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Dendritic Spine Density and Morphology in the Dorsolateral Striatum Following a High Fat Diet

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DENDRITIC SPINE DENSITY AND MORPHOLOGY IN THE DORSOLATERAL STRIATUM FOLLOWING A HIGH FAT DIET

by

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Abstract

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Obesity rates have been dramatically rising in recent years and in 2017-2018 more than 42% of adults in the United States were obese. Obesity is associated with numerous health problems, including cardiovascular disease, stroke, insulin resistance, and type II diabetes. The prevalence of highly palatable and calorically dense foods high in fats and sugars is a significant factor in the increase in obesity rates. Many suggest that palatable food affects the brain in ways similar to drugs of abuse, reinforcing the consumption of highly palatable foods in the same way drugs reinforce drug use. While numerous weight loss programs and diets suggest that simply reducing caloric intake will result in weight loss, the weight loss is usually difficult to maintain long term and results in rebound of weight gain, reflective of a similar behavioral phenotype observed in drugs of abuse. The persistence of the condition suggests that persistent changes in neural circuits may be occurring that result in the long-term maintenance of increased body weight. The neural circuitry involved in drugs of abuse and obesity overlaps as well, with the dopamine pathway system being critically implicated in both. In this study, we focused on the dorsolateral striatum, an area in the dopamine system known to be involved in habit formation, and addictive and compulsive behaviors. We sought to investigate the effect of a high fat diet on alterations in dendritic spines, a synaptic

modification that may be involved in shaping dorsolateral striatal activity and properties, contributing to the behavioral phenotype observed in obesity. To do this we used DiOlistics to label the two main cell types in the dorsolateral striatum, D1 and D2 medium spiny neurons, and analyze the differences in dendritic spines between high fat diet fed mice and control chow fed mice.

Acknowledgements

To my parents and family, thank you for always supporting and encouraging me, I couldn't have done this without you. To my advisor, Dr. Jeff Beeler, thank you for the guidance and support throughout this project. To everyone in the lab who worked on this project with me, Allison, Federico, Eddy, thank you for all the time spent training and helping me complete this project.

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Chapter 1: Introduction

General Background

Obesity rates have been dramatically rising in recent years and in 2017-2018 more than 42% of adults in the United States were obese (Hales et al., 2020). The health consequences associated with obesity include cardiovascular disease, stroke, insulin resistance, and type II diabetes (American College of Cardiology). There are a number of proposed causes for this rise in obesity, with biological sex, genetics, and socioeconomic status all potentially contributing to this trend (Darmon & Drewnowski, 2008). In addition, the prevalence of "western diets," of easily accessible foods that are highly palatable and calorically dense is also proposed as a significant factor in the increase in obesity rates (Swinburn et al., 2011). These highly palatable foods typically contain high amounts of sugars and fats, often in addition to high amounts of salt and flavoring.

While numerous weight loss programs and diets suggest that simply reducing caloric intake will result in weight loss, the weight loss is usually difficult to maintain long term and results in rebound of weight gain (Hall & Guo, 2017). When rodents are temporarily given an obesogenic diet, then switched to a healthier diet, they still maintain a higher weight than rodents who had only been given the healthy diet and had never temporarily received the obesogenic diet (Guo et al., 2009). The persistence of the condition suggests that persistent changes in neural circuits may be occurring that result in the long-term maintenance of increased body weight. A neuroplasticity theory of obesity addresses how obesogenic diets are associated with neural plasticity in brain circuits that result in the persistent changes in behavior and ultimately maintenance of obesity (Matikainen-Ankney & Kravitz, 2018). Neuroplasticity in this theory refers to synaptic level modifications (e.g., alterations in neurotransmitter release, neurotransmitter receptor insertion/deletion on the post synapse, density and morphological changes in dendritic spines, etc.) in response to the obesogenic diets, which persist

even when the diet is no longer being consumed (Morin et al., 2017). These synaptic modifications therefore may be shaping neural circuit's response properties, altering ingestive drives, compulsive behaviors, rewarding/reinforcement of food consumption, or energy expenditure; ultimately resulting in the observed difficulty of maintaining weight loss, and the rebound of weight gain.

Furthermore, many suggest that palatable food affects the brain in ways similar to drugs of abuse (Brown et al., 2017; Meule, 2015; Morris et al., 2015; Volkow et al., 2013), reinforcing the consumption of highly palatable foods in the same way drugs reinforce drug use. The neuroplasticity theory of obesity and the theory of "food addiction," both discuss the overlap between drugs of abuse and obesity regarding underlying mechanisms, brain circuitry, and behaviors. For example, behavioral phenotypes typical of both drug and food addiction include periods of abstaining followed by relapse, engaging in compulsive behaviors for the substance, withdrawal symptoms, and a build-up of tolerance for the substance (Morris et al., 2015). The neural circuitry overlaps as well, with the dopamine pathway system being implicated in both (Blum et al., 2011; Leigh and Morris, 2018; Volkow et al., 2017). In particular, the striatum is an important neural substrate in addiction involved in regulating reinforcement of behavior, habit formation and reward sensitivity (Yager et al., 2015) and is implicated in obesity as well (Wang et al., 2011; Guo et al., 2014; van de Giessen et al., 2014).

The striatum is divided in two regions, the dorsal striatum and the nucleus accumbens. The dorsal striatum is further divided into two regions: the dorsomedial striatum (DMS), which is implicated in goal-directed behaviors, and the dorsolateral striatum (DLS), which is implicated in habit behaviors (Everitt & Robbins, 2005; Pennartz et al., 2011). A shift in activity from the DMS (goal-directed) to the DLS (habit formation) is implicated as a neural substrate involved in the emergence of compulsive, habitual behaviors typical in drugs of abuse (Gremel & Costa, 2013). For example, when rats are trained to lever press for cocaine, lever pressing will decrease when a dopamine receptor antagonist is applied to the DMS *early* in training or to the DLS following

overtraining (Murray et al., 2012). Regarding obesity, data shows that rodents fed an obesogenic diet demonstrated alterations in dorsal striatal glutamate, dopamine, and opioid transmission (Fritz et al., 2018), demonstrating the overlap between the circuitry involved in drug addiction and obesity.

