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Studies have revealed that diets high in saturated fats are linked to alterations in the dopaminergic reward system that ultimately impede normal satiety signals responsible for regulating homeostatic food intake. High saturated fat (HF) consumption has also been shown to play a role in the development of a metabolic syndrome-like phenotype characterized by weight gain, insulin resistance and chronic inflammation. Further, long-term intake of a high saturated fat diet diminishes dopamine release and reuptake and impairs dopamine receptor signaling in the nucleus accumbens (NAc), which could result in overeating as a means of stimulating the dopamine pathway. The specific physiological mechanisms by which HF intake blunts dopamine neurotransmission to influence food behaviors have not been fully characterized. However, one such aspect could involve dopaminergic neuronal responses to glucose, given that neurons of the NAc have been characterized as glucose-responsive. Moreover, previous data from our lab revealed that metabolic state (as dictated by a 12-h fast) could impact synaptic control of dopamine. We therefore sought to determine whether a HF diet, in contrast to a standard low fat (LF) diet, would differentially alter dopamine neurotransmission in the NAc in response to changes in glucose concentrations and whether changes were noticeably different between NAc subregions. Results presented herein suggest that in HF animals, neuronal energy requirements for energetically demanding phasic release in the NAc core are being met by other substrates other than glucose. Moreover, enhanced tonic and phasic release in the core of LF males but not HF could indicate decreased sensitivity of glucose-responsive neurons in HF animals. Noticeable differences in treatment effect between males and females are also suggestive of sex-based differences in metabolic circuitry and energy balance, as well as alterations in estrus cycling in

females due to consumption of a HF diet. Lastly, we report higher baseline dopamine release in the NAc core compared to the shell, as well as enhancements in phasic release after exposure to hypoglycemic conditions that intimate a negative energy state might prime reward-seeking behaviors in the core by preferentially enhancing phasic dopamine release. Overall, negative energy states could promote increased food-seeking and hyperphagia resulting from changes in dopamine neurotransmission within NAc subregions. Ultimately, given that food intake relies on an elaborate interplay of signaling mechanisms and other stimuli, further evaluation of changes in feeding behavior resulting from alterations in dopamine neurotransmission in the NAc are warranted, particularly in relation to metabolic states.

THE EFFECTS OF CHANGING ARTIFICIAL CEREBROSPINAL FLUID GLUCOSE CONCENTRATION ON DOPAMINE NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS

by

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DEDICATION

I dedicate this thesis to my parents, Rafael and Maria Hurtado, who have always given me their unwavering love and support. Dad, thank you for believing I can do anything I set my mind to and for showing me the value of hard work. You never clipped my wings and encouraged me to go on every adventure, especially this one.

APPROVAL PAGE

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CHAPTER I: LITERATURE REVIEW

Introduction

Obesity and its associated metabolic dysfunction have spiraled into a global epidemic affecting millions of children and adults and financially straining public health systems. According to the World Health Organization, more than 1.9 billion adults worldwide are overweight, and nearly 650 million of them are categorized as obese (BMI > 30 kg/m^2) [1]. Hyperglycemia and insulin resistance are commonly associated with obesity and coupled with abnormal β-cell function, result in an inability to effectively modulate elevated blood glucose levels. There are glucose sensors in the brain and peripheral regions that are responsible for coordinating autonomic and neuroendocrine responses to physiological changes in extracellular glucose [2]. Prolonged changes in circulating blood glucose levels as seen in obesity can alter insulin and glucose levels in the brain and potentially affect expression of glucose transporters and influence circuitry in neuronal populations – this has been demonstrated in neurons that alter their action potential frequency in response to extracellular glucose changes [3]. Additionally, reduced aCSF glucose levels elicited a reduction in action potential firing rates of neurons in the nucleus of the solitary tract [4]. It is not fully known how diet-induced obesity (specifically via saturated fats) and resulting changes in glucose levels affect dopaminergic neurons in the nucleus accumbens, given the previous supposition that metabolic state could modulate synaptic dopamine. Therefore, it is critical to understand how glycemic changes resulting from diet-induced obesity can affect neuronal adaptation and synaptic dopamine neurotransmission.

Obesity & Dopamine

Obesity

Obesity is generally defined as an excess of body fat that negatively impacts an individual's health. Excessive food intake along with increased availability of energy-dense, highly palatable foods are considered contributors to the significant rise in obesity rates. In industrialized nations, obesity rates have been correlated with an increase in consumption of high fructose corn syrup and saturated fats, in addition to a decrease in physical activity [5]. Obesity is considered problematic given that it contributes to a variety of disorders. For example, excess free fat mass is associated with hypertension and dyslipidemia, and individuals with obesity display increased metabolic and cardiovascular risk factors compared to those with healthy weights [6, 7]. Being overweight or obese increases the risk of some cancers, respiratory conditions, non-alcoholic fatty liver disease, and various mental health conditions [8]. Despite research and community-based efforts devoted to understanding obesity and reducing its prevalence, it has become a public health issue in numerous countries. Recent data shows that between 1986 and 2010, the percentage of Americans with a BMI greater than 40 kg/m^2 has quadrupled, while those with a BMI greater than 50 kg/m^2 have increased more than ten-fold [9].

How saturated fat contributes to obesity

Dietary fats exert different metabolic, endocrine, and behavioral effects. Saturated fats are foods that are primarily lipids and are typically solid at room temperature; the term *saturated* is used to designate fatty acid chains with all single bonds. The typical Western diet consists primarily of simple sugars and fats, particularly saturated fats. This is important given how excessive consumption of saturated fats in palatable foods can contribute to obesity. Saturated fats have been found to be more metabolically harmful to the liver [10 11] via fat accumulation [12 13].

Excess saturated fat intake has been linked to greater metabolic dysfunction and increased accumulation of visceral fat and peripheral inflammation [14]. A review by Kennedy et al [15] discusses how overconsumption of saturated fats can lead to increased white adipose tissue inflammation and metabolic disease. In mice, high saturated fat intake induces insulin resistance [16] [17]. Diets high in saturated fat therefore enhance weight gain and lead to metabolic complications and eventual systemic inflammation [18].

Links between obesity and brain health

Obesity has been linked to a greater risk of developing dementia [19] and inflammation induced by overnutrition has been found to play a role in neurodegeneration [20] [21]. Obesity and high fat diets cause systemic inflammation that results in localized inflammation in the brain, causing synaptic remodeling and disrupting cognitive function [22]. This perpetuates the cycle of overeating and ultimately damages homeostatic satiety signals. Moreover, a higher BMI has been associated with lower brain volumes in addition to atrophy in overweight and obese elderly human subjects [23]. Obesity is also associated with decreased hippocampal volume and significant atrophy over time [24]. Increased saturated fat consumption in mid and later years in life has been correlated with a reduction in global cognitive function in addition to increased susceptibility to neurological diseases such as dementia and Alzheimer's [25] [26] [27].

Links between obesity and the dopamine system

Gradual changes in the firing rates of dopamine neurons and the resulting changes in extracellular dopamine levels are associated with motivational changes that influence food seeking. This is evidenced by postingestive nutrient sensing that modifies habitual food seeking behaviors [28]. Moreover, foods that are rich in fat and sugar serve as strong rewards that encourage eating even when there is no energy requirement, leading to learned associations

between palatable foods (i.e., the stimulus) and reward [29]. Moreover, ingestion of palatable foods that are high in sugar and/or fat activates brain reward circuitry [30]. Individuals with obesity have reduced D2 receptor activity and a hypofunctional dorsal striatum in comparison to lean individuals [31] . D2 receptors are essential to postsynaptic receptor–mediated behavioral activity and have a higher affinity for dopamine than D1 receptors [32]. In murine models, diet-induced obesity elicits a reduction in dopamine transporter function and expression but more extracellular dopamine in the striatum [33]. The development of habitual behaviors has been linked to a transference in control from the NAc to the striatal dopaminergic pathways [34]. Thus, obesity can result in dysregulation of dopaminergic signaling mechanisms that are distinguished by lack of perseverance and increased reward-seeking behaviors.

Dopamine & Food Intake

Dopamine's role in reward learning and motivation

Dopamine is a regulator of both learning and motivation, originally thought to be only a precursor of catecholic neurotransmitters or an intermediate in tyrosine degradation [35]. It was later recognized to be an independent neurotransmitter and its first receptor was discovered [36]. Dopamine is sometimes labeled a neuromodulator because of how it regulates sensitivity to other neurotransmitters. Dopamine neurons have been found to code for error between the actual reward received and the reward predicted, but fire only when the reward is unexpected, which makes them adept at detecting the value of environmental events in relation to their learned predictions [37]. Predictors of reward become affiliated with the resulting rewards and elicit burst firing of dopaminergic neurons. The subsequent phasic dopamine release in the striatum results in synaptic adaptations such as alterations in sensitivity to presynaptic input (also known as long-term potentiation and long-term depression) that are the neural foundation for mechanisms that

dictate reward-seeking behavior [38]. Thus, rewarding food triggers dopaminergic transmission in the NAc [39] [40]. Dopamine projections from the ventral tegmental area (VTA) to the NAc are involved in fostering reward-seeking behavior [41]. Animals with reduced NAc dopaminergic function exert less effort in procuring rewards [42], in addition to having blunted responses to reward-predictive cues [41] [43] [44].

Dopamine and food approach

Seeking motivation increases the cognitive ability to discern environmental information and store it for future use, stimulating approach behavior [45]. Hungry rats have been found to participate in continuous food seeking behaviors, regardless of whether the seeking behavior is reinforced under extinction conditions [46]. In aversive learning, extinction is a weakening of a previously conditioned response that results in an adaptation in behavior to previously conditioned stimuli [47]. Animals display increased motivation and put forth more effort in reward acquisition when exposed to palatable foods [48]. Obesity-prone rats have increased anticipatory and impulsive behavior following exposure to palatable foods. Moreover, limited access to palatable foods led to development of binge-like overconsumptions in rats [48]. While dopamine depletion does not stop the acquisition and consumption of food, it does alter behavior to seek out the simplest locomotive path to food [49]. Rats with NAc dopamine depletion display a shift towards reduced effort expenditure [50]. This shows that interference with brain dopamine suppresses active complex food seeking [51] [52]. Further studies show that rats with NAc dopamine depletion display a shift from high value food rewards to low value food reinforcers [53] [54] [55] [56].

Dopamine and food eating

D1 and D2 receptors are G-protein coupled receptors most abundant in the central nervous system. Dopaminergic innervation of the basal ganglia is divided into direct and indirect pathways, where D1 receptors comprise the direct pathway and D2 receptors the indirect pathway and act in opposition to one another [57]. In the striatum, the primary cell type is the GABA-ergic medium spiny neuron (MSN). MSNs expressing D1 receptors send primarily inhibitory projections to the substantia nigra pars reticulata [58]. In contrast, MSNs expressing D2 receptors send chiefly inhibitory projections to the globus pallidus externa and to the sub-thalamic nucleus, which then sends excitatory projections back to the basal ganglia [58]. When D1 receptors are bound to dopamine, they generally produce an excitatory response that increases the likelihood of D1 MSNs firing, while D2 receptors reduce the likelihood of D2 MSNs firing [57]. Tonic release of dopamine is continuous and pacemaker-like, while phasic release consists of short bursts followed by pauses. D1 receptors are preferentially sensitive to phasic dopamine release and have a lower affinity for dopamine [59], whereas D2 receptors are more sensitive to changes in tonic release and have a higher affinity for dopamine [60]. GABA-ergic neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons stimulate hunger responses and reduce energy expenditure [61]. Control of consummatory behavior occurs via inhibition of GABA neurons in the lateral hypothalamus by D1 receptor MSNs projecting from the NAc shell [62,63]. Dopaminergic projections from the NAc to the VTA involving the indirect pathway are mediated by both D1 and D2 MSNs, while D1 MSNs directly project to the VTA [64 65]. The ventral palladium projects to the VTA via GABA-ergic neurons [65], where optical activation of D2 MSNs shows inhibition of these neurons and an increase in VTA dopaminergic activity [66 67]. Brief stimulation of D2 MSNs increases VTA dopaminergic activity but prolonged stimulation significantly reduces

VTA dopamine signaling[67]. Variation in synchronized burst fire activity can regulate average activity of different signaling pathways in the mesolimbic dopamine pathway [59]. Dopamine neurons fire phasically in response to salient stimuli in behavioral tasks and return to baseline after hundreds of milliseconds [68, 69 70] .

Dopaminergic signaling after food ingestion signals the nutritive value of the food consumed [71 72]. Obesity has been associated with reduced dopaminergic function [73 74], suggesting that impaired striatal dopamine leads to compensatory overeating [75 76 77] and ultimately, obesity. Rats with DIO display lower basal and evoked dopamine levels in the NAc[78]. Animals with DIO exhibit a blunted response to standard chow but not palatable food, with reduced dopamine release in the NAc and dorsal striatum [79]. This could result from continuous and sustained activation of dopaminergic signals by chronic over-eating that downregulates dopamine receptors [80]. Thus, alterations in the dopaminergic system can produce changes in food consumption and preference that can play a role in the development of obesity.

