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High-fructose diet initiated during adolescence does not affect basolateral amygdala excitability or affective-like behavior in Sprague Dawley rats.

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Abstract

Patients with type-2 diabetes, obesity, and metabolic syndrome have a significantly increased risk of developing depression. Dysregulated metabolism may contribute to the etiology of depression by affecting neuronal activity in key limbic areas. The basolateral amygdala (BLA) acts as a critical emotional valence detector in the brain’s limbic circuit, and shows hyperactivity and abnormal glucose metabolism in depressed patients. Furthermore, administering a periadolescent high-fructose diet (HFrD; a model of metabolic syndrome) to male Wistar rats increases anxiety- and depressive-like behavior. Repeated shock stress in Sprague Dawley rats similarly increases anxiety-like behavior and increases BLA excitability. We therefore investigated whether a metabolic stressor (HFrD) would have similar effects as shock stress on BLA excitability in Sprague Dawley rats. We found that a HFrD did not affect the intrinsic excitability of BLA neurons. Fructose-fed Sprague Dawley rats had elevated body fat mass, but did not show increases in metabolic efficiency and fasting blood glucose relative to control. Finally unlike Wistar rats, fructose-fed Sprague Dawley rats did not show increased anxiety- and depressive-like behavior. These results suggest that genetic differences between rat strains may affect susceptibility to a metabolic insult. Collectively, these data show that a periadolescent HFrD disrupts metabolism, but does not change affective behavior or BLA excitability in Sprague Dawley rats.

Keywords

Fructose; Amygdala; Anxiety; Depression; Sprague Dawley

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1. Introduction

Major depressive disorder (MDD) is one of the most common psychiatric illnesses, with a lifetime prevalence of 17% [1,2]. However, the etiology of depression remains largely unknown. Recent evidence suggests dysregulated metabolism may play a role in MDD development. For example, patients with type 2 diabetes are twice as likely to develop MDD than the general population [3,4]. Obesity and metabolic syndrome similarly increase the risk of developing MDD [5,6]. Dietary high-fructose corn syrup in turn increases the risk of developing metabolic disorders such as obesity, metabolic syndrome, and diabetes [7–9]. Indeed, increased consumption of high-fructose corn syrup [10] correlates alarmingly with rising rates of both metabolic diseases [11–13] and MDD [14,15]. This correlation further increases the need to establish the nature of the relationship between fructose, dysregulated metabolism, and mood disorders.

Some studies suggest dysregulated metabolism could contribute to MDD development by dysregulating the neuronal activity in key limbic regions. One such region is the basolateral amygdala (BLA), which serves as a critical emotional valence detector in the brain's limbic circuit [16]. BLA principal neurons form reciprocal connections with brain regions involved in cognition, motivation, and stress responses, the dysregulation of which are core symptoms of MDD [17]. Furthermore the BLA is hyperactive during MDD, which then normalizes with successful pharmacotherapy [18]. This hyperactivity correlates with abnormal glucose metabolism in depressed patients [19–21]. Dysregulated metabolism may therefore contribute to MDD by increasing the excitability of BLA principal neurons.

In this study, we examined the relationship between dysregulated metabolism and BLA hyperexcitability in rats. Administration of a high-fructose diet in rats is a common model of metabolic challenge in rats. HFrD-fed rats develop abdominal obesity, insulin resistance, and dyslipidemia, and are commonly used as a model of metabolic syndrome [22,23]. In male Wistar rats administration of a high-fructose diet during adolescence and adulthood also increases anxiety- and depressive-like behavior [24]. The behavioral effects of the HFrD are similar to those of repeated shock stress, which in Sprague Dawley rats causes increased anxiety-like behavior and increased BLA excitability [25]. We therefore hypothesized a metabolic challenge (HFrD) would also cause increased BLA excitability in Sprague Dawley rats. After HFrD administration we tested the excitability of BLA principal neurons and assessed the impact of HFrD on anxiety- and depressive-like behavior and metabolism in Sprague Dawley rats.