Within the striatum, there are two main cell types: D1 and D2 medium spiny neurons (MSNs). The cells differ in dopamine receptor type and projection patterns (Wall et al., 2013). D1 MSNs have D1 dopamine receptors and make up the direct pathway (dMSN). D2 MSNs have D2 dopamine receptors and make up the indirect pathway (iMSN). Generally, activating the D1 direct pathway results in excitation of the thalamus, engaging in a positive cortical feedback loop, initiating behavior, while activating the D2 indirect pathway results in inhibition of the thalamus, engaging in a negative cortical feedback loop, inhibiting behavior (Yager et al., 2015). Regarding drug addiction, investigating the pathways has shown that exposure to different drugs (opioid vs. stimulant) can produce different synaptic alterations in D1 or D2 cell types (Lobo & Nestler, 2011; Graziane et al., 2016). Regarding obesity, initial research supported an association between reduced D2 activity and obesity (Huang et al., 2006; Hajnal et al., 2008; Johnson & Kenny, 2010), but subsequent studies did not support those findings (Einstein et al., 2013; Karlsson et al., 2015; Janssen et al., 2022). Additionally, recent evidence also points to a role of D1 MSNs in obesity as well (Matikainen-Ankney et al., 2022).

The behavioral implications of striatal dopamine's direct and indirect pathways association with obesity, though, isn't entirely elucidated. Some interpret the reduction of striatal D2 activity as supporting a reward deficiency hypothesis, in which individuals will engage in compulsory reward seeking behaviors to make up for the loss of dopamine, in the same way drug addiction is proposed to work (Blum et al., 2011). Others find evidence to support an energy expenditure theory, in which the low dopamine isn't associated with increased motivation for food, but rather with an altered energy expenditure, where conservation and exploitation are increased (Beeler et al., 2012; Beeler et

al., 2016; Kravitz et al., 2016). Therefore, while the striatal dopamine system is implicated in obesity, the mechanism behind the associated behavioral outputs of the system remains to be elucidated.

In conclusion, high rates of rebound weight gain and poor long-term success in weight loss suggest a food addiction model of obesity. This model posits that behaviors observed in both conditions are the result of neuroadaptations that persist even after the drug/obesogenic diet is abandoned. Specifically, the dopamine pathway system is implicated as a site for these alterations, with a focus on how dorsal striatal direct and indirect pathways are involved in obesity. A substrate of neuroadaptations, dendritic spines, are heavily implicated in drug addiction (Spiga et al., 2014), and by extension of the above theories, relevant to obesity as well.

Dendritic Spines

Dendritic spines are the small protrusions extending off of dendrites that serve as the point of first contact between neurons. Spine plasticity is the process during which neural activity leads to alterations in spine density and/or morphology. Spine remodeling is associated with learning and memory and a number of neurological disorders are associated with dysregulation of spine dynamics (Khanal & Hotulainen, 2021; Runge et al., 2020). Drug addiction studies indicate that alterations in spines occur differently in the striatum (NAc) in response to different drugs. For example, investigating spine density and morphology following withdrawal, Graziane et al. (2016) found that there was an increase in spine density with cocaine withdrawal, but a decrease in spine density following morphine withdrawal, consistent with findings from previous studies (Robinson et al., 2002). Regarding obesity, studies have found obesogenic diets associated with spine alterations in the nucleus accumbens (Klenowski et al., 2016), lateral orbitofrontal cortex (Thompson et al., 2017), and the medial prefrontal cortex (Dingess et al., 2017).

Allowing rats to consume sucrose in a binge-like manner for either 4 (short-term) or 12 (long-term weeks), Klenowski et al. (2016) found that MSNs in the nucleus accumbens shell had decreased

dendritic length and dendritic complexity but increased mean spine density following long-term but not short term consumption of sucrose. They propose that their results demonstrate direct neurological consequences of long-term sucrose consumption, highlighting the importance of investigating dendritic plasticity in the same circuitry addressed in drugs of abuse, in the context of food rewards like sucrose as well.

Another group gave rats three groups of rats a 'cafeteria diet,' with differential access (either 0, 1, or 24 hours per day) in addition to ad libitum access to chow for 40 days (Thompson et al., 2017). The cafeteria diet was comprised of Kirkland beef hotdogs, Froot Loops, Timbit donut holes, Kraft peanut butter, and Doritos. Extended access (24 hours/day) to the cafeteria diet resulted in a significant decrease in spine density in the lateral orbitofrontal cortex (OFC), while restricted access (1 hour/day) to the cafeteria diet resulted in a significant increase in spine density in the ventral orbitofrontal cortex. The OFC is associated with impaired decision making (Wilson et al., 2014) and Thompson et al. suggest that the altered dendritic spines in the OFC is involved in obesity behaviors, where individuals continue overeating despite knowing the health consequences.

Additionally, another study exposed rats to either ad libitum standard chow, ad-libitum 60% high fat diet or 60% calorically matched (food restricted so that total caloric intake didn't significantly differ from the standard chow group) for three weeks (Dingess et al., 2017). Analyzing spine density and morphology, they found a significant reduction in both high fat diet groups spine density in the medial prefrontal cortex (mPFC), specifically in the infralimbic region. The infralimbic region plays a role in reward related behavior, with studies showing that the region is involved in the inhibition of drug seeking in extinguished rats; when the region was inactivated there was an increase in cocaine reinstatement (Peters et al., 2008). Dingess et al. (2017) were the first to specifically investigate dendritic morphological changes under exposure to a high fat diet. Interestingly, though, they did not find dendritic spine alterations in the nucleus accumbens, an area critically involved in drug addiction,

which they attribute to their lack of differentiating between the direct and indirect pathway cells. Dingess et al. (2017) found that the decrease in spine density in the mPFC was due to a decrease in a specific type of spine, known as thin spines.

Dendritic spines are heterogenous in both length and shape. Spines are therefore characterized by their morphological features, which are proposed to have functionally relevant significance. The length of a spine neck (the distance of the protrusion from the base of the dendrite) is one functionally relevant morphological feature of the spine. The spine head (the bulbous end of the neck of the spine) is a critical feature used to characterize spines as well. The morphology of the spine (i.e. neck and head) affects the cellular responses that ultimately get transferred to the dendrite (Serrano et al., 2022). The length of the head from the dendrite ultimately impacts the response enacted by the neuron, as the head contains the postsynaptic density, which contains the relevant neurotransmitter receptors and proteins involved in postsynaptic responses. Therefore, certain morphological features are associated with increasing or decreasing synaptic communication. For example, Risher et al. (2014) describes the classifications of dendritic spines used to reflect their associated functional significance.

One, filopodia spines have the longest necks (at least longer than 2 μm) and do not contain a head. Two, thin spines are slightly shorter than filopodia spines and may have a small head. Three, stubby spines lack a neck, are the shortest spines and do not contain a true head. Four, mushroom spines are characterized by containing a neck and a large spine head (head to neck ratio). Going from one to four, spines progress from earlier immature spines, which are not as stable and hypothesized to aid in the initiation of synaptic connections (filopodia, thin), to spines that develop as a synapse stabilizes and reflect a more mature spine that contains an abundance of neurotransmitter receptors and can therefore sustain higher levels of activity (stubby, mushroom).