Mechanisms on how saturated fats and obesity influence dopamine

Palatable foods evoke dopamine release in the striatum of animals and humans [30 81 82 83], which leads to the acquisition of habitual behaviors [84]. DIO rats have reduced striatal expression and function of dopamine transporters (DAT) [33], which help regulate extracellular dopamine. Adult obese rats display markedly decreased extracellular dopamine in the NAc [85]. Moreover, a HFD significantly reduces NAc dopamine turnover rate [78]. These alterations in dopamine levels can in part be attributed to receptor changes resulting from food intake. Changes in presynaptic DAT levels and function affect the degree of influence dopamine has in addition to striatal function [86 59]. BMI is negatively correlated with DAT availability in the human striatum [87] and reduced binding and availability of DAT in high fat fed mice [88]. In chronic high fat consumption

and DIO, VTA dopaminergic activity is altered at synaptic terminals in the NAc [89], resulting in hyperphagia and blunted dopamine reuptake [90]. Saturated fats significantly decrease dopamine reuptake V_{max} while also reducing phasic dopamine release [91]. Moreover, a HFD reduces D1 and D2 receptors, though they return to normal once the HFD is removed [92]. This suggests that repeated exposure to a HFD induces reversible changes to D1 receptor signal transduction leading to diminished D1 and D2 receptor availability.

Ex vivo fast scan cyclic voltammetry (FSCV) in mice fed a diet high in saturated fats showed reduced clearance of synaptic dopamine and decreased phasic dopamine release in the NAc, an effect not seen in mice fed unsaturated fats [91]. Moreover, bingeing on high fat food decreases dopamine uptake while increasing phasic release and heightening D2 receptor sensitivity [93], showing that there is increased synaptic dopamine signaling following a HF binge. In the NAc core, dopamine clearance of obese high fat fed mice was diminished while striatal D2R gene expression was elevated [94]. In an obesogenic state, cerebral insulin transport and sensitivity are blunted [95, 96 97], decreasing DAT uptake but increasing DAT surface expression [98 99]. Prolonged HFD consumption causes repeated overstimulation of dopamine receptors, diminishing dopaminergic neurotransmission [100 92 90].

Blood Glucose Regulation

Related to brain function

Diet-induced obesity leads to insulin resistance [101 102 103]. Insulin is secreted in accordance with body fat mass, crosses the blood brain barrier, and affects food consumption and body composition. Insulin receptors in the VTA and hypothalamus control food consumption [104]. In plasma and brain glucose levels at steady-state, individuals with obesity and T2DM have significantly reduced rises in brain glucose concentrations compared to lean controls during

hyperglycemia [105]. Glucose is the main source of energy in the brain, accounting for approximately 20% of total glucose consumption [106]. Neurons have the highest energy demands and need a constant supply [107]. There are glucose transporters (GLUTs) in neurons, astrocytes, and microglial cells, though astrocytes contain the highest amount and can act as metabolic sensors [108] [109] [110]. Magnetic resonance spectroscopy studies found cerebral glucose levels increase linearly in accordance with rising serum glucose [111] [112], with an approximate 30-minute delay relative to blood glucose [113]. The energy produced from glucose metabolism in the brain is used for two primary neuronal functions: action potentials and postsynaptic potentials. This is done through maintenance of ion gradients within neuronal cells that modulate resting membrane potential [114]. Glucose utilization is reduced in projection zones of inhibitory neurons but increased in zones of excitatory neurons[115]. Additionally, glucose supplies energy for neurotransmitter release and can be a precursor in neurotransmitter synthesis [108]. Glucose has also been found to modulate learning and memory in the hippocampus via activation of neurotrophic factors [116]. Moreover, poorly controlled T2DM can cause cognitive decline due to mediators such as oxidative stress, inflammation, dyslipidemia and more [117] [118].

Glucose-sensing neurons

In the CNS there are glucose-sensing neurons that play a role in regulating cerebral glucose concentrations and subsequent metabolic demands. Originally discovered in the hypothalamus [119] [120], they have also been found in other brain regions [121]. These glucose-sensing neurons are hypothesized to influence food intake and cessation and regulate responses to changes in cerebral glucose. They also regulate thermogenesis, β cell proliferation, insulin and glucagon secretion, as well as glucose-seeking behavior [122]. Glucose-sensing neurons modify their electrical activity in response to changes in extracellular glucose. Glucose-excited (GE)

neurons increase their activity when glucose rises, while glucose-inhibited (GI) neurons increase their activity when glucose levels decrease. Data from various studies suggest that changes in glucose above or below 2.5 mM are detected by these neurons [123] [124] [125]. Researchers found that orexin, NPY, and GABA neurons in the lateral hypothalamus are GI [126] [127], whereas melanin-concentrating hormone (MCH) neurons are GE [128]. Additionally, pro-opiomelanocortin (POMC) neurons that are the functional antagonist of NPY/AgRP neurons, are believed to be GE [129]. Non-neuronal cells may also play a role in detecting glucose levels. Astrocytes can directly sense nutrients or metabolically partner with neighboring neuronal cells [130] [131]. Ultimately, multiple mechanisms for glucose-sensing exist in the CNS and though not all have been characterized, they likely have significant roles in glucose homeostasis and overall metabolic outcomes.

Can the NAc be glucose-responsive?

As previously discussed, dopamine is involved in controlling motivated and habitual behavior, including hedonic responses to palatable foods. The possibility of the NAc being responsive to changes in glucose that alter dopamine signaling is likely, given that neurons in the core and shell have been reported to be glucose responsive and they account for approximately 25% of neurons [132]. This response is likely to be impaired in diet-induced obesity, as illustrated by three days of high fat diet consumption suppressing brain glucose uptake by downregulating GLUT1 expression, independent of blood glucose levels [133]. Therefore, this begs the question of whether impairments in glucose-sensing in the NAc can affect dopamine neurotransmission. In order to evaluate whether a HFD can impair the glucose-sensing capabilities of neurons in the NAc and ultimately influence dopamine transmission, we will manipulate glucose levels of aCSF during *ex vivo* fast scan cyclic voltammetry and measure dopamine release and reuptake in high-fat fed animals compared to control.

Conclusion

Understanding how dopamine pathways regulate motivation and habitual behavior in relation to food is vital to understanding the underlying causes of obesity and obesity-related metabolic dysfunction resulting from excessive or compulsive food intake. Evidence has been presented that obesity caused by elevated intake of saturated fat is linked with changes in dopamine neurotransmission and that these findings are important given that dopamine neurons contribute to motivation and food behavior by modulating synaptic dopamine in the NAc. Moreover, obesogenic weight gain and impaired glucose metabolism are hallmarks of metabolic syndrome in humans and diet-induced obesity models in rodents. Mice consuming high fat diets exhibit higher fasting blood glucose levels, and previous reports from our lab show impaired synaptic dopamine clearance and reduced dopamine release in high fat-fed mice [91]. Thus, CSF glucose concentration could be important to dopaminergic neurons given that mice fed a high fat diet have elevated serum glucose levels, and CSF glucose is reflective of plasma levels. Extended hyperglycemia could affect CSF glucose over time and potentially result in neuroadaptation and changes in dopamine signaling. Understanding how CSF glucose levels affect dopaminergic neurons in mice fed diets high in saturated fats is of importance to evaluate the extent to which neurological function is impacted in obesogenic states. Given that reduced dopamine uptake rate in hyperglycemic high-fat-fed mice can be restored by a 12-hr fast [134], testing whether changes in blood glucose levels resulting from energy state may be affecting synaptic dopamine regulation is warranted. Further, identifying the effects of localized glucose concentrations on dopamine signaling can help shed light on the impact of diet on neurological function and further elucidate on factors influencing dopamine signaling and subsequent food intake.

CHAPTER II: THE EFFECTS OF ARTIFICIAL CEREBROSPINAL FLUID GLUCOSE CONCENTRATION ON DOPAMINE NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS

Introduction

Diets high in saturated fat play a significant role in the development of metabolic syndrome and are associated with increased total body and trunk fat deposition [135]. In rodents, high dietary fat consumption leads to development of phenotypes similar to metabolic syndrome that are characterized by weight gain, insulin resistance, and chronic inflammation [18 136 137]. Hyperglycemia and insulin resistance are commonly associated with obesity and result in an inability to effectively modulate elevated blood glucose levels. There are glucose sensors in the brain and peripheral regions that are responsible for coordinating autonomic and neuroendocrine responses to physiological changes in plasma glucose [2]. Evidence also suggests that lowering blood glucose with an overnight fast can change the kinetics of dopamine neurotransmission [134]. Obesity caused by elevated intake of saturated fat is linked with changes in dopamine neurotransmission, specifically in the nucleus accumbens (NAc). Dopamine release in the NAc core and shell is stimulated following consumption of palatable foods[138], and has been illustrated in clinical models where dopamine dysregulation is implicated in hedonic food intake [139]. Mice consuming high fat diets exhibit higher fasting blood glucose levels, and previous reports from our lab show impaired synaptic dopamine clearance and reduced dopamine release in high fat-fed mice [91]. Interestingly, a 12-hr fast restored dopamine uptake rate [134] , suggesting metabolic

state could impact synaptic control of dopamine, and highlighting a role for glucose potentially impacting dopaminergic neuronal function.

Dopaminergic signaling after food ingestion signals the nutritive value of the food consumed [71] [72]. Obesity has been associated with reduced dopaminergic function [73] [74], suggesting that impaired striatal dopamine leads to compensatory overeating [75] [76] [77] and ultimately, obesity. Rats with diet-induced obesity display lower basal and evoked dopamine levels in the NAc[78]. *Ex vivo* fast scan cyclic voltammetry (FSCV) in mice fed a diet high in saturated fats showed reduced clearance of synaptic dopamine and decreased phasic dopamine release in the NAc, an effect not seen in mice fed unsaturated fats [91]. Moreover, bingeing on high fat (HF) food decreases dopamine uptake rate while increasing phasic release and heightening D2 receptor sensitivity [93], showing that there is increased synaptic dopamine signaling following a HF binge. Ultimately, prolonged consumption of HF food causes increased sensitivity of dopamine D2 autoreceptors that diminish dopaminergic neurotransmission, possibly serving as a countermeasure to increased synaptic dopamine [100] [92] [90].

Glucose is the main source of energy in the brain, accounting for approximately 20% of total body glucose consumption [106]. Neurons have the highest energy demands and therefore need a constant supply [107]. There are glucose transporters (GLUTs) in neurons, astrocytes, and microglial cells, though astrocytes contain the highest amount and can act as metabolic sensors [108] [109] [110]. Moreover, glucose-responsive cells in the NAc change synaptic dopamine by modulating their firing rates in response to changes in glucose [132], and have been linked to rapid changes in food intake control [140]. Previous work published by our lab showed that in a fasted state, high-fat fed animals displayed increased dopamine uptake rate as well as increased dopamine release, in a manner similar to low-fat fed animals [134]. Essentially, a 12-hour fast was

able to normalize previously established dopamine transmission abnormalities in HF fed mice by increasing phasic release and uptake rate, suggesting that metabolic status can impact dopamine release. Neurons in the core and shell were first reported to be glucose responsive by Papp et al, and were found to account for approximately 25% of neurons in the NAc [132]. This response is likely to be impaired in diet-induced obesity, as illustrated by three days of HF diet consumption suppressing brain glucose uptake by downregulating GLUT1 expression, independent of blood glucose levels [133]. A gap in the literature is whether impairments in glucose availability in the NAc can affect dopamine neurotransmission. In order to evaluate whether a HF diet can impair the glucose-responsive capabilities of neurons in the NAc and ultimately influence dopamine transmission, we manipulated glucose levels of artificial cerebrospinal fluid (aCSF) during *ex vivo* fast scan cyclic voltammetry and measured resulting dopamine release and uptake rate in HF fed animals and low-fat (LF) fed controls. We hypothesized that manipulating aCSF glucose concentrations would induce changes in dopamine signaling in the NAc, where brain slices from HF fed mice would exhibit increased dopamine release and faster uptake rate when placed in hypoglycemic 3mM glucose aCSF, whereas LF brains placed in hypoglycemic aCSF would display lesser effects on synaptic dopamine kinetics. The reasoning for this is that previous work from our lab showed that fasting enhanced dopamine release and uptake rate in the NAc core, specifically increasing release and uptake rate in high-fat-fed males [134]. This suggests that the NAc is responsive to energy state and that a HF diet shifts dopaminergic signaling, which could be impacted by fasting due to reducing blood glucose levels [141], which would subsequently impact brain glucose.

Methods

Animals and Diet

Six-week old male (n = 12) and female (n = 12) C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in groups of three per cage on a reversed 12-hour light/dark cycle (lights off 0600, lights on 1800). Mice were given free access to water and a purified nutrient-matched diet with 10% (LF, n=12; 6 males, 6 females) or 60% (HF, n=12; 6 males, 6 females) kcals from fat, densities of 3.8 and 5.2 kcals/g (DIO series D12450J and D12492, Research Diets Inc.). Body weight data were recorded once a week with food intake monitored and refreshed bi-weekly. The amount of diet consumed was calculated by food disappearance measurements averaged over the number of mice in each cage. Mice remained on their respective diets for 6 weeks prior to experimental tests. All experiments conducted were in compliance with the University of North Carolina at Greensboro Animal Care and Use Committee.

Intraperitoneal Glucose Tolerance Test

Following completion of the 6-week dietary protocol, all mice underwent an intraperitoneal glucose tolerance test (IPGTT) to measure glucose clearance as proxy for metabolic dysfunction. Briefly, mice were placed in clean cages two hours into the dark cycle with ad libitum access to water, but no food, for a 12-hr fast. Blood glucose levels were then measured from the tail vein using a glucometer and blood glucose test strips (CVS Pharmacy, Woonsocket, RI) to establish fasting blood glucose levels. Next, an i.p. bolus of glucose (2 g/kg in 20% w/v saline) was delivered and blood glucose measurements were performed at 15, 30, 60, and 120 min thereafter.