We hypothesized HFrD administration to Sprague Dawley rats would mimic the effects of HFrD administration to Wistar rats. Specifically, we hypothesized a HFrD would increase body fat mass independently of body weight, increase metabolic efficiency, increase fasting blood glucose, and increase anxiety- and depressive-like behaviors. Finally, we hypothesized HFrD administration would also increase intrinsic BLA principal neuronal excitability, which has been shown to increase after chronic adolescent stress [26,27]. We found that HFrD administration increases fat mass, but not metabolic efficiency or fasting blood glucose. We also found HFrD administration did not increase the intrinsic excitability of BLA principal neurons. We also did not observe increased anxiety- and depressive-like

behaviors in the HFrD group. These data suggest that the effects of a HFrD are influenced by genetic strain differences between Sprague Dawley and Wistar rats, or by environmental variables.

2. Methods

2.1 General Housing

All animal experiments followed the guidelines described in the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023). Animals were housed in the Yerkes National Primate Research Center vivarium at Emory University. A total of 48 male Sprague Dawley rats were used in the following experiments. Animals were ordered from Charles River (Wilmington, MA). Rats arrived in the vivarium on postnatal day (P) 25, and were immediately randomly assigned to either a control or high-fructose diet (HFrD, described below). Rats were kept on an artificial regular 12:12-h light cycle, and were housed in ventilated cages kept at 22° C. Rats were either housed in groups of four (n=32) or pair housed (n=16). All experimental protocols conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Emory University.

Three cohorts of animals (16 rats each, 8 per diet) were used during this study. Metabolic efficiency, body weight, fasting blood glucose were measured in the first cohort. In addition to these measurements, the second and third cohorts (32 animals total) underwent behavioral testing as described below, and then were euthanized for assessment of body fat (Cohorts 1+2) and electrophysiology (Cohort 3) measurements respectively. Unless otherwise specified, no cohort differences were detected and cohorts were combined for appropriate analyses.

2.2 Diet

Consistent with previous work in Wistar rats [24,28], we used a HFrD to induce metabolic challenge in Sprague Dawley rats. Sprague Dawley rats were randomly assigned to either a “control” diet (Lab Rodent Diet 5001, 0.30% kcal from fructose) or a HFrD (Research Diets D05111802, 55% kcal from fructose). Both diets contained sufficient vitamins and minerals to maintain rodent health. Food consumption per cage was measured daily, and was used to calculate the total weekly caloric consumption per cage. Total weekly caloric consumption per cage was divided by the number of rats per cage to determine average weekly caloric intake per rat, which was used to determine metabolic efficiency. Once a week, animals were subjected to an overnight fast before fasting blood glucose measurements (described in section 2.4). Food was removed 1–2 hours before the onset of the dark cycle, and returned after blood glucose measurements approximately 16 hours later. Except for weekly overnight fasts, animals had *ad libitum* access to food and always had *ad libitum* access to water.

2.3 Behavior

Rats used for behavior in this study underwent three behavioral tasks over three days in order of least to most stressful: the open field maze task, elevated O maze task, and forced

swim task. Starting on P83, animals were habituated to the behavioral room for 7 days ($n = 16$ per group). On habituation days, each animal was individually handled for 10 minutes each. All behavior started approximately 3 hours after the beginning of the light cycle, and ended three hours before the end of the dark cycle. Control and HFrD rats were alternated throughout the day to control for circadian effects. All behavioral apparatuses were cleaned thoroughly with Quatricide (PRL Pharmacal, Naugatuck, CT) before and after each behavioral task. Animals were subjected to the following behavioral tasks:

2.3.1 Open field maze: The open field maze allows for assessment of both motor behavior and provides a metric of anxiety-like behavior [29]. Rats were placed in the center of the open field maze illuminated by red light, and were free to move throughout the open field for 6 minutes during the task. Control and HFrD rats were alternated throughout the day to control for circadian effects. An overhead video camera captured the animal's movement within the open field maze to measure baseline motor activity. Videos were analyzed using CleverSys Topscan software (Reston, VA). The open field apparatus used in this experiment was composed of 91×91 cm black plexiglass floor, surrounded by 28 cm walls.

2.3.2 Elevated O Maze: The elevated zero (O) maze is a widely validated test of anxiety-like behavior [30], with slight advantages over the elevated plus maze while retaining comparable results [31]. Rats were subjected to an elevated O maze task to assess anxiety-like behavior under red light illumination. Rats were free to move between the open and closed arms of the maze for 6 minutes during the task. An overhead video camera was used to track the animal's movement for later analysis. Using CleverSys Topscan software (Reston, VA), we measured the time the rat spent in the open arm, closed arm, and "stretch attend" position of the maze (consisting of the first 3" of the open arm immediately outside the closed arm). The specifications for the elevated O maze are as follows: 46.5" diameter, 4" wide track, 19.5" height (open arms), 30" height (closed arms).

