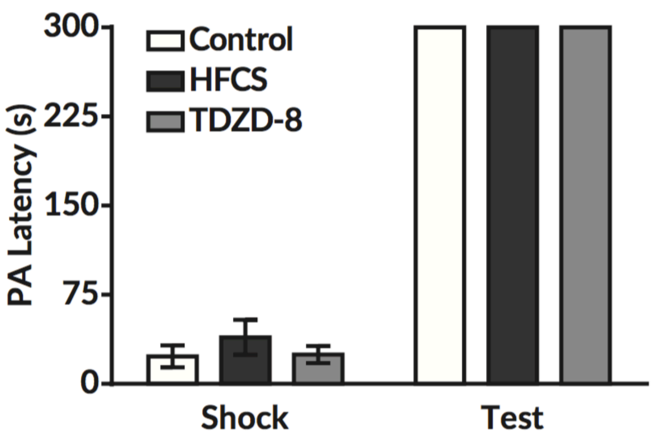

Figure 4.9. Passive Avoidance Test.

4.10. Morris Water Maze. Morris water maze has been considered as one of the best methods to assess hippocampus-dependent spatial learning and memory as well as the general normality of the brain. The mean swim speeds of each group were not statistically significant from each other (Control 0.24±0.005 m/s, HFCS 0.24±0.005 m/s, TDZD-8 0.23±0.004 m/s, ANOVA, $F(2,456)=0.474$, $p=0.622$), suggesting that the rats did not suffer from any motor disability and the differences in latency to platform were indeed due to alterations in cognitive functions (Figure 4.10. B). A two-way ANOVA detected a significant group difference through all four acquisition days (two-way ANOVA, group effect, $F(2,420)=6.90$, $p=0.0011$), however

Tukey's multiple comparisons test revealed a statistically significant difference only in the first acquisition day (Figure 4.10. A). In the first acquisition day, HFCS-fed rats were significantly worse at finding the hidden platform (Tukey's multiple comparisons test, Control 64.0±6.8 s vs. HFCS 84.2±7.4 s, p<0.01). In addition, TDZD-8 failed to restore this HFCS-induced deficit and resulted in a mean latency of 87.6±7.0 s (Tukey's multiple comparisons test, Control vs. TDZD-8, p<0.01; HFCS vs. TDZD, p>0.05). Even though there was a trend of greater mean latency periods of the HFCS group throughout the remaining three acquisition days, no statistical significance was reported. On the final acquisition day, all three groups demonstrated a similar mean latency period to the platform (Control 12.6±1.8s, HFCS 16.3±2.4s, TDZD-8 17.9±3.2s)

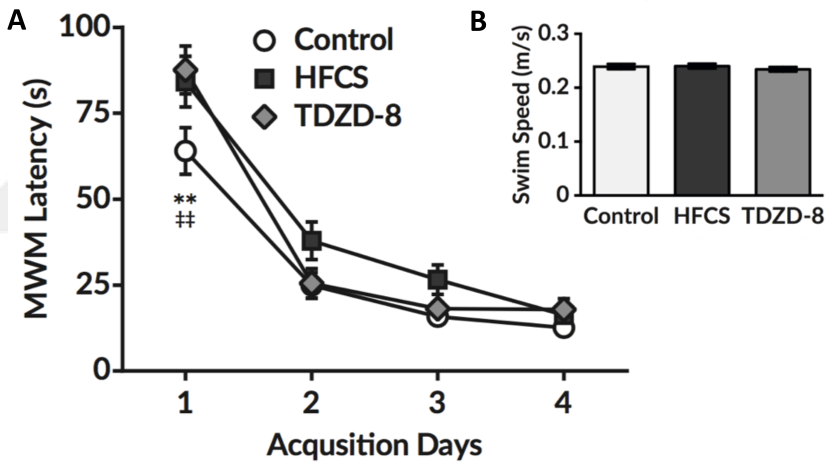

Figure 4.10. Morris Water Maze. (A) Latency to find the hidden platform in Morris water maze. (B) Averaged swim speeds of groups. **p<0.01 between control vs. HFCS, ‡‡p<0.01 between control vs. TDZD-8. (n=9/group).

In concordance with the last acquisition day, the probe trial, which was performed 48 hours the last acquisition trial, yielded similar results. Each group spent a similar duration in the target quadrant, where the platform was located during the acquisition trials (Figure 4.11.) (Control 26.4±2.37 s, HFCS 25.3±1.97 s, TDZD-8 25.1±2.75 s). Thus, ANOVA was unable to detect a statistically significant difference in between groups at the probe test (ANOVA, F(2,24)=0.0901, p=0.9141).

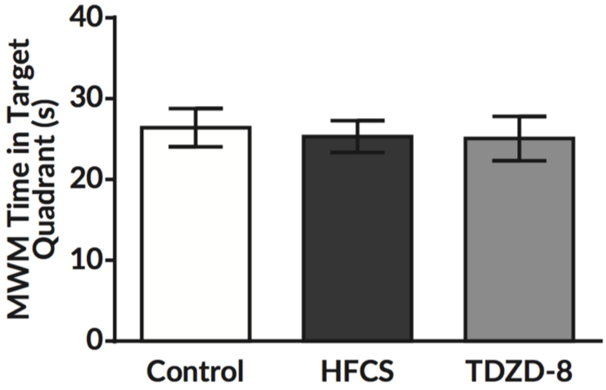

Figure 4.11. Time spent in target quadrant in the probe trial of Morris water maze.

4.11. Input-Output Curves. In order to assess the changes in the synaptic strength of HFCS-fed rats, Schaffer collaterals were stimulated and field potentials from the *stratum radiatum* (synaptic layer) of CA1 region were recorded. The input-output curve of fEPSP slopes shifted significantly towards left in the HFCS group, whereas TDZD-8 caused a rightward-shift, turning HFCS-induced hyperexcitability back to normal (two-way ANOVA, group effect, $F(2,23)=4.66$, $p=0.02$) (Figure 4.12.). In addition, when electrode was placed into *stratum pyramidale* (soma layer), the input-output curves yielded a similar trend to that observed in *stratum radiatum* (Figure 4.13.).

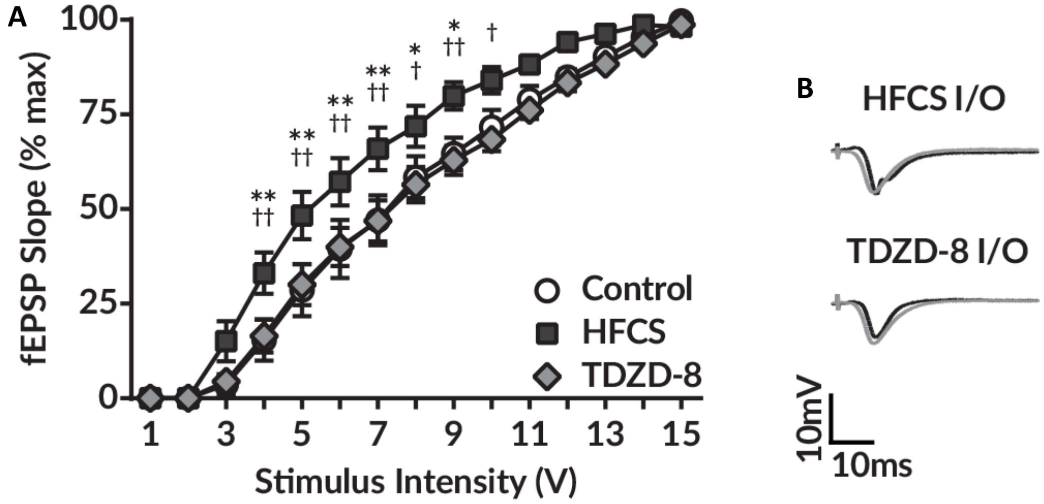

Figure 4.12. (A) Input-output curve recorded from *stratum radiatum*. (B) Representative recordings from *stratum radiatum*. I/O traces were selected from the

responses to stimuli of 7V. Traces from control group were depicted grey, whereas traces from HFCS and TDZD-8 groups were depicted black.

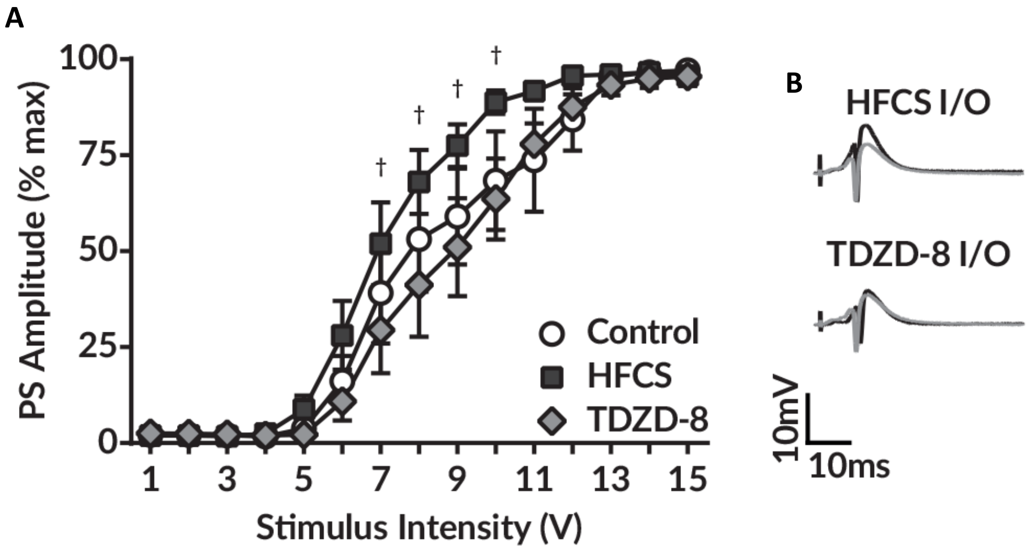

Figure 4.13. (A) Input-output curve recorded from *stratum pyramidale*. (B) Representative recordings from *stratum pyramidale*. I/O traces were selected from the responses to stimuli of 7V. Traces from control group were depicted grey, whereas traces from HFCS and TDZD-8 groups were depicted black.

4.12. Paired Pulse Facilitation/Depression. As a form of short-term plasticity, paired pulse facilitation/depression phenomena were studied. When two stimuli with 20 ms of interpulse interval were applied to the hippocampal CA3-CA1 synapses of control rats, the second fEPSP slope was higher than the first, demonstrating the presynaptic Ca^{2+} accumulation caused by two sequential stimuli, causing increased glutamate release after the second stimulus. However, HFCS group showed significantly less facilitation after the second stimulus than the control group, suggesting an increased presynaptic release probability causing depletion of glutamate containing vesicles when no time was given to replenish the stores. In addition, TDZD-8 treatment was unable to restore this impairment in *stratum radiatum* paired-pulse facilitation (Figure 4.14.) (at 20 ms interpulse interval, Control 1.86$\pm$0.08, HFCS 1.52$\pm$0.12, TDZD-8 1.58$\pm$0.13; Tukey's multiple comparisons test, Control vs. HFCS and Control vs. TDZD-8, p<0.0001). With greater interpulse intervals, no significant differences were found between groups (Figure 4.14.).