Conclusion

There is a growing understanding of the commonality between drugs of abuse and obesogenic diets/compulsive overeating, though there is less known about the latter, specifically regarding synaptic alterations. My project sought to address this gap in the literature regarding dietary effects on synaptic alterations; specifically, I investigated the effect of a diet high in fat on dendritic spine density and morphology in the dorsolateral striatum in the direct and indirect pathways, as this hasn't been investigated before despite the strong implications for the DLS's involvement in obesity and the focus on fat content in the highly palatable foods contributing to obesity.

To address this question, I used DiOlistics, which involves the use of a "gene gun" (PDS-1000/He particle delivery system) to introduce fluorescent dye into brain tissue. The lipophilic dye is weakly fluorescent until incorporated into the plasma membrane of cells. The dye then diffuses throughout the membrane, allowing for imaging of whole, well defined neurons using confocal microscopy. The dye was introduced into brain tissue collected from mice that were fed either one of two diets: ad libitum chow or ad libitum high fat diet. Immunohistochemistry was then performed to label D2 cells, for differentiation between the direct and indirect MSNs. Spine density and morphology were then quantified using Neurolucida to detect differences between the direct and indirect pathways between the two diets.

We hypothesized that direct and indirect cell pathways in the dorsolateral striatum should undergo alterations in spine density and morphology, as both pathways have been shown to be altered due to high fat diet consumption (Meyers, 2022). Additionally, we proposed that the spine alterations would be the result of alterations in filopodia and thin spines, since they are the spines that undergo change most rapidly and are highly labile compared to mushroom and stubby spines.

Chapter 2: Methods

Feeding Protocol

To differentiate between D1 and D2 medium spiny neurons, mice were hemizygous for a transgene expressing green fluorescent protein (GFP) under the DrD2 promoter (D2EGFP), on a C57B1/6 background. Adult mice of a minimum of 10-11 weeks old were used. Mice were housed with 3-5 littermates of the same sex. A total of 11 mice were used, with 5 control mice (3 female, 2 male) and 6 experimental mice (2 female, 4 male). Experimental mice were fed ad libitum high fat diet (60% total calories from fat, TD.06414 Envigo) for a minimum of 6 weeks with access to regular drinking water. This experiment was done in the context of a larger project in the lab investigating the role of silent synapses in the dorsolateral striatum during obesity. Previous work from the lab had found that high fat diet induced alterations in silent synapses are observed after 6 weeks of high fat diet feeding. Therefore, that time course was used in this study as well to determine spine morphology alterations that may be involved in those alterations (Meyers, 2022). Control mice were fed standard chow ad libitum with access to regular drinking water. All experiments were approved by Queens College, Institutional Animal Care and Use committee in accordance with the National Institutes of Health (NIH) guidelines for use of animals in research.

Perfusions

Mice were anesthetized by peritoneal injection of 0.05 ml Euthasol Euthanasia (Virbac AH, Inc.) and perfused transcardially with 1x phosphate-buffer solution (PBS) followed by followed by 4℃, 1.5% paraformaldehyde (PFA) in PBS (pH 7.4). The 1.5% PFA was prepared by dissolving 3.75 g of paraformaldehyde in 250 ml of 1x PBS. Generally, for fixing tissue, 4% PFA is used. Here we used 1.5% because 4% PFA results in unclear illumination and perfusion of the dye throughout the soma and the dendrites, as the stronger concentration of PFA can damage the spines, while 1.5% allows for clear perfusion of dye throughout intact structures for optimal imaging (Staffend &

Meisel, 2011). 1.5% is widely used throughout the literature in DiOlistics protocols for imaging of dendritic spines (Graziene et al., 2016; Dingess et al., 2017).

The brains were extracted and post-fixed in 1.5% PFA for an hour at room temperature (around 20℃) before being transferred to 1x PBS. Then, coronal slices of 150 μm thickness were prepared using a vibratome (Leica) at room temperature and slices were collected in 1x PBS solution in a 12-well plate. We collected slices from rostral portions of the dorsolateral striatum as here is evidence for a rostral-caudal gradient in the dorsal striatum (Vogelsang & D'Espositio, 2020). For each mouse we took around 6 coronal slices from the rostral dorsolateral striatum (Figure 1).

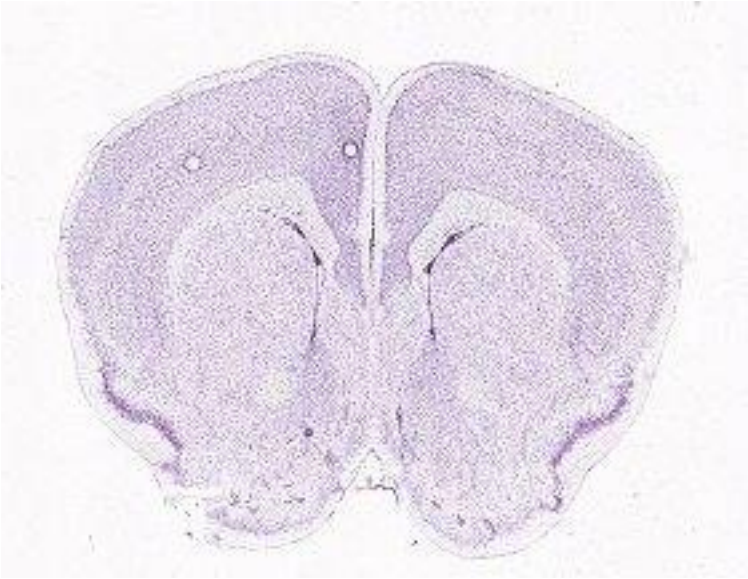

Figure 1. Sample coronal brain slice Nissl stain (left) anatomical labels (right), taken from Allen Mouse Brain Atlas.

Ballistic Dye Delivery

Fluorescent dye (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate ('DiI'; $DiIC_{18}(3)$), Invitrogen) was introduced to the tissue by ballistic deliver using the PDS-100/He

System (Figure 2), also known as a “gene gun.” Prior to using the gene gun to deliver the dye, the dye is prepared on microcarriers, or “bullets.”