2.3.3 Forced Swim task.—The forced swim task is a well characterized antidepressant screen, and has also been used to study the neurological underpinnings of depression [32,33]. We utilized the one-day forced swim task, which is an effective antidepressant screen [34,35], has been used to validate rodent models of depression such as the Flinders Sensitive Strain [36–39], and captures similar immobility behavior compared to the two-day forced swim task [40]. Rats underwent the forced swim task on P93. Animals were placed in clear plexiglass cylinders (20.5" tall, 8.5" diameter) containing 30 cm of 25 °C water for six minutes. Two animals (one from each group) underwent behavior simultaneously in two adjacent plexiglass containers separated by an opaque plastic barrier. The location of each group (left or right) was alternated throughout the day to avoid bias. The animals' movement was captured using a video camera and analyzed using CleverSys ForcedSwimScan software (Reston, VA). Immobility was defined as the animal's hind limbs remaining motionless for over 2 seconds. After the six-minute task, animals were immediately removed from the water, thoroughly dried using paper towels, and moved to recovery cages containing food, water, and heating pads. Animals recovered for at least an hour before being moved back to general housing.

2.4 Metabolic Outcomes

Fasting blood glucose and body weight were measured weekly after an overnight fast. Fasting blood glucose was measured via tail prick using a Freestyle glucometer (Abbot Laboratories, Chicago, IL). Fasting blood glucose was not collected during week 9 to avoid stressing the animals prior to behavioral testing. The weekly metabolic efficiency of each rat was determined by dividing the individual weight gained by the average weekly caloric intake per rat. After 10 weeks on the diet, animals were anesthetized using open-drop exposure to isoflurane. After 90-120 seconds of isoflurane exposure, rats entered a deep plane of anesthesia as verified by decreased respiratory rate and loss of the righting reflex. Once the loss of spinal reflexes was verified using a toe-pinch, rats were sacrificed via decapitation. In cohorts 1 and 2 (16 rats per group), the epididymal and perigonadal fat pads were extracted post- euthanasia and immediately weighed to measure fat pad mass. Rats in cohort 3 (8 rats per group) were instead used for electrophysiology as described below.

2.5 Slice Electrophysiology

Electrophysiological recordings started 7 days after the forced swim task (P100) to allow rats to recover after the forced swim tasks. Rats (n=8 per group) were decapitated under isoflurane anesthesia, and prepared for slice electrophysiology. One to two rats were euthanized per recording day over the course of the next 21 days. Rats remained on the assigned diet during the time period. The date of recording did not affect the observed electrophysiological properties (see section 3.6).

Their brains were removed and processed for slice electrophysiology as described previously [25]. Brains were immersed in ice-cold “cutting solution” consisting of (in mM): NaCl (130), NaHCO₃ (30), KCl (3.50), KH₂PO₄ (1.10), MgCl₂ (6.0), CaCl₂ (1.0), glucose (10), ascorbate (0.4), thiourea (0.8), sodium pyruvate (2.0), and kynurenic acid (2.0). Coronal sections (350 μm thick) were obtained using a Leica VTS-1000 vibrating-blade microtome. Slices were left to incubate in cutting solution for an hour, before being transferred to room-temperature “regular artificial cerebrospinal fluid” (aCSF) consisting of (in mM): NaCl (130), NaHCO₃ (30), KCl (3.50), KH₂PO₄ (1.10), MgCl₂ (1.30), CaCl₂ (2.50), glucose (10), ascorbate (0.4), thiourea (0.8) and sodium pyruvate (2.0). Cutting solution and aCSF were perfused with a 95% oxygen / 5% carbon dioxide gas mixture.

Individual slices were transferred to a recording chamber and were visualized using a Leica DM6000 FS microscope (Leica Microsystems Inc., Bannockburn, IL, USA) as described previously [25]. Slices were continuously perfused by gravity-fed oxygenated 32 °C aCSF at a flow rate of 2–3 ml/ min. Thin-walled borosilicate glass patch electrodes (WPI, Sarasota, FL, USA) were then used to acquire whole-cell patch-clamp recordings of projection neurons in the basolateral amygdala (BLA). Electrodes had a resistance of 4–6 MΩ, were filled with a “patch solution”, containing (in mM): K⁺-gluconate (130), KCl (2), HEPES (10), MgCl₂ (3), K-ATP (2), Na-GTP (0.2), and phosphocreatine (5), adjusted to pH 7.3 with KOH, and having an osmolality of 280–290 mOsm. BLA projection neurons were identified according to their characteristic size, shape, and electrophysiological properties [41,42].

