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Assessing Central Lipid Uptake and Impact in the Mesolimbic Dopamine System

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Assessing Central Lipid Uptake and Impact in the Mesolimbic Dopamine System

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RÉSUMÉ

L'obésité, caractérisée par une prise de poids excessive et un état inflammatoire, est une maladie métabolique qui devrait être prévenue car elle constitue un facteur de risque pour les maladies cardio-métaboliques. Les régimes riches en énergie et en graisses (*high fat diet* - HFD) sont une des causes de l'obésité. Plusieurs études suggèrent que les lipides alimentaires peuvent modifier la signalisation neuronale, l'excitabilité et la connectivité dans les aires cérébrales impliquées dans l'homéostasie énergétique. Dans le cerveau, les acides gras (AG) modulent la prise alimentaire, la prise de poids, et, plus récemment décrit, la motivation à obtenir de la nourriture. Ces effets semblent être médiés par l'incorporation des AG et/ou par leur métabolisme intra-neuronal, des mécanismes qui sont facilités par les protéines de transport, en particulier FATP1 (*fatty acid transport protein-1*). Il a aussi été montré que l'obésité altère l'intensité de l'effet de la dopamine (DA) et la neurotransmission dopaminergique dans le système mésolimbique, bien reconnu pour son implication dans les systèmes de récompense. Certaines études suggèrent que les neurones DA sont capables d'intégrer les AG, et que l'oléate, un acide gras à longue chaîne (AGLC), agit sur les neurones dans l'aire tegmentale ventrale (*ventral tegmental area* - VTA) d'où sont issus les neurones DA. L'oléate inhiberait l'activité neuronale dopaminergique, et donc les comportements de récompense.

Cependant, notre compréhension du métabolisme des AG et de leur incorporation dans le VTA est encore trop partielle. Nous avons voulu : 1) mesurer la captation d'un analogue radiomarqué d'AGLC, le *FTHA, dans diverses régions du cerveau impliquées dans la régulation de la prise alimentaire; 2) vérifier si le blocage pharmacologique de l'entrée des AG dans les cellules, via un inhibiteur de FATP1, module les effets suppresseurs de l'oléate sur la récompense alimentaire lorsqu'injecté dans le VTA; et 3) évaluer les effets d'une longue HFD

sur l'accumulation de graisse dans le cerveau, la neuroinflammation, et sur la barrière hémato-encéphalique (*brain blood barrier* - BBB) dans les régions impliquées dans la récompense.

Pour l'objectif 1, nous avons élaboré un protocole permettant de quantifier l'incorporation et l'accumulation d'AG dans le cerveau. Après une injection de *FTHA dans la veine de la queue des souris, leurs cerveaux ont été disséqués, et la radioactivité était mesurée avec un compteur gamma. Nous avons montré que le cortex préfrontal et certaines zones associées au système de récompense (striatum dorsal-DS, VTA et noyau accumbens-NAC), mais pas l'hypothalamus médiobasal (*mediobasal hypothalamus* – MBH), incorporent le *FTHA. De plus, le VTA et le DS affichaient des taux de radioactivité plus importants et, plus rapidement, que les autres zones d'intérêt. Nous avons aussi déterminé que 15 minutes est le temps d'incubation optimal pour mesurer le *FTHA. Pour l'objectif 2, nous avons confirmé *in vitro* l'action de l'inhibiteur de la FATP1. Cependant, contrairement à des travaux antérieurs de notre équipe, nous n'avons pas trouvé de différence dans les comportements de récompense suite à des injections intra-VTA d'oléate et/ou de l'inhibiteur de la FATP1. Pour l'objectif 3, des souris ont été nourries soit avec une HFD riche en AG saturées, soit avec une diète contrôle durant 20 semaines. Elles sont devenues obèses et, via des techniques immunohistochimiques, nous avons montré que la HFD avait induit une plus grande activation microgliale dans le VTA et le NAC, ainsi qu'une plus grande perméabilité de la BBB au niveau du VTA. En revanche, nous n'avons pas trouvé de différences pour la teneur en lipides, le nombre de microglies, ou les protéines de jonction de la BBB.

L'interprétation de nos résultats tient compte de certaines limites dues à nos approches méthodologiques et à la petite taille de nos échantillons. Néanmoins, s'ils sont confirmés, nos travaux pourraient contribuer à mieux comprendre comment les AG circulants sont incorporés

dans le cerveau. Nous avons démontré que les AGLC traversent la BBB et s'accumulent dans plusieurs zones de récompense (DS, VTA) de façon plus importante que dans le MBH, une région réputée pour être associée à l'homéostasie énergétique et à la détection des nutriments. Nous avons aussi montré que l'obésité induite par l'HFD est associée à une augmentation de la perméabilité de la BBB dans le VTA, et que l'on peut étendre au VTA la relation entre l'obésité et la neuroinflammation.

Notre travail apporte de nouvelles données dans le domaine du métabolisme et de l'incorporation des AG circulants dans le cerveau ainsi que sur les conséquences potentielles d'une exposition prolongée à une HFD. Comme les AG semblent s'accumuler dans le système de récompense et qu'ils pourraient modifier le comportement alimentaire des humains, nos résultats pourraient avoir des implications en obésité.

Mots clés : obésité, acides gras, métabolisme, dopamine, système dopaminergique mésolimbique, récompense, homéostasie énergétique, inflammation

ABSTRACT

Obesity is a preventable metabolic disorder characterized by excessive weight gain and inflammation, which predisposes to numerous cardiometabolic diseases. One of the causes of obesity is the continued consumption of an energy dense, high-fat diet (HFD). Increasing evidence suggests that lipid nutrients can modify neural signaling, excitability and connectivity in brain areas involved in energy homeostasis. Moreover, fatty acids (FA) in the brain have been shown to modulate food intake, weight gain, and, more recently, food-motivated behavior. These effects seem to be mediated by FA uptake and intra-cellular metabolism, which is facilitated by FA transport proteins such as FATP1. Obesity has been shown to induce alterations in dopamine (DA) tone and signaling in the mesolimbic system, well known for its implication in reward. Evidence suggests that DA neurons detect FAs and that oleate, a long chain fatty acid (LCFA), acts on neurons in the ventral tegmental area (VTA), where DA neurons originate, to suppress DA neural activity and food-seeking.

However, our understanding of FA metabolism and its uptake into VTA is still to be refined. We sought to evaluate whether: 1) the incorporation of a radiolabeled LCFA analog, *FTHA, in brain regions implicated in the regulation of food intake; 2) blocking FA entry into cells of the VTA, using a pharmacological inhibitor of FATP1, modulates the suppressive effects of oleate on food-motivated behavior; and 3) prolonged HFD has effects on fat accumulation, neuroinflammation, and blood brain barrier (BBB) integrity and leakage in reward-related areas.

Under objective 1, we developed a protocol to allow the quantification of FA uptake in the brain using tail-vein injections of *FTHA, brain dissections, and gamma counter. We found that the prefrontal cortex and reward-related areas (dorsal striatum [DS], VTA and nucleus accumbens [NAC]), but not the mediobasal hypothalamus (MBH), incorporate *FTHA, that the

VTA and DS emitted proportionally more radioactivity, and may do so more rapidly, than the other brain regions assessed. We also determined that a 15-minute incubation was optimal for *FTHA detection. Under objective 2, we showed in vitro a reduction in lipid accumulation in neurons after FATP1 inhibition. However, contrary to previous experiments conducted in our lab, we found no significant difference in food-motivated behavior following an intra-VTA oleate and/or FATP1 inhibitor injection. Under objective 3, mice were fed either a HFD (high saturated FAs) or a control diet for 20 weeks. They became obese, and via immunohistochemical techniques, we found that HFD induced greater microglial activation in the VTA and NAC, and greater BBB permeability in the VTA. However, we did not find differences in cerebral lipid content, number of microglial cells, or changes in BBB tight junction proteins.

Interpretation of these experiments are discussed within certain methodological limitations and the small size of our samples. Nonetheless, if confirmed, our data may provide additional insight in the transport of peripheral FAs into the brain. We showed that LCFA pass through BBB and accumulate in reward-related areas. The VTA, and DS had significantly greater accumulation of *FTHA compared to the MBH, a region traditionally associated with energy homeostasis and nutrient sensing. We also showed that diet-induced obesity is associated with increased BBB permeability in the VTA, and we extended the established relationship between obesity and neuroinflammation to the VTA.

This work brings forth new insights in the realm of FA uptake and metabolism in the brain, as well as their potential impacts after prolonged exposure. Our data may have potential implications for obesity, as this facilitates this macronutrient uptake in the reward system, and may alter postprandial food-seeking behaviors in humans.

Key words: obesity, fatty acids, metabolism, dopamine, mesolimbic dopamine system, reward, energy homeostasis, neuroinflammation

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LIST OF ACRONYMS AND ABBREVIATIONS

α-MSH	α-melanocyte stimulating hormone
AgRP:	Agouti-related peptide
ARC:	Arcuate nucleus
BMI:	Body mass index
CD36:	Cluster of differentiation 36
CLAMS:	Comprehensive lab animal monitoring system
CNS:	Central nervous system
CoA:	Coenzyme A
CPT1:	Carnitine palmitoyltransferase I
CRP:	C-reactive protein
CTRL:	Control
D1R	Dopamine receptor 1
D2R	Dopamine receptor 2
DA:	Dopamine
DHA:	Docosahexaenoic acid
DIO:	Diet-induced obesity
DMH:	Dorsomedial hypothalamus
DS:	Dorsal striatum
FA:	Fatty acid
FFA:	Free fatty acid
FABP:	Fatty acid binding protein
FATP:	Fatty acid transport protein
FR:	Fixed ratio
*FTHA:	14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid
GABA	Gamma aminobutyric acid
HFD:	High-fat diet
IC50:	Half maximal inhibitory concentration
ICV:	Intracerebroventricular
IP:	Intra-peritoneal
KATP:	ATP-sensitive potassium channel
LCFA:	Long chain fatty acid
LD:	Lipid droplet
LH:	Lateral hypothalamus
MBH:	Mediobasal hypothalamus
MC4R:	Melanocortin receptor type 4
ME:	Median eminence
NAC:	Nucleus accumbens
NPY:	Neuropeptide Y
PBS:	Phosphate buffer solution
PFC:	Prefrontal cortex
POMC:	Proopiomelanocortin
PR:	Progressive ratio
PVN	Paraventricular nucleus

TG:	Triglyceride
TJ:	Tight junction
TNFα:	Tumor necrosis factor-α
VMH:	Ventromedial hypothalamus
VTA:	Ventral tegmental area
WHO:	World health organization
WT:	Wild type

À ma famille, biologique et choisie, mes amis,

Votre support et amour inconditionnel sont la base de qui je suis et ce que j'accomplis.

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… Et puis, finalement, ça a finit par finir…

CONTRIBUTIONS

All experiments were conducted under the supervision and guidance of Dr. Fulton. I performed the redaction and editing of this manuscript.

Experiment 1: Assessing regional differences in acute fatty acid uptake

This experiment was done in collaboration with the Da Silva Lab, Alquier Lab, and the Animal Facility Platform of the CRCHUM.

I participated in all the meeting pertaining to experiment protocol development. During the experiment, I aided Khalil Bouyakdan in overseeing experimental time management for injection and sacrificing. I also processed samples and collected data gamma counter. I re-analyzed and organized the data.

*FTHA was synthesized by the Da Silva Lab. Daniil Petrenyov (Da Silva Lab) prepared the syringes for injections, and performed data extraction, cleaning, and processing. The Animal Facility performed the tail vein injections. Demetra Rodaros performed mouse sacrifice, blood and peripheral tissue collection. Cecile Hryhorczuk performed brain dissections.

Experiment 2: Assessing the impact of fatty acid metabolism in the ventral tegmental area on the rewarding properties of food

I communicated with the Daiichi Sankyo Co. to obtain FATP1 inhibitor 5k. I performed the *in vitro* experiment in full, took the images, analyzed them and performed statistical analyses. For the *in vivo* studies, I performed all the operant conditioning testing, stereotaxic surgeries, intra-VTA injections, and sacrifices. I did the data collection and analysis.

Experiment 3: Assessing the impact of a 20-week high-fat diet on brain inflammation, lipid accumulation and blood brain barrier integrity

I undertook the weekly weighing of mice and food, manipulations, conducted all of brain slicing, adjusted and performed immunohistochemistry protocols on all samples. I took and analyzed all the images for this work.

Khalil Bouyakdan (Rodent Metabolic Phenotyping Platform) aided me in setting up the CLAMS chambers. For the CLAMS experiment, Demetra Rodaros performed saline and SKF injections while I prepped mice, syringes and operated the CLAMS chambers. Léa Décarie-Spain assisted me with sacrifices (I took blood and performed the perfusion while she injected mice with pentobarbital, weighed them and retrieved brains). Mélanie Guèvremont (Cellular Physiology and Metabolomic Platform) performed the plasma analyses.

INTRODUCTION

1. Obesity

Obesity is a global problem of increasing magnitude. The World Health Organization (WHO) states the prevalence of obesity in humans has tripled between 1975 and 2016.[1] Moreover, at this later date, 1.9 billion, or 39% of the worldwide adult population, were overweight or obese.[1] The prevalence of childhood obesity is also rising at alarming rates: from 4% of children aged 5-19 in 1975, to 18% in 2016.[1] Indeed, the economic burden of these conditions is significant; in Canada, it was estimated that 4.1% ($6 billion) of health expenditures were directly attributable to overweight and obesity in 2006.[2] This number has likely since increased.

Overweight and obesity are largely preventable conditions characterized by excessive adipose (fat) tissue accumulation which increases multiple risks for an individual's health. More specifically, these conditions increase the risk of developing chronic diseases such as diabetes mellitus, cardiovascular and respiratory diseases, sleep and breathing related disorders, mood disorders, cancers, and reduced life expectancy.[3–5]

To measure obesity, health professionals principally use the body mass index (BMI). This measure, given by the ratio of weight (kg) divided by height squared (m^2), allows the categorization of patients into normal weight (18.5-24.9), overweight (25-29.9), and obesity categories (class 1: 30-34.9, class 2: 35-39.9, class 3: 40+). Morbid obesity is defined either by a BMI > 35, or a lower score with comorbid cardiometabolic disease.[3] While it is a highly useful and readily accessible measure, it does not take into account the type of mass (adipose tissue or muscle tissue), nor does it specify how fat mass is distributed (visceral or subcutaneous).[6,7]

1.1 Etiology and Pathophysiology

The main reason for the high prevalence of obesity in our societies overall is that we eat too much and expend too little energy. Energy balance is the equilibrium reached between energy intake (caloric consumption) and energy expenditure (thermogenesis, basal metabolic rate, and physical activity). In an obesogenic state, individuals are in positive energy balance – wherein they consume more energy than they expend. This excess energy is stored as fat, mostly in adipose tissue, where adipose cells increase in number and/or size. This tissue is mainly located subcutaneously, around the abdominal or thigh areas, or viscerally, around internal organs.[8] Studies assessing sex differences in fat deposition show that while women tend to have higher body fat composition, their tendency toward gluteal-femoral adipose tissue seems to reduce cardiovascular risk.[9]

Adipose tissue acts as an endocrine organ, releasing multiple factors playing a role in appetite, food intake, glucose and lipid metabolism, insulin sensitivity, inflammation and cardiovascular function. It contributes to the low-grade inflammation associated with obesity by releasing adipokines such as tumor necrosis factor-alpha (TNFα) and interleukin-6. These cytokines have pro-inflammatory properties and contribute to systemic metabolic dysfunction.[10] They seem to facilitate the development and maintenance of obesity as well as associated metabolic diseases.[11]

In an obesogenic state, fat not only accumulates in adipose tissue, but also in organs such as skeletal muscles, liver, heart, and pancreas, which has profound impact on metabolic health, insulin signaling and insulin resistance.[12–14] Conversely, and expectedly, a hypocaloric diet (negative energy balance) and weight loss will reduce fat content in organs.[15,16] Fat metabolism

also appears to be altered in obesity, where postprandial fats accumulate in the skeletal muscles and liver of obese individuals to a greater extent than in lean individuals.[17]

Numerous factors have been associated with obesity: ethnic, metabolic, hormonal, genetic, and socio-cultural environments (food availability). There are several risk factors pertaining to the individual; there is an overall greater prevalence of obesity in women than men; non-Hispanic blacks and Mexican American children and adults tend to be at higher risk compared to white Americans.[18] However, recent evidence points to ethnic differences in abdominal fat distribution between visceral and subcutaneous white adipose tissue, which may account for these outcomes.[19] As well, growing evidence attests that the gut microbiota – the bacteria present in our digestive tract – plays an important role in metabolism, and concordantly, in obesity. Indeed, there are numerous reports of differences in microbiome lineages in lean and obese individuals.[20]

Many genetic markers have been related to obesity, most of which are involved in energy homeostasis and metabolic pathways.[21,22] Twin studies imply the heritability of BMI and body fat ranges between 48-70%.[23–25] However, a study showed that the 32 most common genetic loci associated with obesity account for less than 1.5% of inter-individual variation in BMI.[26] Indeed, "high-risk" individuals (with all 32 genetic variations) only have a 2.7kg/m^2 higher BMI than "low-risk" individuals (none of the genetic loci).[26] This data highlights the importance of taking individual factors within their context.

Obesity prevalence has been related to low income, education, and socio-economic status,[27] although this relationship seems inversed when assessing middle-income countries.[28] It cannot be assumed that lower income or education are direct causes of obesity. Rather, highly processed foods tend to be of greater availability and at lower costs, rendering them a favorable

economic option in low-income households, despite promoting the development of obesity. Indeed, 20-50% variation in BMI can be explained by an interaction between diet and genetics.[29] This indicates the substantial role the environment may play in obesity development and maintenance. Indeed, it is said that we live in an "obesogenic environment", wherein our built environment influences physical activity and dietary behaviors in ways that promotes obesity.[30] With immediate access to a wide variety of cheap, processed, high-fat, high-sugar, and salty foods, the cost of obtaining food has decreased substantially both in terms of physical energy and in monetary value. This further tips the energy balance scale towards a positive energy state. Thus, multiple factors which facilitate an increased accessibility to high calorie foods (and increase food intake) combined with those promoting a sedentary lifestyle (reduced energy output) seems to drive the increased prevalence of obesity in Western civilizations.