Fast Scan Cyclic Voltammetry

FSCV was used to measure dopamine release and uptake rate in the NAc core and shell. FSCV was performed a minimum of three days post IPGTT and began 3 hours into the dark cycle. Mice were anesthetized using 5% isoflurane prior to removing the brains. Brains were then sectioned into 300 μm thick coronal brain slices using a compresstome (Precisionary Instruments; Greenville, NC) in oxygenated (95% O_2/5% CO_2) aCSF (in mM, for 11mM: 126 NaCl, 25 $NaHCO_3$, 11 D-glucose, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 0.4 L-ascorbic acid; for 3mM: 126 NaCl, 25 $NaHCO_3$, 3 D-glucose, 8 sucrose, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 0.4 L-ascorbic acid). All slices equilibrated for 60 min at 37^oC with oxygenated aCSF flowing at 2 mL/min. Voltammetric recordings used a triangular waveform that scanned from −0.4 to +1.2 and back to −0.4 V vs. Ag/AgCl at 400 V/s every 100 ms. The scanning voltage was applied to a carbon fiber electrode (100-150 μM length, 7 μM diameter (Goodfellow, C005722; Huntingdon, UK) placed at a depth of ~75 μM into the NAc core. Dopamine release was evoked with a single 4 ms pulse stimulation (1p) or a train of five pulse stimulations at 20hz (5p20hz) (monophasic, 350 μA) from a bipolar stimulating electrode (Plastics One, Roanoke, VA, 8IMS3033SPCE) every 3 min. Data reported are stable baseline recordings using the criteria of three consecutive 1p recordings with <5% variation in nA peak height. Recordings typically stabilized 60 min after collections began. Once a stable 1p baseline was established, a 5p20hz stimulation was collected from the same location to mimic physiological burst firing. Subsequently, 1p baseline was established again, after which slices were switched from standard aCSF (11mM) over to 3mM hypoglycemic aCSF. After a 60-minute perfusion followed by a 5p20hz stimulation, slices were switched back to standard aCSF and collections continued for a 30-minute washout period with subsequent 5p20hz stimulation.

All recordings were obtained from the NAc core or shell. Dopamine signals were acquired and kinetically modeled using Demon Voltammetry Software, based on Michaelis–Menten kinetics, holding the Km at 160 nM with the assumptions that Km in mice is similar to the well-documented affinity of dopamine for the DAT in rats [142], and that sex or diet does not alter Km.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism (v. 9.4.0). T-tests were used to determine changes in body weight between treatment groups over time without taking sex into consideration, while two-way ANOVAs or mixed-effects analysis were used to analyze bodyweight, food intake, and fasting glucose differences between male and female groups. Two-way ANOVAs were used to analyze fasting blood glucose and area under the curve data for IPGTTs between male and female groups. Subsequently, two-way ANOVAs were used to determine effects of glucose changes in aCSF on dopamine release and uptake rate. Two-way ANOVAs or mixed-effects analysis were used to detect sex effects of hypoglycemic aCSF exposure on dopamine release and uptake rate. All post-hoc analyses utilized Tukey's or Sidak's multiple comparisons tests where appropriate. Group data are presented as means ± standard error of the mean; statistical significance was set at $p \leq 0.05$.

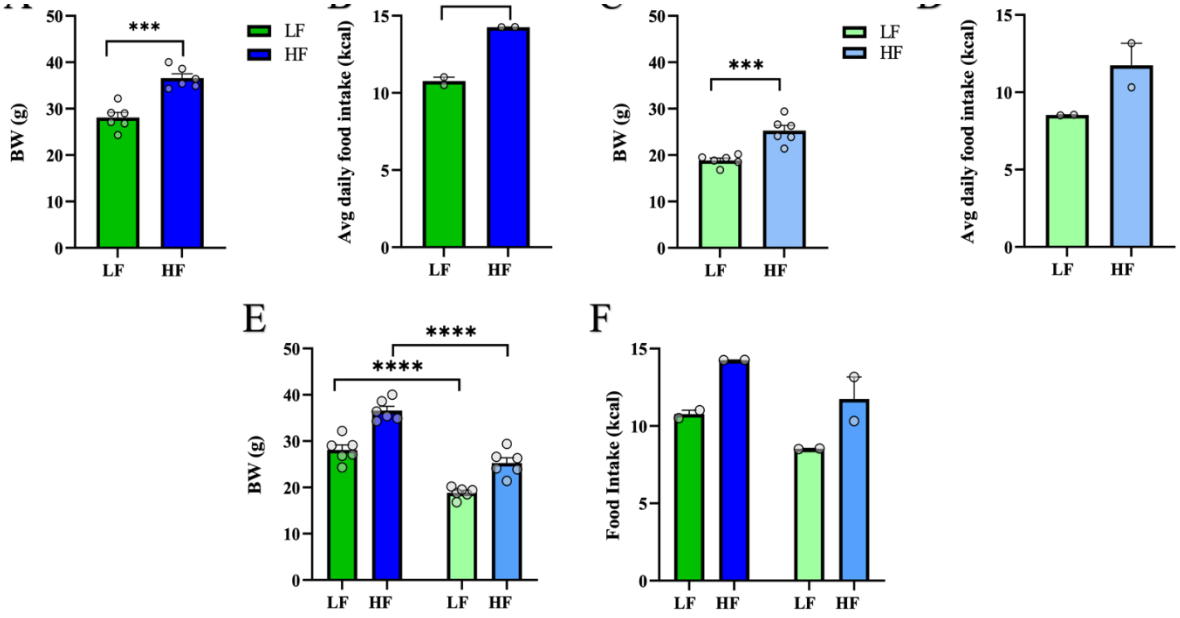
Results

Food intake and body weight

Male mice fed a HF diet consumed significantly more calories (kcals) per day than LF males (HF (n = 6): 14.26 ± 0.26 g/d, LF (n = 6): 10.76 ± 0.26 g/d; $p < 0.005$) (Figure 1-B). In contrast, there was no difference in average daily food intake between females fed either diet (Figure 1-D). Following completion of the 6-week diet intervention, HF males gained significantly more body weight than LF males (HF: 36.60 ± 1.43 g, LF: 28.08 ± 1.43 g; p <

0.0001), with the same trend observed in females (HF (n = 6): 25.28 ± 1.22 g, LF (n = 6): 18.87 ± 1.22 g; $p < 0.0004$) (Figure 1-A, 1-C). No difference in caloric intake was observed between LF and HF females; however, the final body weight of HF females was significantly higher than LF controls, similar to males. Moreover, a two-way ANOVA identified a significant effect of dietary treatment on body weight ($F_{(1,10)} = 58.04$; $p < 0.0001$), as well as sex ($F_{(1,10)} = 130.9$; $p < 0.0001$) (Figure 1-E). Sidak's multiple comparisons test revealed a significant difference in body weight between LF males and females (Males: 28.08 ± 1.33 g; Females: 18.87 ± 1.33 g; $p < 0.0001$). A significant difference in body weight between HF males and females was also observed (Males: 36.60 ± 1.33 g; Females: 25.28 ± 1.33 g; $p < 0.0001$), and both LF and HF males had higher body weights than females. A significant effect of diet on food intake between males and females was identified by two-way ANOVA ($F_{(1,2)} = 10.96$; $p = 0.04$) (Figure 1-F).

Figure 1 - Body Weight and Food Intake



(A) Male mice fed a HF diet (n = 6) had a final body weight that was significantly higher than those in the LF group (n = 6). **(B)** HF males consumed significantly more kilocalories (kcals) on average than LF males. **(C, D)** While HF females (n = 6) did not consume significantly more kcals on average than LF females (n = 6), they did gain significantly more weight. **(E)** Diet had a significant effect on body weight in males compared to females ($p < 0.0001$); there was a significant effect of sex on body weight ($p < 0.0001$). Males had higher body weights than females, with HF males having a significantly higher body weight than HF females. **(F)** No significant difference in average daily food intake was found between males and females.
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; (A-D) Paired t-test; (E, F) Two-way analysis of variance, Sidak's multiple comparisons test)

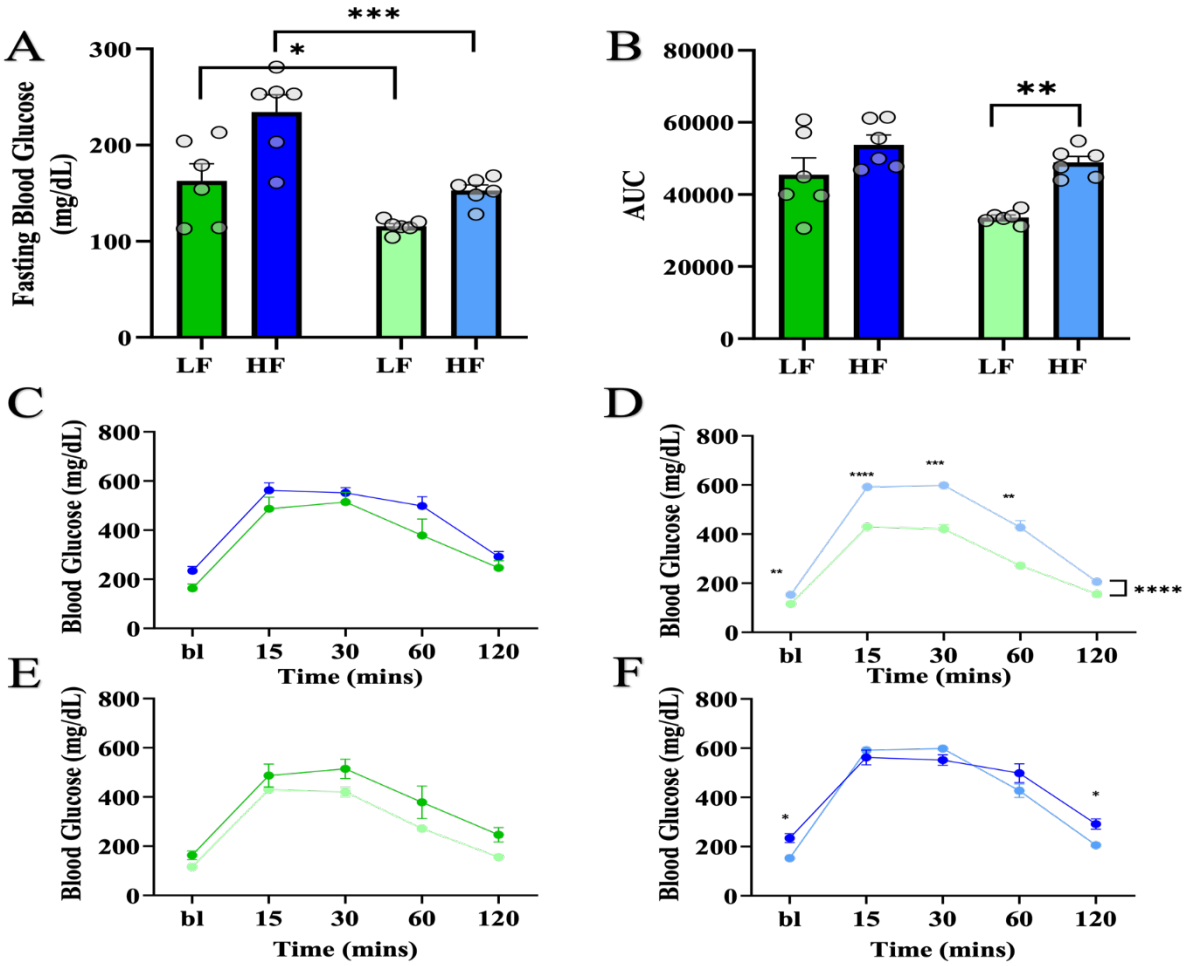
Blood Glucose Regulation

To examine the metabolic phenotypes, fasting blood glucose and blood glucose clearance were measured using IPGTT. Fasting blood glucose was significantly elevated in HF males compared to LF controls (HF: 234.3 ± 25.22 mg/dL; LF: 162.8 ± 25.22 mg/dL; $p = 0.018$) (Figure 2-A), with the same trend observed in females (HF: 152.8 ± 6.40 mg/dL; LF: 115.7 ± 6.40 mg/dL; $p = 0.0002$) (Figure 2-A). A significant effect of sex was also identified in fasting blood glucose levels between males and females ($F_{(1,10)} – 35.33$; $p = 0.0001$), in addition to diet ($F_{(1,10)}$ n= 13.34; $p = 0.004$). Analysis of glucose area under the curve (AUC) revealed impaired