Data acquisition and analysis were performed using a MultiClamp700B amplifier in conjunction with pClamp 10.2 software and a DigiData 1320A AD/DA interface (Molecular Devices, Sunnyvale, CA, USA). Whole-cell patch-clamp recordings were obtained and recorded voltages were low-pass filtered at 5 kHz and digitized at 10–20 kHz. Pipette offset and capacitance were automatically compensated for using Multiclamp software. Series resistance was compensated for manually, and recordings with series resistances of >30 MOhms were discarded.

2.6 Statistical Analysis

Measurements were analyzed using Graphpad Prism 7 (La Jolla, CA). Unpaired two-tailed Student's t-tests or 1-way and 2-way repeated measures Analysis of Variance tests were performed using $\alpha=0.05$. Turkey and Holm-Sidak post-hoc testing was performed when appropriate. Two levels of the independent factor "diet" (control and HFrD) were used for both 1- and 2-way ANOVAs. For 2-way ANOVAs, eight levels of the dependent factor "Time" (Weeks 2-9) were used. We also calculated simple linear regression to predict BLA electrophysiological properties (membrane resistance, spike threshold, and number of action potentials per pA of positive current) based on recording date. All values are reported as mean $\pm$ standard deviation.

3. Results

3.1 Peri-Adolescent High-Fructose Diet Increases Visceral Fat Mass but not Metabolic Efficiency

Prior to the initiation of the dietary intervention, body weight was equivalent between the control (58.81 g $\pm$ 6.35) and HFrD rats (59.94 g $\pm$ 8.04; $t_{30}=0.44$, $p=0.66$). The high fructose diet did not alter body weight and weights remained similar between groups throughout the study ($F_{1,46}=2.4$, $p=0.13$, Figure 1A). Although body weight was similar, HFrD rats had a greater body fat percentage relative to control, defined as the weight of the epididymal ($t_{30}=4.4$, $p=0.0001$; Figure 1B) and perigonadal fat pads ($t_{30}=4.1$, $p=0.0003$; Figure 1C), divided by final total body weight. This effect cannot be explained by alterations in metabolic efficiency as HFrD rats were no more metabolically efficient (weight gained / average cage kCal consumed) than control rats ($F_{1,46}=1.387$, $p=0.25$, Figure 1D). However, we did find a main effect of cohort on metabolic efficiency for both control ($F_{2,21}=9.1$, $p=0.0014$) and HFrD rats ($F_{2,21}=10.87$, $p=0.0006$) consistent across diet, explaining 3.2% and 6.6% of variation respectively. The cohort effect was not explained by the number of rats per home cage, as pair housed rats were not significantly different from group housed rats for either control ($F_{1,22}=0.08$, $p=0.77$) or HFrD ($F_{1,22}=0.87$, $p=0.36$) groups.

3.2 HFrD Decreases Fasting Blood Glucose

At the start of the experiment, fasting blood glucose was equivalent between the groups of rats that would be assigned to standard chow (73.13 mg/dl $\pm$ 14.65) or HFrD (64.44 mg/dl $\pm$ 11.15; $t_{30}=1.87$, $p=0.07$). After administration of the diet, HFrD rats had lower fasting blood glucose values relative to control-fed rats ($F_{1,46}=25.41$, $p<0.0001$, Figure 1E). In addition, fasting blood glucose was influenced by time (defined as # of weeks on the diet, $F_{7,322}=4.201$, $p=0.0002$), but no interaction between diet and time ($F_{7,322}=0.9881$,

$p=0.4397$). Therefore, this effect was likely driven by effects of developmental age of the rats or repeated blood sampling.