1.2. Diet composition and energy density

Energy comes from food as one of three macronutrients: protein, carbohydrates, and lipids. Proteins are large molecules made of amino acids; they are structural components to tissues such as muscles and collagen, and function as enzymes and other essential components to healthy metabolic, immune and endocrine systems. Carbohydrates are chains of sugars of varying lengths and complexity which can be broken down to glucose, the body's primary energy source. Glucose is used in cells to generate ATP via a process called glycolysis. Lipids are composed of fatty acids (FA) and serve many essential bodily functions, which will be discussed in greater detail below. Micronutrients are another essential class of compounds found in our diet, although they are not a source of energy. They include vitamins (organic compounds) and minerals (inorganic substances), which aid in a host of essential bodily functions such as

energy production, immune function, healthy bone and teeth formation, fluid balance, oxygen carriers, neuronal transmission, and heartbeat.[31–33]

Energy density, or the amount of energy per weight of food, is a crucial factor in energy balance. Proteins and carbohydrates each provide about 4kcal per gram while lipids provide approximately 9kcal/g. Energy dense food usually includes highly processed foods, rich in simple sugars and fats, and have high caloric content per gram. These foods also tend to be highly palatable and enjoyable to eat. The WHO stated that, alongside a sedentary lifestyle, an energy-dense diet low in micronutrients is a convincing risk factor for obesity.[34] Indeed, an experiment comparing 29 diets of varying macronutrient content in multiple mouse strains have shown that a high-fat diet (HFD), but not a high sucrose diet, leads to obesity in mice. [35] This finding has been replicated in obese patients,[36] suggesting the importance of diet composition in weight control. Indeed, there is an overwhelming amount of evidence supporting the role of dietary fat in obesity, both in animal models and clinical trials.[37,38] Meals rich in fat appears to have a lower satiating effect, which would promote overeating and weight gain.[39] Conversely, diets lower in fat content, or those which target fat reduction, have shown to effectively promote weight loss to a greater degree than those with more typical fat content.[40]

Thus, our built environment has facilitated access to energy dense foods which favors the adoption of HFDs. These in turn facilitate the development of obesity through their palatability and low satiation properties. Given the relevance of fats in obesity and the objectives of this work, we will now discuss fatty acids, their metabolism, and implication in obesity.

2. Fatty Acids

2.1 Structure

Along with carbohydrates and proteins, fat is one of the three main macronutrients in the diet. FAs are a type of lipid, they are the building blocks for fat in our body and food. FAs are structures composed of a hydrocarbon chain with a carboxylic acid residue on one end. The hydrocarbon chains occur in varying lengths (short chain: 2-6 carbons; medium chain: 8-12; long chain: 14-18; or very long chain: 20-26), and saturation (saturated FA: no double bonds in the hydrocarbon chain; monounsaturated: one double bond; and polyunsaturated: two or more double bonds). FAs are frequently complexed as triglycerides (TG), structures composed of three FAs bound to a glycerol molecule.[41] TGs are transported throughout the body in lipoproteins.

2.2 Functions

In the organism, FAs serve many roles. As fat contains more energy per gram than carbohydrates or proteins, it is the preferred form of energy storage. Carbohydrates act more as an immediate, post-prandial energy source. Once postprandial glucose and glycogen stores are depleted, fat stores begin to be broken down, releasing intermediates used in energy production via a process called β-oxidation. After prolonged fasting or chronic carbohydrate restriction, ketones - a product of FA breakdown - become a significant source of energy in the body. Carbohydrate and fat metabolism work together to maintain a stable amount of available energy for bodily functions and can adapt to states of privation.

In addition to being a form of energy, FAs are important constituents of cell membranes, ensuring their structure and fluidity. They also have roles such as carriers for fat-soluble

vitamins (vitamin A, D, E, and K), facilitate cell signaling, and protein adhesion.[42] Moreover, 60% of the brain is composed of lipids. This high proportion is due to the presence of FAs in both cell membranes and in myelin sheaths (an insulating layer which surrounds neuronal axons to ensure rapid and efficient electrical signaling).[43]

2.3 Digestion and metabolism

Starting in the mouth, fats and TGs start being broken down into diglycerides, monoglycerides, and free fatty acids (FFA) by enzymes called lingual lipases. This continues in the stomach with gastric lipases. However, the majority of lipid hydrolysis occurs in the small intestine, facilitated by pancreatic lipases and bile. The products of fat digestions – FFAs, free cholesterol, and 2-monoacyl glycerol – along with bile salts form structures called micelles. The hydrophilic surfaces of micelles facilitates the transport of lipids across the brush border membranes and into intestinal enterocytes, although FAs can also be absorbed via simple diffusion or facilitated transport.[44] Inside enterocytes, FFAs and monoglycerides are re-packaged as TGs, and then into larger compounds named chylomicrons with other lipid structures such as cholesterol and lipoproteins. Newly formed chylomicrons are then expelled from enterocytes via exocytosis. They are transported either into the hepatic portal circulation and delivered to the liver, or into the lymphatic system which drains directly into the brachiocephalic vein. Chylomicrons circulate throughout the body and are hydrolyzed to release TG, glycerol, and FAs by lipoprotein lipase.[45] Liberated lipids can be taken up by muscle or adipose cells (by simple or protein-mediated diffusion) and used either as energy or stored as lipid droplets (LD). What is left of chylomicrons is returned to the liver and they are eliminated 12-14 hours after meal termination.[46]

Cluster of differentiation 36 (CD36; also known as fatty acid translocase) and fatty acid transport proteins (FATP) are the main classes of proteins which enable FA entry into cells.[47,48] FATP1 is expressed in insulin-sensitive tissues, is specific to LCFAs and is essential to postprandial lipid serum concentrations. Indeed, FATP1 has been shown to regulate tissue distribution of dietary lipids results, making this protein relevant in the context of diet-induced obesity (DIO).[49] However, it does not appear to contribute to the adverse metabolic outcomes associated with HFD consumption.[50]

Most dietary FAs are packaged as TG (95%) and enter circulation, while the remaining 5% can be found as diglycerides, phospholipids, cholesterol esters, or FFAs.[51] FAs can also be generated endogenously. This de novo synthesis is initiated by the transformation of acetyl-coenzyme-A (CoA) to malonyl-CoA by the enzyme acetyl-CoA carboxylase. Malonyl-CoA is a key intermediate molecule, which can undergo a series of reactions catalyzed by fatty acid synthase (FAS), resulting in the structure palmitoyl-CoA, which can then undergo further elongation or desaturation steps to form other FAs.

To produce energy, FFAs in the cytosol (derived from circulation or liberated from LD by lipolysis) must be "activated". To do so, acyl-CoA synthetase adds CoA to long-chain FA (LCFA). This LCFA-CoA then enters the mitochondria with the aid of carnitine palmitoyltransferase I (CPT1). Inside the mitochondria, the LCFA-CoA will enter β-oxidation. In this catabolic process, two carbon atoms from the carboxyl terminal of the FA chain are removed to generate acetyl-CoA. Acetyl-CoA then enters the citric acid cycle (Krebs cycle), a series of enzymatic reactions which generates CO_2, GTP and NADH. CO_2 is expelled as waste and NADH is fed to oxidative phosphorylation (the electron transport chain) to produce ATP.

In conditions where the rate of β-oxidation exceeds the capacity of the citric acid cycle, acetyl-CoA is converted into ketosis products.[52]

2.4 Fatty acid abundance & impact of diet

While a HFD is robustly associated with obesity, not all FAs have the same impact.[42] Common dietary FAs include stearic and palmitic acid (palmitate; saturated LCFAs), oleic acid (oleate; monounsaturated LCFA), and linoleic acid (polyunsaturated omega-6 FA).[53] Oleate appears to protect against insulin resistance and atherosclerosis, while palmitate promotes inflammation and insulin resistance in adipose tissue, muscle, liver and pancreas.[54] Omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid are important for optimal development, neural, immune, and cardiovascular functions, and appear to have protective properties for weight gain and cognitive function.[55]

The industrial revolution has brought about a shift in diet in Western countries. Namely, there is an overall increased carbohydrate intake, increased proportion of saturated FAs and imbalanced omega-6 to omega-3 FA ratio. These changes increase risk for cardiovascular diseases, diabetes and cancer.[56] While the high proportion of fat in the diet is an important factor to consider, the FA composition of diet must also be taken into account. For instance, men fed high-fat or low-fat diets with equal *proportions* of saturated, monounsaturated and polyunsaturated FAs had similar plasma cholesterol levels.[57] Moreover, balancing dietary fat composition (one third of each type) improves vascular function, inflammation, and body composition in obese women.[58] Conversely, manipulating FA proportions in isocaloric HFDs has shown that diets rich in polyunsaturated FA attenuates weight gain, hepatic steatosis and mitochondrial oxidative stress.[59] They also ameliorate blood lipid profiles in rats, compared to

those fed diets higher in saturated FA.[59] Moreover, the deleterious effects of HFD such as hyperphagia and glucose intolerance can be rescued by fish oil supplementation (rich in omega-3).[60] Indeed, it is well recognized today that diets rich in saturated and trans FAs increase risk of poor cardiovascular health and impaired cardiometabolic profiles, while those rich in unsaturated FAs have a protective effect.[61,62] This highlights the importance of fat composition and quality of the diet on the metabolic profile of obese individuals.

In sum, energy dense diets rich in fats tend to promote the development and maintenance of an obesogenic state. However, dietary FA composition can have a profound impact on cardiometabolic health, where saturated and trans FAs have pro-inflammatory properties and tend to worsen metabolic profiles. Contrarily, unsaturated FAs can protect individuals from developing risk factors or even reverse adverse profiles in conditions of obesity. Metabolic health and the control of weight are indeed in part attributable to diet. These are also modulated by the brain, the organ which regulates bodily functions, behavior, and thought.

3. Role of the Central Nervous System in Energy Balance and Metabolism

The control of energy balance and appetite is in large part attributed to the brain, both in metabolically healthy individuals and in conditions of overweight or obesity. This organ comprises various neural circuits and nuclei which, among other functions, can regulate basic, conserved homeostatic mechanisms, eating behavior, process food-related cues and appetitive preferences.[63] Indeed, it plays a vital role in regulating energy homeostasis, which in turn is maintained with bidirectional communication between the CNS and peripheral tissues. This is achieved *via* neuronal signals (peripheral nervous system), gut-derived hormones, adiposity signals, and nutrients. Moreover, a genome-wide association study in nearly 340’000

individuals identified that BMI-related loci are highly enriched in the central nervous system (CNS) compared to other organ systems.[22] This supports the role of the brain in metabolism, weight control, and obesity pathophysiology.

The brain is comprised of five main cell types: neurons, oligodendrocytes, astrocytes, microglia, and ependymal cells. Neurons transmit information both within the brain or between the CNS and periphery. Oligodendrocytes are a type of glial cell which produce myelin sheaths, a structure which insulates axons to increase neuronal transmission speed.[64] Astrocytes, another kind of glia, create and maintain the brain micro-environment, partake in nutrient sensing and metabolism, and act as the primary nutrient provider for neurons.[65] Astrocytes have been shown to utilize FA and glucose to a larger extent than other neural cells.[66–69] They can also facilitate neurotransmission,[70] and are a component of the blood brain barrier (BBB),[71] and appear to be involved in immune protection.[72] Microglia provide an immune and protective role in the CNS by clearing debris, fighting infection, or removing toxins.[73] Microglia are activated in a neuroinflammatory state. Ependymal cells line the ventricles and the central canal of the spinal cord. They make cerebrospinal fluid and are responsible for electrolyte and solute transport between this fluid and the brain parenchyma.[74]

The brain is a complex organ made of many kinds of cells, and their interplay is what gives rise to both basic, autonomic functions as well as higher order processing. We have previously discussed diet and FAs in relation to obesity and have introduced the idea of a central control of weight and eating by the brain. The interplay between the brain and the body is complex, and it is partially mediated by the BBB.

3.1 The Blood Brain Barrier

Before dietary FA or other peripheral signals may impact neural cells, they first need to be transported into the CNS from the blood. The BBB is a highly selective, semi-permeable membrane between neural tissue and the rest of the body. It controls which substances can enter the brain micro-environment from the blood, protecting it from circulating toxins or pathogens.

All blood vessels are lined by endothelial cells. However, the particularity of the BBB is that they are very close together and the gaps between them are "sealed" by tight junctions (TJ). TJ are multiprotein complexes which bind adjacent endothelial cells with transmembrane proteins such as occludins and claudins.[75] This prevents molecules from passing between them. As a result, larger molecules must use specific transporters to get across the BBB. The type and quantity of such transporters is a means by which the BBB can exert control over molecule entry into brain parenchyma. Exceptions to this barrier include the circumventricular organs, areas around the third and fourth ventricles characterized by their small size, and their extensive, permeable, and fenestrated capillary beds (i.e. the absence of TJ).[76] These structures allow the facilitated, bidirectional exchange of nutrients and hormones between brain and periphery *via* the cerebrospinal fluid.[77]

The structure and integrity of the BBB is maintained by the interplay of many components (see Figure 1). The basement membrane (or basal lamina), composed of the protein laminin, contributes to the BBB integrity by creating a protein mesh network, supporting the interactions between the endothelial cells and other structures.[78] One of these includes pericytes, a type of glia which wrap around endothelial cells and whose contractile capabilities can modulate cerebral blood flow.[79] Astrocytic end-feet also encircle the BBB endothelial cells to facilitate nutrient and hormone uptake and availability. In response to neuronal activity,

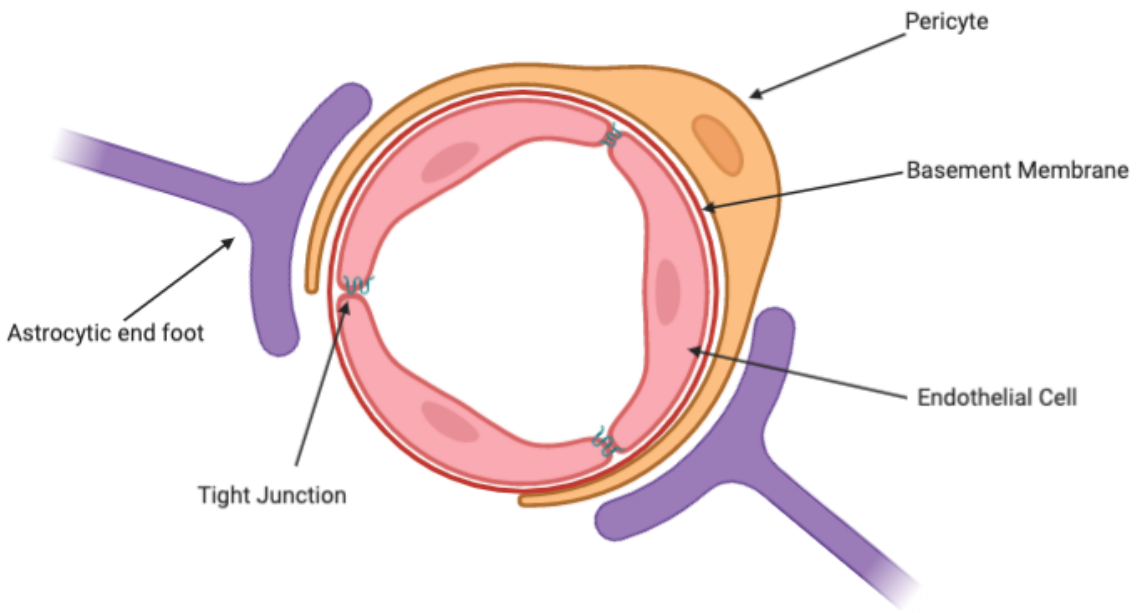

Figure 1. Diagram of the different components of the blood brain barrier. Endothelial cells are joined together by tight junction proteins and supported by the basement membrane. These structures are further supported by pericytes and are in contact with astrocytic end feet. Image created in BioRender.com.

astrocytes will participate in blood flow regulation to enhance oxygen and glucose delivery to metabolically active brain regions.[80] All of these components work together to support the functions of the brain and prevent pathogens from passing through.

There are two main mechanisms by which molecules cross the BBB: transmembrane diffusion and facilitated transport.[81] Simple diffusion is non-saturable, but it is slow and dependent on molecular characteristics. As a general rule, small and fat-soluble molecules can pass through the endothelial cell membranes. Conversely, transport proteins greatly increase uptake of ligands, although their quantity or structural quality can be modified in disease states.[81] Indeed, BBB dysfunction has been described as a basis for pathology of neurological diseases such as multiple sclerosis, stroke, traumatic injuries, neurodegenerative disorders, epilepsy, intracerebral hemorrhage and tumors.[71,82,83] Specifically, loss of function in some or most BBB

properties can lead to altered homeostatic signaling, ion dysregulation, and immune cell or molecule penetration from periphery.[84]

The BBB is an important and complex structure involving the interplay of many cell types. It has several mechanisms to protect the brain, although alterations in its function or integrity could induce, maintain or be a result of diseased states.

3.1.1 Fat transport across the blood brain barrier

As with tissues in periphery, dietary FAs influence brain FA composition both in hypothalamic and mesolimbic regions.[85–88] Indeed, a HFD has been shown to increase the levels of saturated FAs and reduce the amount of polyunsaturated FAs in the forebrain of mice compared to those fed a chow diet.[60] Moreover, in conditions of DIO, this adverse neural FA profiling can be reversed by unsaturated FAs (omega-3) supplementation.[60] This mimics the rescue effect seen in periphery.

FA transport from periphery into brain parenchyma is not well understood. Approximately 5% of circulating FAs can be captured by the brain,[89] at concentrations that are proportional to plasma.[90–92] Studies assessing FA uptake in the brain have mostly employed radiolabeled FAs injected into the bloodstream.[93,94] Early work has shown that peripheral palmitate, octanoic and myristic acids (saturated), and linoleic acid (unsaturated) accumulate in the brain of rats and monkeys,[95–97] which is indicative of their transport across the BBB. Indeed, human brain microvascular endothelial cells have been shown to express relevant FA transporters and carriers FATP-1, FATP-4, FABP-5, and CD36 to a lesser extent.[98,99] FA uptake appears to be dependent on chain length and saturation,[98] and transporters are likely involved in FA transport across the BBB. The transport of oleate is enhanced in the presence of serum

albumin, a FA carrier in the blood, and decreased when FATP1 or CD36 is knocked down.[100] In fact, CD36 has been shown to plays a prominent role in the transport of most FAs across the BBB.[98] FABP5 has also been shown to be significantly involved in the uptake and transport of oleate, DHA, stearic acid (saturated LCFA), lauric acid (saturated MCFA), both in human brain microvascular endothelial cells and in mice.[101,102] Moreover, FATP1 plays a role in the transport of DHA, although this effect is inhibited in the presence of oleate – it's preferred substrate, and is enhanced by insulin which promotes the translocation of FATP1 to the apical membrane.[103] This last finding points to the dynamic interplay between peripheral hormones and cerebral FA metabolism. The disadvantage of these radiolabeled FA studies is that the half-life of the commonly used radionuclide (^{11}C) is relatively short – only 20 minutes.[104] Despite this, these findings highlight the relevance of transport proteins in the uptake of FAs from periphery.