glucose clearance in HF females, but not males, compared to LF controls (HF, females: 48871 ± 1837; LF, females: 33638 ± 1837; $p < 0.0001$; HF, males: 53789 ± 5378; LF, males: 45521 ± 5378) (Figure 2-B). Moreover, AUC for males versus females identified significant effect of diet ($F_{(1,10)} = 21.47$; $p = 0.0009$) and sex ($F_{(1,10)} = 7.26$; $p = 0.023$), though only HF females had significantly higher AUC than low fat controls ($p = 0.003$). Mixed effects analysis identified a significant effect of time ($F_{(1.992, 19.42)} = 79.03$; $p < 0.0001$) in blood glucose levels measured during IPGTT in male mice (LF: 427.7 ± 37.66 mg/dL; HF: 360.4 ± 37.66 mg/dL) (Figure 2-C). Tukey's post hoc analysis found a significant increase in blood glucose levels from baseline ($p < 0.0001$), 15 min ($p < 0.0001$), 30 min ($p < 0.0001$), 60 min ($p = 0.0002$) and 120 min ($p = 0.004$) time points in both diet treatment groups. Two-way ANOVA analysis of IPGTT in females revealed a significant effect of time ($F_{(2.525, 25.25)} = 477.5$; $p < 0.0001$) and dietary treatment ($F_{(1,10)} = 109.8$; $p < 0.0001$), as well as a time x dietary treatment interaction ($F_{(4,40)} = 17.33$; $p < 0.0001$) (Figure 2-D). Moreover, Sidak's test revealed that HF females had significantly higher blood glucose levels than LF females at baseline ($p = 0.03$), 15 min ($p = < 0.0001$), 30 min ($p = 0.001$), and 60 min ($p = 0.01$). A two-way ANOVA analysis of males versus females revealed a significant effect of sex between LF groups ($F_{(1,10)} = 6.169$; $p = 0.03$) as well as time ($F_{(2.021, 19.70)} = 106.1$; $p < 0.0001$), with LF males exhibiting significantly higher blood glucose levels from baseline ($p < 0.0001$), 15 min ($p < 0.0001$), 30 min ($p < 0.0001$), 60 min ($p = 0.0012$), and 120 min ($p = 0.0126$) (Figure 2-E). A significant effect of time was identified between HF males and females ($F_{(2.134, 21.34)} = 218.6$; $p < 0.0001$), as well as a time x sex interaction ($F_{(4, 40)} = 7.043$; $p = 0.0002$) in blood glucose levels. Sidak's multiple comparisons test showed HF males had significantly higher blood glucose levels than HF females at baseline (Males: 234.3 ± 18.85

mg/dL; Females: 152.8 ± 18.85 mg/dL; p = 0.02) and 120 min (Males: 291.8 ± 25.46 mg/dL; Females: 205.5 ± 25.46 mg/dL; p = 0.04) (Figure 2-F).

Figure 2 - Blood Glucose Regulation

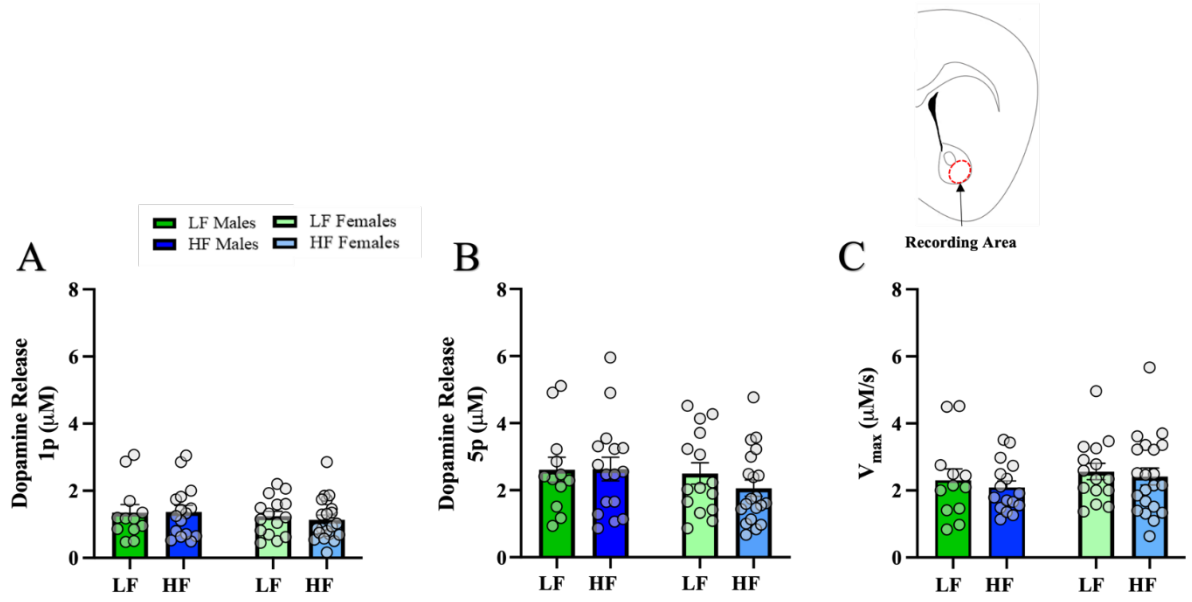


(A) Fasting blood glucose was significantly elevated in both males and females consuming a high fat diet as compared to controls fed a low-fat diet. Moreover, males displayed higher blood glucose levels than females, regardless of dietary treatment. **(B)** Glucose area under the curve was significantly elevated in HF females compared to LF, and no significant difference was found in males regardless of diet treatment. **(C)** A significant effect of time was identified in male blood glucose levels measured during IPGTT, with significant differences between all time-points. **(D)** HF females showed significantly reduced blood glucose clearance compared to low fat controls, indicating greater metabolic dysfunction. **(E)** Among LF males and females, significant differences in blood glucose levels measured during IPGTT were found, with males displaying higher glucose levels than females. **(F)** No significant effect of sex was identified in HF groups, but blood glucose clearance was significantly lower in HF females at baseline and 120 min (p = 0.02 and 0.04, respectively).
(*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; (A, B) Two-way ANOVA, Tukey's post hoc; (C) Mixed-effects analysis, Tukey's post hoc; (D) Two-way ANOVA, Sidak's multiple comparisons test; (E, F) Two-way ANOVA, Sidak's multiple comparisons test)

Dopamine release and uptake rate in the NAc core

Ex-vivo slice voltammetry was utilized to characterize differences in dopamine release and uptake rate in the NAc core and shell following 6-week exposure to respective diets. Two-way ANOVAs revealed no significant differences in single pulse (1p) baseline dopamine release in the NAc core among males or females (1.35 ± 0.31 μM, LF males; 1.37 ± 0.31 μM, HF males; 1.25 ± 0.3 μM, LF females; 1.13 ± 0.3 μM, HF females) (Figure 3-A). Furthermore, no significant differences in baseline dopamine release using five pulse stimulation trains at 20hz (5p20hz) were found among males or females in any treatment group (Figure 3-B). No difference was observed in dopamine uptake rate (Vmax) among either males or females (Figure 3-C).

Figure 3 - Baseline 1p release, 5p release, and Vmax in the NAc core of males and females

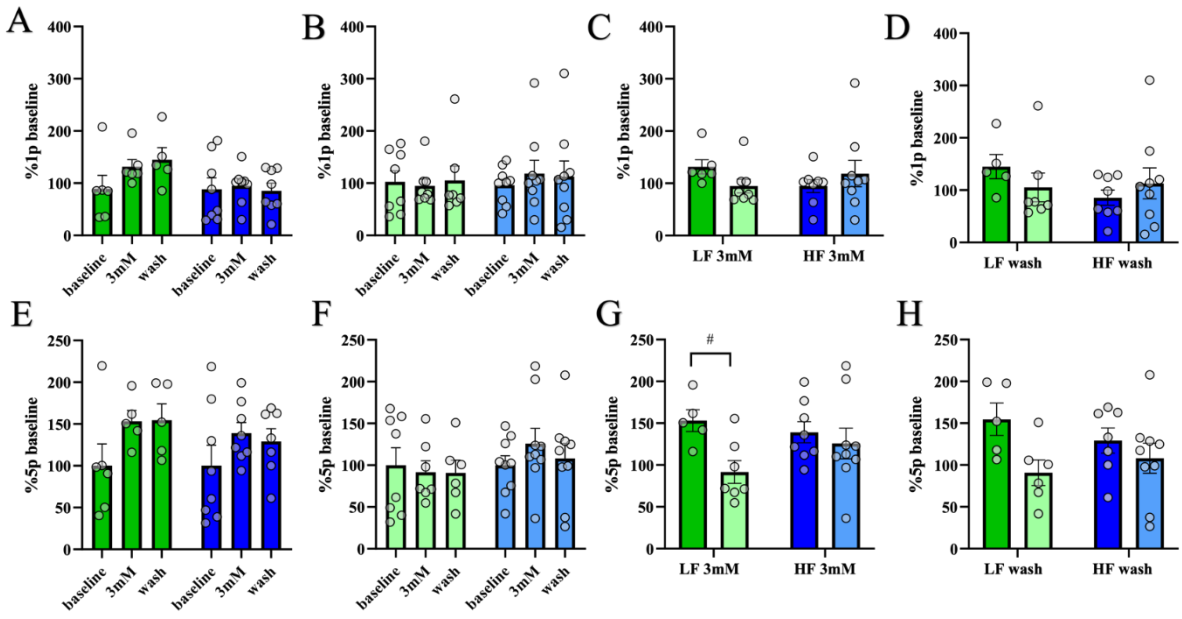


Ex-vivo fast scan cyclic voltammetry was performed in brain slices containing the nucleus accumbens core to characterize differences in dopamine release and uptake rate (Vmax). **(A)** Analysis of dopamine release evoked by a single pulse stimulation (1p) in males and females revealed no significant differences. **(B)** 5p20hz baseline dopamine release was not significantly different in males or females, regardless of dietary treatment. **(C)** No significant differences in Vmax within male groups or females was found, though females tended to trend slightly higher than males.
((A-C) Two-way ANOVA)

Analysis of the change from baseline caused by exposure to hypoglycemic aCSF and washout revealed a significant effect of diet in males ($F_{(1, 35)} = 4.34$; $p = 0.04$) (Figure 4-A). In females, no significant effects of diet or glucose on 1p release subsequent to 3mM exposure and washout were observed (Figure 4-B). No effect of 3mM aCSF was observed on 1p dopamine release in males or females (Figure 4-C, D). A significant main effect of glucose concentration was observed on 5p20hz release after exposure to 3mM and washout in males ($F_{(2, 21)} = 3.53$; $p = 0.05$) (Figure 4-E). 3mM glucose exposure significantly increased 5p20hz release in LF and HF males, which persisted through washout, although no post-hoc differences were detected between timepoints. No main effects of glucose or diet on 5p20hz release were observed in females, regardless of diet treatment (Figure 4-F). For 5p20hz release after 3mM exposure, a

main effect of sex was found between LF males and females ($F_{(1, 25)} = 5.49$; $p = 0.03$) (Figure 4-G). Furthermore, Sidak's test revealed dopamine release was significantly higher in LF males than in females after exposure to 3mM aCSF (Males: 153.2 ± 18.85%; Females: 91.63 ± 18.85%; $p = 0.04$). A main effect of sex was identified in 5p20hz release after washout in males and females ($F_{(1, 23)} = 5.70$; $p = 0.03$) (Figure 4-H). Interestingly, male dopamine release trended higher in comparison to females, particularly in LF males, though not significant.

Figure 4 - Changes in dopamine release resulting from exposure to 3mM aCSF and washout in NAc core of males and females

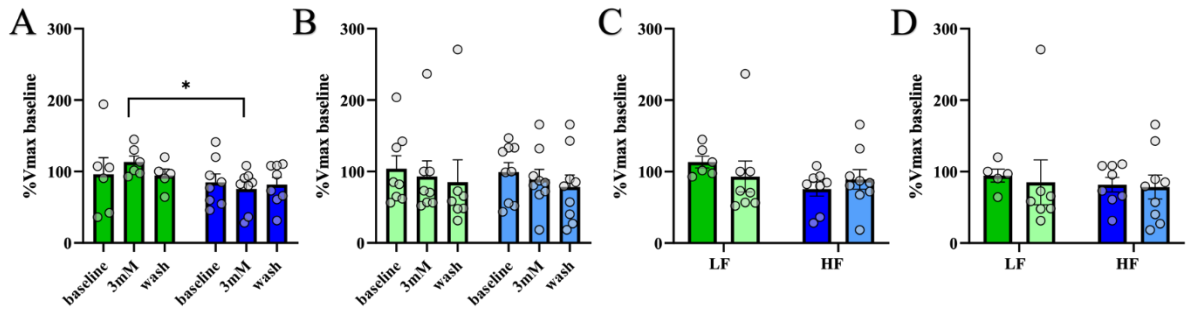


The change of dopamine release from baseline caused by exposure to hypoglycemic aCSF and subsequent washout was calculated for male and female data. **(A)** Diet had a significant effect on 1p dopamine release in males ($p = 0.04$) after 3mM exposure and washout, with LF males trending higher than HF males. **(B)** Females showed no significant differences in 1p release after exposure to 3mM aCSF and washout. **(C)** No significant differences in single pulse dopamine release were observed after exposure to 3mM aCSF in males and females. **(D)** LF groups showed greater tonic release in the core than HF groups after exposure to washout. **(E)** Phasic dopamine release was enhanced in both LF and HF males after exposure to 3mM glucose and washout. **(F)** Only HF females showed slight enhancements in phasic release after treatment with 3mM glucose. **(G)** LF males showed significantly greater phasic dopamine release than females after 3mM glucose exposure. **(H)** While LF males trended highest for phasic release, exposure to washout showed no significant differences between groups.
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; (A-H), Two-way ANOVA; (G) Sidak's multiple comparisons test)

No significant effects of diet or glucose concentration were found for change in Vmax following 3mM aCSF exposure and washout in males. However, Sidak's test showed Vmax was significantly increased in LF males compared to HF after 3mM exposure (113.4 ± 2.91% and 75.54 ± 2.91%, respectively; $p = 0.04$) (Figure 5-A). In females, no significant effects of glucose or diet were observed on Vmax (Figure 5-B). Analysis of change in Vmax following exposure to 3mM in males and females revealed no significant differences between groups (Figure 5-C).