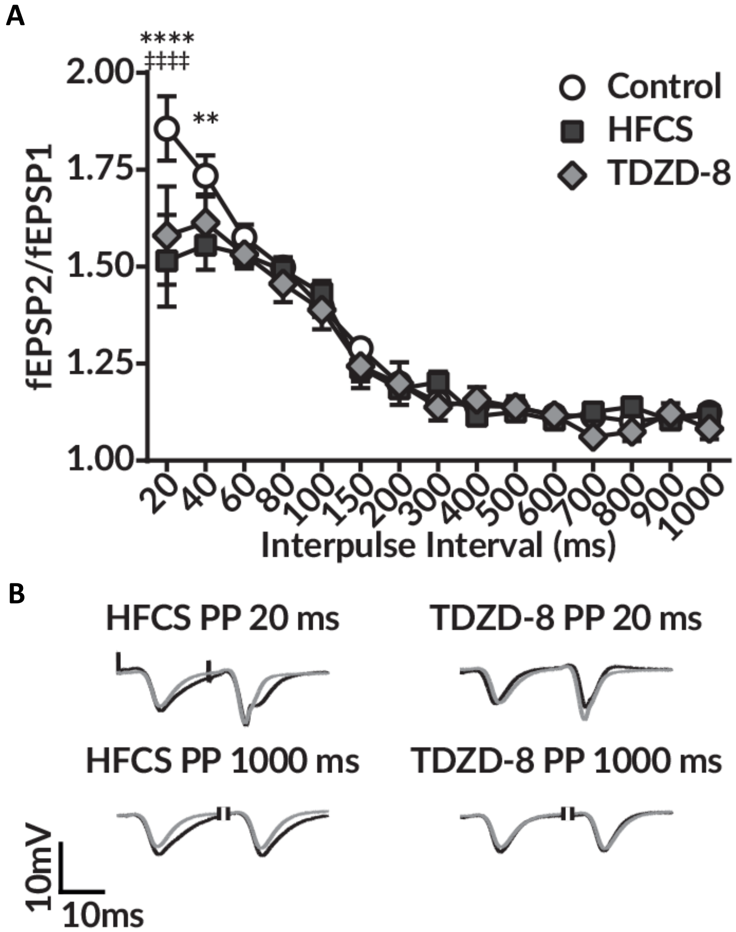

Figure 4.14. (A) Paired-pulse paradigm in *stratum radiatum*. (B) Representative recordings from *stratum radiatum.* Paired-pulse traces were given for interpulse intervals of 20 ms and 1000 ms. Traces from control group were depicted grey, whereas traces from HFCS and TDZD-8 groups were depicted black in their corresponding columns.

In order to assess the activity of GABAergic interneurons fine-tuning the cumulative response of the pyramidal neurons, the recording electrode was placed into the *stratum pyramidale* (soma layer) to record field pSpikes. In paired-pulse paradigm, there were no significant differences between groups in any of the interpulse intervals applied (Figure 4.15.), suggesting that GABAergic, the main inhibitory system, was spared from the effects of HFCS consumption in this study.

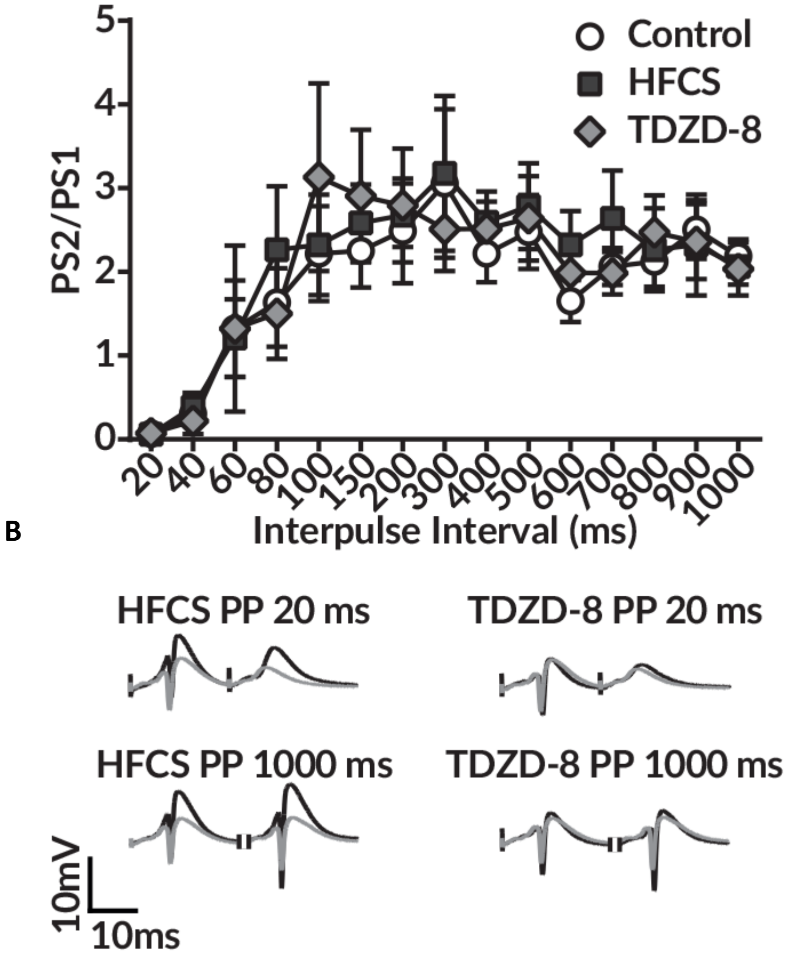

Figure 4.15. (A) Paired-pulse paradigm in *stratum pyramidale*. (B) Representative recordings from *stratum pyramidale.* Paired-pulse traces were given for interpulse intervals of 20 ms and 1000 ms. Traces from control group were depicted grey, whereas traces from HFCS and TDZD-8 groups were depicted black in their corresponding columns.

4.13. AMPA and NMDA Receptors. Ser845-phosphorylation of GluR1 subunits of AMPA receptors create a readily available pool of AMPA receptors to be incorporated to the postsynaptic density. In hippocampi of HFCS fed adolescent rats, the ratio of ser845-phosphorylated GluR1 to GluR1 was significantly higher compared to control group, which was reversed by TDZD-8 treatment (Figure 4.16.). In addition, HFCS consumption caused a significant decrease in total AMPA receptors, which was not reversed with TDZD-8 treatment. In contrast, NMDAR2A and NMDAR2B subunits of NMDA receptors were found to be unchanged.

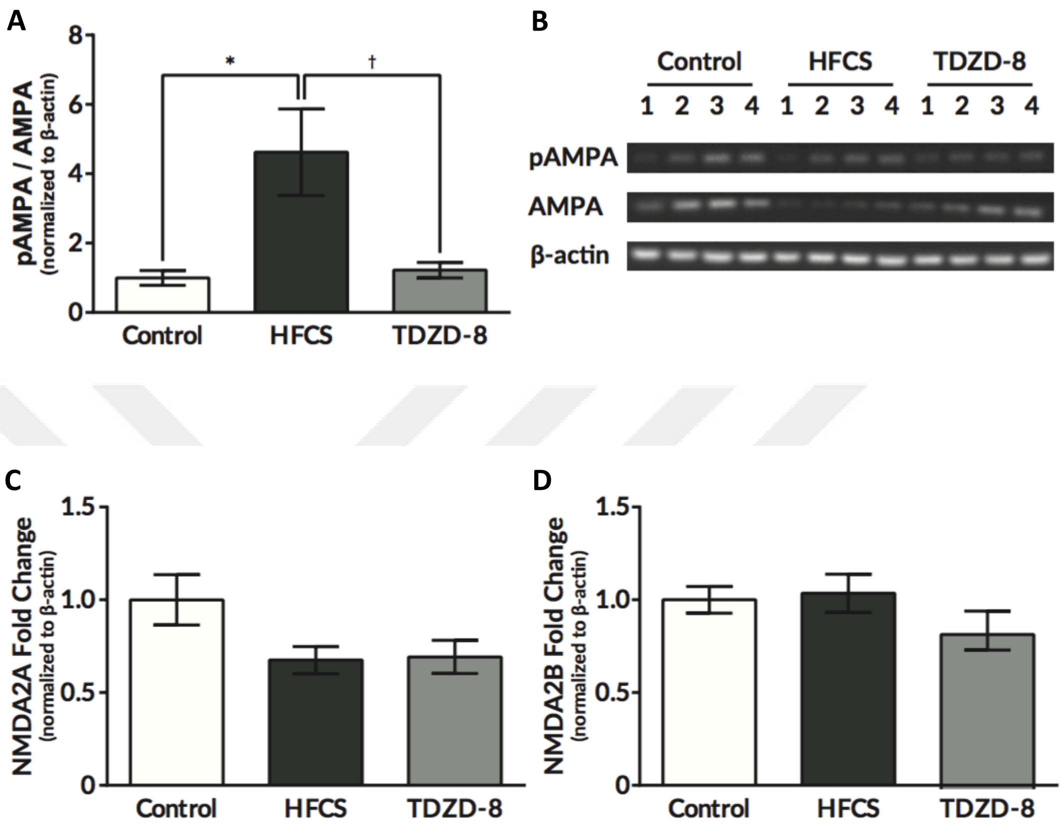

Figure 4.16. Protein levels of AMPA and NMDA receptor subunits. (A) Relative protein levels of ser845-phosphorylated GluR1 subunit of AMPA receptor to GluR1 subunit of AMPA receptor normalized to beta actin compared to control group (Control 1±0.213, HFCS 4.62±1.252, TDZD-8 1.22±0.223, ANOVA $F_{(2,9)}$=7.45, $p<0.05$). (B) Western blot bands of ser845-phosphorylated GluR1, GluR1 and beta actin. (C) Relative protein levels of NMDA2A normalized to beta actin compared to control group. (D) Relative protein levels of NMDA2B normalized to beta actin compared to control group.

4.14. Na^+/K^+-ATPase Subunit Expression. As diabetes was shown to cause decreased NKA levels/activity in hippocampi, which was also associated with the pathophysiology of BD, whether there is a similar decrease in the levels of α-subunits of NKA in the hippocampi of HFCS-fed adolescent rats was tested. While no significant differences in the mRNA levels of α1- and α2-subunits were observed, a significant reduction in the transcription of the neuron-specific α3-subunit was detected in the hippocampi of HFCS-fed rats. Moreover, TDZD-8 was unable to restore this reduction in transcription of α3-subunit (Figure 4.17.).

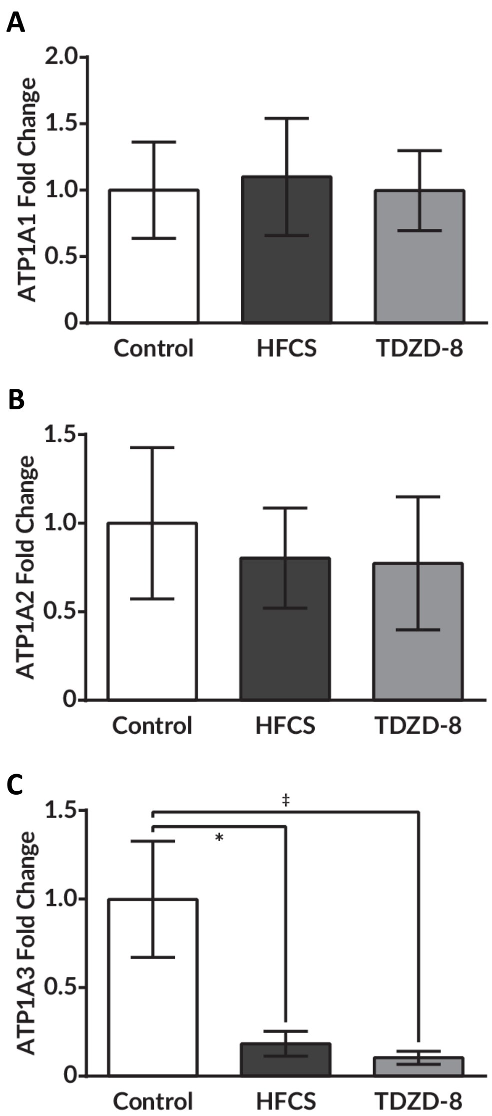

Figure 4.17. Changes in expression of NKA α-subunit genes. (A) Relative expression of *ATP1A1* (α1-subunit) normalized to control group. (B) Relative expression of *ATP1A2* (α2-subunit) normalized to control group. (C) Relative expression of *ATP1A3* (α3-subunit) normalized to control group.