To counter this limitation, the radiolabeled LCFA analog 14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid (*FTHA) was designed to undergo mitochondrial metabolic trapping within the β-oxidation pathway.[105,106] As well, *FTHA has a 109 minute half-life, allowing an improved quantification of FA uptake by tissues. It has been shown that over 90% of *FTHA given to rats by gavage reaches circulation in form of TG,[107] which mimics FA digestive pathways. Moreover, its metabolism appears to be similar to other LCFAs,[108] and has been shown to accumulate in the brain.[109]

These findings support that FAs are indeed transported into the brain micro-environment from periphery, and that this process could be facilitated by transport proteins. The implication of FA transport across the BBB in diseased states is not yet fully understood. However, preliminary evidence suggests the BBB becomes more permeable to lipids in conditions of overweight and obesity.

3.1.2. Blood brain barrier and fatty acid transport in obesity

As seen in other neurodegenerative disorders, the transport of various peptides, FAs and hormones across the BBB is altered in obesity. Different studies assessing these changes suggest an increase in FA uptake, and a decrease in leptin, insulin and ghrelin transport.[110] Notably, in obese human patients with metabolic syndrome, *FTHA uptake into the brain was increased by 50%, and palmitate transport was increased by 86%, compared to control, normal weight subjects.[111]

In humans, overweight/obese patients appear to have impaired BBB permeability as evidenced by greater infiltration of fibrinogen, a blood-bound glycoprotein, into the amygdala and parietal regions.[112] This association was correlated with adiposity.[112] In a rat model of DIO, a western diet (high-fat, high-sugar) appears to increase BBB permeability in the dorsal striatum (DS) and the hippocampus. [113] This effect seems to worsen with prolonged diet duration and in mice with an obesity-prone phenotype (compared to DIO-resistant mice).[113] As well, rats who consumed a HFD for 90 days (13 weeks) showed a significant reduction in BBB TJ protein mRNA, and an increased permeability to sodium fluorescein in the hippocampus, but not in the striatum or the prefrontal cortex.[114] Impairments to the BBB appear to be specific to long-term HFD: one week exposure to *ad libitum* western diet was not sufficient to alter BBB permeability (diffusion) or structure (TJ and glucose transporter expression) in the hypothalamus of both fed and fasted rats.[115] In addition to prolonged intake, increased age also appears to exacerbate the deleterious effect of obesity on the BBB and neuroinflammation. Older HFD-fed mice displayed increased levels of immunoglobin G, a blood-bound antibody, and activated microglia in the hippocampus.[116]

Thus, although the mechanisms are not well understood, obesity appears to affect both the permeability and structure of the BBB. Impairments seem to be related to HFD duration and age. The implication of this increased leakiness to peripheral molecules and FAs into brain parenchyma has yet to be explored. Moreover, how this can affect food intake and body weight is of increasing relevance as the prevalence of obesity continues to grow.

3.2 Hypothalamus

The hypothalamus is a small region located near the base of the brain around the third ventricle and is involved in regulating endocrine, autonomic and behavioral functions. The hypothalamus controls energy balance in part by controlling food intake.[63] It can detect nutritional states and elicits appropriate behavioral and metabolic responses in consequence.

This thesis relates to FA metabolism and their impact on the mesolimbic dopamine system. However, there is a more extensive body of research on the hypothalamic control of feeding and how FA metabolism in this region influences body weight and food intake. To illustrate that FAs are metabolized by neural cells and have functional consequences on energy balance and behavior, these will first be discussed in the hypothalamus. The topic will then be introduced in the context of the mesolimbic dopamine system and reward-mediated feeding.

3.2.1 Hypothalamic regulation of energy balance

There are multiple nuclei involved in hypothalamic control of feeding. In rodents, early studies showed that lesions of the ventromedial hypothalamus (VMH) and the paraventricular nucleus causes hyperphagia and associated obesity,[117,118] while lesions to the lateral hypothalamus (LH) and dorsomedial hypothalamus (DMH) induced severe weight loss and

eventual death, without inducing sensorimotor deficits.[119,120] More recent studies have shown the VMH plays an anorectic role in food intake, as well as prevents the development of insulin resistance and hypercholesterolemia in HFD-induced obese rats.[121] Similarly, DMH promotes physical activity and peripheral metabolism, and reduces food intake and body weight through its response to leptin.[122,123] As well, the LH has been shown to be extensively connected to reward centers, cortical areas, and the amygdala, and is thereby critical in motivation and food intake. Notably, its stimulation can induce voracious feeding in animals, even when fed, and can induce relentless work to obtain food rewards in operant conditioning paradigms.[124]

Of particular relevance, the arcuate nucleus (ARC) is a major contributor to the control of energy balance as it integrates both systemic nutritional and hormonal signals from the periphery, as well as peripheral and central neuronal signals.[125] It does so in part due to its connection to the median eminence (ME), a slight prominence on the ventral surface of the hypothalamus. The ME is a circumventricular organ, a highly vascularized and permeable brain structure which facilitates nutrient and hormone transport and sensing.[126] A schematic of the hypothalamic nuclei involved in the neural control of food intake is seen in Figure 2.

In brief, the ARC contains two functionally opposite neuron populations which regulate appetitive behavior: the anorexigenic proopiomelanocortin (POMC)-expressing neurons, and the orexigenic neuropeptide Y (NPY)- and agouti-related peptide (AgRP)-expressing neurons.[127–129] POMC neurons are activated postprandially to reduce food intake and increase energy expenditure. At this time, POMC is converted to α-melanocyte stimulating hormone (α-MSH), which is released to activate melanocortin receptors (MC4R) on neurons in the paraventricular nucleus (PVN), ultimately resulting in increased energy expenditure and reduced food intake.[130] POMC neurons also project to the DMH, LH, and VMH for further

processing, and these second order neurons project to extra-hypothalamic sites for an integrated response in regulating food intake and energy expenditure.[131]

Contrarily, fasting activates the NPY/AgRP circuit, which also project to the PVN and LH.[132] NPY directly stimulates food intake,[133,134] and reduces energy expenditure,[135] while AgRP is an inverse agonist antagonist of MC4R,[136] thereby preventing the anorexigenic effects of second-order melanocortin neurons, and directly inhibit POMC neurons with the inhibitory

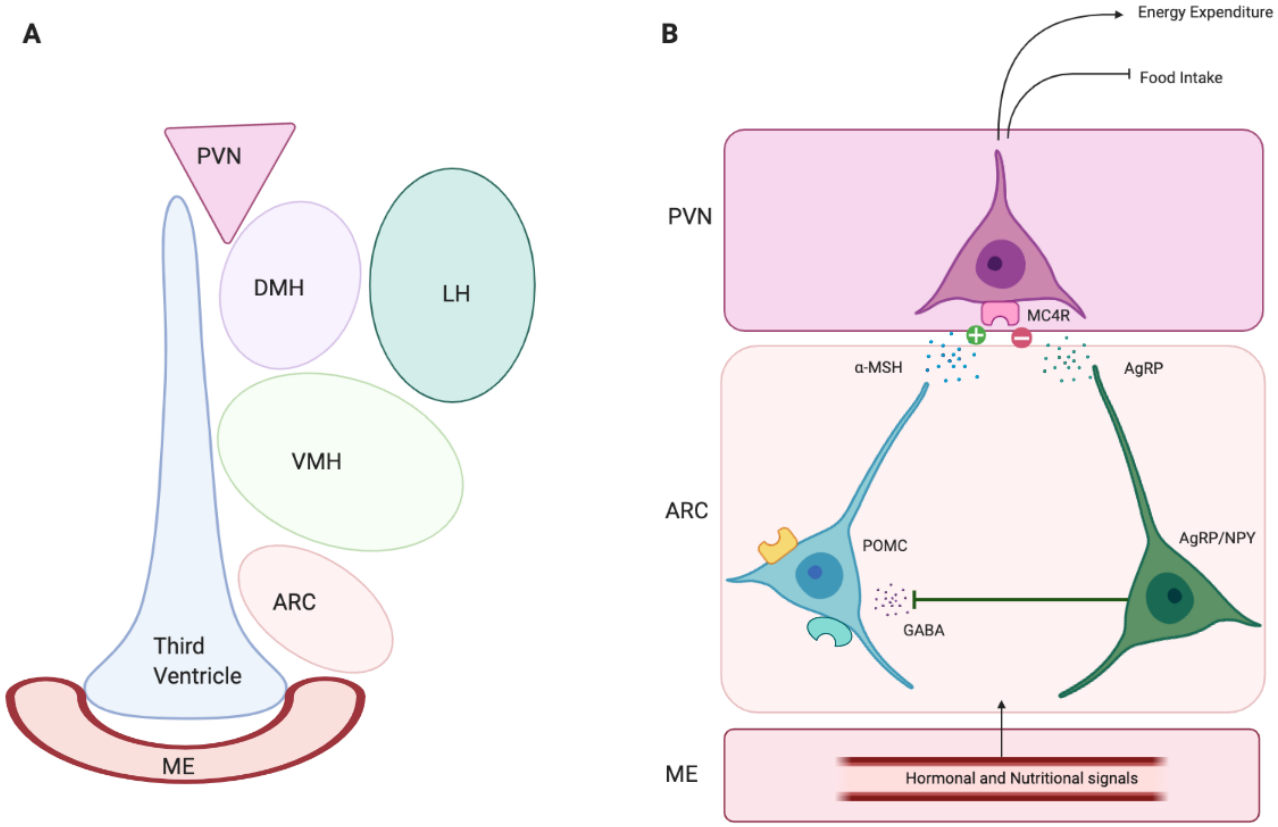

Figure 2. Hypothalamic control of eating. **A**. Hypothalamic nuclei relevant in the control of food intake in relation to the third ventricle. **B** Principle neural circuits underlying food intake and energy expenditure. Nutrient and hormone signals are sensed in the median eminence (ME) and act on proopiomelanocortin (POMC) and neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons in the arcuate nucleus (ARC). POMC neurons have an orexigenic effect by releasing α-melanocyte stimulating hormone (α-MSH) on melanocortin receptors (MC4R) on neurons in the paraventricular nucleus (PVN). NPY/AgRP neurons exert an anorectic effect either by inhibiting MC4R or acting directly on POMC neurons. DMH: dorsomedial hypothalamus; LH: lateral hypothalamus; VMH: ventromedial hypothalamus. Image created in BioRender.com.

neurotransmitter gamma aminobutyric acid (GABA).[137] Additionally, AgRP ablation in mice induces comfort-like eating behaviors and palatable food preference, suggesting a close relation between the energy balance control and emotional, stress-related neural circuits in feeding-related behavior.[138]

In addition to the POMC and AgRP/NPY circuitry, there is a dynamic interplay between the various hypothalamic nuclei.[139] For instance, the LH is a richly interconnected area which, through numerous neuropeptides, is implicated both in regulating feeding, reward, but also metabolism. For instance, orexin in the LH are thought to regulate arousal,[140] increase food intake,[141] while their antagonism causes reduced consumption in ob/ob mice.[142] In opposition, stimulation of melanin-concentrating hormone neurons of the LH induces hyperphagia, obesity, and insulin resistance in rodents,[143,144] while their ablation induces hypophagia and a lean phenotype.[145,146] Another related peptide in the LH is neurotensin, which appears to suppress feeding and increased resting behavior.[147] This demonstrates the complexity of neural control of food intake, and highlights the numerous interconnected systems which must work together.

3.2.2 Extra-hypothalamic control of food intake

We have discussed some ways the hypothalamus regulates food intake, but eating behavior is not strictly limited to the control of caloric and nutrient requirements. Indeed, the role of the LH extends beyond the homeostatic control of food intake, via its numerous connections. Namely, it is widely connected to the mesolimbic dopamine system, a pathway involved in reward and the reinforcement aspects of eating. Orexin projections to the implicated regions participate in reward, their activation can re-instate extinguished drug- or food-seeking behavior, and this was blocked by orexin-A inhibition.[148] This interplay between the

hypothalamus and other regions is also mediated by leptin, which produces overall anorectic effects via neurotensin neurons, ultimately resulting in altered orexin projections from LH to the ventral tegmental area (VTA) of the mesolimbic system.[149]

There are multiple other neural structures and systems which participate in the sensorial, cognitive, emotional, motivational and even hedonic properties of eating behavior.[150] In humans, visual and olfactory senses not only aid in identifying food in our environment, they also guide food choices and are linked to memory. Taste and texture also appear to influence meal size and satiety.[151] Cognitive function has also been related to appetite and eating regulation. The dorsolateral prefrontal cortex (PFC) has been associated to appetite control, food cravings, as well as executive decision making.[152] Indeed, DIO appears to dampen PFC responsivity to food cues, and is thought to drive the maintenance of overconsumption and unhealthy eating behaviors.[153] The involvement of the hippocampus in regulating food intake in humans is another emerging field. Hippocampal dysfunction appears to be induced by a Western diet, and this may in turn lead to long-term positive energy balance by impairing enteroception, timing of food intake, cravings, and nutrient-related learning.[154] Namely, impairments in working or episodic memory have been related to increased adiposity and BMI.[155] It is thought that these are related to the ability to sense internal states and make food-related decisions, such that impairments in these capabilities predispose to uncontrolled eating and weight gain.[155]

Other neurotransmitter systems are also involved in regulating food intake. Serotonin acts as an appetite suppressant, acting both in the hypothalamus (ARC and paraventricular nucleus) and in extrahypothalamic areas (nucleus of the solitary tract and parabrachial nucleus).[156] As well, glutamate within the mesolimbic pathway is thought to be involved in regulating rewarding properties and behavior in the context of drug addiction and overeating.[157]

3.2.3 Hypothalamic fatty acid metabolism

When the brain is exposed to FAs, both local and global responses are observed. Intracerebroventricular (ICV) infusions allow for substances to be delivered into the cerebrospinal fluid, leading to the whole brain being exposed and affected. In fact, ICV delivery of unsaturated FAs (oleate and DHA) acutely reduces food intake, body weight, plasma insulin and glucose concentrations, and endogenous glucose production, while saturated FAs (palmitate and octanoic acid) seem to have no effect on these outcomes.[158,159] Contrarily, ICV palmitate induces a pro-inflammatory response in the hypothalamus as well as local insulin and leptin resistance.[160–163] More localized infusions of FAs to the mediobasal hypothalamus (MBH), which encompasses the ARC, DMH, VMH and ME, replicate the effects seen in ICV while also inhibiting hepatic glucose production.[164] These effects were also found to be induced by gavage,[162] and independent of caloric intake,[161] suggesting they are due to dietary fat composition. Moreover, TGs infused directly into the carotid artery induced a state of hypothalamic inflammation.[165] Interestingly, oleate can block the inflammatory state induced by palmitate in POMC neurons.[163] As well, long-term DHA infusions protects against liposaccharide (LPS)-induced neuroinflammation and neuron loss.[166] These findings suggest that saturated dietary FAs can act on the nervous system and induce neuroinflammation, and that unsaturated FAs may have anti-inflammatory or protective properties both in the brain and in periphery.

The effect of these FAs on food intake and peripheral metabolism may in part be due to the ability of FAs to alter neuropeptide expression and firing rate. Indeed, oleate and palmitate given through ICV significantly alters hypothalamic expression of NPY and POMC. [158,159,163,165]

Moreover, it appears that FAs can modulate hypothalamic neurons' firing rate in a dose- and glucose-dependent manner, both *in vivo* and in live brain slices.[167–170] This interaction between glucose and FA sensing and metabolism is a potential mechanism by which nutrient sensing elicits appropriate responses to metabolic states.[169]

While the mechanism is not fully understood, hypothalamic manipulations of transporters or enzymes involved in FA catabolism or anabolism have shown to induce metabolic and behavioral changes. For instance, the effect of ICV oleate seems to be mediated by its ability to close ATP-sensitive potassium channels (KATP) on POMC neurons, and thereby depolarizing them.[158,170] KATP channels are mainly found on plasma membranes and are sensitive to metabolic changes: opening during states of low metabolic activity, causing an efflux of potassium and hyperpolarizing the cell, and closing during high metabolic activity.[171] This suggests extracellular oleate may serve as an indicator of metabolic state.

Intracellular FA metabolism also plays a role on these systemic effects. The ability of FAs to alter neural firing rate and their suppressive effect on food intake is principally mediated by CD36 and acylCoA synthase, as well as by CPT1 and acetyl-CoA synthetase.[169,172] This suggests the effects of FAs are dependent on their entry into neurons and mitochondria.

These data support the idea that FAs can directly act on neurons and have an effect on food intake and metabolic regulation. These appear to be dependent on FA chain length and saturation, which will have differential influence on neural protein expression, firing, and behavior. Importantly, these elicited effects seem dependent on FA transport into neurons and intracellular lipid metabolism.

3.2.4 Obesity, lipid metabolism, and hypothalamic neuroinflammation

We have discussed the ability of hypothalamic neurons to process FAs and their functional consequences. As discussed previously, obesity is associated with systemic inflammation and impaired BBB integrity. It is thought that obesity-induced damage to the BBB allows peripheral cytokines, FAs, and immune cells to infiltrate brain tissue, thereby inducing local inflammation as well as microglial activation and proliferation, i.e. neuroinflammation.[173] Importantly, hypothalamic inflammation has been linked to impaired energy homeostasis, insulin resistance, overeating and consequent weight gain.[174] In short, obesity and neuroinflammation may be two conditions which are functionally linked, worsening the pathogenicity of the other.