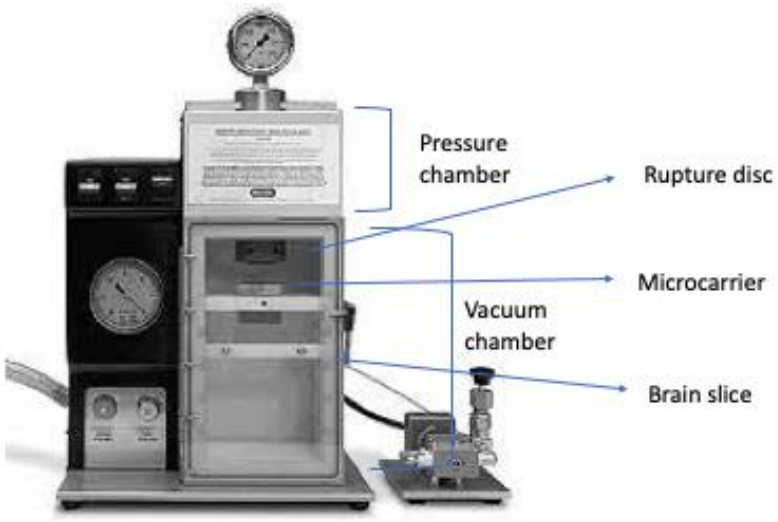

Figure 2. PDS-1000/He Ballistic Delivery System

To prepare the bullets, 1.5 mg of DiI was dissolved in 50 μL of methylene chloride. Then 12.5 mg of tungsten microcarrier particles were spread on a glass slide, and the DiI solution was poured onto the tungsten particles and allowed to dry. Once the dye was dried onto the tungsten microcarrier particles, the dye was scraped off the glass slide using a blade and transferred to weighing paper and then transferred into a 5 ml tube covered with aluminum foil (to keep the fluorescent dye away from light). 3 ml distilled water was added to the tube. The tube was then paced in a sonicator for 20 minutes to disrupt large clumps of dye.

Lastly, the dye is placed onto microcarrier films. The microcarrier films are prepared in 6-well plates that are light sealed with aluminum foil. Desiccant, which helps facilitate drying of the dye solution, is placed into the wells and the film is placed over the desiccant. Using a plastic pipette, a drop of dye solution is placed on the center of the microcarrier film. Both the dye solution and the microcarriers are stored at 4℃.

The ballistic delivery system works in the following way: a vacuum is created and a rupture disc placed in the system bursts at a certain pressure (psi), releasing a high pressure burst of helium gas, which propels the dye off the microcarrier film and onto the tissue slice below. We used 1,100 psi rupture discs which get placed in the retaining cap, while the microcarrier film was placed in the launch assembly at the highest slot, a quarter of an inch away from the rupture disc retaining cap. The brain slice is placed in the center of a dish two slots away from the microcarrier launch assembly. The dye is propelled by the shock wave created by burst of helium gas when the rupture disc bursts.

Slices were then resuspended in PBS in a 12-well plate, covered in aluminum to preserve the fluorescence. The tissue was then stored at 4℃ for at least 24 hours before imaging to allow the dye to diffuse through the neuronal membranes (Foster Olive et al., 2018).

Immunohistochemistry

After ballistic delivery of the dye, immunohistochemistry (IHC) was performed. One of either two IHCs were used: (1) chicken anti-GFP to enhance the endogenous expression of GFP in D2 neurons or (2) rabbit anti-D2R to label D2 neurons. Both IHCs used an Alexa Fluor 488 secondary antibody as well: (1) goat anti-chicken Alexa Fluor 488 or (2) donkey anti-rabbit Alexa Fluor 488. Both IHCs utilized the following stages. Tissue was permeabilized in 0.01% Triton-X in PBS for 15 minutes. Then, tissue was blocked in 0.01% Triton-X and 10% normal serum in PBS for 30 minutes. Next, the tissue was incubated in the primary antibody at 1:1000 in the blocking solution (Triton-X, normal serum, PBS) for 2 hours. After the two hours, the tissue was washed three times for ten minutes each in PBS. Then, the tissue was incubated in the secondary antibody at 1:1000 in blocking solution, followed by 10 minute washed in PBS, four times.

Confocal Imaging

Following the immunohistochemistry, tissue was mounted onto glass slides and coverslipped using Vectashield Antifade mounting medium, which protects the fluorescence and inhibits rapid photobleaching of fluorescent proteins and dyes. Clear nail polish was used to seal the coverslip. An Olympus Fv10i confocal microscope was used for imaging. Imaging was done with Alexa Fluor 488 (Excitation 495 nm; Emission 519 nm) and DiI (Excitation 549 nm; Emission 565) filters using 60x oil immersion lens.

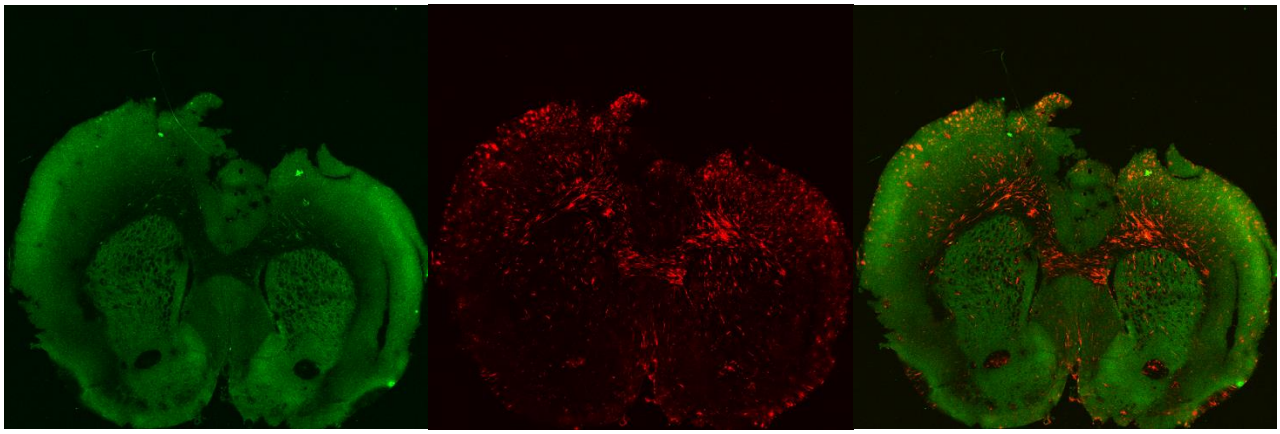

Figure 3. Representative images of green filter (left most), red filter (middle), and overlay images taken of brain slices from a control mouse.

Cells were selected with the following considerations. For purposes of analyzing dendritic segments of 60 μm – 90 μm with clear dendritic spines capable of being quantified for both density and spine type/morphology were selected. Cells needed to be clearly diffused with the DiI along the dendritic branch with a clear trace to the cell body from which the dendrite is originating for verification, using the IHC, that the cell is either D1 or D2. Cells were sampled from the dorsolateral striatum of both left and right hemispheres, as there is currently no indication in the literature that there is lateralization in the DLS.