4.15. *In vivo* Hippocampal Insulin Responsiveness. After intracerebroventricular administration of insulin, the ratios of ser473- and thr308-phosphorylated Akt to total Akt protein levels were found to be similar between groups (Figure 4.18.). Thr308-phosphorylated Akt cannot be detected by western blot unless hippocampi are stimulated by insulin, which confirms the appropriateness of intracerebroventricular insulin administration. In addition, no significant difference was observed for the ratio of ser9-phosphorylated GSK-3 to total GSK-3 protein levels (Figure 4.18.), suggesting PI3K-Akt-GSK-3 pathway coupled to insulin receptor was not impaired in HFCS-fed adolescent rats.

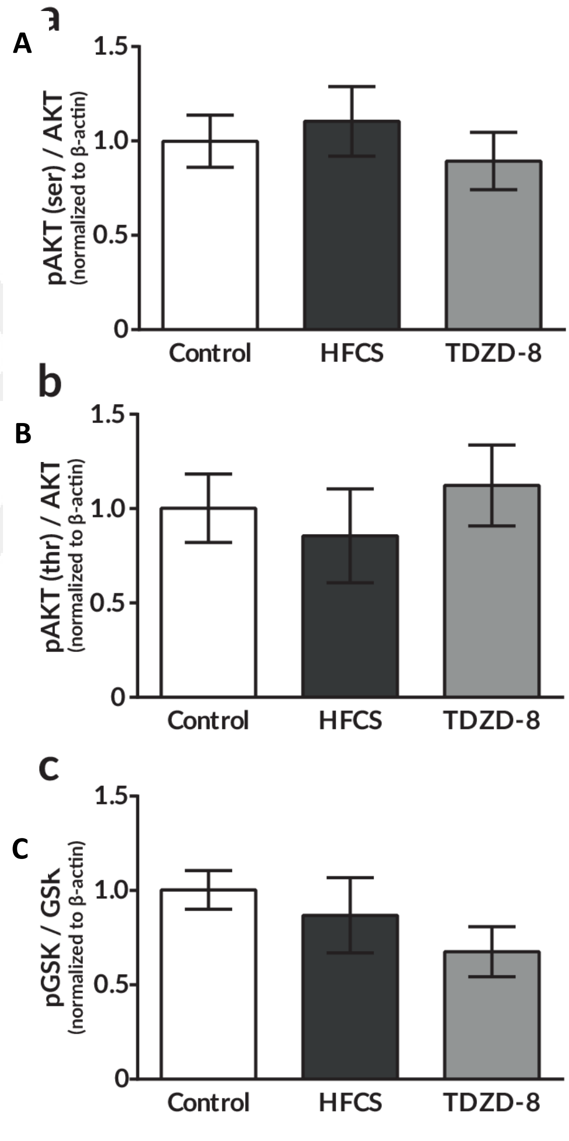

Figure 4.18. Phosphorylated to total protein ratio of Akt and GSK-3 after intracerebroventricular insulin administration. (A) Relative protein levels of ser473-phosphorylated Akt to Akt normalized to beta actin compared to control group. (B) Relative protein levels of thr308-phosphorylated Akt to Akt normalized to beta actin compared to control group. (C) Relative protein levels of ser9-phosphorylated GSK-3 to GSK-3 normalized to beta actin compared to control group.

4.16. Proinflammatory Markers in Serum and Hippocampus. Here, whether HFCS consumption has caused a similar increase in serum and hippocampus was tested, indicating systemic inflammation and local neuroinflammation, respectively. As expected, the levels of all three proinflammatory markers were found to be increased in serum of HFCS-fed rats and returned back to normal in TDZD-8-treated rats (Fgure 4.19. A-C). However, the differences of hippocampal levels of TNF-α, IL-1β, and IL-6 were statistically insignificant between groups (Figure 4.19. D-F), excluding hippocampal neuroinflammation in spite of the presence of systemic inflammation.

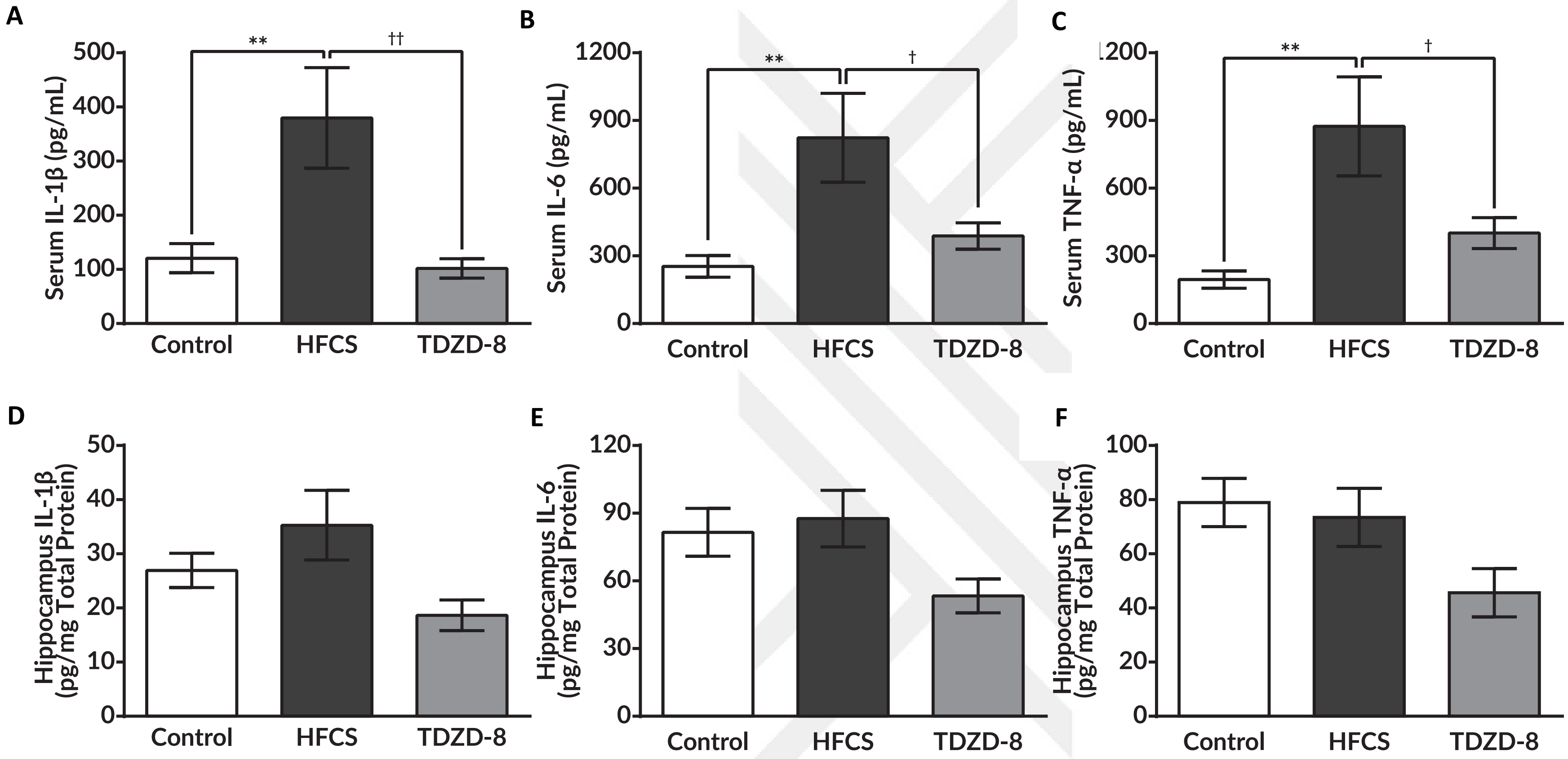

Figure 4.19. Levels of proinflammatory markers in serum and hippocampus. (A) IL-1β levels in serum. (B) IL-6 levels in serum. (C) TNF-α levels in serum. (D) IL-1β levels normalized to total protein in hippocampus. (E) IL-6 levels normalized to total protein in hippocampus. (F) TNF-α levels normalized to total protein in hippocampus.

5. DISCUSSION

5.1. Metabolic Characterization of HFCS Consumption in Adolescent Rats

Adolescent rats were fed with either tap water or 11 w/v carbohydrate containing HFCS solution to mimic the content of sugar-sweetened beverages of human consumption. As HFCS is highly palatable (233), the rats consumed more HFCS solution when compared to tap water intake. Interestingly, HFCS-fed rats decreased their chow intake significantly, which was also previously observed in a similar study done with mice (204). Increased HFCS solution with a reduction in chow intake may be the underlying reason of why HFCS-fed rats gain significantly less weight after the second week of tracking period. This is interesting and not compatible with human consumption, because humans tend to drink sugar-sweetened beverages without reducing their solid food intake, creating a *"caloric surplus"*. The reason for this discrepancy should be further investigated.

The HFCS group had gained significantly less weight compared to the control group. Nevertheless, HFCS-fed rats had a significantly higher ratio of fat pads to total body weight. As the majority of fat pads dissected were visceral instead of subcutaneous, this difference in fatty body mass was due to increased visceral adiposity in HFCS-fed rats. This is parallel with previous studies showing increased visceral adiposity upon high fructose feeding. Interestingly, TDZD-8 treatment had no effect on the weight ratio of fat pads to total body mass and failed to restore HFCS-induced visceral adiposity. To the best of our knowledge, there is no previous data on how TDZD-8 treatment affects adiposity.

Many studies so far showed that high fructose feeding, including HFCS, caused insulin resistance in both rodent, non-human primates and humans (208, 209, 224, 234). Here, the mean fasting blood glucose level of HFCS group was significantly higher compared to that of control group. Even though there are no objective criteria to diagnose a rat as insulin resistant or diabetic, increased fasting blood glucose levels were shown to be associated with impaired insulin sensitivity and T2DM. In addition, TDZD-8 improved insulin sensitivity and reduced fasting blood glucose levels, as expected from a GSK-3 inhibitor (126-131). After the oral glucose load, the control and TDZD-8 groups were relatively successful in handling the glucose load, thus returning the 2-hour postprandial mean blood glucose levels back to fasting levels. However, HFCS group had consistently elevated mean blood glucose levels, related to impaired insulin sensitivity. The analysis of area under the OGTT curves further showed an impairment in glucose tolerance in HFCS group, which was reversed by TDZD-8 treatment.

To sum up, HFCS consumption in adolescent rats resulted in impaired glucose tolerance, insulin resistance and visceral adiposity with a reduction in lean body mass. The only discordant finding with the human consumption of HFCS was less weight gain of HFCS-fed rats. This finding can be explained by the HFCS-induced reduction of chow intake, causing a decrease in total energy intake. Inhibition of GSK-3 partially restored HFCS-induced impairments that are evident in OGTT.