A mechanism connecting these two conditions may be cellular lipid metabolism. Neural cells appear to be capable of lipid storage in the form of LDs, which also have local and systemic effects. Indeed, similar to adipocytes, LDs are found in the hypothalamic neurons, their number and size vary depending on whether cells are fasted or not.[175] This supposes these neurons can both store and utilize lipids according to nutritional state. Interestingly, HFD increases LD abundance in mice, but human patients with type 2 diabetes were shown to have a reduced number of LDs compared to control, non-type 2 diabetes patients.[176] However, the validity of this data is debatable. This study analyzed brain tissue of deceased human patients. It is possible the energy stored in the LD would have been used or otherwise decayed during the postmortem delay (between 2.1 and 24.0 hours). Between time of death and tissue sampling, the parenchyma would have been in a fasting state, and this impaired nutrient availability could have impacted lipid storage, utilization, and handling. Nonetheless, this suggests hypothalamic neurons are

capable of intracellular energy storage and that they can utilize this energy when resources are depleted.

Glia have emerged as critical players in the regulation of whole-body energy homeostasis in DIO.[177] Astrocytes can accumulate LDs in conditions of DIO,[178] or via a complex interplay between other neural cells and FA accumulation. Novel evidence suggests that hyperactive neurons increase their ROS production and release peroxidized FAs,[179] which are captured and stored in astrocytes as LDs. This triggers astrocytic upregulation of β-oxidation and transcription genes involved detoxification, thereby protecting neurons from oxidative stress and toxicity in periods of increased activity.[180] This relationship has yet to be evaluated in the hypothalamus specifically. However, FA metabolism in hypothalamic astrocytes regulates nutrient sensing, glucose metabolism, and weight regulation,[181] while astrocytic inflammatory pathways appear to be necessary to induce HFD-induced hyperphagia and DIO.[182] Indeed, astrocytes with LDs stimulate the activation of microglia and release pro-inflammatory cytokines.[178]

The involvement of microglia in DIO is important. Microglia appears to have a protective and anti-inflammatory response to palmitate.[183] As well, the degree of HFD-induced microgliosis (microglia activation and accumulation) appears to predict metabolic outcomes and weight gain in a gender-specific manner.[184,185] Increased microgliosis in male rodents seems to predict weight gain, while females appear to be resistant to both gliosis and obesity.[174] However, pharmacological blocking of microglial proliferation during an HFD experiment abolished the typically-observed increase in food intake, body weight, adiposity, and markers of inflammation.[186] The relationship between diet and neuroinflammation can also be protective.

Indeed, fish oil supplementation (rich in unsaturated FAs) has been shown to reverse DIO-induced gliosis.[60]

Together, this suggests a complex interplay between neural cells in relation to neuroinflammation and DIO. Increased nutrient availability, specifically FAs, would promote lipid storage both in neurons and astrocytes, which in turn promote neuroinflammation and microgliosis. This inflammatory state would impair systemic metabolism and contribute to obesity, which would continue to nefariously influence these pathways to promote hypothalamic inflammation.

Neuroinflammation is important to consider as it has also been hypothesized to initiate neurodegeneration. Altered neural signaling within the hypothalamus due to an inflamed state would result in the disruption of function in the regions connected to it.[187] Specifically, areas such as the hippocampus, cortex, brainstem, amygdala, and reward areas would indirectly suffer from obesity-induced neuroinflammation, which would in turn impair executive function and cognitive processing.[188]

Overall, the presented evidence suggests there is a neural control of food intake, peripheral metabolism, and energy expenditure. These are regulated by complex interplays between brain regions, and operate in response to peripheral hormonals and nutritional signals, which reflect of the body's fed or fasting state. However, these systems can be compromised by obesity, which in turn has a recognized bidirectional link with inflammation. Both obesity and inflammation are linked to overconsumption, and specifically diets high in saturated FAs. These interactions would have synergistic and nefarious effects on brain tissue, and consequently, on energy homeostasis and cognitive function. As alluded to above, the brain is also involved in

the pleasurable aspects of eating. The discussion that follows will describe the brain reward system, and its relationship with eating, obesity and FAs.

3.3 Mesolimbic dopamine system

Reward is the driving factor underlying the effort, motivation, or willingness to work to achieve or obtain a goal. This concept is relevant as it promotes and reinforces behavior which has previously resulted in something of value or was pleasurable. Behaviors such as eating, drinking and reproduction all promote species survival, and thus humans have evolved to find these things to be naturally rewarding. The mesolimbic dopamine system is one such pathway involved in neural circuitry of reward. It is implicated in motivation, goal-directed behavior, and is the focus of this thesis.

3.3.1 Dopamine and the mesolimbic system

Dopamine (DA) is a neurotransmitter within the catecholamine family; it is the precursor to the neurotransmitters adrenalin and noradrenalin. DA has strongly been associated with reward, motor control, memory, learning and addiction.[189] Indeed, DA neuron excitability appears to have a certain specificity for reward, as they have been shown to be inhibited by aversive stimuli.[190]

3.3.1.1 Dopamine synthesis, metabolism, and mechanism of action

The precursor of DA is the amino acid tyrosine. DA synthesis begins by the rate-limiting step involving the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine by the enzyme tyrosine hydroxylase. L-3,4-dihydroxyphenylalanine is then decarboxylated by L-DOPA

decarboxylase to form DA. After synthesis, DA is stored in vesicles which are transported to the synaptic area of axons. Presynaptic vesicles can be released into the synaptic cleft by exocytosis both in the absence of stimuli or following action potentials. Depolarization induces calcium influx and crosslinking of vesicles to the plasma membrane with SNARE proteins.

Most DA is recaptured by dopamine transporter (DAT) on the pre-synaptic neuron, and repackaged into vesicles. DA degradation is accomplished principally by monoamine oxidase on the external mitochondrial membrane, and to a lesser extent by cathechol-O-methyltransferase. Glia and astrocytes can also participate in DA recapture and degradation.[191]

DA exerts its effects on post-synaptic neurons via G protein coupled receptors. There are 5 DA receptors (D1R to D5R), which fall under two main classes: D1-like (D1R and D5R) and D2-like (D2R, D3R, D4R). These have facilitative and inhibitory properties on adenylyl cyclase, respectively.[189] The two most abundantly expressed DA receptors are D1R and D2R.

Within the D1-like class of DA receptors, D1R is highly expressed in target areas such as the PFC, striatum, thalamus, hypothalamus, hippocampus, and substantia nigra. D5R is also expressed in target areas, although to a lesser extent.[192] The D2-like class of receptors can be found in many target and limbic regions.[193] D2R, specifically, are localized on presynaptic neurons and act in an autoregulatory fashion, dampening basal dopaminergic tone,[194] reducing DA synthesis,[195] and limiting DA release.[196] These receptor classes have functionally opposite consequences, wherein DA has a stimulating effect when binding to D1-like receptors, and an inhibitory effect when acting on D2-like receptors. DA receptors are also found in glial cells, which aids it DA retrieval and metabolism.[197]

One way DA is thought to exert its effects is in the pattern of its release. Tonic, or basal, pattern is characterised by low-frequency firing which maintains low but constant levels of

DA.[198] Meanwhile, phasic or short bursts of neural activity produce acute increase in extracellular DA concentrations. It is believed these transient increases in DA are associated with goal-directed behaviours,[199] and play essential roles in reward circuitry.[199,200] These patterns of firings would differentially affect D1 and D2 receptors. Where D1 has lower affinity for DA, they are thought to be preferentially affected by phasic bursts, while D2, with their high affinity, may respond to low levels of the basal concentrations.[201]

3.3.1.2 Mesolimbic dopamine system anatomy and reward

Midbrain DA neurons are located in three main nuclei: the VTA, substantia nigra pars compacta, and the retrorubral field (see Figure 3). There are three main DA pathways. The mesolimbic pathway consists of DA neurons originating from the VTA and projecting to the NAC (ventral striatum). Dopaminergic circuits from the VTA also project to the prefrontal cortex (PFC) via the mesocortical pathway. These first two are sometimes grouped under the term mesocorticolimbic pathway. In addition to these, the VTA also send DA projections to the amygdala, hippocampus, cingulate cortex, olfactory bulb, and subthalamic nucleus.[202] DA neurons in the VTA and retrorubral field have been shown to be implicated in cognitive functions such as reward, emotion, motivation and addiction.[203] Lastly, the nigrostriatal pathway originates from the substantia nigra and projects to the DS, and aids in regulating voluntary control, goal-directed behaviours and learning.[203]

More specifically, the VTA is located medially around the midbrain floor and is an important component of the reward system as it is the main site of DA production. The degree to which VTA DA neurons are activated predicts the acquisition of a reward-associated learning.[204] The VTA projects and releases DA mainly in the NAC. The NAC is an integral part

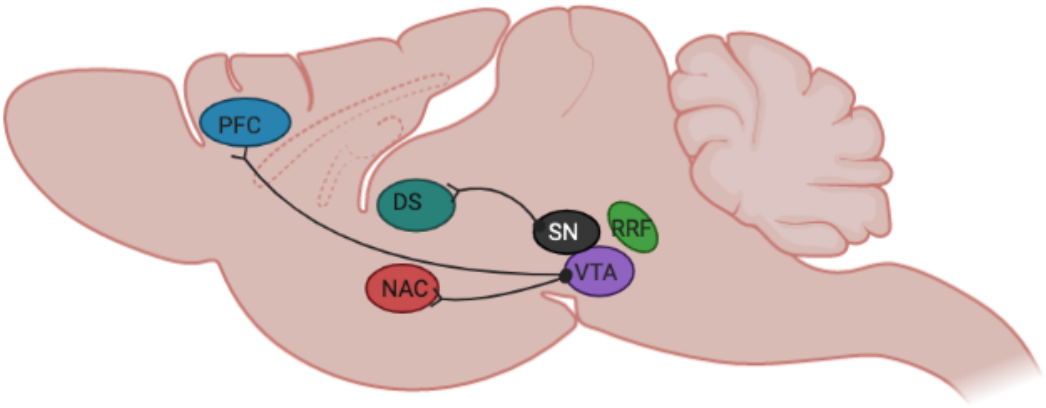

Figure 3. Overview of the reward system. The main dopaminergic nuclei and their projections. The three main dopaminergic nuclei are the ventral tegmental are (VTA), substantia nigra (SN), and the retrorubral field (RRF). The nigrostrial pathway originates from the SN and projects to the dorsal striatum (DS); the mesolimbic pathway projects from the VTA to the nucleus accumbens (NAC), and the mesocortical pathway arises from the VTA and extends to the prefrontal cortex (PFC). Image created in BioRender.com.

of the reward system as it has numerous connections to various brain regions. Accordingly, it is seen as an integration center. It is located in the ventral striatum, and is divided into two principle regions, the core, which is the central component, and the shell, which surrounds it. While these two regions play roles in reward and motivation, their connections with other regions provide them with distinct functional characteristics. It has been reported that the shell primarily partakes in positive reinforcement, while negative reinforcement elicits DA release in the core.[205] These appear to be reflected in studies assessing behavioral flexibility, where the core was shown to be implicated in learning and behavior adjustments, while the shell encodes outcome value.[206]

The importance of the mesolimbic DA system with regards to behavior was first reported in the context of addictive drugs, which induce large DA release as well as long term changes to this pathway's circuitry and sensitivity.[207–209] The mesolimbic DA system is often described as a single pathway, yet the relationships between the VTA and NAC are more intricate. Indeed,

understanding this system as multiple DA projections is useful, even critical, to understand the neural basis of reward.[210] It has been demonstrated that there is a special mapping between the VTA and NAC projections. Specifically, the ventromedial aspects of the VTA maps onto the medial olfactory tubercle and the medial shell of the NAC, while the ventrolateral VTA send projections to the ventrolateral striatum (core, lateral shell, lateral tubercle), and the dorsal VTA synapses with the DS. [210] This system appears to be characterized by DA neuron subtypes: the VTA has fast-firing DA neurons specific for the PFC, NAC core, NAC shell, and amygdala, while the conventional slow-firing midbrain neurons only project to the lateral shell and DS.[211]

At this point, a note on aversion should be raised. Aversion and reward are functional opposites, but are intrinsically and neurobiologically linked. Like reward, aversion promotes species survival by avoiding deleterious stimuli and situations. Traditionally, studies assessing the individual role of DA receptors have reported that, in the NAC, D1R neuron activation underlies reward signals and D2R neurons direct aversion.[212–214] However, this assumption has been challenged by recent studies which have demonstrated that, in fact, both DA receptor subtypes are involved in mediating motivation.[215,216]

3.3.2 Mesolimbic dopamine system, food reward and obesity

It is now widely recognized that many substances with rewarding properties, both natural and synthetic, such as drugs, alcohol, sex and food share an underlying dopaminergic origin. Of relevance, eating and the effort required to obtain food are crucial components to our survival as a species, and pleasure derived from eating aids in perpetuating this behavior. Indeed, food and food cues have shown to activate the different neural areas and circuits involved in reward such as the NAC, VTA, hippocampus, amygdala and PFC.[217] The implication of this

system in obesity is functionally relevant. Indeed, obesity is strongly related to heightened food reward, food anticipation, and neural reactivity to food cues.[218,219] These food-motivated factors are linked to the mesolimbic system, whereby there are well documented alterations in neural circuits and connectivity in obese patients.[220,221] In terms of overall brain activity, positron emission tomography studies have found that satiety produces differential regional cerebral blood flow patterns in lean and obese individuals. In obese men and women, satiation induced greater increases in blood flow to the PFC, and reduced blood flow in the hypothalamus, NAC and amygdala (and other paralimbic areas).[222,223]

More specifically, food has been known to induce DA release in the NAC of rats, [224] and this effect is of greater magnitude with foods high in fat or sugar.[225–227] DA release has likewise been seen in the DS of humans in response to palatable foods or cues.[228,229] This relationship has been further strengthened by studies where mice unable to synthesize DA (tyrosine hydroxylase knockout) gradually become hypoactive and stop feeding, despite their neural architecture remaining intact.[230] These deficiencies were rescued when administering L-DOPA.[230,231]

These effects appear to be altered in obesity. Indeed, there is a well-recognized decrease in DA sensitivity, availability and release within the mesolimbic system in conditions of DIO.[232] There is documented over- or under-active DA neural activity in response to food cues in these reward-related areas, which increases risk of overeating and future weight gain.[233,234] These changes may be due to genetic susceptibility: there appears to be a basal hyper-responsiveness in the NAC of adult obesity-prone rats compared to obesity-resistant animals, both in terms of intrinsic excitability and cocaine-induce locomotor activity.[235] This is suggestive of a predisposing phenotype for obesity linked to the reward system. One study showed there are

opposite neural adaptions after exposure to a high-fat high-sugar diet. Namely, the excitability of NAC neurons was enhanced in obesity-resistant animals and a reduced in the obesity-prone group.[236] Thus, mesolimbic variations appear to be related to obesity both in terms of neuropathological predisposition as well as altered neural adaptations to western-style diets.

The effects underlying mesolimbic activity in relation to food, and the adaptations seen in obesity seem to be mediated by DA receptors. The relevance of mesolimbic D1R has traditionally been dismissed as underlying locomotor function, as knockout studies showed mice pups had stunted growth and low survival rate.[201,237] However, their role in food reward should not be discounted. As the aforementioned D1R-dependent deficits are rescued by easy access to palatable food.[238] Moreover, a group of D1R neurons in the NAC medial shell have been shown to represent food reward, their activity being proportional and predictive of the amount of food consumption.[239] Although less studied in the context of obesity, D1R appears necessary for the development of DIO.[240] Prolonged HFD, specifically those high in saturated fat, diminishes D1R-induced locomotor activity both in basal and in amphetamine-sensitized conditions.[241,242] Moreover, hyperphagic, obesity-prone rats have been shown to have reduced D1R expression.[239] As well, when infused into either the NAC core or shell, D1 and D2 antagonists increased feeding time, but not total consumption, and locomotor activity was suppressed.[243] It is possible that increased locomotor effort following D1R blockade could outweigh their effect on enhanced palatability of food.[244] In this way, impairing the ability to move may mask the dopamine-dependent food motivation.

The relevance of D2R may be greater in the context of food reward and obesity. Indeed, D2R has been more extensively shown to be involved in the reinforcing properties of food.[245] As well, in the context of drug reward, D2R has been linked to drug-seeking behavior and self-

administration of drugs.[201] In the context of obesity, DIO reduces D2R availability in the striatum, and this worsens with increasing BMI.[246] Moreover, experimental manipulation of striatal D2R by viral knockdown accelerates the development of addiction-like reward deficits and compulsive food-seeking behavior in rats under a HFD.[247] Thus, DIO appears to induce D2R-mediated alterations in the striatum which in turn induce a behavioral phenotype akin to addiction. This supports the idea of an interconnectedness between food intake and the reward system, with DA being the underlying mechanism. As well, the DA release typically observed in the NAC in response to food is blunted in DIO, where only palatable food (not chow) could elicit a response.[248] This may be explained by an increased D2R auto-inhibition in VTA DA neurons,[249] despite basal neural activity not being affected in DIO.[250] Moreover, a higher density of DAT and D2R in the striatum, NAC and hypothalamus is associated with resistance to obesity, and D2R density is reduced in the DS of DIO mice.[251]

Our lab has also previously demonstrated that a diet high in saturated fats (palm oil) can induce obesity, increase food-motivated behavior and triggers a state of neuroinflammation in the NAC.[252] Of note, viral knockdown of inflammatory pathways in the NAC significantly reduces HFD-induced food-motivated behavior and abolishes compulsive sucrose-seeking. These findings further highlight the relevance of the mesolimbic system and mimics the findings of hypothalamic neuroinflammation in the pathogenicity of obesity.[252]

Beyond reward, DA within the mesocorticolimbic pathways have also been shown to play essential roles in cognitive processes such as attention, motivation – both positive and negative – as well as encoding value and salience of rewards.[253] As well, DA neurons in the VTA have been shown to respond directly to insulin, leptin, and ghrelin.[254,255] This supports the

idea that the mesolimbic pathway could also sense peripheral adiposity and nutritional state signals.

Indeed, the increase in palatable food ingestion for their rewarding properties can provoke neural and behavioral adaptations similar to those seen in drugs of abuse.[247] This can contribute to the development and maintenance of an obesogenic state. We have shown here that there are neural differences in dopaminergic signaling and neuroinflammation within the mesolimbic pathway in conditions of DIO. Given the relevance of FAs in obesity and their ability to alter hypothalamic neural regulation of energy homeostasis, we will discuss the available evidence for FA metabolism in the mesolimbic pathway.