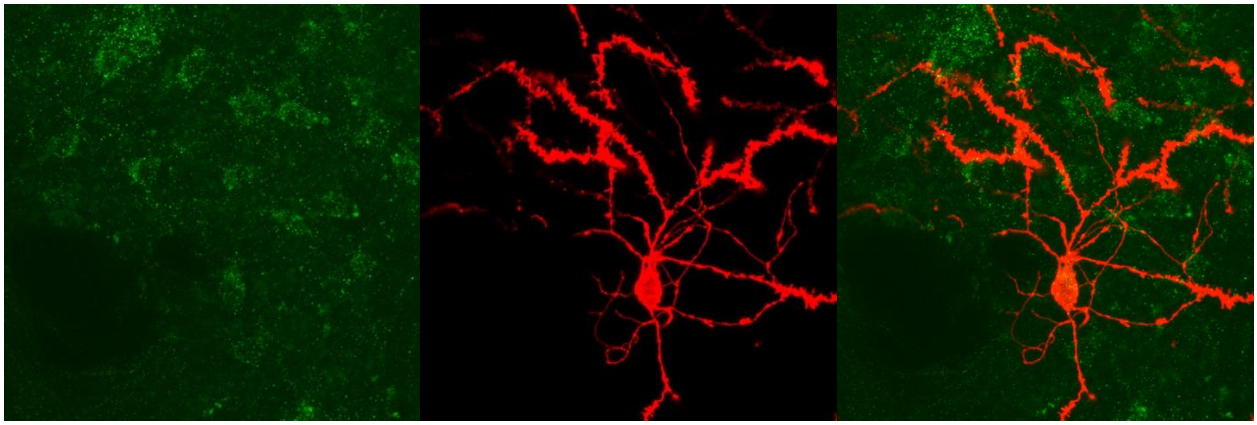

Figure 4. Representative images of green filter (left most), red filter (middle), and overlay images taken of a D2 MSN from the DLS of a control mouse (right). Images taken using 60x oil immersion lens.

Dendrite Tracings

Next, collected images were opened in Neurolucida and dendrites between 60 μm – 90 μm were traced. Secondary, tertiary, or quaternary branch order were selected. Primary dendrites were not sampled as they tend to contain little to no spines, while the higher order branches contain the majority of the dendrites spines. For each cell a maximum of 3 dendrites were sampled, with the majority of cells containing one segment capable of being sampled. In total, there were 57 dendrites sampled from the control group (34 D1, 23 D2) and 68 segments sampled in the high fat diet group (38 D1, 30 D2).

After the branch is traced, the spines along the dendrite are traced as well with the following criteria: (1) stubby spines: less than 1 um, no neck protrusion from dendrite (2) mushroom spines: between 1-2 um, with neck and head (3) thin spines: length between 2-3 um with neck and head (4) filopodia: neck greater than 3 um, no head. These classifications were chosen based on Graziane et al., 2016, Risher et al., 2014, and Dingess et al., 2017.

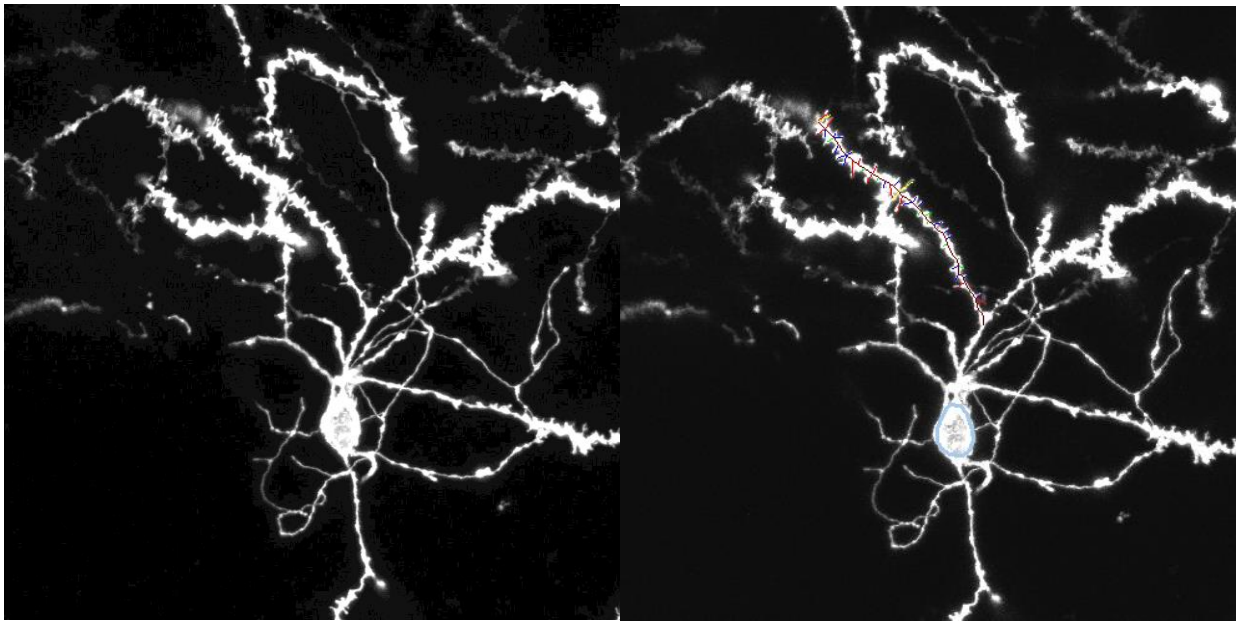

Figure 5. Image opened in Neurolucida (left), dendrite segment being traced (right), the different colored spines indicate morphology (green = stubby, purple = mushroom, red = thin, yellow = filopodia).

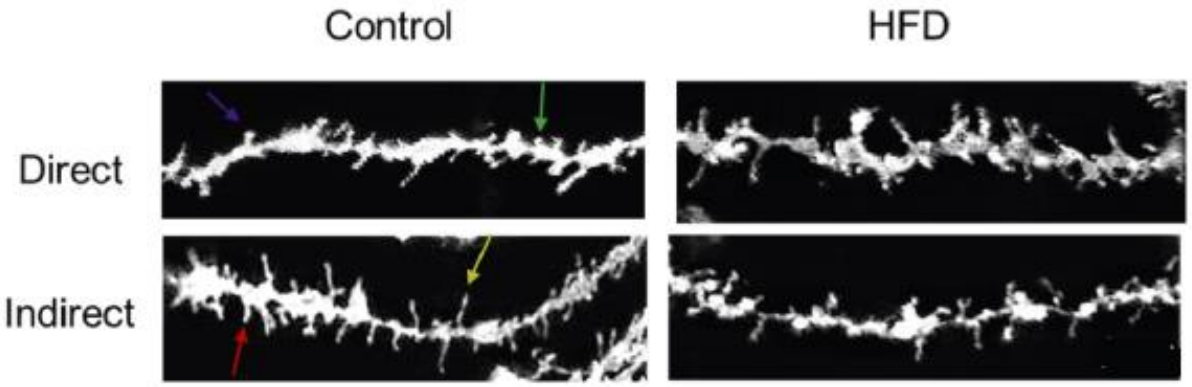

Figure 6. Sample dendritic segments from control and high fat diet mice, with arrows pointing towards: yellow-filopodia, red-thin, purple-mushroom, and stubby-green spines.