Lastly, no significant differences in Vmax after washout were observed between groups (Figure 5-D).

Figure 5 - Changes in uptake rate resulting from exposure to 3mM aCSF and washout in NAc core of males versus females

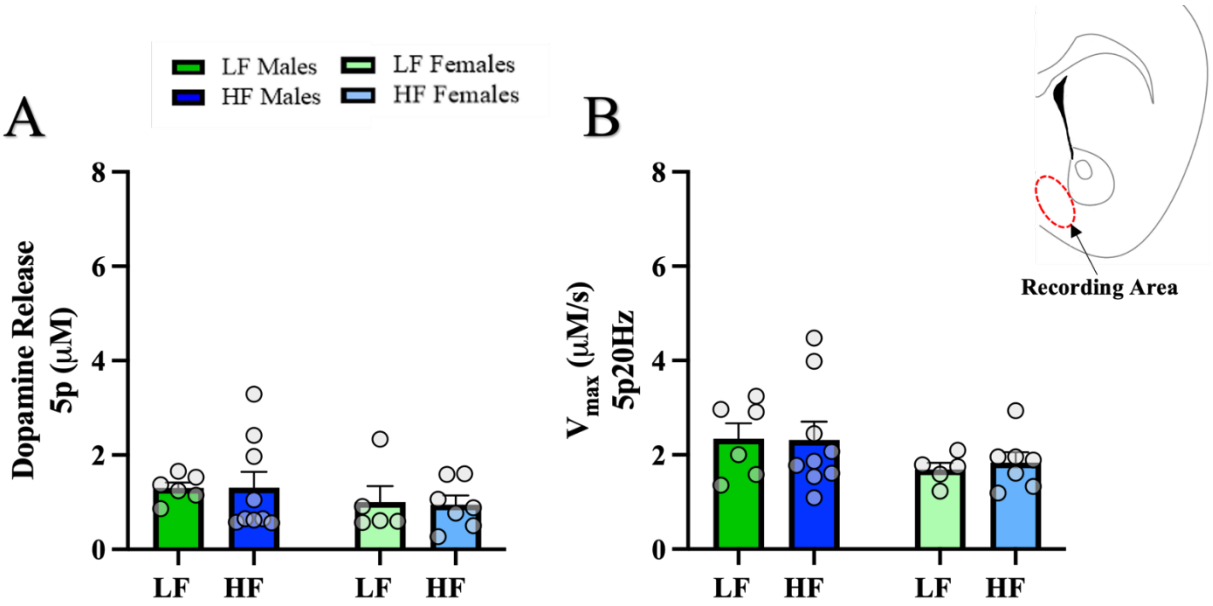


(A) In LF males, Vmax was significantly higher than in HF males after exposure to 3mM aCSF. **(B)** No significant effects of glucose or diet were observed on Vmax in females. However, LF females trended higher than HF females. **(C)** Though not significant, LF groups showed faster dopamine uptake rate subsequent to 3mM exposure compared to HF groups. **(D)** No significant difference in dopamine uptake rate following exposure to washout were observed. Despite some data variability, HF females seemed to trend lower compared to other groups.
(*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; (A-C) Two-way ANOVA; (A) Sidak's multiple comparisons test)

Dopamine release and uptake rate in the NAc shell

In the NAc shell, all dopamine release data was collected using five pulse stimulations trains at 20hz (5p20hz). No significant differences in baseline 5p20hz dopamine release was found between males and females, regardless of dietary treatment (Figure 6-A). Furthermore, no significant effects of sex or diet were identified in baseline Vmax between groups (Figure 6-B).

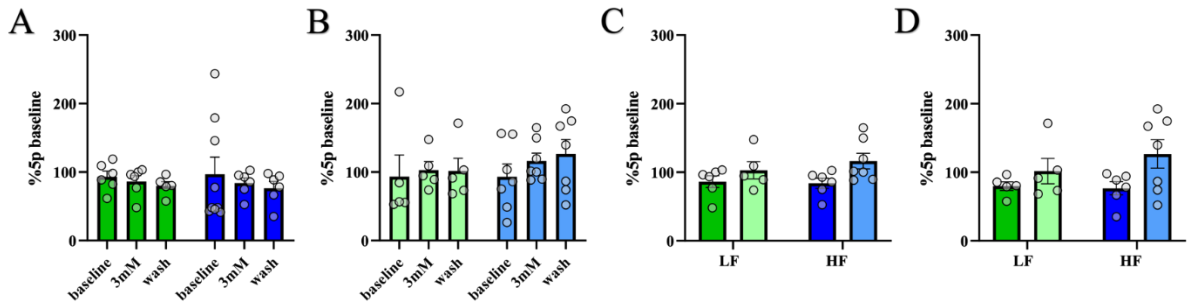
Figure 6 - Baseline dopamine release and uptake rate in the NAc shell of males and females



(A) No significant differences in baseline 5p20hz dopamine release were observed in the NAc shell of males and females, regardless of dietary treatment. However, males groups trended higher in dopamine release than females. **(B)** Neither diet nor sex had significant effects on baseline Vmax in the shell. Male groups showed a trend of faster uptake rates compared to females, however.
((A, B) Two-way ANOVA)

Analysis of the change from baseline caused by exposure to hypoglycemic 3mM aCSF and washout in the shell revealed no significant effects of glucose or diet on 5p20hz dopamine release in the NAc shell of male mice (Figure 7-A) or females (Figure 7-B). However, HF females showed increases in dopamine release that were sustained at washout, a trend not seen in LF females. Mixed-effects analysis of exposure to 3mM aCSF in males and females revealed a significant effect of sex on dopamine release ($F_{(1,11)} = 5.62$; $p = 0.04$) (Figure 7-C). After washout, no significant differences in dopamine release between males and females were detected (Figure 7-D).

Figure 7 - Change from baseline resulting from exposure to 3mM aCSF and washout in NAc shell of males and females

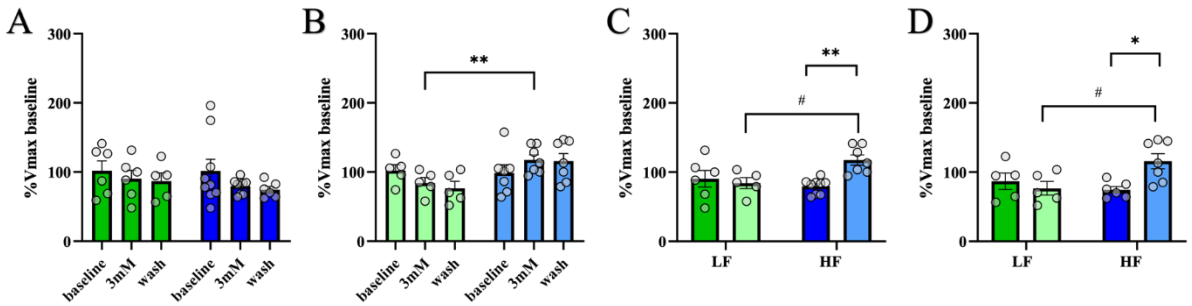


(A) In male mice, no significant differences in dopamine release were detected after exposure to 3mM aCSF and washout. **(B)** Similarly, females also showed no significant effects of hypoglycemia on 5p20hz dopamine release. However, HF females showed slight enhances in dopamine release after exposure to hypoglycemia and washout, a trend not observed in LF females. **(C)** A significant effect of diet ($p = 0.04$) on dopamine release was observed between males and females after exposure to hypoglycemia. HF females trended higher than males, though this was not significant. **(D)** There were no significant differences in dopamine release after exposure to washout. However, HF females showed noticeably enhanced phasic release compared to all other groups. ((A-D) Two-way ANOVA)

Analysis of the change from baseline caused by exposure to hypoglycemic 3mM aCSF and washout in the shell revealed no significant effects of glucose or diet on Vmax in the NAc shell of male mice (Figure 8-A). In females, however, two-way ANOVA analysis identified a significant glucose concentration x diet interaction ($F_{(2, 20)} = 7.73$; $p = 0.003$), as well as a significant effect of subject ($F_{(10, 20)} = 6.76$; $p = 0.002$) (Figure 8-B). Moreover, Sidak's multiple comparisons test revealed HF females had significantly higher Vmax than LF females after exposure to 3mM aCSF (HF: 117.5 ± 10.75% ; LF: 84.2 ± 10.75%; $p = 0.04$). This increase in Vmax was sustained after washout in HF females, but not lean controls. Mixed-effects analysis of males and females after exposure to 3mM revealed a significant interaction of diet x sex on Vmax ($F_{(1, 23)} = 8.23$; $p = 0.01$) (Figure 8-C). Further, LF females had significantly slower Vmax than HF females after 3mM (LF: 84.2 ± 10.75% ; HF: 117.5 ± 10.75% ; $p = 0.04$). In contrast, HF females had significantly higher Vmax than HF males (Females: 117.5 ± 9.84%; Males: 79.66 ± 9.84%; $p = 0.002$). Additional analysis of Vmax in males and females after exposure to washout revealed a significant diet x sex interaction ($F_{(1,19)} = 6.79$; $p = 0.02$) (Figure 8-D).

Moreover, Sidak's test showed that LF females had significantly slower Vmax at washout compared to HF females (LF: 76.69 ± 13.73%; HF: 115.8 ± 13.73%; p = 0.02). HF females also displayed significantly faster Vmax at washout than HF males (Females: 115.9 ± 13.04%; Males: 74.44 ± 13.04%; p = 0.01).

Figure 8 - Change in Vmax after exposure to 3mM aCSF and washout in NAc shell of males and females



(A) In males, there were no significant differences in Vmax after exposure to hypoglycemic aCSF and washout, regardless of dietary treatment. In HF males, there was a slight decrease in Vmax, though not statistically significant. **(B)** HF females showed significantly faster dopamine uptake rate (Vmax) after exposure to 3mM aCSF compared to LF females. This enhancement seemed to be sustained throughout washout in HF females as well. **(C)** HF females had significantly faster Vmax after exposure to hypoglycemic aCSF compared to LF females (p = 0.04). Moreover, HF males had significantly slowly Vmax compared to HF females (p = 0.01). **(D)** HF females had significantly faster dopamine clearance at washout compared to LF females (p = 0.02). Furthermore, HF males had significantly reduced dopamine clearance at washout compared to HF females (p = 0.01).
(*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; (A, B) Two-way ANOVA, Sidak's multiple comparisons test; (C, D) Mixed-effects analysis, Sidak's multiple comparisons test)

Discussion

This study sought to evaluate metabolic phenotypes using a model of high saturated fat feeding and their effect on dopamine release and uptake rate in the nucleus accumbens via glucose-responsive cells. Interestingly, our glucose clearance data replicate previous studies in females, but the male HF group did not have delayed blood glucose clearance like we observed in other studies. Interestingly, this occurred despite significant weight gain in the HF male mice compared to the LF group. In contrast to historical data from our lab [143] [91] [144], a diet high in

saturated fat had no effect on baseline tonic (1p) or phasic (5p) dopamine release and uptake rate in the nucleus accumbens core and shell of either males or females. However, we report a novel observation of hypoglycemic conditions enhancing phasic release in the NAc core of HF animals without changing dopamine uptake rate, in addition to enhancing both tonic and phasic release in LF groups. Moreover, we demonstrated that hypoglycemic aCSF significantly increased phasic dopamine release in the core of LF males. In the NAc shell, dopamine uptake rate after exposure to 3mM and washout was significantly enhanced in HF females compared to LF, supporting the possibility of sexually dimorphic control of reward circuitry.

High-fat diet stimulates food intake and leads to obesogenic weight gain

Both males and females fed a high fat diet gained significantly more weight than their LF counterparts. This was consistent with previous work from our lab showing that increased saturated fat consumption stimulates weight gain [91 134 144]. However, a metabolic syndrome-like phenotype was only observed in HF-fed females. Both male and female HF groups had significantly elevated fasting blood glucose levels compared to LF groups, but reduced blood glucose clearance was only observed in HF females. Blood glucose clearance measured via IPGTTs is an indirect measure of insulin receptor function and is a well-established proxy to evaluate glucose homeostasis and metabolic impairments in rodent models [145 146]. Diets high in saturated fat have been shown to decrease insulin sensitivity [147] and promote insulin resistance via increased production of pro-inflammatory cytokines [137]. Furthermore, saturated fat can lead to increased levels of compounds such as TNFα and proinflammatory adipose tissue macrophages that can contribute to metabolic abnormalities [148]. The lack of impaired glucose clearance in males that contradicts historical data from our lab could be attributed to reported

differences in glucose metabolism, insulin sensitivity and lipogenesis, where females showed increased insulin sensitivity and lipogenic capacity compared to male mice [149] [150].