3.3.3 Mesolimbic dopamine system and fatty acid metabolism

There is some evidence to suggest parts of brain reward circuitry and the mesolimbic pathway can incorporate FAs derived from the periphery. In one study, radiolabeled oleate infused into the carotid of mice was significantly detected in the striatum, hypothalamus, hippocampus, and cortex.[241] As well, acute Intralipid infusion was shown to reduce spontaneous and amphetamine-induced locomotion, abolished preference for palatable food, and reduced motivation to obtain food rewards,[241] all behaviors in which DA neurons are involved. Indeed, recent evidence has shown dietary TG alters D2R excitability, VTA DA activity, and acts as a strong reinforcer and modulator for food-seeking behavior.[256] It appears TGs target the mesolimbic system via lipoprotein lipase, upstream of D2R.[241,256] Building on this, in addition to developing a worsened metabolic phenotype, mice fed with a HFD rich in saturated FAs had reduced capacity for phasic DA release and slower synaptic DA clearance in the NAC, compared to those fed a HFD rich in unsaturated FAs or a blend of both types.[257] This data

mirrors what was reviewed above, where the type of FA has important implications in neural signaling and adaptations.

Despite the above findings, less is known about the capacity of the mesolimbic DA system to sense and metabolize FA. Short-term exposure to HFD has been shown to activate c-FOS, a marker of neuronal activity in the VTA, NAC, amygdala and LH.[258] As well, 30-min lingual exposure to LCFA linoleic acid increases c-FOS expression in the VTA, ARC, amygdala, hippocampus and gustatory system.[259] This suggests that FA may activate taste, reward, nutrient sensing and memory networks.

Moreover, knockout of lipoprotein lipase in the NAC, which blocks FA hydrolysis, increased appetite, weight gain, preference for palatable food and motivation to obtain food rewards.[241] Our lab has recently demonstrated that oleate dampens midbrain DA neuron firing, and that in an *in vitro* model, DA neurons of the VTA appear to incorporate fluorescent FAs into LD-like structures.[260] This study also demonstrated that oleate (but not palmitate) injected directly into the VTA can suppress food intake and food-motivated behavior, while stimulating locomotor activity. All of these effects were attenuated or abolished when phloretin (a general transporter blocker) was co-administered. This mimics what has been observed in the hypothalamus and following ICV infusions of FAs. Moreover, these neurons also express FATP-1, -4, which transport FAs into cells, and FABP3, which transports FAs intracellularly, supporting the idea that dopaminergic neurons in the VTA have the necessary machinery for FA uptake and metabolism. Taken together, these findings suggest that DA neurons in the VTA are capable of FA uptake, that oleate has a functional effect on their firing rate, and that the suppressive effect of oleate on eating behavior may in part be due to FA uptake in VTA dopaminergic neurons.

RATIONALE

The regulation of energy homeostasis requires that the CNS receives nutritional and hormonal signals from periphery and send out anorexigenic or orexigenic indications accordingly. It is well established that hypothalamic cells respond to and metabolize FAs, and that this has an impact on food intake, body weight, and peripheral metabolism. Prolonged overeating and DIO appears to induce long term alterations in the hypothalamus, reward systems, and other regions, which facilitates an obesogenic state.

The role of the mesolimbic dopamine system (VTA to NAC pathways), and other regions involved in reward (DS, PFC, hippocampus and amygdala) are increasingly evoked and linked to eating behavior. Some preliminary evidence advances that mesolimbic DA neurons can incorporate FAs from periphery, and that FA metabolism in the NAC impacts food-related behavior. This suggests that FAs, which are an increasingly important component of modern diets, would be major signals for both energy homeostasis and the rewarding properties of food. The short- and long-term impact of lipids on the brain, specifically in the reward centers, remains unclear, but may have significant implications in the development and maintenance of obesity. Understanding these pathways may influence nutritional recommendations, inform weight loss and management strategies, and may prove to be the basis for new pharmacological avenues.

There remains some questions as to whether and how FAs from the periphery can reach the regions of interest in the brain. Specifically, little information is available for this phenomenon in reward-related areas including the VTA, NAC, PFC, DS, as well as the hippocampus and the MBH. In the first part of this Master's, we sought to evaluate the entry of LCFAs into these regions by injecting *FTHA, a radiolabeled analog directly into peripheral

circulation of WT mice. This would give a relative measure of FA uptake and accumulation into different brain regions by comparing their levels of emitted radioactivity.

After assessing FA entry into the brain from periphery, we considered other remaining questions pertaining to whether modulating FA entry into cells in the VTA could affect food-motivated behavior. It was previously published that oleate injected into the VTA suppresses motivation to work for food rewards in rats; this effect was blocked by a general transporter blocker, phloretin.[260] We sought to develop on this finding by increasing the specificity in the modulation of FA uptake or metabolism. Namely, by targeting a specific protein. FATP1 facilitates FA transport into cells and is expressed on dopaminergic VTA neurons. We thought the targeted inhibition of FATP1 with a pharmacological agent in the VTA, in conjunction with oleate, would aid in determining whether FA uptake – and potentially, subsequent metabolism – is involved in regulating food motivation.

Little is known on the effects of DIO on the mesolimbic system, despite its relevance in food motivation and behavior. As discussed above, obesity, which is in large part caused by prolonged HFD, is related to increased fat accumulation in peripheral tissues, inflammation, and is thought to induce damage at the level of the BBB. These effects are worsened by HFDs rich in saturated FAs. The link between DIO and fat accumulation in the brain has yet to be evaluated. As well, the relationship between HFDs, neuroinflammation and BBB damage has seldom been explored in mesolimbic areas. Therefore, we sought to assess in mice the effects of a long-term HFD on cerebral fat accumulation, neuroinflammation, BBB permeability and tight junction integrity.

HYPOTHESES

Based on the presented evidence, we hypothesized that:

1. LCFAs are incorporated into selected reward-related brain regions: VTA, NAC, DS, PFC, and into the hippocampus and MBH;
2. Inhibiting FATP1-mediated FA transport in the VTA diminishes the suppressive effect of oleate on food-motivated behavior;
3. In the mesolimbic dopamine system, a long term HFD increases fat content in brain tissue, induces cerebral inflammation and alterations in BBB integrity.

OBJECTIVES

Thus, the objectives of this work were:

1. To verify whether tail-vein injected *FTHA is incorporated into reward-related brain areas, more specifically into VTA, NAC, DS, PFC, hippocampus and MBH;
2. To assess whether pharmacological inhibition of FATP1 decreases immunofluorescent FA C1-BODIPY 500/510-C12 uptake in neurons, and whether it modulates the suppressive effect of oleate on food-motivated behavior when injected into the VTA;
3. To evaluate the effect of a 20-week HFD on cerebral lipid accumulation, neuroinflammation, and BBB integrity in the VTA and NAC.

METHODS

A series of experiments were conducted to assess FA uptake into different nuclei (Experiment 1), the behavioral consequence of modulating FA entry into cells of the VTA (Experiment 2), and the long-term effects of a HFD on the BBB, lipid accumulation in brain parenchyma and neuroinflammation (Experiment 3).

All experiments were approved by and conducted in accordance to the CRCHUM Animal Care Committee and the Université de Montréal Animal Ethics Committee. Adult male C57Bl/6 mice (Charles River, Saint-Constant, Quebec) were used for all experiments. This strain was chosen for inter-experiment consistency and as there are many genetic models available with this strain for replication studies. Male mice were employed to assess whether findings from the literature could be replicated; studies with female mice are planned. After arrival, animals were given at least 7 days to habituate to their new environment. They were housed in a 12:12h reverse light-dark cycle (lights off at 10h00 AM), where temperature and humidity were controlled.

Experiment 1: Assessing regional differences in acute fatty acid uptake

1.1 Compound

We employed 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (*FTHA), a radiolabeled analog of LCFA with a long half-life (109 minutes). *FTHA was chosen as it's tissue uptake is similar to palmitate.[107] Moreover, unlike other radiotracers such as 13C-LCFA or deuterated FAs, *FTHA is designed to be taken up by the mitochondria and is trapped after 2 cycles of β-oxidation, allowing for the detection of regional uptake and metabolism of FAs.[105] Findings

with these types of radiotracers may be more accurate for a complete mapping of FA metabolism, but using these with our methodology would be confounded by radioactive metabolites. As we wanted to assess which regions took FAs from periphery, *FTHA was the appropriate choice of tracer. *FTHA was synthesized by the DaSilva Lab the morning of experiments, as previously described.[261,262]

1.2 Tail-vein injection and tissue collection

To assess central uptake of peripheral FA, WT mice (N=12, age= 8-10 weeks) were briefly placed in a restrainer and received a tail-vein injection of 50μCi *FTHA. Total volume injected was 50uL. The *FTHA solution had a concentration of 20.9mCi/mL at time zero. This was diluted in 0.9% saline in syringes, adjusting volumes of *FTHA according to the decay rate and elapsed time. Following injection, mice were placed under isoflurane-induced anesthesia. Mice were sacrificed after a delay of 5, 15, or 30 minutes post-injection. Trunk blood was collected in EDTA-coated pre-weighed tubes and centrifuged at 8000RPM for 5 minutes to obtain plasma. Heart, liver and brain were then collected. The brain was placed in an adult mouse brain matrix and discrete slices of 0.5 and 1mm containing our regions of interest were obtained. Slices were retrieved further dissected to isolate the VTA, NAC, PFC, DS, hippocampus, and MBH with a blade, according to the mouse brain atlas.[263] Tissues were placed in pre-weighed tubes, and radioactivity was measured in each region. This measure was used as an indicator of FA uptake.

1.3 Radioactivity assessment

Samples were analyzed in an automatic gamma-counter (Wizard^2, PerkinElmer 3470) over 30 seconds. Output data for level of radioactivity in each sample was adjusted for decay with a 1 % (V/V) internal standard and standard curve. Data is expressed as percent of the injected dose (%ID), controlled for sample weight (g).

1.4 Statistical analyses

Statistical analyses were performed on GraphPad Prism (version 7 for Windows GraphPad Software, CA, USA). Differences between incubation time and brain regions were assessed with a two-way ANOVA (factors: time and region of interest [ROI]). Tukey's multiple comparison post hoc tests with adjusted *p* value were performed. Statistical significance was set at $\alpha = 5\%$ ($p < .05$).

Experiment 2: Assessing the impact of fatty acid metabolism in the ventral tegmental area on the rewarding properties of food

2.1 Compounds

We used FATP1 inhibitor 5k, which was developed by and obtained from Daiichi Sankyo Co. (Tokyo, Japan).[264] The compound was dissolved in heparinized saline 0.9%. Pharmacological inhibition of FATP1 was chosen over genetic knockout as this method allowed for time-sensitive modulation of FA metabolism. Moreover, the approach was judged adequate for proof of concept.

C1-BODIPY 500/510-C12 was purchased from Invitrogen (CA, USA). C1-BODIPY 500/510-C12 is a fluorescent LCFA analog designed to be incorporated into cells and can be

visualized with a microscope. 4', 6-diamidino-2-phenylindole (DAPI) non-hardening mounting media, used to stain nuclei and preserve fluorescence, was purchased from Vectashield (Vector Labs, CA, USA).

Hydroxypropyl-β-cyclodextrin (HPB) was used as it is a non-toxic substance that increases lipid solubility in aqueous solutions (CTD Holdings, Inc., FL, USA). Oleic acid (Sigma-Aldrich) was complexed to HPB in 0.9% saline. Oleate concentration (2nM) was determined based on a previous study in our lab in which 6nmol/side was used in rats.[260] The concentration was reduced according to dose conversion between animal models.[265]

2.2 Cell Culture

Immortalized hypothalamic neuronal cell line (GT1-7, Sigma Aldrich, MO, USA) was used to evaluate the FATP1 inhibitor 5k. Reported half maximal inhibitory concentration (IC50; 600nM)[264] was employed as a basis to generate a standard curve relating inhibitor 5k concentration to C1-BODIPY 500/510-C12 entry into GT1-7 neurons. Cells were exposed to the inhibitor at given concentration (IC50/100: 6nM; IC50: 600nM; IC50x100: 60μM) for 2 hours, followed by 1 hour of co-exposure of the inhibitor and 10μM BODIPY. Reagents were diluted in cell culture complete media (DMEM with 10% heat inactivated FBS, supplemented with 1% antibiotics (Penicillin G (10,000U/ml)-Streptomycin Sulfate (10,000 μg/ml)). Each condition was performed in duplicate. Following the incubation period, cells were rinsed with 1% bovine albumin serum in phosphate buffer solution (PBS) for 1 minute, with PBS alone (1 minute), and then fixed for 10 minutes with 4% paraformaldehyde. Coverslips were mounted onto microscope slides with DAPI mounting media.

2.3 Microscopy and image analysis

All images were acquired with Apotome microscope (ZEISS Axio Imager 2, ApoTome.2, Germany). Image analyses were conducted with ImageJ/FIJI for Windows. 40X-magnification images were obtained of the GT1-7 cells (n=5/condition). The number of LD and nuclei were counted in each image using the "analyze particles" function. Average number of LD per cell was obtained by dividing total LDs by number of nuclei in the image. Background incorporation in neurons was assessed by thresholding, with LD signal being subtracted from neuron signal.

2.4 Stereotaxic surgeries

Stereotaxic surgeries were conducted after mice completed progressive ratio (PR) training and achieving stable performance in operant task. Surgeries were performed under isoflurane anesthesia (2% isoflurane, 0.5% oxygen), and coordinates were derived from the mouse brain atlas.[263]

In an ultraprecise mouse stereotaxic frame (Kopf; Tujunga, CA, USA), mice were implanted with bilateral 26-gauge guide cannula (HRS Scientific, QC). Coordinates for the injector site: 3.4mm posterior to Bregma, ±0.4mm from midline, -4.7mm from dura (Figure 4). Injection site and stereotaxic coordinates were verified during pre-experiment practice surgeries. Cannula viability was maintained by removable dummy cannula extending 1mm past the guide cannula. Mice were monitored and allowed to recover for 5 days prior to the start of operant conditioning. Weight was monitored post-surgery to ascertain proper recovery.

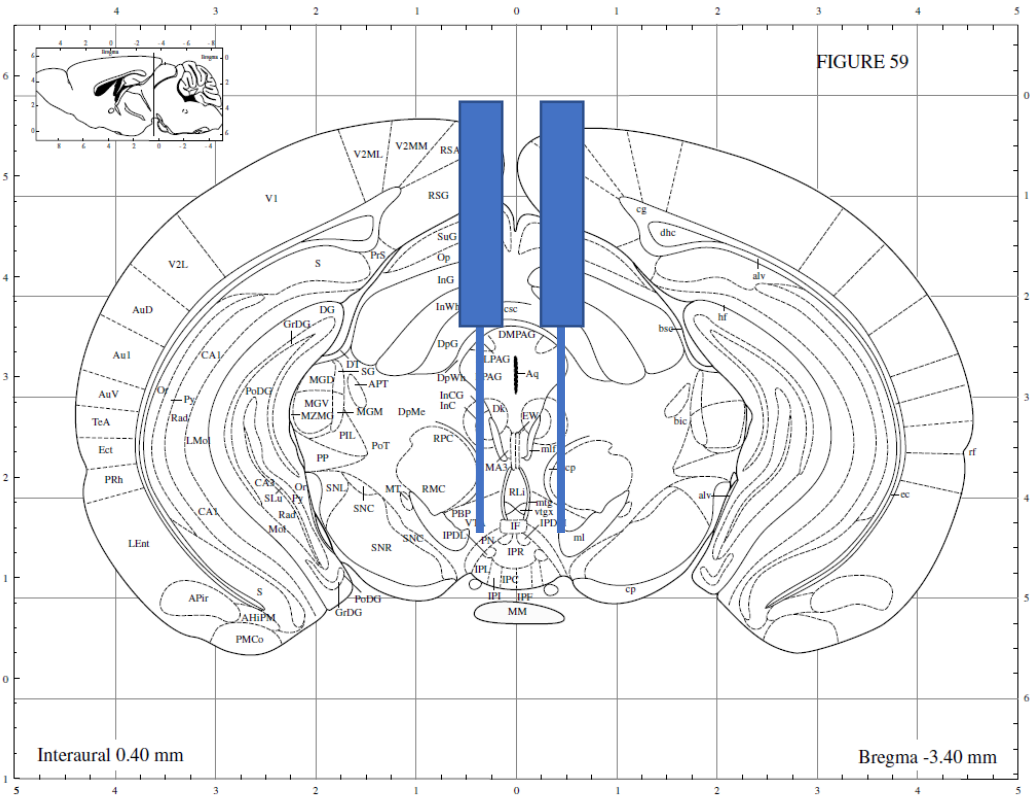

Figure 4. VTA guide cannula and injector placement. Image derived from the mouse brain atlas. [263]

2.5 Operant conditioning for food rewards

Mice were trained, as previously described,[266] to respond to 20mg high-fat, high-sugar pellets (TestDiet, MO, USA) on a progressive ratio schedule of reinforcement. Mice were first food restricted to increase their motivation to obtain food pellets. Basal chow intake was measured over 3 days, and from this, food restriction was calculated as 70% of basal average. During this time, weight was monitored daily so that it did not drop under 90% of baseline. They were placed in sound-attenuated operant chambers with two retractable levers and pellet dispenser with receptacle place midway between levers (Med Associates Inc., VT, USA). Mice first learned on a fixed-ratio 1 (FR-1) that one lever in the chamber is “active”, meaning pressing it once causes the obtention of 1 pellet, and one is “inactive”, meaning no pellet. The active

lever (left or right) was randomly allocated within groups. Lever press data (number on each, timing) was calculated by computer-controlled software (Med Associates Inc.). Each session lasts a maximum of 1 hour, until mice are inactive (no lever pressing) for 10 minutes, or when 50 pellets have been obtained. Acquisition criteria of food-maintained operant responding was determined to be discrimination for the active lever over the inactive level of ≥3:1 and obtention of at least 20 pellets per session over 3 consecutive days. When acquisition criteria were met, mice were no longer food restricted and allowed to return to normal weight while they were exposed to fixed ratio-5 for 3-5 days. Any metabolic adaptations to weight loss would return to baseline at this time. Mice were then placed on a PR schedule of reinforcement. In PR reinforcement, the number of required presses to obtain 1 pellet increases after each reward, following the formula = [5e (R*0.2)]-5 (rounded to the nearest integer; R is equal to the number of food rewards already earned plus 1 [i.e., next reinforcer]).[267] All behavioral training and testing were conducted once a day during the dark cycle, 3 hours after lights out (13:00). Chambers and levers were washed and disinfected between each mouse.