We then export a data sheet containing the overall spine density of the dendritic segment, as well as the individual density for each of the spine types along the dendrite.

Type	Qty 1	Density(1/µm)
Spines	42	0.6507
none	0	0.0000
thin	11	0.1704
stubby	9	0.1394
mushroom	19	0.2943
filopodia	3	0.0465
branched	0	0.0000
detached	0	0.0000
other	0	0.0000

Figure 7. Data Sheet exported from Neurolucida after tracing dendrites and spines.

Data Analysis

One way ANOVAs were used to determine the differences in spine density and morphology in the high fat diet mice compared to the control mice.

Chapter 3: Results

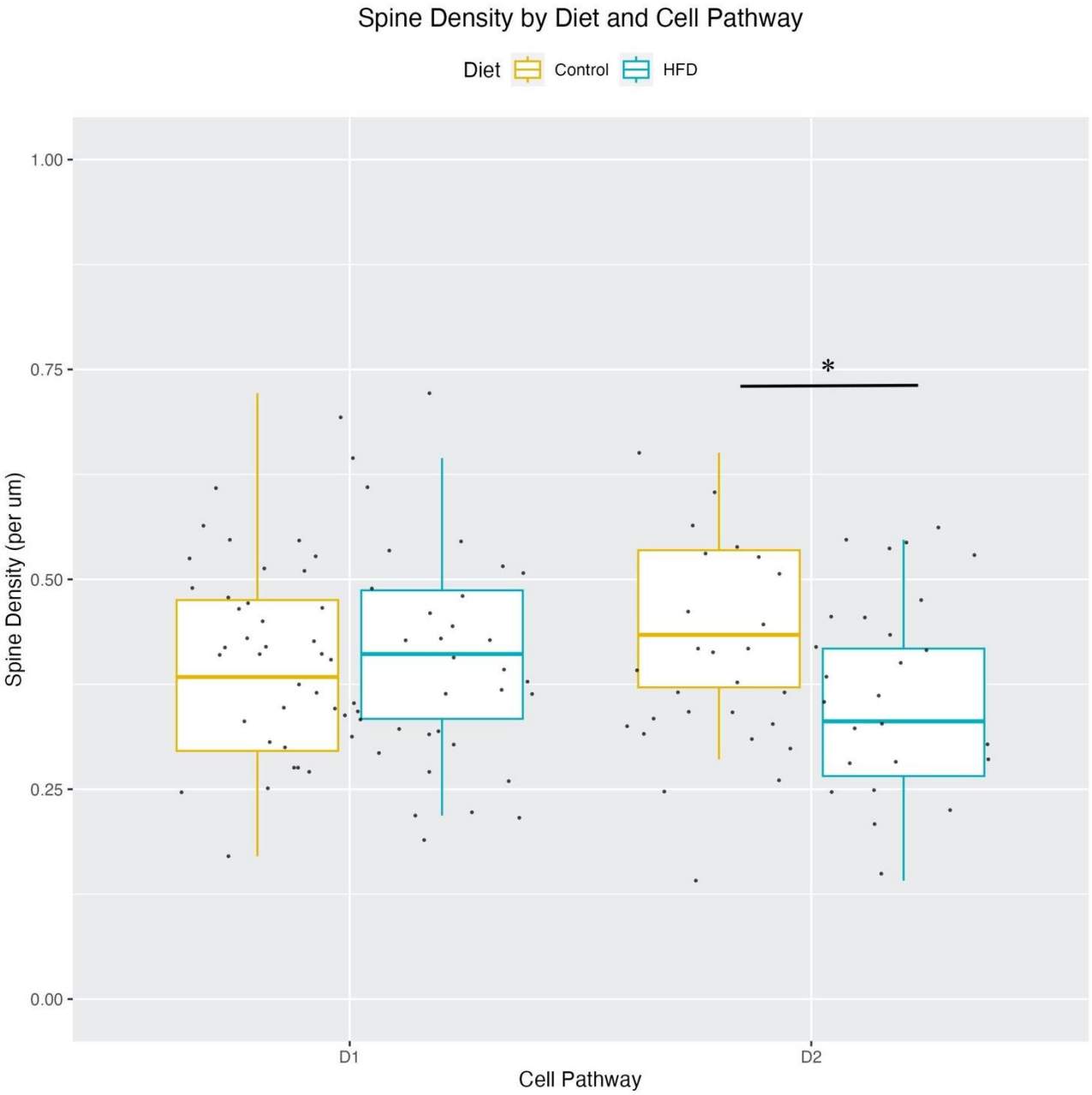

Figure 8. Spine Density in direct (D1) and indirect (D2) pathways of medium spiny neurons due to high fat diet consumption. Black dots indicate spine density of each dendritic segment sampled.