5.2. Behavioral Characterization of HFCS Consumption in Adolescent Rats

HFCS fed adolescent rats displayed spontaneous hyperlocomotion in the open field arena experiments as well as increased risk-taking behavior and decreased anxiety in elevated plus maze experiments. Female urine sniffing testing was significant for increased sexual hedonic behavior in HFCS fed rats. All of these behaviors emerged upon HFCS feeding are considered as spontaneously mania-like. In contrast, forced swim test showed an increased duration of immobility when HFCS fed rats were exposed to behavioral despair, which is traditionally known as depressive-like. Even though all the other behavioral changes were readily observable spontaneously, increased immobility was only evident after rats were exposed to a pre-experiment, in which stress and despair were induced. Broadly, these behavioral changes are compatible with bipolar disorder. Unfortunately, both

Morris water maze and passive avoidance experiments were failed to show any alterations in memory except a mild disturbance in the first acquisition day of Morris water maze.

Inhibition of GSK-3 has recently been shown to be the mechanism of action of lithium and other commonly used mood-stabilizers for their mood-stabilizing effect. (134-139, 146). Recently developed highly specific GSK-3 inhibitors were proven to have mood-stabilizing effects on animal models of BD, suggesting that the primary mechanism of action of lithium is, indeed, inhibition of GSK-3 (141-144). In addition to pharmacological interventions, genetic manipulations of GSK-3 activity further support this hypothesis (145, 147, 150). Because of these evidence suggesting the critical role of GSK-3 and its inhibition by various drugs used in the treatment of BD, we decided to treat HFCS fed rats with TDZD-8, a highly specific novel inhibitor of GSK-3 (177). As expected, TDZD-8 treatment reversed the impairments evident in all settings except in Morris water maze experiments.

HFCS consumption in comparable amounts to human consumption in adolescent rats led to spontaneous hyperlocomotion, decreased anxiety, increased risk-taking behavior, increased social/sexual reward-seeking behavior and increased sensitivity to behavioral despair paradigm with subtle learning deficits and preserved long-term memory; all of which are characteristically seen in patients with bipolar disorder. In addition, these behavioral changes associated with HFCS consumption were readily reversible with the inhibition of GSK-3, which is known as the most probable mechanism of action of lithium for its mood-stabilizing effects. Even though there are limitations to define bipolar disorder in a rat (235), spontaneous mania-like behavior with susceptibility to behavioral despair, combined with rats being responsive to the inhibition of GSK-3 are remarkably suggestive of bipolar disorder.

Only a handful of studies investigated the effects of fructose on different domains of mood. For instance, prenatal fructose exposure was found to cause an increase in locomotion and impulsivity with a decrease in attention in offspring mice

(236). When rats were given bee honey, which is known to be rich of fructose, rats displayed hyperlocomotion and decreased anxiety-related behaviors (237). Adult rats fed with a solution similar to HFCS (a 10% w/v solution containing 50/50 fructose/glucose) displayed no significant alterations in locomotion and anxiety-related behaviors (238). These data suggest that early developmental periods are especially vulnerable, whereas adults are spared from the negative outcomes of high fructose consumption. This suggestion was confirmed by a study that showed periadolescent consumption of fructose led to behavioral impairments whereas these impairments were not observed in adult rats fed with fructose (239).

5.3. Electrophysiologic and Molecular Characterization of HFCS Consumption in Adolescent Rats

In this study, HFCS consumption resulted in hyperexcitability of CA3-CA1 synapses as evident by a leftward shift of the input-output curve. It can be explained by two different mechanisms. (I) a decrease in the amount of α3-subunit of NKA resulting in reduced Ca^{2+} clearance through reverse function of Na^{+}/Ca^{+2} exchanger pumps in presynaptic terminals and increasing the glutamate release and (II) increased Ser845-phosphorylation of GluR1 subunit of AMPA receptor, facilitating its relocation on the postsynaptic terminal. Paired-pulse facilitation and western blot data suggested that HFCS consumption caused increased presynaptic release probability and increased available pool of AMPA receptors ready to be incorporated into the postsynaptic membrane, both of which explain the HFCS-induced neuronal hyperexcitability. However, TDZD-8 failed to normalize both impaired HFCS-induced paired-pulse facilitation and reduced expression of α3-subunit of NKA even though it restored HFCS-induced hyperexcitability with attenuation of HFCS-induced phosphorylation of GluR1. When one considers that the ketogenic diet, a low carbohydrate diet, attenuates neuronal hyperexcitability by modulating a wide variety of different cellular processes (92), no single mechanism should be expected to explain how HFCS consumption leads to hyperexcitability.

Previous literature showed that lithium did not alter the presynaptic glutamate release probability as suggested by previous paired-pulse facilitation experiments (81). Similar to lithium, TDZD-8 also did not alter paired-pulse facilitation response of HFCS-fed adolescent rats and failed to restore fEPSP2/fEPSP1 ratio towards control levels. This is in concordance with the failure of restoration of the reduced transcription of α3-subunit in TDZD-8 treated rats. One interesting behavioral correlation of impaired paired-pulse facilitation, a form of short-term plasticity, would be the subtle learning deficit evident in the first acquisition day of MWM (240).

Evidence supporting NKA hypothesis of BD has been accumulating since early 1950s, including that with Myshkin mice carrying an inactivating mutation in the α3-subunit of NKA, showing bipolar-like behavioral phenotype (112). It is still not known whether this decrease in NKA levels/activity is the primary cause of BD or a secondary outcome of an unknown primary process. In addition, the casual relationship between the decreased NKA levels/activity with neuronal hyperexcitability should be further studied. In our study, the α3-subunit is the only subunit whose expression was found to be decreased. This subunit is the only neuron-specific subunit and was previously shown to be closely associated with BD. The exact role of this finding in the pathophysiology of the observed changes should be further investigated.

Insulin resistance was reported in whole brain tissue as well as in the hippocampus in high fructose and high fat fed rats (75, 76). As insulin receptors are coupled to PI3K-Akt-GSK-3 pathway, which is known to be a crucial secondary signaling pathway related to synaptic plasticity, synaptic structure, learning-memory and mood processes. Here, the alterations in phosphorylation levels of Akt and GSK-3 were assessed through immunoblotting after stimulated with intracerebroventricularly administered insulin. No changes were shown, eliminating the presence of insulin resistance locally in the hippocampi of HFCS-fed rats. Even though HFCS-induced hyperexcitability and associated presynaptic and postsynaptic changes were not due to hippocampal insulin resistance, the PI3K-Akt-GSK-3 pathway may still be involved in the process. However, the primary activating ligand/stimulus is most possibly something other than insulin. As NKA was also shown to be linked to PI3K-Akt pathway, decreased expression of the α3-subunit of NKA may cause alterations in this pathway leading to observed changes. Unfortunately, the underlying signaling mechanism couldn't be unrevealed in this study and requires further focus in the future.

It is known that both western diet consumption and BD are associated with inflammation. Here, we showed that HFCS consumption caused a systemic inflammatory response, which was readily reversible with GSK-3 inhibition. Even though the results of this study showed many functional changes related to the hippocampus, we did not observe local neuroinflammation in the hippocampi of rats fed with HFCS for 6 weeks. This finding does not exclude the existence of inflammation in other brain regions, which might be involved in the pathogenesis of BD, it simply showed that the electrophysiological and molecular changes in the hippocampus cannot be explained by the inflammation by itself.

To sum up, HFCS consumption in adolescent rats resulted in hyperexcitability of CA3-CA1 synapses of hippocampus, which may explain the behavioral changes strikingly similar to those in patients with bipolar disorder. Both presynaptic and postsynaptic mechanisms contributed: (I) HFCS consumption led to increased presynaptic release probability and (II) increased ser845-phosphorylation of GluR1 subunit of AMPA receptors, leading to synaptic translocation of AMPA receptors. Even though systemic inflammation was evident in HFCS-fed adolescent rats, which was reversed by TDZD-8, local hippocampal neuroinflammation was not significant.

5.4. Conclusion

This study demonstrated that short-term HFCS consumption in adolescent rats caused bipolar-like behavioral changes, including spontaneous hyperlocomotion, decreased anxiety, increased risk-taking behavior, increased social and sexual reward-driven behavior, increased sensitivity to behavioral despair and subtle learning deficits without impairments in long-term memory. Furthermore, these changes were responsive to the inhibition of GSK-3, which further supports that the observed changes are closely associated with bipolar disorder. The mechanism responsible for these changes was suggested to be neuronal hyperexcitability, which was due to both presynaptic and postsynaptic alterations. Presynaptic alterations include increased presynaptic release probability, which was caused by increased intracellular Ca^{+2} levels activating the release machinery secondary to decreased levels of neuronal NKA. GSK-3 inhibition was failed to restore both decreased expression of NKA and impaired paired pulse facilitation. Postsynaptic alterations include increased ser845-phosphorylation of GluR1 subunits, leading to a readily available pool of AMPA receptors to be incorporated into the postsynaptic membrane. GSK-3 inhibition successfully reversed increased phosphorylation of GluR1 and overall hyperexcitability. Even though previous literature showed inflammation is a contributor to insulin resistance, T2DM, major depression and bipolar disorder in humans, HFCS consumption did not result in significant hippocampal neuroinflammation that might have explained the local changes in CNS. However, systemic inflammation or local neuroinflammation in areas other than hippocampus may still cause or exacerbate the observed changes. The underlying signaling pathway linking all these observed changes could not be revealed in this study, however it’s for sure that mechanisms other than hippocampal insulin resistance should be looked for.

6. BIBLIOGRAPHY

1. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, et al. Origins and evolution of the Western diet: health implications for the 21st century. Am J Clin Nutr. 2005;81(2):341-54.

2. WHO. Global report on diabetes.2016.

3. Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. Diabetes Res Clin Pract. 2014;103(2):150-60.

4. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol. 2013;28(2):169-80.

5. Marriott BP, Cole N, Lee E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. J Nutr. 2009;139(6):1228S-35S.

6. Bray GA. Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. Adv Nutr. 2013;4(2):220-5.

7. Gross LS, Li L, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. Am J Clin Nutr. 2004;79(5):774-9.

8. Goran MI, Ulijaszek SJ, Ventura EE. High fructose corn syrup and diabetes prevalence: a global perspective. Glob Public Health. 2013;8(1):55-64.

9. Aeberli I, Hochuli M, Gerber PA, Sze L. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. Diabetes Care. 2013;36(1):150-6.

10. Baena M, Sanguesa G, Davalos A, Latasa MJ, Sala-Vila A, Sanchez RM, et al. Fructose, but not glucose, impairs insulin signaling in the three major insulin-sensitive tissues. Sci Rep. 2016;6:26149.

11. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol. 2010;7(5):251-64.

12. Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. Medscape J Med. 2008;10(7):160.

13. Marriot BP, Olsho L, Hadden L, Connor P. Intake of added sugars and selected nutrients in the United States, National Health and Nutrition Examination Survey (NHANES) 2003-2006. Crit Rev Food Sci Nutr. 2010;50(3):228-58.

14. Ventura EE, Davis JN, Goran MI. Sugar content of popular sweetened beverages based on objective laboratory analysis: focus on fructose content. Obesity (Silver Spring). 2011;19(4):868-74.

15. Reichelt AC. Adolescent Maturational Transitions in the Prefrontal Cortex and Dopamine Signaling as a Risk Factor for the Development of Obesity and High Fat/High Sugar Diet Induced Cognitive Deficits. Front Behav Neurosci. 2016;10:189.

16. Park S, Blanck HM, Sherry B, Brener N, O'Toole T. Factors associated with low water intake among US high school students - National Youth Physical Activity and Nutrition Study, 2010. J Acad Nutr Diet. 2012;112(9):1421-7.

17. The NS, Suchindran C, North KE, Popkin BM, Gordon-Larsen P. Association of adolescent obesity with risk of severe obesity in adulthood. JAMA. 2010;304(18):2042-7.