Following stereotaxic surgery recovery, mice underwent 3 PR training sessions (1/day) without any manipulation before experiment initiation.

2.6 Intra-VTA injections

Prior to experimental conditions, mice experienced 3 habituation sessions with bilateral intra-VTA injections of vehicle followed by PR training. Each experiment was conducted at one-day intervals.

Cannula blockers were replaced by bilateral injectors (HRS Scientific, QC) extending 2 mm below guide cannula. In a within subject design, mice underwent PR testing under each of

4 injection conditions. Animals received the vehicle (VEH) condition first (3% HPB dissolved in 0.9% saline), followed in counterbalanced order: oleate alone (2nM), FATP1 inhibitor alone (60μM), and oleate + FATP1 inhibitor.

500nL was delivered on each side at a rate of 100nL/min. Following this, injectors were left in place 3 minutes to allow substance penetration into brain tissue. After the time elapsed, injectors were replaced once again with cannula blockers. Injectors and blockers were sanitized before each introduction. Following each injection, mice recovered for 30 minutes before being placed in the operant chamber for behavioral testing.

2.7 Statistical analyses

Statistical analyses were performed on GraphPad Prism (version 7 for Windows GraphPad Software, CA, USA). Results are expressed as mean ± SEM. One-way analyses of variance (ANOVA) were conducted to assess differences between groups. Tukey's multiple comparison post hoc tests with adjusted *p* value were done. Statistical significance was set at α = 5% ($p \leq .05$).

Experiment 3: Assessing the impact of a 20-week high-fat diet on brain inflammation, lipid accumulation and blood brain barrier integrity

3.1 Animals and diet

Mice were received in the lab at 6-7 weeks of age and were singly housed. At 7-8 weeks (young adults), they were given free access to one of two ingredient-matched custom diets (Table 1; obtained from Dyets, Bethlehem, PA, USA) for 20 weeks: a 50% kcal palm oil saturated high-fat diet (HFD; n=5), or an ingredient-matched 16.8% kcal soybean oil low-fat

control diet (CTRL; n=4). Mice and food were weighed once a week. Choice of diet was determined based on a previous study from our lab which demonstrated this diet high in saturated FA induced a blunting of dopaminergic tone and neuroinflammation in the NAC.[242,252] Moreover, the Western diet consumed by humans includes high proportions of saturated FAs,[268] as reflected in the composition of the diets below.

Table 1. Diet composition

	CTRL	**HFD**
Fat source	Soybean oil	Palm oil
Fat (g/kg)	70	270
Casein (g/kg)	200	200
L-Cystine (g/kg)	3	3
Sucrose (g/kg)	100	100
Cornstarch (g/kg)	397.5	197.5
Dyetrose (g/kg)	132	132
Mineral Mix (g/kg)	35	35
Vitamin Mix (g/kg)	10	10
% Kcal Fat	17	50
% Kcal carbohydrates	62	43
% Kcal proteins	21	7
Total Kcal/g	3.8	4.8
% palmitic acid (C16:0)	10.2	44.5
% stearic acid (C18:0)	4.5	4.2
% oleic acid (C18:1)	22.7	39.4
% linoleic acid (C18:2)	54.8	9.5
% linolenic acid (C18:3)	7.8	N/A
% saturated fat	15	51.1
% monounsaturated fat	23.4	38.8
% polyunsaturated fat	61.2	9.7

CTRL: control; HFD: high-fat diet; N/A: not available.

3.2 Drug

The D1R agonist SKF82958 (Santa Cruz, Dallas, Texas, USA) was dissolved in 0.9% saline at a concentration of 3mg/mL. Based on a previous study in our lab,[242] mice received intra-peritoneal (IP) injections of 10mL/Kg SKF.

3.3 Metabolic profiling and locomotor activity assessment

After 18.5 weeks on diet, mice were placed in Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, OH, USA) chambers for 72h. The CLAMS can measure oxygen consumption, carbon dioxide production, locomotor activity, energy expenditure and core body temperature. For this study, locomotor activity and energy expenditure were assessed. Over the first 24 hours, mice were allowed to habituate. The following days, 3h prior to the initiation of the dark cycle (7:00AM), mice were injected IP with 0.3mL saline (day 2) or SKF-82958 (day 3). Locomotor activity (total X-ambulatory beam breaks) was assessed over the 3 hours post-injection.

Immediately after the CLAMS experiment, body mass composition, including percent lean and percent fat mass, was measured by Echo MRI (Echo Medical Systems, Houston, TX, USA).

3.4 Blood collection and sacrifice

After 20.5 weeks of diet, sacrifices were performed between 10h00 and 13h00 (start of dark cycle). Mice were anesthetized with 0.2mL IP sodium-pentobarbital. Once deeply anesthetized, blood was collected by cardiac puncture, placed in EDTA-coated tubes and centrifuged at 11000g for 15 minutes at 4°C. Plasma was retrieved and stored at -80°C until use.

Plasma levels of glucose (Autokit, Wako, CA, USA), insulin and TNFα (AlphaLISA, PerkinElmer, USA), CRP (ELISA kit, Abcam, Cambridge, UK), and TG and FFA (dosage assay, Ensight, PerkinElmer, USA) were assessed.

After blood collection, mice were perfused transcardially with 10mL of chilled PBS (rate: 2 mL/minute), followed by 10mL of 10% neutral buffered formalin. Brains were immediately retrieved and kept in 10% formalin overnight (~20h) and cryoprotected in 30% sucrose solution for 24h. They were then washed three times with PBS, flash frozen, and kept at -80°C.

3.5 Immunohistochemistry

To assess the long-term effect of DIO on the mice' brains, we evaluated the impact of prolonged HFD on brain lipid accumulation, neuroinflammation, as well as BBB permeability and structural integrity. Whole-brain coronal sections (10µm) were collected in series of 10 with a cryostat (Leica CM 3050 S, Leica Biosystems, IL, USA). Brain regions were determined *via* cross-referencing with the mouse brain atlas coordinates.[263] For each animal, 3-4 sections were collected per ROI to represent the rostral, middle and caudal areas. Sections were mounted on microscope slides at -80°C, wrapped in aluminum foil, until use. Before any manipulations, slides were allowed to thaw for 30 minutes.

To assess lipid accumulation, brain slices were rinsed with PBS, and then incubated with 10µM BODIPY 493/503 (Invitrogen, CA, USA) for 15 minutes. BODIPY 493/503 is a nonpolar structure used as a stain for neutral lipids. Slides were then washed three times with PBS for 3 minutes. The choice in favor of BODIPY 493/503 over other commonly used lipid stains such as oil red O is twofold. Firstly, the protocol for oil red O requires isopropanol,[269] a substance

used for lipid extraction from tissues.[270] In addition, this approach can cause the fusion of adjacent LDs,[271] thereby influencing their morphology and total lipid content. This makes oil red O a risky method to visualize lipids in neural tissue with much smaller LD content than adipocytes and hepatocytes.

For immunohistochemistry, slides were washed in PBS, cold (-20 °C) acetone 100% (Fisher Chemicals A949-4) for 10 mins, cold ethanol 70% for 5 mins, and again with PBS. A hydrophobic barrier was drawn around brain slices with Super PAP pen Liquid Blocker Mini (ProSciTech PEL22311). Slices were blocked in 10% normal goat serum (NGS; Sigma Aldrich, MO, USA) for 30 mins at room temperature, and then incubated overnight at 4°C in 3% NGS with a primary antibody: anti-Iba1 (1:500, Cedarlane 019-19741); anti-Fibrinogen (1:1000, Innovative Research IASMFBGN); anti-Laminin (1:1000, Sigma-Aldrich L9393); anti-ZO-1 (1:100, Thermo Fisher Scientific 33-9100); or anti-Claudin-5 (1:100, Thermo Fisher Scientific 34-1600). The next day, slices were washed 6 times with PBS with 0.1% Tween20 (3 minutes) and incubated for 1h at room temperature with appropriate secondary antibodies: Goat-anti-Rabbit IgG (H+L) Alexa Fluor 488 (1:500, Thermo Fisher Scientific A-11008); Alexa Fluor 568 (1:500, Thermo Fisher Scientific A-11011).

After staining, all slices were coverslipped with DAPI mounting media (Vectashield, Vector Labs, CA, USA) and kept in microscope slide boxes at 4°C until imaging.

3.6 Microscopy and image analysis

All images were acquired with an Apotome microscope (ZEISS Axio Imager 2, ApoTome.2, Germany). Maximum projections were done on all Z-stack images. Analyses were conducted with ImageJ/FIJI for Windows. For each ROI, the values of the three brain sections

(rostral, middle, caudal) were averaged into a single representative, composite value. Outliers were removed from analysis (determined as values greater or less than 2.5 times the mean of other values).

For BODIPY 493/503 lipid accumulation, images were obtained at 40X magnification within the ROI (for both left and right hemispheres). Mean pixel intensity was used as a measure of signal strength, where greater values indicate greater fat accumulation.

For microglia analyses, 10X magnification images were obtained over whole ROI as well as 63X magnified Z-stack images (0.25μm stacks) of individual microglial cells. Iba-1 stained microglial cell bodies were counted by mask thresholding followed by the "analyze particles" function in the 10X images. The perimeter, maximum ferret and minimum ferret for microglial somas (n=1 per ROI for each mouse) were derived from the 63X images. An indicator of circularity (0.00-1.00) was used to generate a soma ratio by dividing the minimum ferret by the maximum ferret. Total visible processes were counted, and their length was measured as distance from soma border to end of process.

For both BBB stains, 40X Z-stacks (0.52μm stacks) of the ROIs were obtained. Average intensity and total area were measured for laminin, fibrinogen, ZO-1 and claudin-5 signals. To assess BBB leakage, the laminin mask was subtracted from the fibrinogen. Additionally, the fibrinogen leakage area was divided by total laminin area to create a normalized ratio value of leakage for individual images.

3.7 Statistical analyses

All statistical analyses were performed on GraphPad Prism (version 7 for Windows GraphPad Software, CA, USA). Results are presented as mean ± SEM. Differences between

HFD and CTRL plasma concentrations, percent fat and lean mass were analyzed with independent samples t-tests. Body weight and cumulative food intake were analyzed using one-way repeated-measures analysis of variance (RM-ANOVA), where diet is the fixed factor, and body weight/food intake is the repeated factor over time.

From the CLAMS, locomotor activity was analyzed as total X-ambulatory beam breaks, and energy expenditure is measured as the AUC of heat. Basal differences in locomotor activity and energy expenditure over the first 24h habituation period between HFD and CTRL groups were assessed by a two-way RM-ANOVA, where the fixed factor is diet, and the repeat factor is cycle (light/dark, 12h each). Differences in D1-induced locomotion between HFD and CTRL were assessed by comparing total activity over the 3hr period post-saline and -SKF injection by two-way RM-ANOVA, where the fixed factor is diet, and repeat factor is drug condition. To assess the magnitude of effect and remove any background, delta values (3hr saline value subtracted from SKF) were computed. An independent sample t-test was done on this delta value to assess the difference between HFD and CTRL groups.

For all immunohistochemistry image analyses, relative differences in mean fluorophore intensities, areas, and microglial morphology were assessed by two-way ANOVAs (factors: diet and ROI).

Bonferroni's multiple comparison post hoc test were done where main effects of ANOVAs were significant. Statistical significance was set at $\alpha = 5\%$ ($p < .05$).

RESULTS

Experiment 1: Assessing regional differences in acute fatty acid uptake

Given the relevance of lipids in obesity, the first thing we sought to assess was the regional uptake of peripheral FA in the brain of mice. We injected *FTHA, a radiolabeled analog of LCFA with a long half-life (109 minutes), directly into WT mice peripheral circulation. Relative radioactivity levels (average %ID) controlled for sample weight (g) at each time point (5, 15, and 30-minutes post-injection) was assessed in dissected brain regions (PFC, MBH, DS, hippocampus, NAC and VTA), as well as in plasma, heart and liver with a gamma counter (Table 2). The data therefore represents the relative incorporation of FAs in circulation in various tissues and nuclei.

After accounting for residual *FTHA in syringes, the actual injected dose into mouse circulation was 41.53 ± 1.24 μCi of *FTHA. In terms of peripheral LCFA incorporation into various brain regions, we found region-specific differences in *FTHA signal, $F(6,63) = 10.72$, $p < .001$. Overall, the PFC, hippocampus, DS, NAC, and VTA accumulated *FTHA significantly above circulation levels ($p < .03$). Between the different brain regions, the VTA accumulated more *FTHA than the MBH ($p = .001$), and than the hippocampus ($p = .039$). The DS also had greater uptake than the MBH ($p = .002$). In addition, at the 5 minutes time point, only the VTA and DS had *FTHA levels above plasma ($p < .023$).

We also found a time-dependent effect of *FTHA uptake, $F(2,63) = 17.02$, $p < .001$. There was greater *FTHA accumulated in brain regions at the 15 minutes time point compared to the 5 minutes (adjusted $p = .016$), and to 30 minutes ($p < .001$) timepoints. Moreover, there was greater FA incorporation at 5 minutes compared to 30 minutes ($p = .011$).

Table 2. Average $\%ID_{FTHA}$/g in plasma and selected brain areas according to exposure time in wildtype mice.

	5 mins (n = 4)	15 mins (n = 4)	30 mins (n = 4)
Plasma	1.53 ± 0.17	1.55 ± 0.14	1.42 ± 0.07
PFC	2.23 ± 0.25	2.30 ± 0.19 *	1.94 ± 0.12
MBH	1.74 ± 0.14	2.07 ± 0.14	1.51 ± 0.12
DS	2.31 ± 0.21 *	2.62 ± 0.17 ***	2.08 ± 0.10
Hippo	1.90 ± 0.24	2.29 ± 0.17 *	1.59 ± 0.09 #
VTA	2.35 ± 0.24 *	2.74 ± 0.11 ***	1.96 ± 0.13 ##
NAC	2.16 ± 0.17	2.39 ± 0.19 *	1.87 ± 0.13
Heart	38.20 ± 6.56	41.99 ± 4.75	34.65 ± 3.82
Liver	28.54 ± 1.16	27.07 ± 1.03	22.63 ± 1.32

$\%ID_{FTHA}$: percentage of FTHA injected dose; DS: Dorsal Striatum; Hippo: Hippocampus; MBH: Medio Basal Hypothalamus; NAC: Nucleus Accumbens; PFC: Prefrontal Cortex; VTA: Ventral Tegmental Area. Tukey's within-factor multiple comparisons post-hoc tests are reported. Data is presented as mean (%ID/g) ± SEM. * $p < .05$, ** $p < .01$, *** $p < .001$, compared to plasma; # $p < .05$, ## $p < .01$ compared to 15 minutes.

Experiment 2: Assessing the impact of fatty acid metabolism in the ventral tegmental area on the rewarding properties of food

2.1 FATP1 inhibitor reduces long chain fatty acid uptake in neurons *in vitro*

We have previously shown that intra-VTA infusion of oleate reduces food-motivated behavior and this effect is reversed by a general channel blocker, phloretin.[260] Given this and the relevance of FAs in reward, and the involvement of FATP1 in DIO, we then sought out to assess whether manipulating a specific protein involved in FA metabolism in the VTA similarly influence food-seeking responses. We first tested the ability of FATP1 inhibitor 5k to block FA entry into immortalized GT1-7 neurons. Figure 5A shows representative images for each of the cultured conditions, where cells were exposed either to BODIPY only, or co-exposed with varying concentrations of 5k (6nM, 600nM, 60μM). There was a significant dose-response

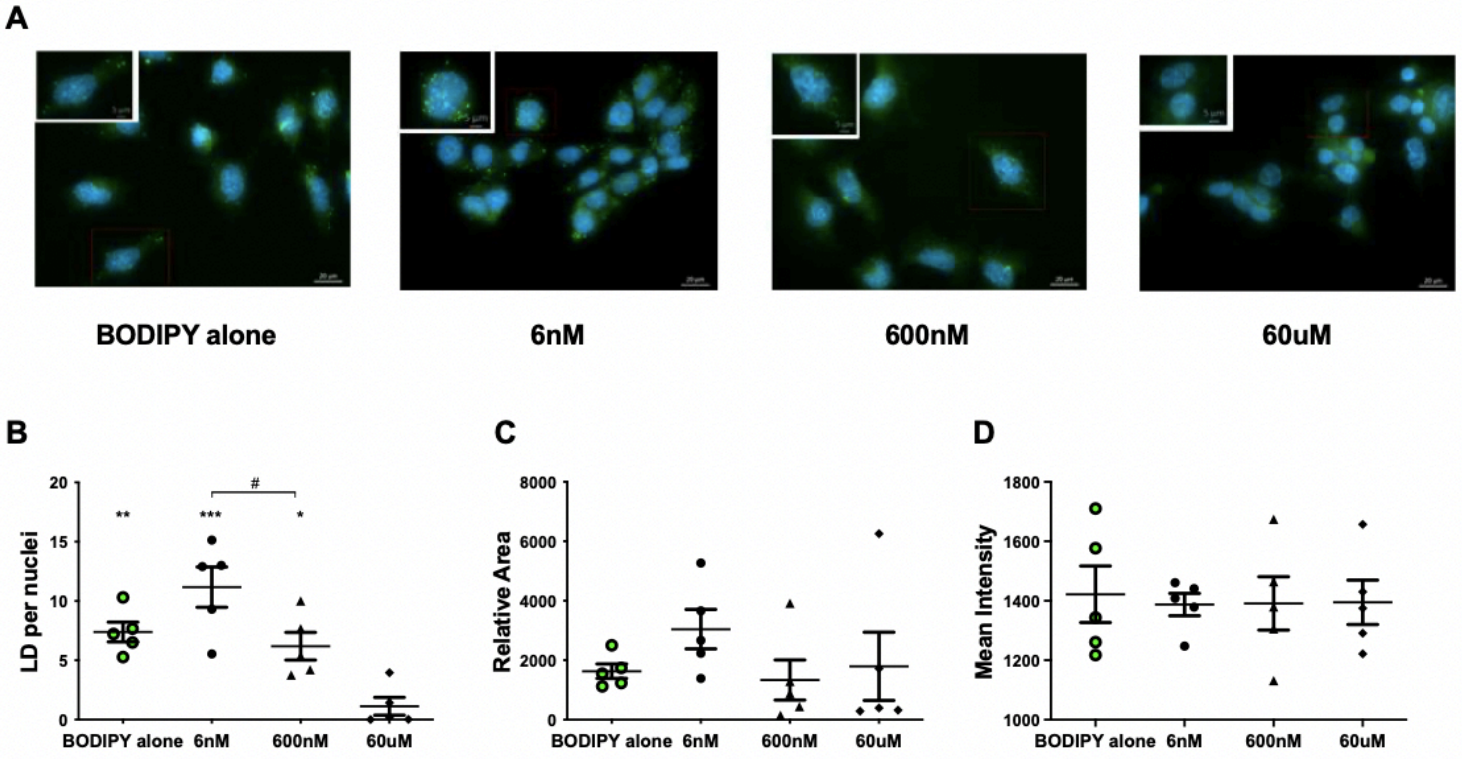

Figure 5. FATP1 inhibitor impact on lipid droplet accumulation in immortalized GT1-7 cells. GT1-7 immortalized hypothalamic neurons were exposed to FATP1 inhibitor 5k for 2 hours, followed by 1 hour of co-exposure of 5k with 10μM BODIPY. **(A)** Representative images of each condition. **(B)** Average number of lipid droplet structures per cell according to 5k concentration and BODIPY alone. **(C)** Relative area of staining. **(D)** Mean intensity of staining after subtracting lipid droplet signal. FATP1 inhibitor 5k concentrations: 600nM, 6nM, 60μM; BODIPY alone: cells were exposed only to C1-BODIPY 500/510-C12 for 1 hr. Results are expressed as mean ± SEM, with individual image data represented by points (n=5 images per condition). * $p < .05$, compared to control; # $p < .05$, ### $p < .001$.

relationship between inhibitor 5k concentration and number of LDs per neuron, $F(3,16) = 12.53$, $p < .001$ (Figure 5B). Specifically, compared to the BODIPY-alone condition (7.39 ± 0.83 LDs), the lowest concentration of 5k (11.18 ± 1.69 LDs) and the medium concentration (6.20 ± 1.17 LDs) did not significantly change the number of LD per cell. However, the highest dose of FATP1 inhibitor 5k significantly reduced the number of LD in neurons (1.12 ± 0.76 LDs), $p < .05$. The 600nM group also had significantly fewer LDs compared to 6nM, $p < .05$.