Dopamine release and uptake rate in the NAc core under changing glucose concentrations

It has been established that high saturated fat diets induce a phenotype of metabolic impairment related to insulin-resistance. We induced this impaired metabolic phenotype in females, but not males, despite both HF groups exhibiting excessive weight gain. Previous studies have demonstrated that this metabolic syndrome-like phenotype induced by saturated fat is affiliated with impaired dopamine neurotransmission [151] [134] [91]. Interestingly, we did not observe a reduction in phasic dopamine release (5p20Hz) or uptake rate (Vmax) that is historically seen with HF feeding. Typically, the HF diet has the largest effect on dopamine parameters in males, with less pronounced effects in females. It is possible that in our study the lack of metabolic impairment in the male HF group didn't impact dopamine neurotransmission as much as HF males in previous studies that had elevated fasting blood glucose and reduced glucose clearance. Phasic release places a high demand on energy metabolism, which is usually met through glucose utilization. One possible explanation for the lack of change in phasic release is that the HF males that didn't exhibit metabolic impairments still had sufficient glucose utilization in tissues to support neuronal glucose demands. Given that hypoglycemic 3mM glucose aCSF had an effect on phasic dopamine release when normal aCSF did not, suggests a negative energy state could preferentially enhance phasic dopamine and prime reward-seeking behaviors. Additionally, LF males displayed increases in tonic release after exposure to hypoglycemia, similar to how HF mice had increased phasic release after fasting for 12-hr in our previous study [134]. In contrast with past studies, our study suggests hypoglycemia could prime mice for tonic and phasic dopamine neurotransmission, enhancing cue-initiated motivation and

reward acquisition in that LF males would display more vigorous food seeking behavior in a negative energy state. This is supported by the theory that tonic dopamine release regulates motivational vigor, given that increased tonic release has been linked to enhanced responsivity [152] [153]. These results build on previous data from our lab, where a 12-hour fast was found to augment dopamine release in the NAc core and had a more pronounced effect on males [134].

The blunted treatment effect in HF females could be attributed to alterations in estrus cycling from consuming a high fat diet, given that 4 weeks on a high fat diet were sufficient to alter cycling until 8 weeks, after which estrus returned to normal [154]; where our study only provided treatment for 6 weeks. Our observation of sustained increases in phasic release following hypoglycemia are pertinent given that the NAc is densely populated with GLUT-1 transporters [155], has a high degree of glucose utilization [156], and has been reported to contain glucose-monitoring neurons that display changes in firing rates in response to changes in blood glucose levels or direct administration of glucose [132] [157]. Phasic burst firing is dependent on afferent input and occurs specifically in response to salient stimuli such as calorically dense, highly palatable foods [158]. Further, energy restriction diminishes dopamine tone [159] [160] and circulating insulin levels [161], potentially priming D1 and D2 receptors in the NAc. The observed increases in phasic dopamine release in response to a reduction in aCSF glucose have implications in driving motivation and consumption when salient stimuli are presented. Such enhancements could increase food attention and food seeking behaviors in a negative energy state, particularly the tendency to choose more palatable foods. Increased synaptic dopamine could correlate to greater sensitivity to reward and implicate food seeking by potentially counteracting blunted dopamine responses seen in HF diet models characterized by hedonic eating. Given that there is differential activation of dopamine receptors in relation to synaptic

dopamine, energy state could impact preferential activation of one receptor type over another. The enhanced tonic release observed in LF males could affect D2 dopamine receptors (D2DRs), as they have been found to be more sensitive to changes in tonic dopamine levels and have increased affinity for dopamine [59]. D2DRs function in an inhibitory fashion, decreasing the likelihood of a given medium spiny neuron (MSN) from firing and induces D2DR internalization. Thus, the changes in tonic release could have implications in reward association, given the theory that high reward association strength results in low D2DR expression [162]. This is pertinent to our study, as tonic dopamine levels have been found to increase in times of hunger [163], underscoring the importance of being able to select highly rewarded actions during times when nutrient needs are high.

Lastly, the observed changes in phasic dopamine release could be the result of glucose-responsive neurons in the NAc core [132] modifying dopamine terminals via cholinergic interneurons, which have been shown to strongly regulate striatal dopamine release via nicotinic acetylcholine receptors on dopamine axons that release acetylcholine and promote dopamine release [164 165]. Thus, hypoglycemia could enhance the dynamic shift in dopamine release from tonic to phasic, given that only phasic release was increased in HF males after exposure to 3mM glucose. This has relevance given that activation of D1DRs is dependent on a dynamic shift in synaptic dopamine concentration and phasic release primarily increases D1 receptor occupancy [59]. Activation of this 'direct pathway', therefore, would hypothetically make HF males more responsive to cues and lead to faster activation of D1 MSNs, perpetuating food-seeking behaviors.

Dopamine release and uptake rate in the NAc shell under changing glucose concentrations

The nucleus accumbens shell is a distinct subregion of the NAc characterized by various morphological and histochemical differences in comparison to the core, including cholinergic neuron density and membrane properties of medium spiny neurons [166]. Much like in the core, glucose-monitoring neurons have also been reported in the shell, albeit to a lesser degree [132]. Both the core and the shell have been linked to incentive salience (i.e., wanting) [167] [168]. We therefore sought to expand on previous work from our lab that showed no effect of fasting on dopamine release or uptake rate in the NAc shell [134]. No significant differences between diet treatment groups or sex were seen in baseline dopamine release or uptake rate in the shell. Interestingly, phasic release remained the same in LF groups after exposure to 3mM aCSF and washout, which is suggestive of neuronal energy requirements being met by other means. HF females had significantly enhanced Vmax compared to males after exposure to 3mM and washout, an effect not seen among LF groups. The lack of enhanced Vmax in males could be attributed to their lack of metabolic impairment. Given reports of heterogeneity of DAT function/expression and extracellular striatal dopamine between males and females [169], it is possible that the observed differences in treatment response are related to sex hormones distinctly affecting dopamine release, synthesis, and/or metabolism [170]. The lack of effect on phasic release after exposure to hypoglycemic conditions could be attributed to differential distribution of glucose-responsive neurons between the core and shell, with the core displaying more neurons responsive to glucose than the shell (29% versus 19%, respectively) [132]. As most glucose-responsive neurons discovered in the shell have been characterized as glucose-sensitive and are inhibited by glucose, this could account for the lack of change in dopamine release.

Conclusion

Overall, this study demonstrated that HF animals gain significantly more weight than LF animals following a 6-week dietary intervention. Moreover, hypoglycemic conditions enhanced phasic dopamine release in the NAc core of HF groups in a manner similar to LF animals. This suggests that in HF animals, neuronal energy requirements for phasic release were being met by other substrates. HF intake for 7 days was sufficient to induce fat oxidation equal to fat intake in humans [171] [172], suggesting that fat oxidation adapts to increases in fat content and that other substrates besides glucose may help meet energy requirements when HF animals are subjected to hypoglycemic conditions. LF males displayed enhanced tonic and phasic release following exposure to 3mM glucose, potentially due to glucose-responsive neurons in the core promoting dopamine release via nicotinic acetylcholine receptors on cholinergic interneurons. The lack of treatment effect in the core of females could also be suggestive of alterations in estrus cycling because of consuming a HF diet. In contrast, uptake rate in the shell was significantly accelerated by hypoglycemic 3mM glucose exposure, most notably in HF females. Given that this effect was not present in the shell of males regardless of treatment group, the possibility of sexually dimorphic anatomy regarding DAT function/expression exists. Ultimately, there are few sex comparisons evaluating energy state, diet, and obesity and their effects on dopamine neurotransmission. Our findings are suggestive of sex-based differences in metabolic circuitry and energy balance, as well as in response to changing glucose conditions, whereby enhancements in phasic release and uptake rate in the core and shell, respectively, could presumably improve satiety mechanisms and sensitivity to reward and cue reactivity.

CHAPTER III: DIFFERENCES IN DOPAMINE NEUROTRANSMISSION AND GLUCOSE-RESPONSIVENESS BETWEEN THE NUCLEUS ACCUMBENS CORE AND SHELL

Introduction

The nucleus accumbens serves as a key area in the brain's reward system and is divided into two major subregions, the core, and the shell. The NAc core and shell have been found to be both functionally and anatomically different, with the core comprising more densely packed cells and the shell composed of a looser cellular arrangement [173]. Differences in innervation between both regions have also been identified, with afferents from the ventral tegmental area (VTA) primarily innervating the shell [174] and a blend of the VTA and substantia nigra cell bodies innervating the core [175]. The primary output neurons from the NAc are medium spiny neurons (MSNs) that project to different areas of the brain. The core projects to the dorsolateral ventral pallidum, which in turn projects to the subthalamic nucleus and substantia nigra, the origin of dopaminergic innervation in the striatum. In contrast, the shell projects to the ventromedial ventral pallidum, in addition to the lateral hypothalamus [176]. As a result, the core and shell each play a role in mediating different behaviors. The NAc core is linked to food seeking and spatial learning, in addition to responses to motivational stimuli. Conversely, the shell has been found to play a role in satiety and responses to rewarding substances, as well as novel stimuli [177]. Foods rich in fats and sugar act as strong rewards that encourage eating when there is no energy requirement [29], as well as activating reward circuitry in the brain [30]. Moreover, mice fed a high fat (HF) diet exhibit dysfunctional dopamine release and reduced uptake rate in the NAc [177 91]. Additionally, neurons have the highest energetic demands in the brain, which could be impacted

by metabolic state, given that the brain can't recruit receptors in a temporal way in diet-induced obesity (DIO) as it would under normal metabolic conditions [108]. Glucose-responsive neurons were originally discovered in the hypothalamus via direct electro-osmotic application of glucose [178] [120] but have also been more recently discovered in the NAc [132]. Given that glucose-responsive neurons modify their electrical activity in response to changes in glucose and non-neuronal cells in the brain have the potential to detect glucose [131] [122], it is logical to question whether glucose-responsive neurons in the NAc can impact dopamine release and uptake rate in response to changes in glucose. If true, this would have downstream implications on food seeking and satiety. Preliminary evidence from our lab suggests that energetic state can change NAc dopamine, where a fasted state enhanced phasic dopamine release and uptake rate in the NAc core of both low fat (LF) and high fat (HF) males [134]. Those data show a fasted state can normalize dopamine release and uptake rate of HF males to their LF counterparts. This suggests energy state impacts dopamine neurotransmission and chronic palatable food consumption could promote salience for food via phasic dopamine release in a fasted state. Given that DIO is associated with hyperglycemia and fasting HF mice improves phasic release and speeds up dopamine uptake, we sought to explore whether experimentally manipulating glucose to mimic a fasted state would explain improvements in synaptic dopamine regulation and whether glucose-responsive neurons in the NAc core respond differently than the shell to changes in glucose. Moreover, it is physiologically important to further characterize the dichotomy between NAc subregions due to their different projections and circuitry functions regarding food behaviors.

Methods

Animals and Diet

Six-week old male (n = 12) and female (n = 12) C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed in groups of three per cage on a reversed 12-hour light/dark cycle (lights off 0600, lights on 1800). Mice were given free access to water and a purified nutrient-matched diet with 10% (LF, n=12; 6 males, 6 females) or 60% (HF, n=12; 6 males, 6 females) kcals from fat, densities of 3.8 and 5.2 kcals/g (DIO series D12450J and D12492, Research Diets Inc.). Body weight data were recorded once a week with food intake monitored and refreshed bi-weekly. The amount of diet consumed was calculated by food disappearance measurements averaged over the number of mice in each cage. Mice remained on their respective diets for 6 weeks prior to experimental tests. All experiments conducted were in compliance with the University of North Carolina at Greensboro Animal Care and Use Committee.

Fast scan cyclic voltammetry

FSCV was used to measure dopamine release and uptake rate in the NAc core and shell. FSCV was performed a minimum of three days post IPGTT and began 3 hours into the dark cycle. Mice were anesthetized using 5% isoflurane prior to removing the brains. Brains were then sectioned into 300 μm thick coronal brain slices using a compresstome (Precisionary Instruments; Greenville, NC) in oxygenated (95% O_2/5% CO_2) aCSF (in mM, for 11mM: 126 NaCl, 25 $NaHCO_3$, 11 D-glucose, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 0.4 L-ascorbic acid; for 3mM: 126 NaCl, 25 $NaHCO_3$, 3 D-glucose, 8 sucrose, 2.5 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 0.4 L-ascorbic acid). All slices equilibrated for 60 min at 37^{o}C with oxygenated

aCSF flowing at 2 mL/min. Voltammetric recordings used a triangular waveform that scanned from −0.4 to +1.2 and back to −0.4 V vs. Ag/AgCl at 400 V/s every 100 ms. The scanning voltage was applied to a carbon fiber electrode (100-150 μM length, 7 μM diameter (Goodfellow, C005722; Huntingdon, UK) placed at a depth of ~75 μM into the NAc core. Dopamine release was evoked with a single 4 ms pulse stimulation (1p) or a train of five pulse stimulations at 20hz (5p20hz) (monophasic, 350 μA) from a bipolar stimulating electrode (Plastics One, Roanoke, VA, 8IMS3033SPCE) every 3 min. Data reported are stable baseline recordings using the criteria of three consecutive 1p recordings with <5% variation in nA peak height. Recordings typically stabilized 60 min after collections began. Once a stable 1p baseline was established, a 5p20hz stimulation was collected from the same location to mimic physiological burst firing. Subsequently, 1p baseline was established again, after which slices were switched from standard aCSF (11mM) over to 3mM hypoglycemic aCSF. After a 60-minute perfusion followed by a 5p20hz stimulation, slices were switched back to standard aCSF and collections continued for a 30-minute washout period with subsequent 5p20hz stimulation. All recordings were obtained from the NAc core or shell. Dopamine signals were acquired and kinetically modeled using Demon Voltammetry Software, based on Michaelis–Menten kinetics, holding the Km at 160 nM with the assumptions that Km in mice is similar to the well-documented affinity of dopamine for the DAT in rats [142], and that sex or diet does not alter Km.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism (v. 9.4.0). T-tests were used to determine changes in body weight between treatment groups over time, while two-way ANOVAs were used to analyze bodyweight, food intake, and fasting glucose differences between male and female groups. One-way ANOVAs were used to analyze fasting blood glucose