18. Pollock NK, Bundy V, Kanto W, Davis CL, Bernard PJ, Zhu H, et al. Greater fructose consumption is associated with cardiometabolic risk markers and visceral adiposity in adolescents. J Nutr. 2012;142(2):251-7.

19. McCrimmon RJ, Ryan CM, Frier BM. Diabetes and cognitive dysfunction. Lancet. 2012;379(9833):2291-9.

20. Regenold WT, Thapar RK, Marano C, Gavirneni S, Kondapavuluru PV. Increased prevalence of type 2 diabetes mellitus among psychiatric inpatients with bipolar I affective and schizoaffective disorders independent of psychotropic drug use. J Affect Disord. 2002;70(1):19-26.

21. Kalmijn S, Feskens EJ, Launer LJ, Stijnen T, Kromhout D. Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. Diabetologia. 1995;38(9):1096-102.

22. Luchsinger JA, Tang MX, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. Neurology. 2004;63(7):1187-92.

23. Kuusisto J, Koivisto K, Mykkanen L. Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study. BMJ. 1997;315(7115):1045-9.

24. Ohara T, Doi Y, Ninomiya T, Hirakawa Y, Hata J, Iwaki T, et al. Glucose tolerance status and risk of dementia in the community: the Hisayama study. Neurology. 2011;77(12):1126-34.

25. Wandell P, Ljunggren G, Wahlstrom L, Carlsson AC. Diabetes and psychiatric illness in the total population of Stockholm. J Psychosom Res. 2014;77(3):169-73.

26. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 2006;5(1):64-74.

27. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease--is this type 3 diabetes? J Alzheimers Dis. 2005;7(1):63-80.

28. Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. Proc Natl Acad Sci U S A. 2003;100(4):2019-22.

29. Vanhanen M, Koivisto K, Kuusisto J, Mykkanen L, Helkala EL, Hanninen T, et al. Cognitive function in an elderly population with persistent impaired glucose tolerance. Diabetes Care. 1998;21(3):398-402.

30. Williamson R, McNeilly A, Sutherland C. Insulin resistance in the brain: an old-age or new-age problem? Biochem Pharmacol. 2012;84(6):737-45.

31. Gunstad J, Paul RH, Cohen RA, Tate DF, Gordon E. Obesity is associated with memory deficits in young and middle-aged adults. Eat Weight Disord. 2006;11(1):15-9.

32. Francis H, Stevenson R. The longer-term impacts of Western diet on human cognition and the brain. Appetite. 2013;63:119-28.

33. Molteni R, Barnard RJ, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience. 2002;112(4):803-14.
34. Kanoski SE, Meisel RL, Mullins AJ, Davidson TL. The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. Behav Brain Res. 2007;182(1):57-66.
35. Darling JN, Ross AP, Bartness TJ, Parent MB. Predicting the effects of a high-energy diet on fatty liver and hippocampal-dependent memory in male rats. Obesity (Silver Spring). 2013;21(5):910-7.
36. Cao D, Lu H, Lewis TL, Li L. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J Biol Chem. 2007;282(50):36275-82.
37. Hsu TM, Konanur VR, Taing L, Usui R, Kayser BD, Goran MI, et al. Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. Hippocampus. 2015;25(2):227-39.
38. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus. 2008;18(11):1085-8.
39. Kanoski SE, Davidson TL. Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. J Exp Psychol Anim Behav Process. 2010;36(2):313-9.
40. Murray AJ, Knight NS, Cochlin LE, McAleese S, Deacon RM, Rawlins JN, et al. Deterioration of physical performance and cognitive function in rats with short-term high-fat feeding. FASEB J. 2009;23(12):4353-60.
41. Calvo-Ochoa E, Hernandez-Ortega K, Ferrera P, Morimoto S, Arias C. Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. J Cereb Blood Flow Metab. 2014;34(6):1001-8.

42. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care. 2001;24(6):1069-78.

43. Cassidy F, Ahearn E, Carroll BJ. Elevated frequency of diabetes mellitus in hospitalized manic-depressive patients. Am J Psychiatry. 1999;156(9):1417-20.

44. Eaton WW, Armenian H, Gallo J, Pratt L, Ford DE. Depression and risk for onset of type II diabetes. A prospective population-based study. Diabetes Care. 1996;19(10):1097-102.

45. Gavard JA, Lustman PJ, Clouse RE. Prevalence of depression in adults with diabetes. An epidemiological evaluation. Diabetes Care. 1993;16(8):1167-78.

46. Lilliker SL. Prevalence of diabetes in a manic-depressive population. Compr Psychiatry. 1980;21(4):270-5.

47. Winokur A, Maislin G, Phillips JL, Amsterdam JD. Insulin resistance after oral glucose tolerance testing in patients with major depression. Am J Psychiatry. 1988;145(3):325-30.

48. Lawrence JM, Standiford DA, Loots B, Klingensmith GJ, Williams DE, Ruggiero A, et al. Prevalence and correlates of depressed mood among youth with diabetes: the SEARCH for Diabetes in Youth study. Pediatrics. 2006;117(4):1348-58.

49. McIntyre RS, Konarski JZ, Misener VL, Kennedy SH. Bipolar disorder and diabetes mellitus: epidemiology, etiology, and treatment implications. Ann Clin Psychiatry. 2005;17(2):83-93.

50. Charles EF, Lambert CG, Kerner B. Bipolar disorder and diabetes mellitus: evidence for disease-modifying effects and treatment implications. Int J Bipolar Disord. 2016;4(1):13.

51. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. Nature. 1978;272(5656):827-9.

52. Hill JM, Lesniak MA, Pert CB, Roth J. Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. Neuroscience. 1986;17(4):1127-38.

53. Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R, Allen AM, et al. Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. Endocrinology. 1987;121(4):1562-70.

54. Marks JL, Porte D, Jr., Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by in situ hybridization. Endocrinology. 1990;127(6):3234-6.

55. Apostolatos A, Song S, Acosta S, Peart M, Watson JE, Bickford P, et al. Insulin promotes neuronal survival via the alternatively spliced protein kinase CdeltaII isoform. J Biol Chem. 2012;287(12):9299-310.

56. Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, et al. Intranasal insulin improves memory in humans. Psychoneuroendocrinology. 2004;29(10):1326-34.

57. Benedict C, Hallschmid M, Schultes B, Born J, Kern W. Intranasal insulin to improve memory function in humans. Neuroendocrinology. 2007;86(2):136-42.

58. Reger MA, Watson GS, Frey WH, 2nd, Baker LD, Cholerton B, Keeling ML, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. Neurobiol Aging. 2006;27(3):451-8.

59. Marks DR, Tucker K, Cavallin MA, Mast TG, Fadool DA. Awake intranasal insulin delivery modifies protein complexes and alters memory, anxiety, and olfactory behaviors. J Neurosci. 2009;29(20):6734-51.

60. Kern W, Peters A, Fruehwald-Schultes B, Deininger E, Born J, Fehm HL. Improving influence of insulin on cognitive functions in humans. Neuroendocrinology. 2001;74(4):270-80.

61. Park CR, Seeley RJ, Craft S, Woods SC. Intracerebroventricular insulin enhances memory in a passive-avoidance task. Physiol Behav. 2000;68(4):509-14.

62. Grillo CA, Piroli GG, Lawrence RC, Wrighten SA, Green AJ, Wilson SP, et al. Hippocampal Insulin Resistance Impairs Spatial Learning and Synaptic Plasticity. Diabetes. 2015;64(11):3927-36.

63. Costello DA, Claret M, Al-Qassab H, Plattner F, Irvine EE, Choudhury AI, et al. Brain deletion of insulin receptor substrate 2 disrupts hippocampal synaptic plasticity and metaplasticity. PLoS One. 2012;7(2):e31124.

64. Zhao W, Chen H, Xu H, Moore E, Meiri N, Quon MJ, et al. Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. J Biol Chem. 1999;274(49):34893-902.

65. Margolis RU, Altszuler N. Insulin in the cerebrospinal fluid. Nature. 1967;215(5108):1375-6.

66. Woods SC, Porte D. J. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. Am J Physiol. 1977;233(4):331-4.

67. Baura GD, Foster DM, Porte D, Jr., Kahn SE, Bergman RN, Cobelli C, et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. J Clin Invest. 1993;92(4):1824-30.

68. Poduslo JF, Curran GL, Berg CT. Macromolecular permeability across the blood-nerve and blood-brain barriers. Proc Natl Acad Sci U S A. 1994;91(12):5705-9.

69. Banks WA, Jaspan JB, Huang W, Kastin AJ. Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. Peptides. 1997;18(9):1423-9.

70. Banks WA, Jaspan JB, Kastin AJ. Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. Peptides. 1997;18(8):1257-62.

71. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes. 2000;49(9):1525-33.

72. Urayama A, Banks WA. Starvation and triglycerides reverse the obesity-induced impairment of insulin transport at the blood-brain barrier. Endocrinology. 2008;149(7):3592-7.

73. Banks WA, Jaspan JB, Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. Peptides. 1997;18(10):1577-84.

74. Huber JD, VanGilder RL, Houser KA. Streptozotocin-induced diabetes progressively increases blood-brain barrier permeability in specific brain regions in rats. Am J Physiol Heart Circ Physiol. 2006;291(6):H2660-8.

75. Mielke JG, Taghibiglou C, Liu L, Zhang Y, Jia Z, Adeli K, et al. A biochemical and functional characterization of diet-induced brain insulin resistance. J Neurochem. 2005;93(6):1568-78.

76. Liu Z, Patil IY, Jiang T, Sancheti H, Walsh JP, Stiles BL, et al. High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. PLoS One. 2015;10(5):e0128274.

77. Plum L, Schubert M, Bruning JC. The role of insulin receptor signaling in the brain. Trends Endocrinol Metab. 2005;16(2):59-65.

78. Schloesser RJ, Huang J, Klein PS, Manji HK. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology. 2008;33(1):110-33.

79. Zarate CA, Jr., Singh J, Manji HK. Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder. Biol Psychiatry. 2006;59(11):1006-20.

80. Du J, Quiroz J, Yuan P, Zarate C, Manji HK. Bipolar disorder: involvement of signaling cascades and AMPA receptor trafficking at synapses. Neuron Glia Biol. 2004;1(3):231-43.

81. Du J, Creson TK, Wu LJ, Ren M, Gray NA, Falke C, et al. The role of hippocampal GluR1 and GluR2 receptors in manic-like behavior. J Neurosci. 2008;28(1):68-79.

82. Du J, Gray NA, Falke C, Yuan P, Szabo S, Manji HK. Structurally dissimilar antimanic agents modulate synaptic plasticity by regulating AMPA glutamate receptor subunit GluR1 synaptic expression. Ann N Y Acad Sci. 2003;1003:378-80.

83. Du J, Gray NA, Falke CA, Chen W, Yuan P, Szabo ST, et al. Modulation of synaptic plasticity by antimanic agents: the role of AMPA glutamate receptor subunit 1 synaptic expression. J Neurosci. 2004;24(29):6578-89.

84. Lan MJ, McLoughlin GA, Griffin JL, Tsang TM, Huang JT, Yuan P, et al. Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. Mol Psychiatry. 2009;14(3):269-79.

85. Dixon JF, Hokin LE. Lithium acutely inhibits and chronically up-regulates and stabilizes glutamate uptake by presynaptic nerve endings in mouse cerebral cortex. Proc Natl Acad Sci U S A. 1998;95(14):8363-8.

86. Hassel B, Iversen EG, Gjerstad L, Tauboll E. Up-regulation of hippocampal glutamate transport during chronic treatment with sodium valproate. J Neurochem. 2001;77(5):1285-92.