There were no statistical differences between conditions for the stained areas (cells), $F(3,16) = 0.99$, $p = .419$, indicating similar number of neurons per image (Figure 5C). There was also no significant difference for mean signal intensities, $F(3,16) = 0.04$, $p = .989$, suggesting similar background-level signal (Figure 5D).

As the 5k concentration of 60μM was the most effective in reducing LD number, we used this concentration for behavioral testing.

2.2 Intra-VTA FATP1 inhibition does not affect the rewarding properties of food

To determine whether FATP1 inhibitor 5k can alter motivation for palatable high-fat high-sugar pellets when injected into the VTA, we assessed its effect on operant responding. After PR training was acquired and stable, mice underwent stereotaxic surgery. The first cohort (n=9) did not survive surgery when cannulas were implanted 1mm above VTA. We adjusted the guide cannula depth such that it ended 2mm above the VTA, thereby increasing survival (n=6).

As shown in Figure 6, there were no differences between the different conditions tested, for any of the assessed food-motivation parameters: breakpoint ratio (Figure 6A, $F[3,15] = 0.85$, $p = .487$); total rewards received (Figure 6B, $F[3,15] = 1.17$, $p = .354$); or proportion of correct responses (Figure 6C, $F[3,15] = 1.29$, $p = .315$). There was a trend towards an increase in the rate of presses when receiving an active substance compared to vehicle during the first 10 minutes of the experiment, (Figure 6D, $F[3,15] = 2.73$, $p = .081$).

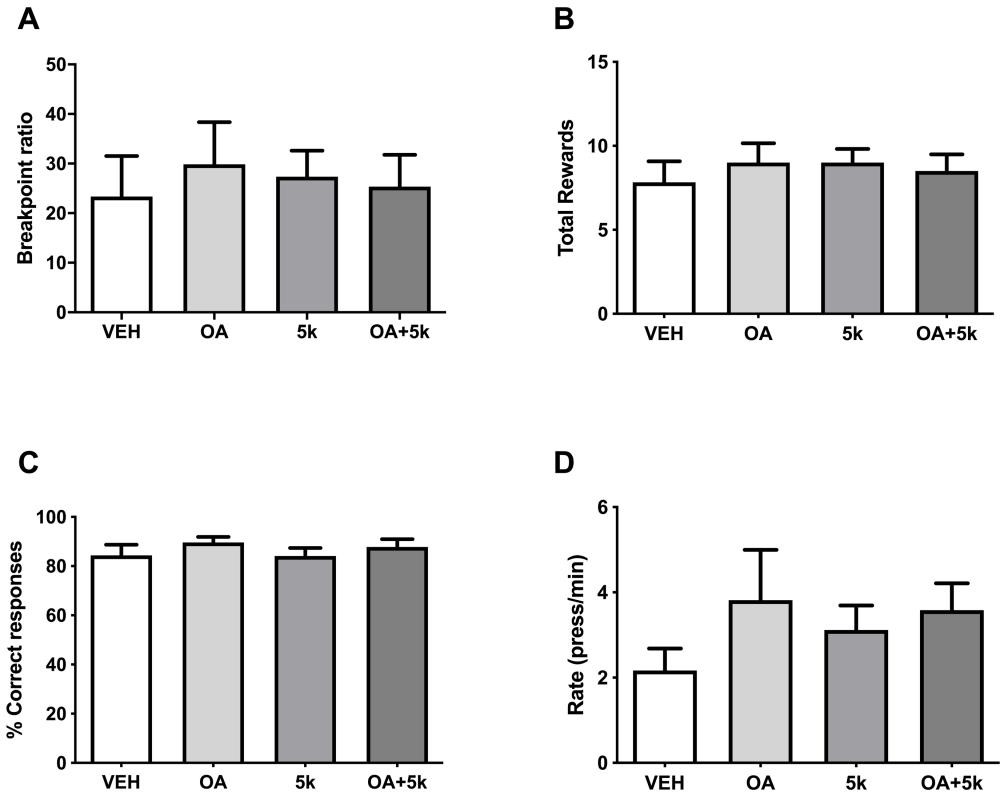

Figure 6. Impact of intra-ventral tegmental area oleate and/or FATP1 inhibitor on the rewarding effects of high-fat/high-sugar pellets in wild type mice. Mice (N=6) surgically implanted with bilateral cannula projecting to the VTA received injection of vehicle (VEH; 3% HPB in 0.9% saline), oleate (OA; 2nM), FATP1 inhibitor 5k (5k; 60µM), or co-injection of both compounds (OA+5k). Each mouse was exposed to all four conditions, first condition was vehicle, followed by the 3 others in a counterbalanced fashion. Figure shows: **(A)** breakpoint ratio values, **(B)** the total rewards received, **(C)** the percentage of correct lever responses, **(D)** the response rate for the first 10 minutes of the experiment (lever presses/minute). Results are expressed as mean ± SEM.

Experiment 3: Assessing the impact of a 20-week high-fat diet on brain inflammation, lipid accumulation and blood brain barrier integrity

3.1 Diet-induced obesity and metabolic outcomes following 20-week high-fat diet

WT male mice were fed a palm oil HFD (n=5) or an ingredient-matched soybean oil control diet (n=4) for 20 weeks. The diet high in palm oil was used as it is found in most processed foods,

has been shown to induce central inflammation and alter dopamine tone.[232,252] Mice on HFD became significantly heavier than the CTRL group from week 7 and over the 13 remaining weeks ($p < .01$, Figure 7A). The HFD mice also ate significantly more (in terms of calories) than the CTRL group from week 6 onwards (week 6 $p < .05$; week 7-20 $p < .001$, Figure 7B). Diet alone accounted for 34.32% of the total variance in body weight $F(1,7) = 13.10$, $p = .007$.

Body composition (percent fat and lean mass) was also analyzed. Compared to the CTRL diet mice, HFD mice had significantly reduced lean mass (51.12±1.71g vs. 64.85± 2.90g; $t[7] = 4.29$, $p = .004$, Figure 7C), and increased fat mass (39.52±1.86g vs. 23.55± 3.76g; $t[7] = 4.08$, $p = .005$, Figure 7D).

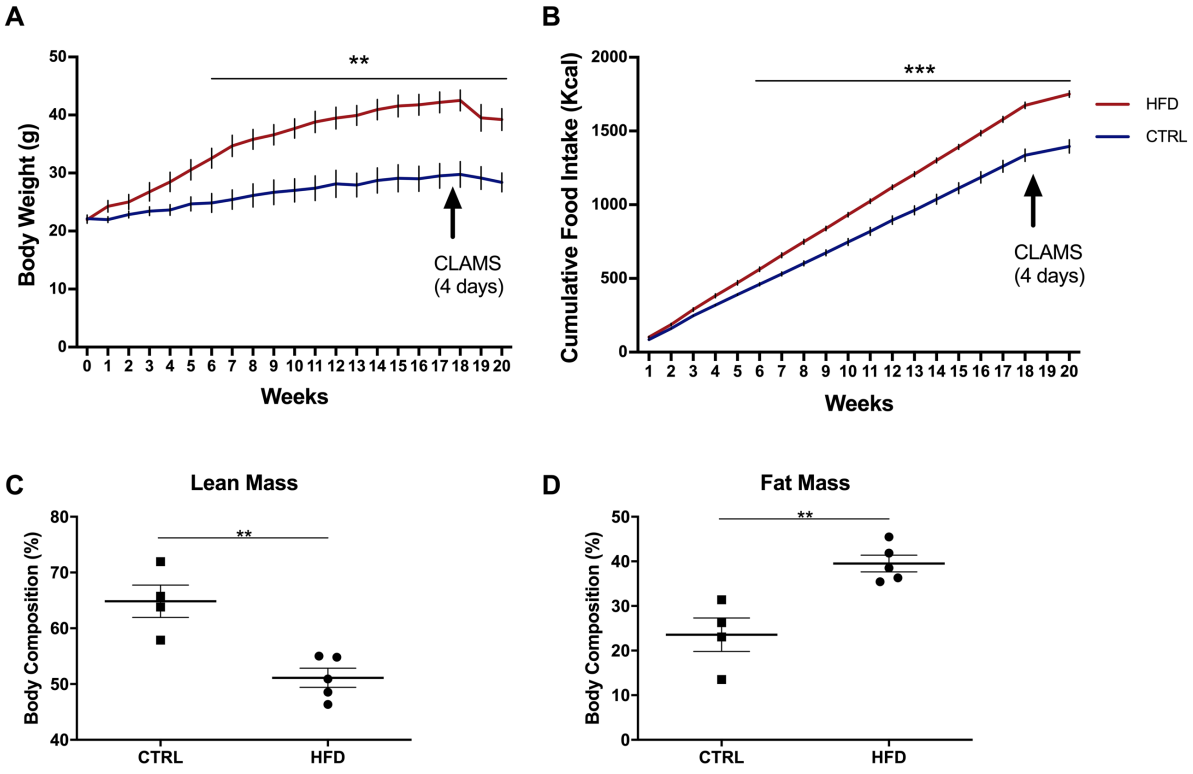

Figure 7. Characteristics of mice on a 20-week high-fat diet. Body weight and food intake were assessed weekly for 20 weeks, while body composition was measured at week 19 (HFD n=5, CTRL n=4). The figure shows: **(A)** body weight over time, **(B)** cumulative food intake, **(C)** percent lean mass and **(D)** percent fat mass. CLAMS: Comprehensive Lab Animal Monitoring System (chambers); CTRL: control; HFD**:** high-fat diet. Results are expressed as mean ± SEM. ** $p < .01$, *** $p < .001$.

3.2 Long term high-fat diet impacts basal energy expenditure, but not locomotor activity or dopamine tone

Basal locomotor activity and energy expenditure for the first 24h in the CLAMS (light and dark cycles) were recorded after 18 weeks on high-fat or control diet (Figure 8A-B and 5C-D, respectively). There were no group differences in basal locomotor activity ($p > .05$), although both diet groups showed significant increases in locomotor activity during the dark cycle compared to the light cycle, $F(1,7) = 32.13$, $p < .001$. Energy expenditure was significantly increased during the dark (active) cycle for the HFD group compared to the CTRL group, $F(1,7) = 10.29$, $p = .015$.

Over the following two days in the CLAMS, the effect of D1R agonist SKF82958 on locomotor activity was assessed (Figure 8E-G). Saline injection did not alter locomotor activity (Figure 8E), and the D1R significantly increased locomotion over the 3 hours post-injection compared to saline, $F(1,7) = 87.06$, $p < .01$ (Figure 8F-G). However, the magnitude of this effect was the same between HFD and CTRL groups ($p > .05$; Figure 8G). A difference value (SKF – saline) for number of beam breaks during the 3 hours following injection confirmed this finding, $t(7) = 1.61$, $p = .152$, (Figure 8H).

3.3 Long term high-fat diet increases plasma insulin and inflammation markers, but not nutrient concentrations

Plasma biochemical analyses from time of sacrifice (after 20 weeks on diet) are presented in Table 3. Levels of insulin, TNFα and CRP were significantly greater in the HFD group compared to CTRL mice at the end of the 20-week diet intervention (all $p < .05$). There were no significant differences between groups in plasmatic levels of glucose, TG or FFA (all $p > .100$).

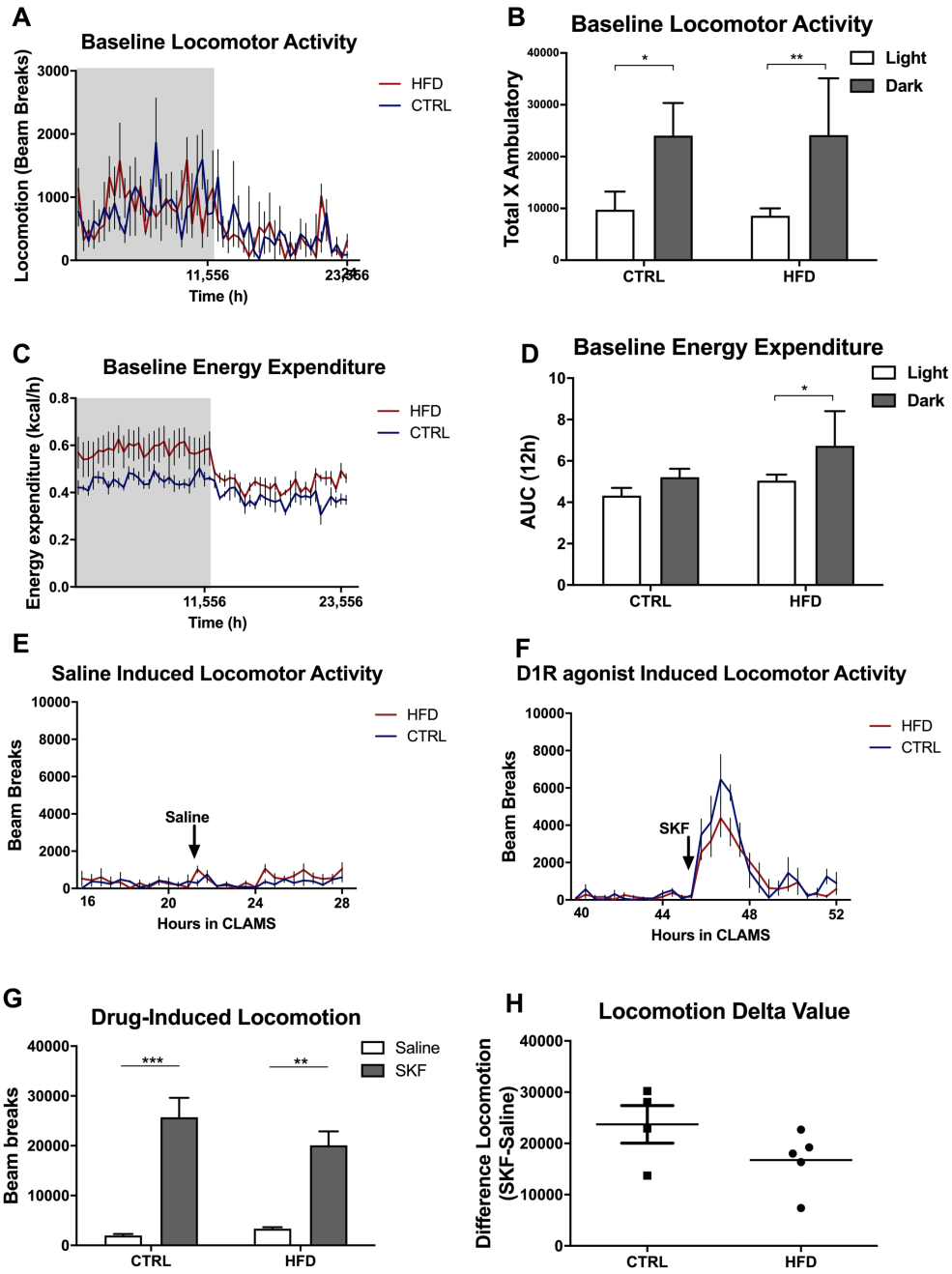

Figure 8. Locomotor activity and energy expenditure under basal conditions and in response to a dopamine-1 receptor agonist in wild type mice after 18-week high-fat diet. HFD n=5, CTRL n=4. Baseline **(A)** locomotor activity and **(C)** energy expenditure during the 24h habituation period. Quantitative assessment of baseline **(B)** locomotor activity and **(D)** energy expenditure in the 24h habituation period during dark and light cycle. **(E)** Saline-induced locomotor activity. **(F)** Drug-induced locator activity. **(G)** Quantification of total locomotor activity during the 3hrs post-injection of saline and SKF in both groups. **(H)** delta values for the difference between total locomotion following SKF and saline injections. CTRL: control diet; HFD**:** high-fat diet; SKF: D1 receptor agonist SKF82958. Results are expressed as mean ± SEM. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 3. Metabolic and inflammation markers after 20-week diet in wildtype mice

	CTRL (n=4)	HFD (n=5)	P value
Glucose (mmol/L)	12.63 ± 2.821	12.43 ± 1.094	.947
Insulin (ng/mL)	0.685 ± 0.226	1.956 ± 0.261	.009
TG (mg/mL)	0.104 ± 0.035	0.089 ± 0.023	.722
FFA (mmol/L)	0.185 ± 0.019	0.224 ± 0.024	.261
TNFα (pg/mL)	4.163 ± 1.747	7.946 ± 0.540	.028
CRP (µg/mL)	1.493 ± 0.214	2.222 ± 0.258	.037

CRP: C-Reactive Protein; CTRL: control; FFA: Free Fatty Acids; HFD: high-fat diet; TG: Triglycerides; TNFα: Tumor Necrosis Factor-alpha. Values presented as mean ± SEM.