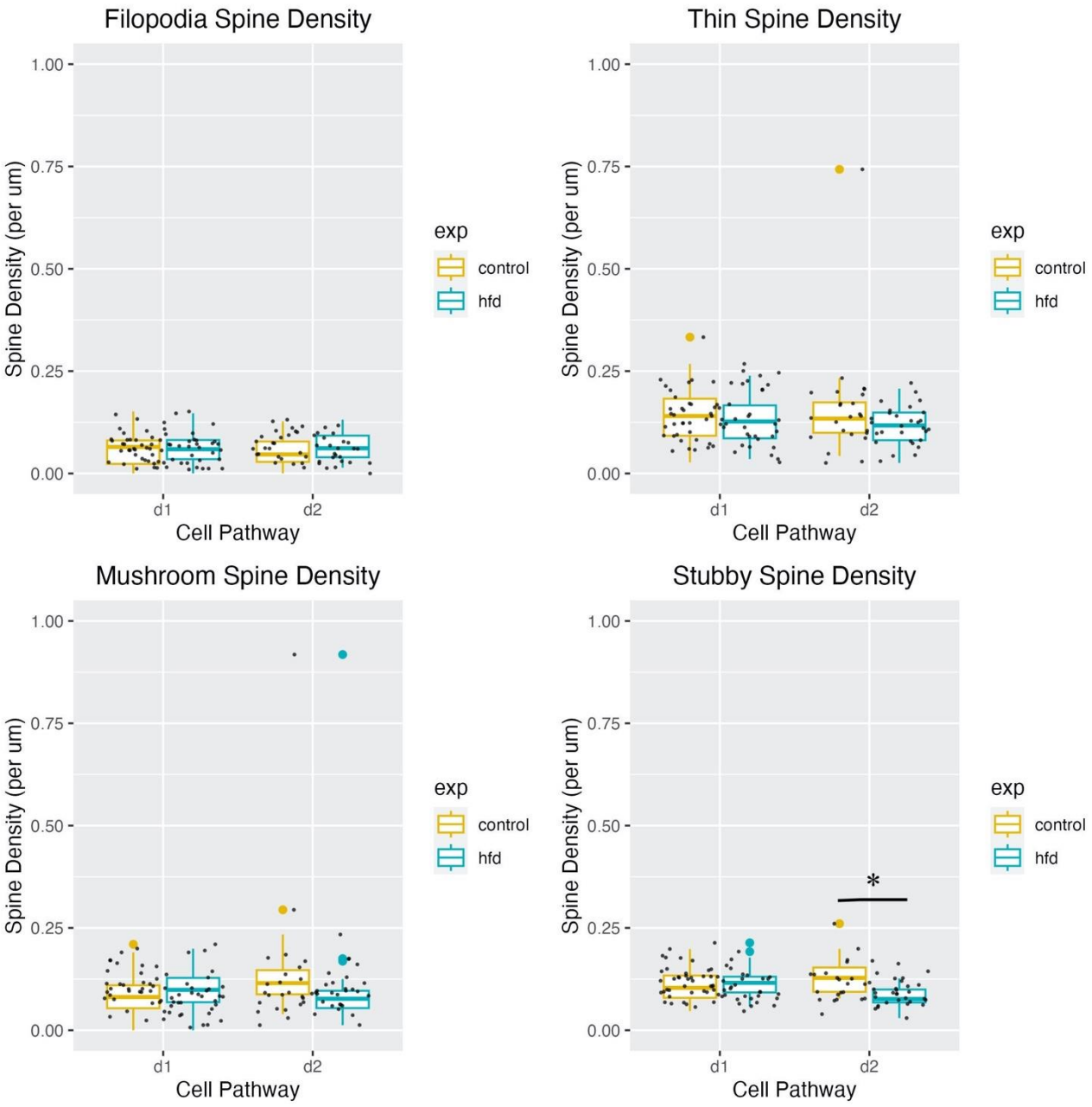

Figure 9. Spine Density by Spine type.

High fat diet fed mice showed a significant reduction in overall spine density ($F_{1,128}$=4.17, p<0.043, figure 8). The decrease in overall density was attributable to a reduction in indirect pathway neurons (F1,58=5.81, p<0.05), with no alterations in direct pathway neurons (F1,70=0.18, p=0.66). The reduction of overall density in the indirect pathway was attributable to a loss of stubby

spines ($F_{1,58}=10.19$, $p<0.05$, figure 9). No significant differences were detected for the other spine types (thin, filopodia, mushroom).

Chapter 4: Discussion

Discussion

With a significant rise in obesity over the past decades, the need to understand underlying neurobiological states associated with diets high in fat and sugars has been of increasing importance. Considering the high prevalence of highly palatable foods in western societies, and the poor long term success of weight loss programs for maintenance of weight loss, with high rates of rebound of weight gain, a food addiction model of obesity has been proposed to address both the behavioral and potential neurobiological similarities between drugs of abuse and obesity. The dopamine system being highly implicated in drugs of abuse, studies have begun investigating dopamine's involvement in obesity as well.

This study utilized DiOlistics, a method for staining whole-neurons, to investigate morphological changes in dendritic spines due to chronic consumption of a high fat diet. We proposed that both direct and indirect cell types in the dorsolateral striatum would display alterations in dendritic spines, and that those alterations would be attributable to changes in filopodia and thin spine types, as those spines are the most labile and quickest to change. Our findings, though, indicate that dendritic spine alterations were only present in the D2 pathway in the DLS, and the decrease in spine density in that pathway was attributable to a decrease in stubby spine type specifically.

Our findings are interesting for a number of reasons. As mentioned, spine types influence synaptic responses and changers are considered critical markers of synaptic plasticity (Runge et al., 2020). Therefore, alterations in spines, which can inform on the strength of synaptic response, can potentially indicate functional capacity of the cell. For instance, thin and filopodia spines exhibit smaller responses compared to the more stable mushroom and stubby spines. Additionally, the time course for alterations of less stable spines like filopodia is as little as ten minutes, while the more

stable spines change over the course of days and weeks (Holtmaan et al., 2005). Thus, our hypothesis may not have aligned with our observations, considering that we were using drugs of abuse research into spine alterations to guide our research into high fat diet-induced alterations. Drugs of abuse, such as cocaine and morphine, can induce changes in both silent synapses and spine morphology over the course of a few days (Graziene et al., 2016). In this study, on the other hand, the time course of a minimum of 6 weeks on a high fat diet was used, this could explain why more stable spine type was altered, when in drugs of abuse alterations are much quicker and therefore work through the more labile spines. A study by Saiyasit et al. (2020) found that rats fed a high fat diet for either 12 or 40 weeks, had significantly different alterations in spines, with the latter having reduced spine density. This implies that that the longer time frame of high fat diet consumption affects alterations in spines, with longer time frame reducing spine density further.

Additionally, our results indicate that only indirect medium spiny neurons underwent alterations, consistent with numerous studies findings that D2 specifically is altered in the context of obesity. This aligns with theories of both reward deficiency and energy expenditure. For instance, the loss of spines in our experiment is consistent with findings that reduction of striatal D2 activity in obesity may be contributing to increased appetitive motivation in the attempt to make up for the loss of dopamine observed in obesity by engaging in reward seeking behaviors (Blum et al., 2011). On the other hand, energy expenditure theories can also work in this context to explain D2's role in obesity, by altering energy expenditure, resulting in animals engaging in less exercise activity, thus, contributing to weight gain (Beeler et al., 2012; Kravitz et al., 2016). For example, a study by Beeler et al. (2016) found that D2R knockdown mice and wildtype mice, when in standard environments did not differ in weight gain or appetitive motivation, but when placed in environments with voluntary exercise opportunity the D2R knockdown mice exhibited less engagement with the exercise than wildtype mice and did gain more weight.