87. Nonaka S, Hough CJ, Chuang DM. Chronic lithium treatment robustly protects neurons in the central nervous system against excitotoxicity by inhibiting N-methyl-D-aspartate receptor-mediated calcium influx. Proc Natl Acad Sci U S A. 1998;95(5):2642-7.

88. Hashimoto R, Hough C, Nakazawa T, Yamamoto T, Chuang DM. Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. J Neurochem. 2002;80(4):589-97.

89. Yildiz-Yesiloglu A, Ankerst DP. Neurochemical alterations of the brain in bipolar disorder and their implications for pathophysiology: a systematic review of the in vivo proton magnetic resonance spectroscopy findings. Prog Neuropsychopharmacol Biol Psychiatry. 2006;30(6):969-95.

90. Mertens J, Wang QW, Kim Y, Yu DX, Pham S, Yang B, et al. Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. Nature. 2015;527(7576):95-9.

91. Augustin K, Khabbush A, Williams S, Eaton S, Orford M, Cross JH, et al. Mechanisms of action for the medium-chain triglyceride ketogenic diet in neurological and metabolic disorders. Lancet Neurol. 2018;17(1):84-93.

92. Simeone TA, Simeone KA, Stafstrom CE, Rho JM. Do ketone bodies mediate the anti-seizure effects of the ketogenic diet? Neuropharmacology. 2018.

93. Phelps JR, Siemers SV, El-Mallakh RS. The ketogenic diet for type II bipolar disorder. Neurocase. 2013;19(5):423-6.

94. Horwood JM, Dufour F, Laroche S, Davis S. Signalling mechanisms mediated by the phosphoinositide 3-kinase/Akt cascade in synaptic plasticity and memory in the rat. Eur J Neurosci. 2006;23(12):3375-84.

95. Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, et al. Control of synaptic strength, a novel function of Akt. Neuron. 2003;38(6):915-28.

96. Dou JT, Chen M, Dufour F, Alkon DL, Zhao WQ. Insulin receptor signaling in long-term memory consolidation following spatial learning. Learn Mem. 2005;12(6):646-55.

97. Man HY, Wang Q, Lu WY. Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. Neuron. 2003;38(4):611-24.

98. Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, et al. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat Neurosci. 2000;3(12):1291-300.

99. Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, et al. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nat Neurosci. 2000;3(12):1282-90.

100. Man HY, Lin JW, Ju WH, Ahmadian G, Liu L, Becker LE, et al. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. Neuron. 2000;25(3):649-62.

101. Huang CC, Lee CC, Hsu KS. An investigation into signal transduction mechanisms involved in insulin-induced long-term depression in the CA1 region of the hippocampus. J Neurochem. 2004;89(1):217-31.

102. Passafaro M, Piech V, Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci. 2001;4(9):917-26.

103. van der Heide LP, Kamal A, Artola A, Gispen WH, Ramakers GM. Insulin modulates hippocampal activity-dependent synaptic plasticity in a N-methyl-d-

aspartate receptor and phosphatidyl-inositol-3-kinase-dependent manner. J Neurochem. 2005;94(4):1158-66.

104. Christie JM, Wenthold RJ, Monaghan DT. Insulin causes a transient tyrosine phosphorylation of NR2A and NR2B NMDA receptor subunits in rat hippocampus. J Neurochem. 1999;72(4):1523-8.

105. Liao GY, Leonard JP. Insulin modulation of cloned mouse NMDA receptor currents in Xenopus oocytes. J Neurochem. 1999;73(4):1510-9.

106. Liu L, Brown JC, 3rd, Webster WW, Morrisett RA, Monaghan DT. Insulin potentiates N-methyl-D-aspartate receptor activity in Xenopus oocytes and rat hippocampus. Neurosci Lett. 1995;192(1):5-8.

107. Singh MM. A unifying hypothesis on the biochemical basis of affective disorder. Psychiatr Q. 1970;44(4):706-24.

108. el-Mallakh RS, Wyatt RJ. The Na,K-ATPase hypothesis for bipolar illness. Biol Psychiatry. 1995;37(4):235-44.

109. Dobretsov M, Stimers JR. Neuronal function and alpha3 isoform of the Na/K-ATPase. Front Biosci. 2005;10:2373-96.

110. Goldstein I, Lerer E, Laiba E, Mallet J, Mujaheed M, Laurent C, et al. Association between sodium- and potassium-activated adenosine triphosphatase alpha isoforms and bipolar disorders. Biol Psychiatry. 2009;65(11):985-91.

111. El-Mallakh RS, El-Masri MA, Huff MO, Li XP, Decker S, Levy RS. Intracerebroventricular administration of ouabain as a model of mania in rats. Bipolar Disord. 2003;5(5):362-5.

112. Kirshenbaum GS, Clapcote SJ, Duffy S, Burgess CR, Petersen J, Jarowek KJ, et al. Mania-like behavior induced by genetic dysfunction of the neuron-specific Na+,K+-ATPase alpha3 sodium pump. Proc Natl Acad Sci U S A. 2011;108(44):18144-9.

113. Kjeldsen K, Braendgaard H, Sidenius P, Larsen JS, Norgaard A. Diabetes decreases Na+-K+ pump concentration in skeletal muscles, heart ventricular muscle, and peripheral nerves of rat. Diabetes. 1987;36(7):842-8.

114. Leong SF, Leung TK. Diabetes induced by streptozotocin causes reduced Na-K ATPase in the brain. Neurochem Res. 1991;16(10):1161-5.

115. Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog Neurobiol. 2001;65(4):391-426.

116. Hur EM, Zhou FQ. GSK3 signalling in neural development. Nat Rev Neurosci. 2010;11(8):539-51.

117. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. Eur J Biochem. 1980;107(2):519-27.

118. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature. 1995;378(6559):785-9.

119. ter Haar E, Coll JT, Austen DA, Hsiao HM, Swenson L, Jain J. Structure of GSK3beta reveals a primed phosphorylation mechanism. Nat Struct Biol. 2001;8(7):593-6.

120. Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci. 2004;29(2):95-102.

121. Schubert M, Gautam D, Surjo D, Ueki K, Baudler S, Schubert D, et al. Role for neuronal insulin resistance in neurodegenerative diseases. Proc Natl Acad Sci U S A. 2004;101(9):3100-5.

122. Eldar-Finkelman H, Schreyer SA, Shinohara MM, LeBoeuf RC, Krebs EG. Increased glycogen synthase kinase-3 activity in diabetes- and obesity-prone C57BL/6J mice. Diabetes. 1999;48(8):1662-6.

123. Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes. 2000;49(2):263-71.

124. Eldar-Finkelman H, Krebs EG. Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. Proc Natl Acad Sci U S A. 1997;94(18):9660-4.

125. Liberman Z, Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. J Biol Chem. 2005;280(6):4422-8.

126. Henriksen EJ, Kinnick TR, Teachey MK, O'Keefe MP, Ring D, Johnson KW, et al. Modulation of muscle insulin resistance by selective inhibition of GSK-3 in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab. 2003;284(5):E892-900.

127. Lochhead PA, Coghlan M, Rice SQ, Sutherland C. Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolypyruvate carboxykinase gene expression. Diabetes. 2001;50(5):937-46.

128. Ring DB, Johnson KW, Henriksen EJ, Nuss JM, Goff D, Kinnick TR, et al. Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization in vitro and in vivo. Diabetes. 2003;52(3):588-95.

129. Nikoulina SE, Ciaraldi TP, Mudaliar S, Carter L, Johnson K, Henry RR. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. Diabetes. 2002;51(7):2190-8.

130. Cline GW, Johnson K, Regittnig W, Perret P, Tozzo E, Xiao L, et al. Effects of a novel glycogen synthase kinase-3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (fa/fa) rats. Diabetes. 2002;51(10):2903-10.

131. Plotkin B, Kaidanovich O, Talior I, Eldar-Finkelman H. Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. J Pharmacol Exp Ther. 2003;305(3):974-80.

132. Crossley NA, Bauer M. Acceleration and augmentation of antidepressants with lithium for depressive disorders: two meta-analyses of randomized, placebo-controlled trials. J Clin Psychiatry. 2007;68(6):935-40.

133. Ahrens B, Muller-Oerlinghausen B. Does lithium exert an independent antisuicidal effect? Pharmacopsychiatry. 2001;34(4):132-6.

134. Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci U S A. 1996;93(16):8455-9.

135. Gould TD, Chen G, Manji HK. In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. Neuropsychopharmacology. 2004;29(1):32-8.

136. Ryves WJ, Harwood AJ. Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. Biochem Biophys Res Commun. 2001;280(3):720-5.

137. De Sarno P, Li X, Jope RS. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. Neuropharmacology. 2002;43(7):1158-64.

138. Chalecka-Franaszek E, Chuang DM. Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proc Natl Acad Sci U S A. 1999;96(15):8745-50.

139. Li X, Zhu W, Roh MS, Friedman AB, Rosborough K, Jope RS. In vivo regulation of glycogen synthase kinase-3beta (GSK3beta) by serotonergic activity in mouse brain. Neuropsychopharmacology. 2004;29(8):1426-31.

140. Jope RS, Yuskaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. Neurochem Res. 2007;32(4-5):577-95.

141. Kalinichev M, Dawson LA. Evidence for antimanic efficacy of glycogen synthase kinase-3 (GSK3) inhibitors in a strain-specific model of acute mania. Int J Neuropsychopharmacol. 2011;14(8):1051-67.

142. Gould TD, Einat H, Bhat R, Manji HK. AR-A014418, a selective GSK-3 inhibitor, produces antidepressant-like effects in the forced swim test. Int J Neuropsychopharmacol. 2004;7(4):387-90.

143. Kaidanovich-Beilin O, Milman A, Weizman A, Pick CG, Eldar-Finkelman H. Rapid antidepressive-like activity of specific glycogen synthase kinase-3 inhibitor and its effect on beta-catenin in mouse hippocampus. Biol Psychiatry. 2004;55(8):781-4.

144. Valvassori SS, Dal-Pont GC, Resende WR, Jornada LK, Peterle BR, Machado AG, et al. Lithium and valproate act on the GSK-3beta signaling pathway to reverse manic-like behavior in an animal model of mania induced by ouabain. Neuropharmacology. 2017;117:447-59.

145. O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S, et al. Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. J Neurosci. 2004;24(30):6791-8.

146. Gould TD, Manji HK. Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. Neuropsychopharmacology. 2005;30(7):1223-37.

147. Prickaerts J, Moechars D, Cryns K, Lenaerts I, van Craenendonck H, Goris I, et al. Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. J Neurosci. 2006;26(35):9022-9.

148. Beaulieu JM, Zhang X, Rodriguiz RM, Sotnikova TD, Cools MJ, Wetsel WC, et al. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A. 2008;105(4):1333-8.

149. Mines MA, Yuskaitis CJ, King MK, Beurel E, Jope RS. GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. PLoS One. 2010;5(3):e9706.

150. Polter A, Beurel E, Yang S, Garner R, Song L, Miller CA, et al. Deficiency in the inhibitory serine-phosphorylation of glycogen synthase kinase-3 increases sensitivity to mood disturbances. Neuropsychopharmacology. 2010;35(8):1761-74.

151. Rowe MK, Wiest C, Chuang DM. GSK-3 is a viable potential target for therapeutic intervention in bipolar disorder. Neurosci Biobehav Rev. 2007;31(6):920-31.

152. Hernandez F, Borrell J, Guaza C, Avila J, Lucas JJ. Spatial learning deficit in transgenic mice that conditionally over-express GSK-3beta in the brain but do not form tau filaments. J Neurochem. 2002;83(6):1529-33.

153. Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J. Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. EMBO J. 2001;20(1-2):27-39.