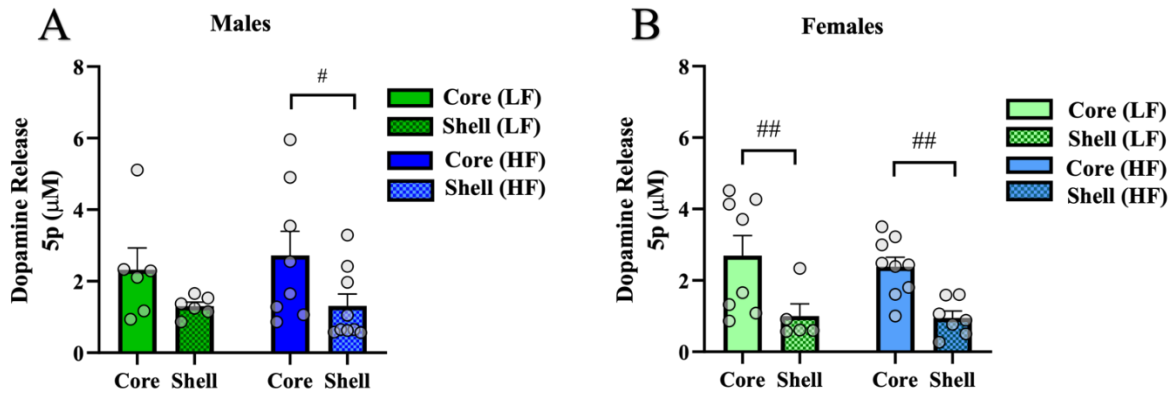
and area under the curve data for IPGTTs. Subsequently, two-way ANOVAs were used to determine effects of glucose changes in aCSF on dopamine release and uptake rate. Two-way ANOVAs or mixed-effects analysis were used to detect sex effects of hypoglycemic aCSF exposure on dopamine release and uptake rate. All post-hoc analyses used Tukey's or Sidak's multiple comparisons tests were utilized where appropriate. Group data are presented as means ± standard error of the mean; statistical significance was set at $p \leq 0.05$.

Results

Effect of dietary fat on baseline dopamine release and uptake rate in the NAc core versus shell

Slice voltammetry was used to characterize differences in baseline dopamine release and uptake rate in the NAc core and shell subsequent to 6-week exposure to either a LF or HF diet. Given that dopamine release in the shell must be evoked using five pulse stimulation trains at 20hz (5p20hz), all subsequent comparisons between the core and the shell were done using 5p20hz data. In males, a main effect of location was identified ($F_{(1, 25)} = 5.81$; $p = 0.02$) for baseline dopamine release (Figure 1-A), indicating a significant difference in dopamine release between the core and shell; however, post-hoc analyses did not reveal any significant differences between region in LF males, only HF males (2.73 ± 0.57 μM, core; 1.31 ± 0.57 μM, shell; $p = 0.04$). Two-way ANOVA analysis of female baseline release showed a main effect of location ($F_{(1, 10)} = 44.56$; $p < 0.0001$) (Figure 1-B). Moreover, Sidak's test showed significantly elevated release in core versus shell in the LF group (2.70 ± 0.39 μM, core; 0.84 ± 0.39 μM, shell; $p = 0.002$). Similarly, there was significantly greater dopamine release in the core of HF females than the shell (2.38 ± 0.34 μM, core; 0.77 ± 0.34 μM, shell; $p = 0.002$) (Figure 1-B).

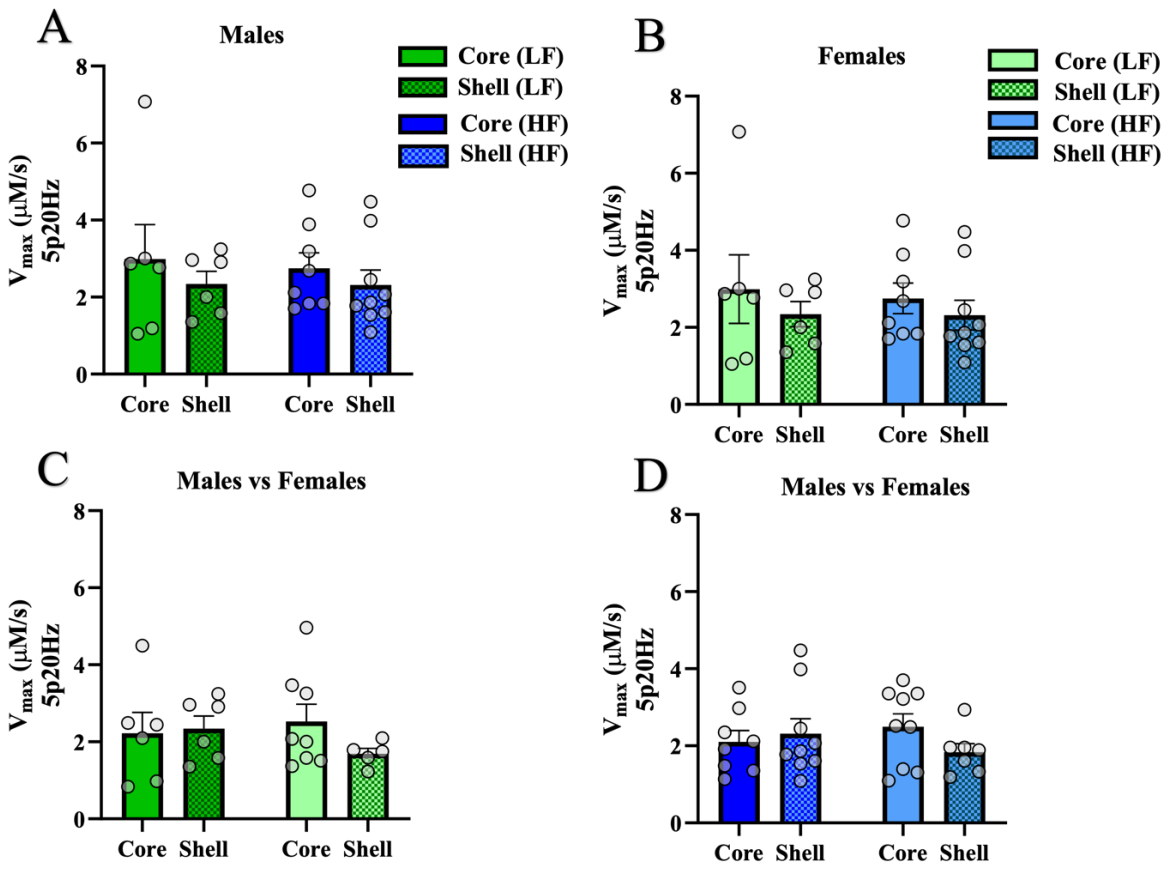
Figure 1 - Baseline 5p20hz dopamine release in the NAc core and shell in males and females



Ex-vivo fast scan cyclic voltammetry was performed in brain slices containing the nucleus accumbens to characterize differences in baseline dopamine release between morphologically distinct regions of the core and shell subsequent to dietary treatment. **(A)** In males, a main effect of location ($p = 0.002$) was found, with HF release in the core being significantly higher than in the shell. **(B)** Females were also observed to have a main effect of location ($p = 0.02$) and had higher baseline release in the core than in the shell ($p = 0.04$).
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Two-way ANOVA, Sidak's multiple comparisons test)

Interestingly, analysis of Vmax in males revealed no significant effects of location on baseline values (Figure 2-A). In contrast, females were identified to have a main effect of location ($F_{(1, 25)} = 8.89$; $p = 0.01$) on baseline Vmax (Figure 2-B). Comparisons between LF groups showed no significant effect of sex or location on Vmax (Figure 2-C). Similarly, no significant differences in baseline Vmax were observed between HF males and females, regardless of location (Figure 2-D).

Figure 2 - Baseline dopamine uptake rate in the NAc core and shell in males and females



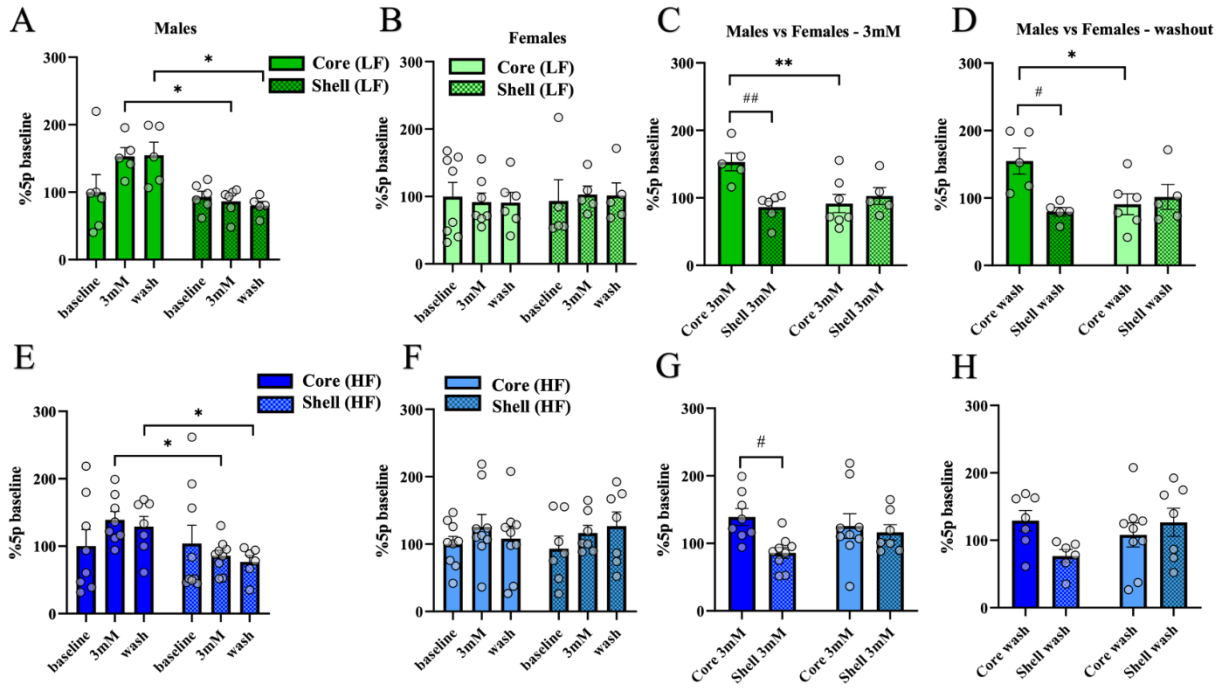
(A) In males, no significant differences in baseline 5p20hz Vmax were observed. **(B)** Females showed a main effect of location ($p < 0.0001$) on baseline Vmax, but no other differences were noted. **(C)** Comparison between LF groups showed no differences in Vmax between males and females, though females seemed to trend higher in the core. **(D)** Similarly, HF groups showed no significant effects on Vmax, though HF males had greater variability in Vmax in the shell.
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Two-way ANOVA)

Effects of hypoglycemic aCSF on dopamine release and uptake rate in the NAc core and shell

Data collected in the NAc core and shell were analyzed to determine the effects of changing aCSF glucose concentration on dopamine release and uptake rate. In LF males, a main effect of location was identified for dopamine release ($F_{(1, 10)} = 12.96$; $p = 0.01$) (Figure 3-A), with Sidak's post-hoc test indicating significantly elevated dopamine release in the core compared to shell at 3mM glucose (153.2 ± 15.57%, core; 86.39 ± 15.57%, shell; $p = 0.01$) and washout (154.8 ± 20.39%, core; 79.80 ± 20.39%, shell; $p = 0.04$). No effect of 3mM glucose

was observed in LF females (Figure 3-B). After exposure to 3mM glucose, a main effect of location ($F_{(1, 19)} = 5.02$; $p = 0.04$) and a location x sex interaction ($F_{(1, 19)} = 9.88$; $p = 0.01$) was identified in dopamine release among LF groups (Figure 3-C). Furthermore, LF males had greater dopamine release in the core than in the shell after 3mM glucose exposure (153.2 ± 17.84%, core; 86.39 ± 17.84%, shell; $p = 0.003$), with males displaying significantly more dopamine in the core than females (153.2 ± 17.25%, males; 91.63 ± 17.84%, females; $p = 0.004$). Analysis of LF groups identified a significant location x sex interaction ($F_{(1, 17)} = 7.33$; $p= 0.01$) on dopamine release after washout, with dopamine release being higher in the core of males than in the shell (154.8 ± 22.86%, core; 79.80 ± 22.86%, shell; $p = 0.01$) (Figure 3-D). Additionally, in the core, males had significantly greater dopamine release at washout compared to females (154.8 ± 21.89%, males; 90.80 ± 21.89%, females; $p = 0.02$). In HF males, there was a main effect of location on dopamine release ($F_{(1, 41)} = 4.84$; $p= 0.03$) (Figure 3-E). There was also significantly greater dopamine release in the core than in the shell after 3mM glucose exposure (139.1 ± 15.02%, core; 85.98 ± 15.02%, shell; $p = 0.01$), and after washout (129.2 ± 17.76%, core; 76.75 ± 17.76%, shell; $p = 0.04$). In contrast, HF females showed no significant differences in dopamine release after exposure to 3mM glucose and washout (Figure 3-F). Comparison of dopamine release in HF males and females after 3mM glucose exposure revealed a main effect of location ($F_{(1, 13)} = 6.98$; $p= 0.02$) (Figure 3-G). Sidak's test identified males as having significantly greater dopamine release in the core than in the shell after exposure to 3mM glucose (139.1 ± 18.7%, core; 85.98 ± 18.7%, shell; $p = 0.03$). Lastly, after washout, a significant location x sex interaction was observed in dopamine release among HF groups ($F_{(1, 11)} = 7.16$; $p= 0.02$) (Figure 3-H).