Therefore, our study's results are consistent with findings on the importance of the D2R-expressing indirect pathway as a critical substrate for obesity-induced changes in the striatum. Though how D2 is modulating aspects of behaviors such as appetitive motivation and energy expenditure needs further elucidating. Though it should be mentioned that some studies do not support the association between D2 and obesity (Janssen et al., 2022), and others implicate D1 MSNs in obesity as well (Matikainen-Ankney et al., 2022). Additionally, considering this research was done in the context of a larger project investigating silent synapses in the DLS during obesity, which found upregulation of silent synapse in both D1 and D2 pathways, the alterations in spines only in the indirect pathway may indicate that while certain alterations under obesity are found in the D2 pathway, other alterations are still occurring in the D1 pathway and contributing to behavioral alterations involved in obesity.

For instance, differences in connectivity between cortical and thalamic input to the DLS may contribute to different adaptations under obesity. Cortical inputs to the DLS tend to innervate the dendritic spines, while thalamic inputs to the DLS tend to innervate the dendritic shaft (Wall et al., 2013). Additionally, cortical afferents preferentially target the indirect pathway, while thalamic inputs target both direct and indirect pathways. Therefore, potentially the cortical inputs to the DLS by acting through indirect cell connections on the dendritic spine are altering spine density and morphology while direct cell connections via the dendritic shaft are altering other aspects of dendritic and synaptic morphology and function.

Lastly, our findings are consistent with silent synapse literature, which shows that opioids specifically (as opposed to cocaine which works through a different mechanism) increase silent synapses in the indirect pathway by removing established synapses via internalization of AMPA receptors from preexisting synapses (Graziane et al., 2017), aligning with our findings on the loss of

stubby spines in the indirect pathway following a high fat diet. Our results, therefore, show a commonality between "food addiction" and drugs of abuse.

Limitations

While this study provides insights into morphological alterations associated with obesity in the direct and indirect pathways in the striatum, there are important limitations to discuss. Most importantly, as mentioned in the introduction, dendritic spines exist on a continuum and the separation of spines in separate classes (thin, filopodia, stubby, mushroom, etc.) while informative in general terms on functional significance of the spine classification, may also be reductive in capturing the full nature of how spine shapes are working in synaptic activity. Pchitskaya and Bezprozvanny (2020) suggest that while the majority of dendritic spine analysis focuses on classifying spines by definitive categories, a newer approach, called "clusterization," may resolve some of the information lost when spines are classified by predetermined qualifications for strict groupings, by clustering the spines on a continuum of shapes and sizes of morphology. Thus, our findings may provide a broad overview of dendritic spine alterations associated with obesity, but cannot inform on dynamic and more nuanced alterations in spines potentially present as we utilized the more common method of classification based on specific criteria into discrete groups.

Additionally, due to the time frame for completing the protocol for each mouse (3 days from perfusion to imaging), the variation in the time the mice spent consuming a high fat diet is less than perfect, and considering the potential factor that the longer time spent consuming high fat diet produces greater alterations in the striatum, less variation between mice for time spent on the diet would have been optimal. Lastly, while both male and female mice were used in the study, the control group consisted of 2 males and 3 females but the high fat diet group had a less even division with 2 females and 4 males, barring analysis for sex as a potential factor modulating the effects of a high fat diet on spines. This was due to technical reasons involved in perfusions and dye delivery,

making the number of females and males in each group not conducive for statistical comparison of sex differences. While there were no apparent trends in the data suggesting sex differences, additional mice would have been required to obtain sufficient power to confirm there were no sex differences mediating the effects of high fat diet.

Future Directions

As mentioned, differences in cortical vs. thalamic input into the dorsolateral striatum direct and indirect pathways can potentially be mediating different dendritic adaptations following high fat diet consumption. Since cortical inputs innervate indirect pathway cells preferentially via the dendritic spines, while thalamic inputs innervate indirect and direct pathway cells via the dendritic shaft, potentially other aspects of dendrites are adapted followed high fat diet. For instance, arborization, which refers to the branching of the dendrites could be altered in the direct pathway, and future studies should address dendritic arborization in the dorsolateral striatum as well. Arborization has been analyzed during drugs of abuse as another potential morphological marker of the alterations occurring under drug use. For example, one study found differences between two genetic strains of rats and the dendritic complexity (arborization) following cocaine self-administration. Additionally, a study investigating the hippocampus found that after 1-week of high fat diet consumption there were reductions in the complexity of dendritic branches (Chiazza et al., 2021). Therefore, future studies should investigate arborization as well as dendritic spines to reveal potentially different alterations occurring following high fat diet consumption.

Additionally, another aspect of drug addiction that may prove relevant to obesogenic diets as well is the period of withdrawal from drugs, during which increased cravings for the substance are observed. During withdrawal spine density and morphology are altered from their states while under drug use. For example, after 28 days withdrawal from cocaine, Graziene et al. (2016) found that total spine density in the nucleus accumbens which had increased after one day withdrawal,

persisted though now the alterations were associated with increases in mushroom spines as well as just filopodia and thin spines as seen after one day withdrawal. Thus, considering the commonality between drugs of abuse and obesogenic diets, especially regarding the poor rates of success of remaining in periods of withdrawal without relapse, spine alterations should be investigated during periods of withdrawal from high fat diets.

Lastly, an important factor regarding obesity is physical activity and exercise. While high fat diet consumption can result in insulin-resistance, when mice exercise they have better insulin responses than mice who don't exercise (whether high fat diet or chow diets) (Bradley et al., 2008). Additionally, as mentioned D2R knockdown mice do not gain more weight than wildtype mice in standard conditions, but when a wheel is introduced, they display reduced physical activity compared to the wildtype mice and do not benefit from the exercise opportunity. Thus, future studies could investigate the role exercise plays during high fat diet consumption and how dendritic spines or arborization are altered in response to high fat diet alone and high fat diet with the opportunity for exercise.

Conclusion

This study sought to investigate high fat diet induced alterations in dendritic spine density and morphology in the direct and indirect pathways in the dorsolateral striatum. We found a significant reduction in spine density in indirect medium spiny neurons in high fat diet fed mice, attributable to a loss of stubby spines, with no differences between spine density in the direct pathway between high fat diet and chow fed mice. These results align with numerous studies that find D2 receptor signaling to be downregulated in obesity, though contradictory studies that do not support D2's role in obesity need to be considered as well. Furthermore, other studies show that D1 is involved in obesity as well. Future studies should further assess parameters of neuroadaptations (e.g., dendritic arborization) to elucidate the potential role of D1 in obesity, as well as investigating

the behavioral implications of the dopamine system (i.e. reward deficiency, energy expenditure, cravings, etc.) to determine how striatal alterations are contributing to obesity behaviors.

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