154. Spittaels K, Van den Haute C, Van Dorpe J, Terwel D, Vandezande K, Lasrado R, et al. Neonatal neuronal overexpression of glycogen synthase kinase-3 beta reduces brain size in transgenic mice. Neuroscience. 2002;113(4):797-808.

155. Zhu LQ, Wang SH, Liu D, Yin YY, Tian Q, Wang XC, et al. Activation of glycogen synthase kinase-3 inhibits long-term potentiation with synapse-associated impairments. J Neurosci. 2007;27(45):12211-20.

156. Hooper C, Markevich V, Plattner F, Killick R, Schofield E, Engel T, et al. Glycogen synthase kinase-3 inhibition is integral to long-term potentiation. Eur J Neurosci. 2007;25(1):81-6.

157. Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, et al. LTP inhibits LTD in the hippocampus via regulation of GSK3beta. Neuron. 2007;53(5):703-17.

158. Wei J, Liu W, Yan Z. Regulation of AMPA receptor trafficking and function by glycogen synthase kinase 3. J Biol Chem. 2010;285(34):26369-76.

159. Martin M, Rehani K, Jope RS, Michalek SM. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat Immunol. 2005;6(8):777-84.

160. Dugo L, Collin M, Allen DA, Patel NS, Bauer I, Mervaala EM, et al. GSK-3beta inhibitors attenuate the organ injury/dysfunction caused by endotoxemia in the rat. Crit Care Med. 2005;33(9):1903-12.

161. Whittle BJ, Varga C, Posa A, Molnar A, Collin M, Thiemermann C. Reduction of experimental colitis in the rat by inhibitors of glycogen synthase kinase-3beta. Br J Pharmacol. 2006;147(5):575-82.

162. Cuzzocrea S, Mazzon E, Di Paola R, Muia C, Crisafulli C, Dugo L, et al. Glycogen synthase kinase-3beta inhibition attenuates the degree of arthritis caused by type II collagen in the mouse. Clin Immunol. 2006;120(1):57-67.

163. Hu X, Paik PK, Chen J, Yarilina A, Kockeritz L, Lu TT, et al. IFN-gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. Immunity. 2006;24(5):563-74.

164. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004;27(3):813-23.

165. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia. 1997;40(11):1286-92.

166. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286(3):327-34.

167. Muller S, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, et al. Impaired glucose tolerance is associated with increased serum concentrations

of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia. 2002;45(6):805-12.

168. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996;271(5249):665-8.

169. Tanaka T, Itoh H, Doi K, Fukunaga Y, Hosoda K, Shintani M, et al. Down regulation of peroxisome proliferator-activated receptorgamma expression by inflammatory cytokines and its reversal by thiazolidinediones. Diabetologia. 1999;42(6):702-10.

170. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. Diabetes. 2003;52(11):2784-9.

171. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem. 2003;278(46):45777-84.

172. Smith RS. The macrophage theory of depression. Med Hypotheses. 1991;35(4):298-306.

173. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. J Clin Psychiatry. 2009;70(8):1078-90.

174. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 2006;27(1):24-31.

175. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000;21(3):383-421.

176. Eldar-Finkelman H, Martinez A. GSK-3 Inhibitors: Preclinical and Clinical Focus on CNS. Front Mol Neurosci. 2011;4:32.

177. Martinez A, Alonso M, Castro A, Perez C, Moreno FJ. First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3beta) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. J Med Chem. 2002;45(6):1292-9.

178. Mazanetz MP, Fischer PM. Untangling tau hyperphosphorylation in drug design for neurodegenerative diseases. Nat Rev Drug Discov. 2007;6(6):464-79.

179. Martinsson L, Westman J, Hallgren J, Osby U, Backlund L. Lithium treatment and cancer incidence in bipolar disorder. Bipolar Disord. 2016;18(1):33-40.

180. Huang RY, Hsieh KP, Huang WW, Yang YH. Use of lithium and cancer risk in patients with bipolar disorder: population-based cohort study. Br J Psychiatry. 2016;209(5):393-9.

181. Reinehr T. Type 2 diabetes mellitus in children and adolescents. World J Diabetes. 2013;4(6):270-81.

182. Klurfeld DM, Foreyt J, Angelopoulos TJ, Rippe JM. Lack of evidence for high fructose corn syrup as the cause of the obesity epidemic. Int J Obes (Lond). 2013;37(6):771-3.

183. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr. 2004;79(4):537-43.

184. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ. 2013;346(e7492):e7492.

185. Hu FB, Malik VS. Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. Physiol Behav. 2010;100(1):47-54.

186. Malik VS, Popkin BM, Bray GA, Despres JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. Circulation. 2010;121(11):1356-64.

187. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr. 2013;98(4):1084-102.

188. Kaiser KA, Shikany JM, Keating KD, Allison DB. Will reducing sugar-sweetened beverage consumption reduce obesity? Evidence supporting conjecture is strong, but evidence when testing effect is weak. Obes Rev. 2013;14(8):620-33.

189. Forshee RA, Storey ML, Allison DB, Glinsmann WH, Hein GL, Lineback DR, et al. A critical examination of the evidence relating high fructose corn syrup and weight gain. Crit Rev Food Sci Nutr. 2007;47(6):561-82.

190. Ebbeling CB, Feldman HA, Chomitz VR, Antonelli TA, Gortmaker SL, Osganian SK, et al. A randomized trial of sugar-sweetened beverages and adolescent body weight. N Engl J Med. 2012;367(15):1407-16.

191. de Ruyter JC, Olthof MR, Seidell JC, Katan MB. A trial of sugar-free or sugar-sweetened beverages and body weight in children. N Engl J Med. 2012;367(15):1397-406.

192. Le MT, Frye RF, Rivard CJ, Cheng J, McFann KK, Segal MS, et al. Effects of high-fructose corn syrup and sucrose on the pharmacokinetics of fructose and acute metabolic and hemodynamic responses in healthy subjects. Metabolism. 2012;61(5):641-51.

193. Welsh JA, Cogswell ME, Rogers S, Rockett H, Mei Z. Overweight among low-income preschool children associated with the consumption of sweet drinks: Missouri, 1999-2002. Pediatrics. 2005;115(2):e223-9.

194. Davis JN, Whaley SE, Goran MI. Effects of breastfeeding and low sugar-sweetened beverage intake on obesity prevalence in Hispanic toddlers. Am J Clin Nutr. 2012;95(1):3-8.

195. Malik VS, Willett WC, Hu FB. Sugar-sweetened beverages and BMI in children and adolescents: reanalyses of a meta-analysis. Am J Clin Nutr. 2009;89(1):438-9; author reply 9-40.

196. Malik VS, Hu FB. Fructose and Cardiometabolic Health: What the Evidence From Sugar-Sweetened Beverages Tells Us. J Am Coll Cardiol. 2015;66(14):1615-24.

197. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest. 2009;119(5):1322-34.

198. Maersk M, Belza A, Stodkilde-Jorgensen H, Ringgaard S, Chabanova E, Thomsen H, et al. Sucrose-sweetened beverages increase fat storage in the liver,

muscle, and visceral fat depot: a 6-mo randomized intervention study. Am J Clin Nutr. 2012;95(2):283-9.

199. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care. 2010;33(11):2477-83.

200. InterAct C, Romaguera D, Norat T, Wark PA, Vergnaud AC, Schulze MB, et al. Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. Diabetologia. 2013;56(7):1520-30.

201. Fung TT, Malik V, Rexrode KM, Manson JE, Willett WC, Hu FB. Sweetened beverage consumption and risk of coronary heart disease in women. Am J Clin Nutr. 2009;89(4):1037-42.

202. Tasevska N, Park Y, Jiao L, Hollenbeck A, Subar AF, Potischman N. Sugars and risk of mortality in the NIH-AARP Diet and Health Study. Am J Clin Nutr. 2014;99(5):1077-88.

203. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. Pharmacol Biochem Behav. 2010;97(1):101-6.

204. Jurgens H, Haass W, Castaneda TR, Schurmann A, Koebnick C, Dombrowski F, et al. Consuming fructose-sweetened beverages increases body adiposity in mice. Obes Res. 2005;13(7):1146-56.

205. Mock K, Lateef S, Benedito VA, Tou JC. High-fructose corn syrup-55 consumption alters hepatic lipid metabolism and promotes triglyceride accumulation. J Nutr Biochem. 2017;39:32-9.

206. Bursac BN, Vasiljevic AD, Nestorovic NM, Velickovic NA, Vojnovic Milutinovic DD, Matic GM, et al. High-fructose diet leads to visceral adiposity and hypothalamic leptin resistance in male rats--do glucocorticoids play a role? J Nutr Biochem. 2014;25(4):446-55.

207. Zavaroni I, Sander S, Scott S, Reaven GM. Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism. 1980;29(10):970-3.

208. Bremer AA, Stanhope KL, Graham JL, Cummings BP, Wang W, Saville BR, et al. Fructose-fed rhesus monkeys: a nonhuman primate model of insulin resistance, metabolic syndrome, and type 2 diabetes. Clin Transl Sci. 2011;4(4):243-52.

209. Panchal SK, Brown L. Rodent models for metabolic syndrome research. J Biomed Biotechnol. 2011;2011:351982.

210. Miller C, Bauer MS. Excess mortality in bipolar disorders. Curr Psychiatry Rep. 2014;16(11):499.

211. Sarris J, Logan AC, Akbaraly TN, Amminger GP, Balanza-Martinez V, Freeman MP, et al. Nutritional medicine as mainstream in psychiatry. Lancet Psychiatry. 2015;2(3):271-4.

212. O'Neil A, Quirk SE, Housden S, Brennan SL, Williams LJ, Pasco JA, et al. Relationship between diet and mental health in children and adolescents: a systematic review. Am J Public Health. 2014;104(10):e31-42.

213. Jacka FN, Ystrom E, Brantsaeter AL, Karevold E, Roth C, Haugen M, et al. Maternal and early postnatal nutrition and mental health of offspring by age 5 years: a prospective cohort study. J Am Acad Child Adolesc Psychiatry. 2013;52(10):1038-47.

214. Steenweg-de Graaff J, Tiemeier H, Steegers-Theunissen RP, Hofman A, Jaddoe VW, Verhulst FC, et al. Maternal dietary patterns during pregnancy and child internalising and externalising problems. The Generation R Study. Clin Nutr. 2014;33(1):115-21.

215. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. Mediterranean diet, stroke, cognitive impairment, and depression: A meta-analysis. Ann Neurol. 2013;74(4):580-91.

216. Lai JS, Hiles S, Bisquera A, Hure AJ, McEvoy M, Attia J. A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. Am J Clin Nutr. 2014;99(1):181-97.

217. Rahe C, Unrath M, Berger K. Dietary patterns and the risk of depression in adults: a systematic review of observational studies. Eur J Nutr. 2014;53(4):997-1013.

218. Opie RS, O'Neil A, Itsiopoulos C, Jacka FN. The impact of whole-of-diet interventions on depression and anxiety: a systematic review of randomised controlled trials. Public Health Nutr. 2015;18(11):2074-93.

219. Jacka FN, O'Neil A, Opie R, Itsiopoulos C, Cotton S, Mohebbi M, et al. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). BMC Med. 2017;15(1):23.

220. Calkin CV, Ruzickova M, Uher R, Hajek T, Slaney CM, Garnham JS, et al. Insulin resistance and outcome in bipolar disorder. Br J Psychiatry. 2015;206(1):52-7.

221. Reininghaus EZ, Lackner N, Fellendorf FT, Bengesser S, Birner A, Reininghaus B, et al. Weight cycling in bipolar disorder. J Affect Disord. 2015;171:33-8.