3.3 HFrD Did Not Alter Motor Behavior or Affective-like Behaviors

Control and HFrD groups traveled the same distance in the open field maze ($t_{30}=69$, $p=0.50$; Figure 2A), and both average velocity (Control: 60.39 mm/s $\pm$ 7.56, HFrD: 63.40 mm/s $\pm$ 6.77, $F_{1,30}=0.44$, $p=0.51$) and maximum velocity (Control: 260.6 mm/s $\pm$ 8.27, HFrD: 269.7 mm/s $\pm$ 12.75, $F_{1,30}=0.27$, $p=0.61$) were similar between the groups. In addition, the zones of the open field in which the rats spent time were equivalent between the control and HFrD groups: outer ($t_{30}=0.63$, $p=0.78$), middle ($t_{30}=0.44$, $p=0.78$), and center ($t_{30}=1.46$, $p=0.40$; Holm-Sidak method, $\alpha=0.05$, Figure 2B).

Control and HFrD rats displayed the same number of crossings between the open arm, “stretch-attend”, and closed arm zones, suggesting similar motor activity ($t_{30}=0.37$, $p=0.72$, Figure 2C). Further, a diet high in fructose did not alter time spent in the open arms ($t_{46}=0.46$, $p=0.88$), closed arms ($t_{46}=0.14$, $p=0.89$), or “stretch attend” zone ($t_{46}=0.79$, $P=0.82$) of the elevated O maze, as compared to behavior of the control diet rats (Holm-Sidak method, $\alpha=0.05$, Figure 2D).

HFrD did not alter time spent struggling in the forced swim test ($t_{30}=0.008$, $p=0.99$, Figure 3A). In addition, neither latency to the first 2 sec immobility event ($t_{30}=0.08$, $p=0.94$, Figure 3B), or total time spent immobile ($t_{30}=0.13$, $p=0.90$, Figure 3C) were impacted by consumption of the high fructose diet.

3.4 HFrD does not Affect BLA principal neuron membrane properties

High fructose diet (Control, $n=20$; HFrD, $n=19$) did not alter membrane resistance ($t_{37}=1.14$, $p=0.26$, Figure 4A) or spike threshold ($t_{37}=1.01$, $p=0.32$, Figure 4B) of BLA principal neurons. Additionally, there was no difference between control and HFrD groups in the evoked firing response: the average number of action potentials fired per pA of depolarizing current ($t_{39}=0.65$, $p=0.52$, Figure 4C). Recording date did not influence membrane resistance (Control: $F_{1,18}=0.06$, $p=0.8$, $R^2<0.01$; HFrD: $F_{1,17}=0.86$, $p=0.37$, $R^2=0.05$), spike threshold (Control: $F_{1,18}=1.208$, $p=0.29$, $R^2=0.06$; HFrD: $F_{1,17}=0.27$, $p=0.61$, $R^2=0.02$) or the average number of action potential fired per pA of depolarizing current (Control: $F_{1,18}<0.01$, $p=0.98$, $R^2<0.01$; HFrD: $F_{1,17}=4.39$, $p=0.051$, $R^2=0.21$).

4. Discussion

Collectively, the data presented here demonstrate that although there are metabolic implications of a high fructose diet initiated in adolescence in male Sprague Dawley rats, neither affective-like behaviors nor BLA excitability were altered by diet exposure. The effects of a HFrD on fat pad mass are consistent with previous work [43], and are indicative that the HFrD disrupted metabolism. However, we did not observe the behavioral changes previously reported in Wistar rats fed a HFrD [24]. We also did not observe changes in BLA physiology, correlating with our behavioral results. The physiological/behavioral response to dietary fructose may therefore be influenced by either strain-dependent genetic variation or environmental influences. Future studies might utilize the strain-dependent effects of a

HFrD to investigate the mechanisms by which a diet high in fructose can elicit neural and behavioral effects.

4.1 Genetic strain differences could affect response to dietary fructose

Consumption of a HFrD has been shown to increase body fat mass, impair insulin sensitivity, and increase plasma leptin and triglyceride levels in rats [22,23,44–46]. However, the physiological outcomes of HFrD administration may be strain-dependent. Differences between Sprague Dawley and Wistar rats could be a major source of variation between this and previous studies. At baseline Sprague Dawley rats are more sensitive to insulin than Wistar rats [47], possibly ameliorating fructose-induced insulin resistance. Higher baseline corticosterone levels have also been reported in Wistar rats compared to Sprague Dawley [48], possibly affecting both fructose-induced hepatic gluconeogenesis [49] and affective behavior via HPA axis activation [24,50]. Cultured hepatocytes from Sprague Dawley rats also exhibit faster glucose and amino acid metabolism than in Wistar rats [51], suggesting Sprague Dawley and Wistar rats may metabolize fructose in the liver at different rates.