Figure 9 - Dopamine release after exposure to 3mM glucose and washout in the NAc core and shell of males and females

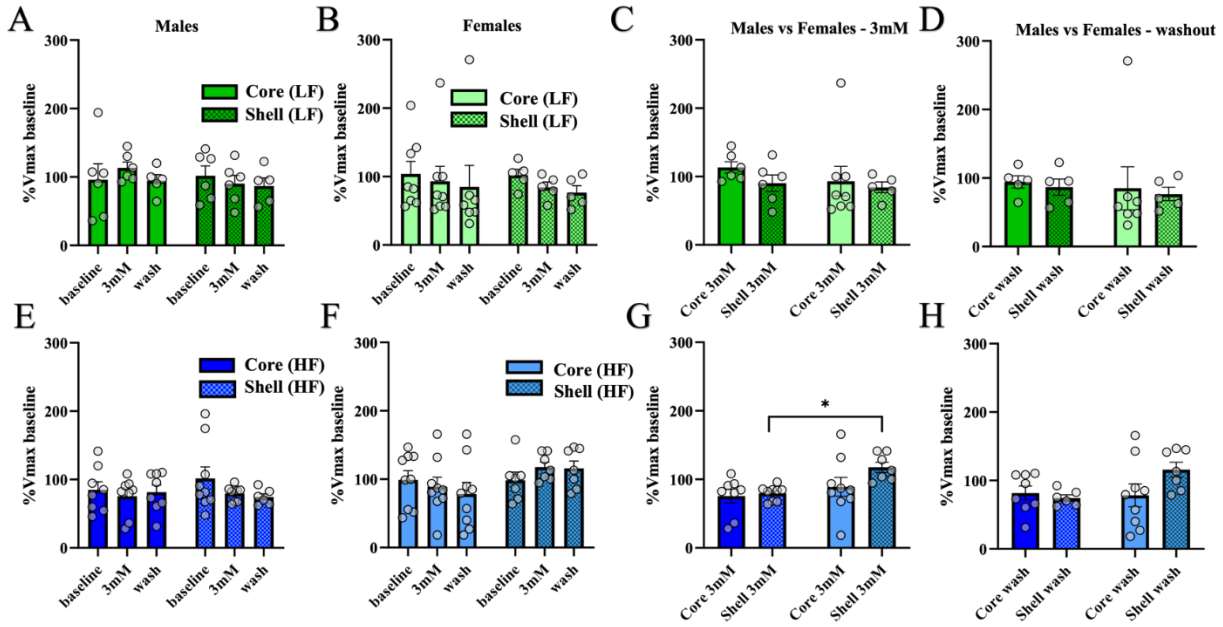


(A) LF males had significantly more 5p20hz dopamine release in the core than in the shell after exposure to both 3mM aCSF and washout. **(B)** In LF females, however, no significant differences in dopamine release were observed. **(C)** Exposure to 3mM significantly increased dopamine release in the core of LF males compared to females. **(D)** Dopamine release was significantly higher in the core of LF males after washout. Sex-based differences in dopamine release were also observed, with males showing higher release in the core compared to females. **(E)** HF males had significantly increased dopamine release in the core after exposure to 3mM aCSF and washout compared to the shell. **(F)** In contrast, no significant differences in dopamine release were observed in HF females after 3mM and washout. **(G)** Between HF groups, only males showed significantly higher dopamine release in the core than the shell after 3mM exposure. **(H)** A significant location x sex interaction was identified in washout among HF groups ($p = 0.02$), with males displaying higher release in the core and females in the shell.
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Two-way ANOVA, Sidak's multiple comparisons test)

Analysis of the percent change in uptake rate within LF males revealed no significant changes from baseline after exposure to 3mM glucose and washout (Figure 4-A). Similarly, LF females also did not exhibit significant changes in Vmax after 3mM and washout (Figure 4-B). Comparison of LF males and females revealed no significant effects of 3mM glucose concentration on Vmax (Figure 4-C). Similarly, no significant differences in Vmax were observed after washout in the core and shell of LF groups (Figure 4-D). In HF males, Vmax was

not significantly different after 3mM and washout, regardless of location in the NAc (Figure 4-E). In HF females, however, a significant effect of subject ($F_{(14, 28)} = 2.89$; $p= 0.01$) was identified (Figure 4-F). Among HF groups, a significant effect of sex ($F_{(1, 16)} = 6.98$; $p= 0.02$) on Vmax after exposure to 3mM was identified (Figure 4-G). Furthermore, Sidak's test revealed females had significantly higher Vmax in the shell compared to males (79.66 ± 14.01%, males; 117.6 ± 14.01%, females; $p = 0.02$). In contrast, no significant differences in Vmax were observed between HF groups after exposure to washout (Figure 4-H).

Figure 4 - Dopamine uptake rate after exposure to 3mM glucose and washout in the NAc core and shell of males and females



(A) LF males did not display significant changes in Vmax after exposure to 3mM aCSF and washout. **(B)** Similarly, LF females showed no main effect of glucose concentration on Vmax, though a downward trend was noted after exposure to 3mM. **(C)** No notable differences in Vmax after exposure to 3mM were observed between LF males and females. **(D)** No significant differences in Vmax after washout were observed in LF groups. **(F)** There were no observed differences in Vmax after exposure to 3mM glucose and washout in HF females. **(G)** HF females were observed to have significantly faster uptake rate in the NAc shell compared to the shell of HF males ($p = 0.02$). **(H)** Exposure to washout resulted in no significant differences in Vmax between regions among HF groups.
(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Two-way ANOVA, Sidak's multiple comparisons test)

Discussion

This study sought to evaluate the effect of metabolic phenotypes on dopamine release and uptake rate in the nucleus accumbens core versus shell via changes in glucose conditions. Our data showed that location had a significant effect on dopamine release, with the NAc core exhibiting greater baseline dopamine release than the shell, regardless of dietary treatment. Interestingly, there were no observed differences in baseline uptake rate between NAc subregions or dietary groups, despite reported differences in cellular configuration [173]. We report a novel observation of phasic (5p) dopamine release being significantly enhanced in the NAc core versus shell of males following exposure to hypoglycemic 3mM aCSF, with no significant

differences observed in females. In contrast to our previously reported findings [134], hypoglycemic conditions did not change uptake rate in males. The observed enhancements in phasic release without changes to dopamine uptake rate are a novel observation, and support not only the possibility of sexually dimorphic control of reward circuitry in response to changes in energy state, but also suggest regional differences in dopamine neurotransmission play a role in modulating reward circuitry and food responses.

Baseline phasic (5p) dopamine release differs between NAc subregions but not uptake rate

Examination of the NAc as a homogenous structure has revealed few sex differences in dopamine neurotransmission. However, when examining anatomical regions of the NAc, we report pronounced differences in baseline phasic dopamine release between the core and shell. Specifically, the NAc core of HF males and females, as well as LF females, showed significantly greater dopamine release than the shell. This is congruent with data categorizing the core as having a greater surface area [176] and more glucose-responsive neurons than the shell [132]. Interestingly, differences in baseline release were more pronounced among HF groups, suggesting that shifts in dopaminergic signaling caused by a HF diet may impact circuitry projecting from the NAc with anatomical origins within the core or shell. This is important when considering the distinct functions each subregion has. Given that the NAc core is essential for associative learning and cue-initiated motivation [179 180], greater dopamine release in comparison to the shell might be more pertinent for discerning physiological stimuli and eventual spatial learning. Conversely, no differences in baseline dopamine uptake rate were observed between the core and shell, regardless of dietary treatment, which suggests a lack of heterogeneity in dopamine transporter (DAT) function/expression between NAc subregions, despite recent reports of differential expression between striosome and matrix [181]. Thus, differences in baseline

electrically-evoked dopamine release were greater in the NAc core relative to the shell and are suggestive of spatial learning and discernment of physiological stimuli playing a greater role in the core.

Dopamine release and uptake rate in the NAc core versus shell under changing glucose conditions

In order to mimic fasting conditions from our previous study [134], we exposed brain slices to hypoglycemic aCSF (3mM glucose). This alteration was meant to imitate changes in energy state resulting from food restriction. We report significant differences in phasic dopamine release in males after exposure to 3mM glucose. Specifically, males showed enhanced dopamine release in the NAc core versus shell following exposure to 3mM glucose and washout. Further, HF males showed similar levels of dopamine release to LF males, suggesting that reduced glucose conditions normalized dopamine release in a manner similar to what was observed when mice underwent a 12-hr fast [134]. HF females showed a general increase in dopamine release but lacked significant subregion differences, indicating that subregion heterogeneity of dopamine neurotransmission is sex-dependent. Another possibility is that the blunted treatment effect in HF females could be attributed to alterations in estrus cycling from consuming a high fat diet, given that 4 weeks on a high fat diet were sufficient to alter cycling until 8 weeks, after which estrus returned to normal [154]; where our study only provided treatment for 6 weeks. The observed enhancements in dopamine release in the core of male animals persisted throughout washout, implying that these glucose-responsive neurons are receptive to localized changes in brain glucose concentrations and that the resulting adaptations may be sex-dependent. Moreover, our results are suggestive of negative energy states inducing food-seeking behaviors via phasic dopamine release in the NAc core and when combined with previous data from our lab, imply

that males have more desensitized NAc dopaminergic responses to negative energy states. Given that phasic dopamine release in the NAc core also relates to effort and vigor of responding [182] [183], it would follow that enhancements in core phasic release in response to negative energy states would translate to more vigorous food-seeking, particularly in males [184]. However, few studies have examined differences in dopamine release between NAc subregions in response to changes in energy state and further work is required to explain why LF mice displayed greater enhancements in dopamine release in the core versus shell.

There were no significant enhancements in dopamine uptake rate in the core or shell, regardless of diet or sex. This was unique to our study, given that the previous fasted study showed increased uptake rates in fasted controls and HF males. The sparse change in uptake rate in males in our study could perhaps be due to the lack of metabolic impairments traditionally seen in HF animals [177] [93] [91], and which typically diminishes DAT function/expression, reducing dopamine uptake rates [184]. Conversely, females also were not observed to have significant changes in uptake rate, which could result from the aforementioned alterations in estrus. However, given that animal work has found that females express greater striatal DAT density than males [185], it is possible that increased DAT expression and function account for the lack of treatment effect on dopamine uptake rate in females. This is further supported by our data showing females having faster uptake rates in the shell compared to males, particularly in HF groups. HF females showed faster uptake rates in the shell versus core after exposure to 3mM glucose and washout, which could imply enhanced sensitivity of pre-synaptic dopamine D2 receptors, given that they control surface expression of DAT along with insulin receptors [186]. Further, 3mM glucose did not increase uptake rate in LF females compared to males, which suggests that the observed differences in Vmax could be sex- and diet-dependent. Long-term HF

diet consumption induces striatal insulin resistance, reduces dopamine release and uptake rate and DAT shuttling in males but not females [151] [160]. Typically, the HF diet has the largest effect on dopamine parameters in males, with less pronounced effects in females. However, males in our study lacking the expected metabolic impairments and typical diet-induced insulin resistance could explain the lack of observed differences in dopamine uptake rate.

Conclusion

Overall, this study showed that baseline dopamine release is significantly higher in the NAc core than in the shell, regardless of dietary treatment. This contributes to the concept that heterogenous control over food intake occurs in specific NAc subregions [187] [188] and in response to energy states. We report that hypoglycemic 3mM glucose aCSF had an effect on phasic dopamine release when normal aCSF did not, which suggests a negative energy state could preferentially enhance phasic dopamine and prime reward-seeking behaviors in the NAc core. Moreover, these enhancements were significant in the core of males, but not females. These differences most likely depend on sexually dimorphic striatal development [169] and alterations to dopamine neurotransmission within NAc subregions resulting from a HF diet. While a HF diet typically the largest effect on dopamine parameters in males, the absence of traditionally seen metabolic impairments such as insulin resistance and diminished glucose metabolism may account for the lack of effect of 3mM glucose on uptake rate, as well as diminished effects on dopamine release in HF males. Given that long-term consumption of palatable foods results in diminished anorectic signaling [151] [160] and impacts both tonic and phasic dopamine release in the NAc [93] [177], negative energy states could promote hyperphagia and increased attention to food, while impeding food cessation. Ultimately, food intake relies on a complex interplay of environmental stimuli, sex hormones, energy state and hedonic value of foods [177] [189] [179]. As such,

further evaluation of feeding behaviors resulting from changes in dopamine neurotransmission within NAc subregions is warranted, though our data provides evidence of possible sex-dependent heterogeneity on NAc dopamine circuitry in response to a HF diet and subsequent changes in energy state.

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