222. Lopresti AL, Jacka FN. Diet and Bipolar Disorder: A Review of Its Relationship and Potential Therapeutic Mechanisms of Action. J Altern Complement Med. 2015;21(12):733-9.

223. Beyer JL, Payne ME. Nutrition and Bipolar Depression. Psychiatr Clin North Am. 2016;39(1):75-86.

224. Cisternas P, Salazar P, Serrano FG, Montecinos-Oliva C, Arredondo SB, Varela-Nallar L, et al. Fructose consumption reduces hippocampal synaptic plasticity underlying cognitive performance. Biochim Biophys Acta. 2015;1852(11):2379-90.

225. Van Meter AR, Moreira AL, Youngstrom EA. Meta-analysis of epidemiologic studies of pediatric bipolar disorder. J Clin Psychiatry. 2011;72(9):1250-6.

226. Gore FM, Bloem PJ, Patton GC, Ferguson J, Joseph V, Coffey C, et al. Global burden of disease in young people aged 10-24 years: a systematic analysis. Lancet. 2011;377(9783):2093-102.

227. Hashimoto K, Sawa A, Iyo M. Increased levels of glutamate in brains from patients with mood disorders. Biol Psychiatry. 2007;62(11):1310-6.

228. Gigante AD, Bond DJ, Lafer B, Lam RW, Young LT, Yatham LN. Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: a meta-analysis. Bipolar Disord. 2012;14(5):478-87.

229. Yuksel C, Ongur D. Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. Biol Psychiatry. 2010;68(9):785-94.

230. de Vasconcellos-Bittencourt AP, Vendite DA, Nassif M, Crema LM, Frozza R, Thomazi AP, et al. Chronic stress and lithium treatments alter hippocampal glutamate uptake and release in the rat and potentiate necrotic cellular death after oxygen and glucose deprivation. Neurochem Res. 2011;36(5):793-800.

231. Hokin LE, Dixon JF, Los GV. A novel action of lithium: stimulation of glutamate release and inositol 1,4,5 trisphosphate accumulation via activation of the N-methyl D-aspartate receptor in monkey and mouse cerebral cortex slices. Adv Enzyme Regul. 1996;36:229-44.

232. Collino M, Thiemermann C, Mastrocola R, Gallicchio M, Benetti E, Miglio G, et al. Treatment with the glycogen synthase kinase-3beta inhibitor, TDZD-8, affects transient cerebral ischemia/reperfusion injury in the rat hippocampus. Shock. 2008;30(3):299-307.

233. Ackroff K, Sclafani A. Rats' preferences for high fructose corn syrup vs. sucrose and sugar mixtures. Physiol Behav. 2011;102(5):548-52.

234. Thorburn AW, Storlien LH, Jenkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am J Clin Nutr. 1989;49(6):1155-63.

235. Gould TD, Einat H. Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. Neurosci Biobehav Rev. 2007;31(6):825-31.

236. Choi CS, Kim P, Park JH, Gonzales EL, Kim KC, Cho KS, et al. High sucrose consumption during pregnancy induced ADHD-like behavioral phenotypes in mice offspring. J Nutr Biochem. 2015;26(12):1520-6.

237. Oyekunle OA, Akanmu MA, Ogundeji TP. Evaluation of anxiolytic and novelty induced behaviours following bee-honey consumption in rats. Journal of Neuroscience and Behavioural Health. 2010;2(4):38-43.

238. Sheludiakova A, Rooney K, Boakes RA. Metabolic and behavioural effects of sucrose and fructose/glucose drinks in the rat. Eur J Nutr. 2012;51(4):445-54.

239. Harrell CS, Burgado J, Kelly SD, Johnson ZP, Neigh GN. High-fructose diet during periadolescent development increases depressive-like behavior and remodels

the hypothalamic transcriptome in male rats. Psychoneuroendocrinology. 2015;62:252-64.

240. Powell CM. Gene targeting of presynaptic proteins in synaptic plasticity and memory: across the great divide. Neurobiol Learn Mem. 2006;85(1):2-15.

APPENDICES

Appendix 1: Hayvan Deneyleri Yerel Etik Kurul Kararı

T.C.
HACETTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu

Sayı : 52338575 -75

HAYVAN DENEYLERİ YEREL ETİK KURUL KARARI

TOPLANTI TARİHİ	: 20.06.2017 (SALI)
TOPLANTI SAYISI	: 2017/06
DOSYA KAYIT NUMARASI	: 2016/12
KARAR NUMARASI	: 2016/12 -04
ARAŞTIRMA YÜRÜTÜCÜSÜ	: Prof. Dr. M. Yıldırım SARA
HAYVAN DENEYLERİNDEN SORUMLU ARAŞTIRMACI	: Prof. Dr. M. Yıldırım SARA, Metin YEŞİLTEPE, Arwa Al BERKDAR
YARDIMCI ARAŞTIRMACILAR	: Prof. Dr. Hayrünnisa Bolay BELEN, Barış ALTEN, Metin YEŞİLTEPE, Arwa Al BERKDAR
ONAYLANAN HAYVAN TÜRÜ ve SAYISI	: ***114 Adet Wistar Sıçan***

Kurulumuzun 26.02.2016 tarihli toplantısında onaylanmış olan 2016/12 kayıt numaralı ***"Yüksek Fruktozla Beslenen Sıçanlarda Gelişen Hipokampal Öğrenme-Bellek ve Duygudurum Bozukluklarında Santral İnsülin Direncinin Rolü ve GSK-3 İnhibisyonu İle Tedavisi"*** başlıklı projeniz için vermiş olduğunuz 06.06.2017 tarihli araştırmacı değişikliği dilekçeniz, Kurulumuzun 20.06.2017 tarihli toplantısında değerlendirilmiş olup, Uzm. Biyolog Canan KURŞUNGÖZ' ün projeden ayrılması ve Arwa Al BERKDAR' ın yardımcı araştırmacı olarak projeye dahil edilmesi **uygun bulunmuş** ve kayıtlarımıza eklenmiştir. Kurul üyesi Prof. Dr. M. Yıldırım SARA çıkar çatışması nedeni ile tartışma ve oylamaya katılmamıştır.

Prof. Dr. Sema ÇALIŞ
Etik Kurul Başkanı

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WORK EXPERIENCE

Postdoctoral Researcher

Kavalali Lab, Vanderbilt University

09/2018 – Present *Nashville, TN, USA*

Assistant to the President of EAS, Prof. Dr. Lale Tokgozoglu

European Atherosclerosis Society

01/2017 – 05/2018 *Göteborg, Sweden*

EDUCATION

Doctor of Philosophy (PhD) in Medical Pharmacology

Hacettepe University Institute of Health Sciences Dept. of Medical Pharmacology

09/2014 – 07/2018 *GPA 3.79*

Doctor of Medicine (MD)

Hacettepe University Faculty of Medicine

10/2010 – 06/2018 *GPA 3.82 (Graduated 3rd of the class of 2018)*

CLERKSHIPS AND OBSERVERSHIPS

Hospital Clinico Universitario Lozano Blesa, Dept. of Vascular Surgery, Zaragoza, Spain (2011)
Clerkship

MD Anderson Cancer Center, Dept. of Experimental Therapeutics, Houston, TX, USA (2013)
Observership

University of Toronto, St. Michael's Hospital, Dept. of Gastroenterology, Toronto, Canada (2015)
Clerkship

Weill-Cornell Medical School, NewYork-Presbyterian Hospital, Dept. of Gastroenterology, New York City, NY, USA (2016)
Clerkship

Cleveland Clinic, Dept. of Internal Medicine and Critical Care Medicine, Cleveland, OH, USA (2018)
Subinternship

HONOR AWARDS

Vehbi Koc Foundation Scholar (2016 – 2017)
Vehbi Koc Foundation

Research Scholar (2016 – 2017)
The Scientific and Technological Research Council of Turkey (TUBITAK)

- The effects of nanoparticles produced during material processing with laser on rat organs, tissues and cell lines. Grant No: 113S223

Prof. Dr. Seref Zileli Memoriam Award for the most successful doctor in tranining of the year (2015)
Dept. of Internal Medicine, Hacettepe University Faculty of Medicine

- Awarded to the top student in the final written and oral examinations of core internal medicine clerkship. This award was named after Prof. Zileli, the founder of the Dept. of Internal Medicine, Hacettepe University Faculty of Medicine.

PUBLICATIONS

Article

High fructose corn syrup consumption in adolescent rats causes bipolar-like behavioral phenotype with hyperexcitability in hippocampal CA3-CA1 synapses

Author(s)

Alten B., Yesiltepe M., Bayraktar E., Tas ST., Gocmen AY., Kursungoz C., Martinez A., Sara Y.

In review

Poster

TDZD-8 attenuates insulin resistance in high fructose corn syrup fed adolescent rats

Author(s)

Alten B., Yesiltepe M., Bayraktar E., Tas ST., Gocmen AY., Kursungoz C., Martinez A., Sara Y.

2017

85th European Atherosclerosis Society Congress, Prague, Czech Republic.

Abstract published in Atherosclerosis vol 263 August 2017 doi: 10.1016/j.atherosclerosis.2017.06.868

Poster

Acute esophageal necrosis causing hematemesis in a patient with diabetic ketoacidosis

Author(s)

Alten B., Cohen-Mekelburg S., Rosenblatt R., Wan D.

2016

American College of Gastroenterology 2016 Annual Scientific Meeting Las Vegas, NV, USA.

ACHIEVEMENTS

Turkish Representative of Children's United Parliament of the World (CUPW) (2005 – 2007)

CUPW is an international non-governmental organization set up in Finland, in 1998. Today, there are children's parliaments in more than 50 countries across the globe and the main goal is to promote mutual understanding and peace. Attended to the General Assembly of the CUPW which was held in Tunisia, organized parallel to the United Nations' World Summit on the Information Society 2005.

European Union Comenius Project (2007 – 2010)

An European Union project titled "Eco-Issues in our e-Neighbourhood: Main Environmental Issues in our Settings" was started with the collaborations of four high schools from Turkey, Norway, Ireland and Sweden. Attended to the annual project meeting held in Egersund, Norway.

Executive Board Member of MEDISEP (2011 – 2013)

MEDISEP is currently representing the International Federation of Medical Students' Associations (IFMSA) and European Medical Students' Association (EMSA) in Hacettepe University, Faculty of Medicine.

Mantar (2010 – 2015)

Member between 2010-2014, President in the term of 2014-2015. Mantar is the first and the oldest student organization in the history of Hacettepe University. It was founded by Prof. Dr. Ihsan Dogramaci, the founder of Hacettepe University, in 1964. Mantar is responsible for the intellectual development of the medical students apart from their academic work. These include an annual journal of humour, organizations related to charity, annual prom of Hacettepe University Faculty of Medicine and more.

PUBLICATIONS

Poster

Three months of laser-generated nanoparticle exposure did not alter cognition and mood in rats

Author(s)

Tas ST., Kursungoz C., Alten B.,Ortac B., Sargon MF., Sara Y.

2016

International Nanotoxicology Congress, Boston, MA, USA.

Poster

Localization and distribution of nanoparticles generated during real-time laser material processing in rats

Author(s)

Tas ST., Kursungoz C., Alten B.,Ortac B., Sargon MF., Sara Y.

2016

International Nanotoxicology Congress, Boston, MA, USA.

LANGUAGES

Turkish
Native or Bilingual Proficiency

English (TOEFL: 116/120)
Full Professional Proficiency

USMLE EXAMINATIONS

Step 1: 259 (08/2016)

Step 2 CK: to be announced (07/2018)

Step 2 CS: to be announced (06/2018)