Strain differences also affect the outcomes of diet administration. Wistar rats fed a high-fat diet had more pronounced weight gain, obesity, hyperinsulinemia, and hyperleptinemia than Sprague Dawley rats [52,53]. Conversely, Sprague Dawley rats fed a 90 day HFrD failed to develop fructose-induced insulin resistance or glucose tolerance [54]. Wistar rats could thus be more susceptible than Sprague Dawley rats to the physiological effects of a metabolic insult. Genetic strain differences may have also influenced the anxiety- and depressive-like behavior observed in this study. Wistar and Sprague-Dawley rats exhibit similar behavior in most tests [55]. However, some studies have reported increased baseline motor [55] and anxiety-like behavior [56] in Wistar relative to Sprague Dawley rats. Studies have even reported significantly different anxiety-like behavior between Wistar rats from different vendors, highlighting the importance of small genetic variations [57,58].

This genetic variation may be of use in future studies investigating genetic susceptibility to a metabolic insult. Indeed, contrasting control and disease-model genomes using QTL mapping has identified genetic loci associated with alcohol-seeking behavior [59], anxiety- and depression-like behavior [60,61], and diabetes [62] in various rodent models. Contrasting Sprague Dawley and Wistar rats in a similar manner may identify genetic loci associated with pathological responses to dietary fructose.

4.2 Laboratory environment could affect response to dietary fructose

Seemingly benign environmental differences between laboratories can also affect both physiology and behavior in rats. For example, bedding material and the frequency of bedding replacement affects body weight and inflammatory markers [63]. Similarly, the level of environmental enrichment can affect stress tolerance [64] and depression-like behavior [65,66]. Controlling these variables can be difficult, as identical mouse strains exhibited different behavior when tested in different laboratories despite rigorous efforts to standardize protocols and environmental variables [67]. A follow up study revealed interaction effects between laboratory environment and mouse strain, with the potential to alter “moderate genetic effects” [68]. Similarly, laboratory environment could interact with

between- and within-strain genetic variability, influencing the anxiety- and depression-like behavior.

It is possible rats in this study were exposed to more environmental stress than in previous studies. Unlike a similar study [24], rats were kept on a 12:12h light cycle, which has been shown to increase anxiety- and depressive-like behavior [69]. Moreover, rats were shipped during adolescence and subjected to a weekly blood glucose test. However, Harrell et al. [24] found no interaction effect between stress and response to dietary fructose in the open field, elevated plus, and forced swim task. Furthermore, control rats in this study exhibited similar anxiety- and depressive-behavior as control groups in previous studies. It is therefore unlikely that environmental stress strongly affected the behavior observed in this study.

4.3 HFrD does not change BLA electrophysiology

HFrD administration did not change the intrinsic excitability of BLA principal neurons (Figure 4). This finding correlates with the lack of behavioral changes observed during this study. The membrane properties of BLA principal neurons observed in this study were comparable to those described previously in control Sprague Dawley rats [42]. It is possible that a HFrD changed more subtle properties of BLA excitability not assessed in this study, such as synaptic plasticity. It is also possible any differences in BLA principal neuron excitability between the HFrD and control groups was lost during the slice procedure, but given our previous experience and success with these techniques, this is an unlikely alternative explanation. We can conclude that under the conditions used in this study, that BLA principal neuron excitability was not altered by exposure to a high fructose diet initiated during adolescence.

4.4 A HFrD Changes Fat Pad Mass, but not Fasting Blood Glucose or Metabolic Efficiency in Sprague Dawley Rats

The increased body fat percentage in the HFrD group confirms that the HFrD disrupted metabolism in Sprague Dawley rats. This result is consistent with multiple studies reporting similar HFrD-induced increases in body fat percentage without increases in body weight [24]. We did not observe the increased metabolic efficiency reported in other studies using Wistar and Sprague Dawley rats [24,70]. However, as adiposity is one of the core symptoms of metabolic syndrome, the increased fat pad mass observed in this study is sufficient confirmation that a HFrD disrupted metabolism in Sprague Dawley rats. However, we did not measure other metabolic outcomes disrupted by HFrD administration such as insulin resistance, hyperleptinemia, dyslipidemia, or liver steatosis [22,23,44–46]. The Sprague Dawley rats in this study could therefore be less susceptible to these fructose-induced metabolic effects, possibly affecting both behavioral and electrophysiological outcomes. Additional study is needed to fully assess the physiological outcomes of fructose administration in Sprague Dawley rats.

We found a significant effect of cohort on metabolic efficiency. This effect was not explained by whether rats were pair or group housed. As Sprague Dawley rats are an outbred strain, genetic variation between cohorts may explain the observed cohort effect. Indeed studies have reported phenotypic differences in Sprague Dawley rats originating from

different vendors and from different colonies within vendors [71,72]. Despite the effect of cohort on metabolic efficiency, there was no interaction effect between cohort and diet on metabolic efficiency. Thus, cohort did not influence the physiological response to dietary fructose.

We observed that HFrD administration significantly decreased fasting blood glucose. Most studies report that HFrD increases fasting blood glucose in Wistar [24,73,74] and Sprague Dawley rats [75]. However, some studies have found no HFrD-induced change in fasting blood glucose in either Wistar or Sprague Dawley rats [54,76]. One possible factor is before the administration of the diet, the HFrD group had an almost significant ($p=0.07$) reduction in baseline fasting blood glucose relative to control. Despite efforts to randomize rats between diet groups, this finding also suggests animals used in this study were genetically heterogeneous.

4.5 Overall Conclusions

Administration of a HFrD from adolescence into adulthood causes elevated fat pad mass in Sprague Dawley rats, which is indicative of disrupted metabolism. However unlike Wistar rats [24], HFrD administration does not affect motor, anxiety-like, or depressive-like behavior in Sprague Dawley rats. HFrD administration also does not change the intrinsic excitability of BLA principal neurons, correlating with our behavioral findings. These findings suggest that the physiological and behavioral effects of HFrD administration are strain-dependent, although environmental variables may also play a role. This study highlights the importance of accounting for strain and subtle environmental variables in diet studies. Furthermore, comparing Sprague Dawley and Wistar strains may offer a strategy to elucidate the mechanisms by which dietary fructose can alter neural endpoints and behavior. In conclusion, HFrD administration changes body fat, but not affective behavior or BLA excitability in male Sprague Dawley rats.

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Abbreviations

HFrD:	High-Fructose Diet
BLA:	Basolateral Amygdala
MDD:	Major Depressive Disorder

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Figure 1:

A) Body weight of control and HFrD rats (Weeks 1-9, n=24 per group. Terminal, N=16 per group.). B) Normalized epididymal fat pad weight (mg fat / g rat body weight) for control and HFrD rats (n=16 per group). C) Normalized perigonadal fat pad weight (mg fat / g rat body weight) for control and HFrD rats (n=16 per group). D) Metabolic efficiency (mg rat body weight / average cage kcal consumed) of control and HFrD rats over nine weeks (Week 1, n = 16 per group; Weeks 2-9, n=24 per group). E) Fasting blood glucose of control and HFrD rats over eight weeks (n=24 per group). *: p<0.05, ***: p<0.001

Figure 2:

A) Distance travelled (in mm) during the six-minute open field maze task for control and HFrD rats (n= 16 per group). B) Time (in sec) control and HFrD rats spent in the center (25% total maze area) of the open field maze (n=16 per group). C) Number of transitions (defined as a crossing between the closed arm, “stretch-attend”, and open arm zones) during the Elevated Zero Maze task for control and HFrD rats (n=16 per group). D) Time (in seconds) spent in the closed arm during the six-minute Elevated Zero Maze task for control and HFrD rats (n=16 per group).

Figure 3:

A) Total time (in sec) spent struggling during the six-minute Forced Swim Task for control and HFrD rats (n=16 per group). B) Latency to a 2-second immobility event (in sec) during the six-minute Forced Swim Task for control and HFrD rats (n=16 per group). C) Total time (in sec) spent immobile during the six-minute Forced Swim Task for control and HFrD rats (n=16 per group).

Figure 4:

TOP: Example recordings from a control BLA principal neuron. LEFT: Hypdep protocol, used to calculate membrane resistance / action potential frequency. RIGHT: Voltage Ramp protocol, used to calculate action potential threshold. BOTTOM: A) Input resistance of BLA principal neurons from control and HFrD rats (n=20 control; n=19 HFrD). B) Action potential threshold (in mV) of BLA principal neurons from control and HFrD rats (n=20 control; n=19 HFrD). C). The average number of action potentials fired per pA of depolarizing current in control and HFrD animals (n=20 control; n=19 HFrD).