3.4 Long term high-fat diet does not significantly increase brain lipid accumulation, but may induce neuroinflammation and alter blood brain barrier permeability

A series of immunohistochemical analyses were conducted to assess brain lipid accumulation, neuroinflammation, and BBB structural integrity and permeability. For the first analysis, fat accumulation was measured in the VTA and NAC of HFD and CTRL mice brain sections (Figure 9A). There was no significant difference in the intensity of the BODIPY 493/503 signal between diet groups in either region, $F(1, 14) = 0.98$, $p = 0.338$. However, there was a region-specific difference, where the VTA expressed greater BODIPY signal than the NAC, $F(1,14) = 5.54$, $p = .034$ (Figure 9B).

As a measure of central inflammation, the number and morphology Iba-1 positive microglial cell bodies was assessed (Figure 10A). The number of microglial cells was greater in the NAC compared to the VTA, $F(1,14) = 13.54$, $p = .003$, but there was no difference in their count between HFD and CTRL mice (Figure 10B). For the morphometric analyses, soma ratio was used to assess whether microglia were of elliptical, i.e. “activated”, shapes. Figure 10C shows that microglia in the HFD group had significantly more activated morphology compared to CTRL mice ($F(1,14) = 8.12$, $p = .013$), and that the NAC displayed significantly more

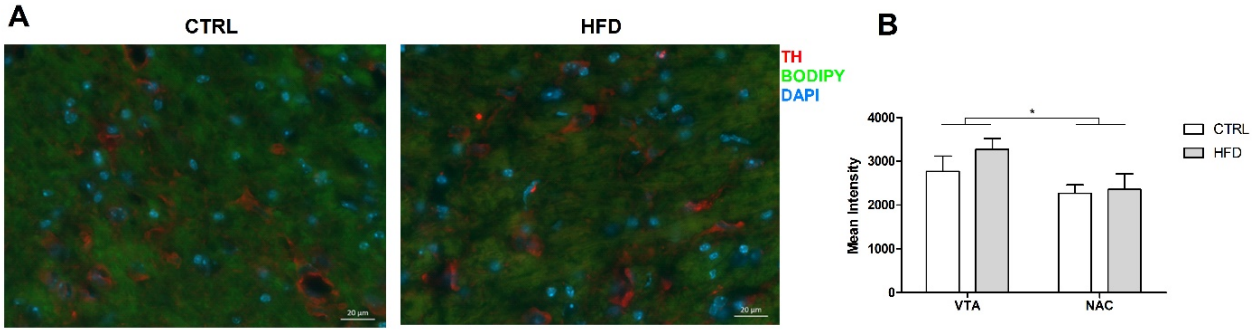

Figure 9. Cerebral lipid content in wildtype mice after 20-week high-fat diet. HFD n=5, CTRL n=4. The figure shows in **(A)** representative immunohistochemistry images of the VTA for both CTRL and HFD brains. **(B)** Mean signal intensity of BODIPY 493/503, a stain for neutral fat, across mesolimbic brain regions. CTRL: control diet; HFD**:** high-fat diet; NAC: nucleus accumbens VTA: ventral tegmental area. Data is presented as mean signal intensity ± SEM. * $p < .05$.

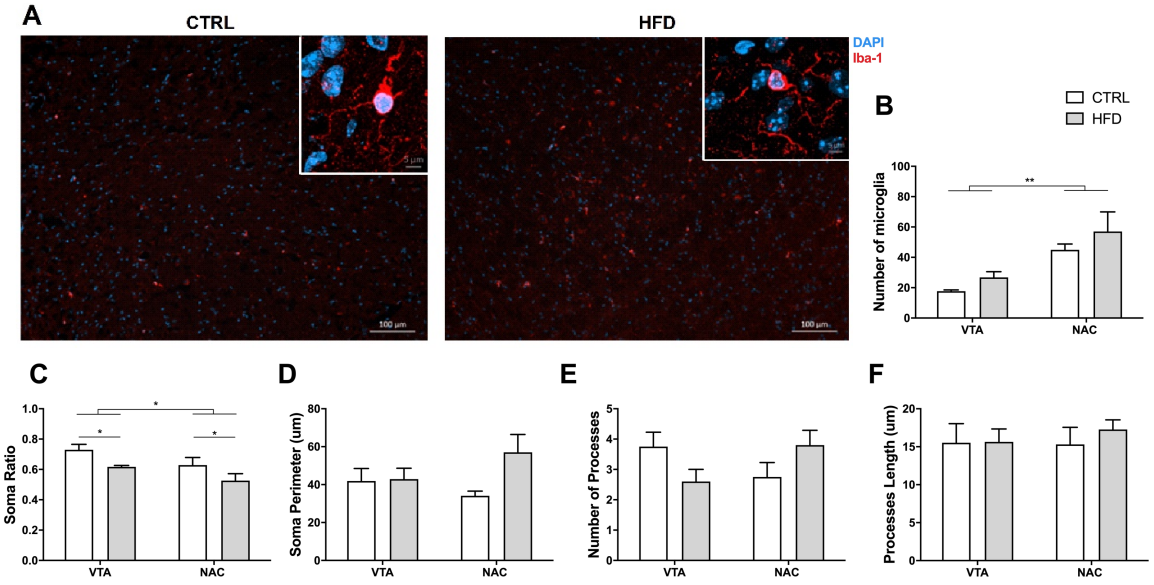

Figure 10. Cerebral inflammation in wildtype mice after 20-week high-fat diet. HFD n=5, CTRL n=4. **(A)** Representative images of Iba-1 (red) immunofluorescence on coronal VTA brain slice. **(B)** Quantification of Iba-1 cells in the VTA and NAC. **(C)** Ratio (minimum ferret/maximum ferret) and **(D)** soma perimeter of Iba-1 stained cells. **(E)** Number and **(F)** length of processes of Iba-1 cells. CTRL: control diet; HFD**:** high-fat diet; NAC: nucleus accumbens VTA: ventral tegmental area. Data is represented as mean ± SEM. * $p < .05$.

activated microglial phenotype compared to the VTA, $F(1,14) = 6.43$, $p = .024$. There were no statistical difference between diet groups or brain regions for microglia soma perimeter (Figure 10D), number of processes (Figure 10E), or the length of processes (Figure 10F), $p > .110$. However, there was a significant interaction between diet and brain region for the number of microglial processes, $F(1,14) = 5.58$, $p = .033$, although there was no significant main effects or trends in the post hoc analyses ($p > .833$).

To assess whether prolonged high-fat diet could affect the BBB in a mouse model, we measured leakage by co-staining endothelial basal lamina and fibrinogen (a blood-bound protein which would not normally be found in cerebral parenchyma; Figure 11A). To control for different amounts of BBB included in the images taken for each region, a ratio was generated

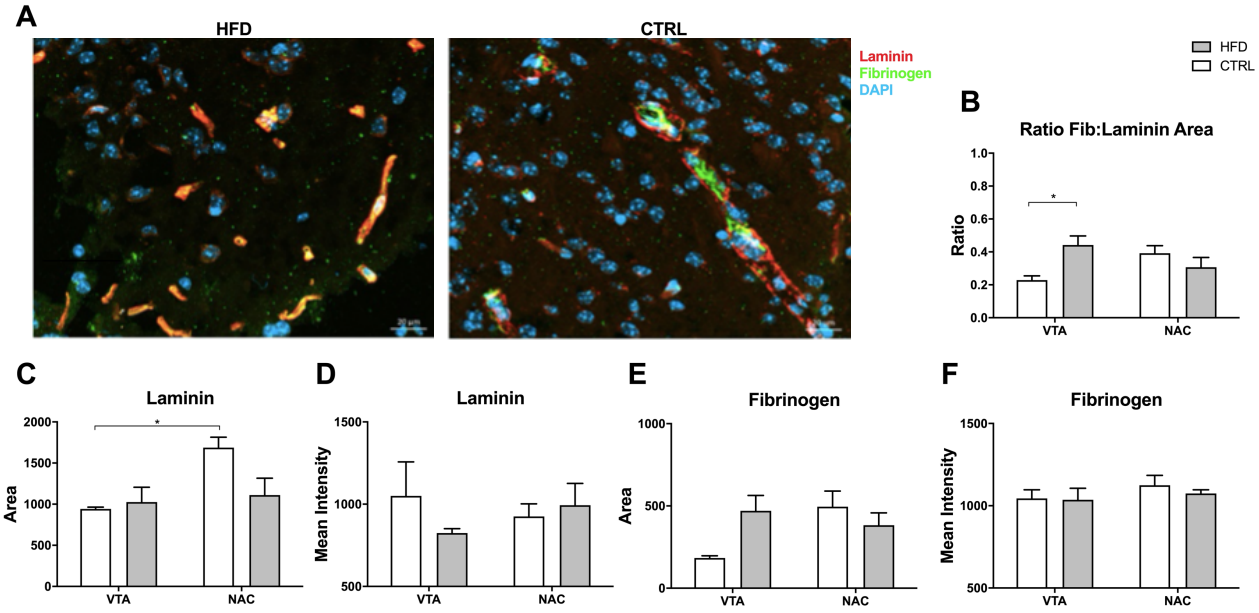

Figure 11. Blood brain barrier leakage in wild type mice after 20-week high-fat diet. (HFD n=4, CTRL n=2). **(A)** Representative images of laminin (red) and fibrinogen (green) immunofluorescence on coronal brain slice of HFD and CTRL mice. **(B)** Ratio of fibrinogen leakage area divided by laminin area to control for amount of blood vessels in each picture. The figure shows laminin signal **(C)** area, and **(D)** mean intensity, as well as excess fibrinogen **(E)** area, and **(F)** mean intensity. CTRL: control diet; HFD**:** high-fat diet; NAC: nucleus accumbens VTA: ventral tegmental area. Data is presented as mean ± SEM. * $p < .05$.

wherein the area of fibrinogen is divided by the area of laminin staining (Figure 11B). There was a significant interaction for this ratio, $F(1,13) = 8.80$, $p = .011$, but no difference for overall effect of the diet condition or brain region. Post hoc analyses revealed that within the VTA, the HFD group had greater amount of fibrinogen leakage than the CTRL group ($p = .024$). In terms of the amount of BBB captured, the area of laminin was greater in the images taken of the NAC compared to the VTA, $F(1,12) = 5.95$, $p = .031$ (Figure 11C). This effect remained significant in post hoc tests only for the CTRL mice, $p = .026$. There was no significant difference for laminin signal intensity between diet groups or brain area, $p > .252$ (Figure 11D). For the area of leaked fibrinogen alone (Figure 11E), there was a significant interaction between brain area and diet group, $F(1,12) = 5.63$, $p = .035$. Although post hoc tests revealed no significant differences between factors, trends were suggestive for increased fibrinogen in the VTA of HFD mice compared to CTRL mice ($p = .086$), and that for CTRL mice, the NAC be leakier than the VTA ($p = .059$). Mean signal intensity of fibrinogen was the same between diet groups and brain regions ($p > .299$; Figure 11F).

The structural integrity of the BBB was assessed by immunohistochemical measures of TJ proteins, ZO-1 and claudin-5 (Figure 12A). There was a trend indicative of reduced overall amount of ZO-1 in the NAC compared to the VTA, $F(1,14) = 3.92$, $p = .068$ (Figure 12B). We found no other differences between diet groups or brain regions in ZO-1 signal intensity (Figure 12C), claudin-5 area (Figure 12D) or claudin-5 signal intensity (Figure 12E), $p > .17$.

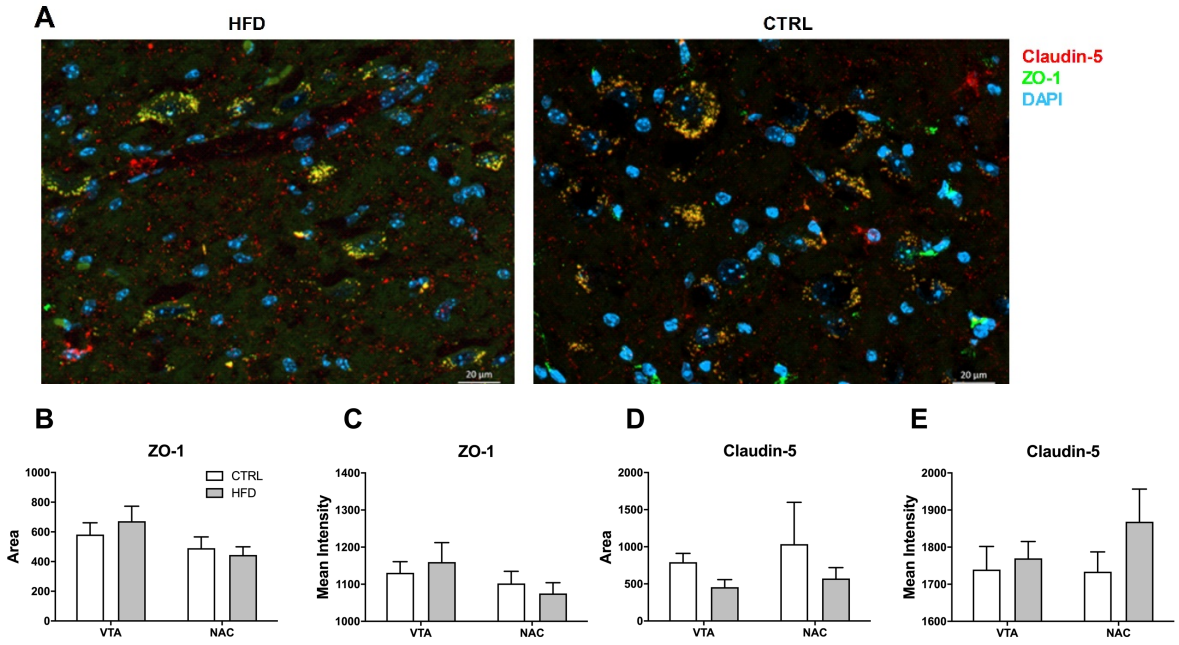

Figure 12. Blood brain barrier structural integrity in wild type mice after 20-week high-fat diet. (HFD n=4, CTRL n=2). **(A)** Representative images of claudin-5 (red) and ZO-1 (green) immunofluorescence on coronal brain slice of HFD and CTRL mice. The figure shows ZO-1 signal **(B)** area, and **(C)** mean intensity, as well as claudin-5 **(D)** area, and **(E)** mean intensity. CTRL: control diet; HFD**:** high-fat diet; NAC: nucleus accumbens VTA: ventral tegmental area.

DISCUSSION

Experiment 1: Assessing regional differences in acute fatty acid uptake

The primary objective of our first experiment was to assess the relative incorporation of peripheral LCFAs into various brain regions in mice, and the subsidiary objective was to determine the amount of time mice should be exposed to the radiolabeled LCFA, *FTHA, for best detection.

*FTHA is a radiolabeled C17 saturated FA which has been shown to be absorbed from food, packaged into TG and reaches peripheral circulation, mimicking usual FA absorption.[107] It is taken up by cells and metabolized in similar ways to other LCFAs.[108] Once in the mitochondria, *FTHA can undergo 2 cycles of β-oxidation, after which it is thought to be metabolized into 8-^{18}F-undecanethiol, and binds to protein metabolites, presumably detected as hydrophilic radioactivity.[105] This is what accounts to the mitochondrial trapping, unlike other FAs which are cleared as they complete β-oxidation. Thus, *FTHA detection reflects principally this mitochondrial retention, but may also reflect glycerol ester activity, such that it is incorporated into complex lipid molecules such as TGs. While metabolic trapping of *FTHA in mitochondria is a limitation for studies assessing metabolite kinetics,[272] this is an advantage for our purposes as it allows the tracking of FA uptake in preferential tissues. Moreover, *FTHA is sensitive to insulin, which increases it's uptake into tissues,[273] although one study reported a reduction in detected radioactivity in the brain of pigs who were co-administered *FTHA and insulin compared to fasted pigs.[109] One limitation of this compound is that it has been shown to have impaired partitioning in oxidative and nonoxidative conditions compared to radiolabeled 14C-palmitate, wherein it is insensitive to the shift of esterification of FA into complex lipids

structures such as TG.[273] This limitation may become an issue in studies where subjects have altered insulin sensitivity, such an those in obesity models. However, in the brain, *FTHA has been shown to principally be distributed in glycerol ester compounds (for instance, TGs) and other hydrophilic phases, with lower fractions unchanged or in phospholipids.[109] For these reasons, *FTHA was deemed a suitable choice of radiotracer for our research question.

Our methodology allowed to obtain a relative measure of FA accumulation in brain parenchyma, which could be compared to circulating levels in plasma. Due to the nature of *FTHA, we showed that cells in the mouse brain incorporate LCFAs from circulation. These are likely trapped in mitochondria or complexed into larger structures. Our results are novel in that we have been able to identify region-specific differences in *FTHA detection in WT mice. Other *FTHA studies, such as one in insulin-resistant humans, assessed global brain uptake.[274] Additionally, we have been able to determine that, with our method, waiting 15 minutes after *FTHA injection prior to sacrifice is optimal for signal detection with a gamma counter in mice.

In terms of peripheral LCFA incorporation into various brain regions, our study revealed that the PFC, hippocampus, DS, NAC, and VTA had concentrations of *FTHA above circulation levels. While global brain uptake of *FTHA has been previously reported in pigs and humans,[109,111] we were able to show region-specific differences in mice. Specifically, the VTA and DS appeared to emit greater radioactivity levels when compared to the MBH, and the VTA also had more *FTHA than the hippocampus. This highlights the importance of the VTA in terms of FA incorporation, and possibly in nutrient sensing and food consumption. Studies assessing the effect of peripheral TGs have reported their significant impact on DA-dependent motivated behavior.[241,256] Given the VTA is the source of DA neurons in the mesolimbic system

and it's high uptake of peripheral FAs, our data provide new evidence for a better understanding of the behavioral consequences associated with food reward.

Surprisingly, the levels of *FTHA detected in the MBH, which includes the ARC – the major center for food intake and appetite regulation – was not significantly different than circulation levels. Given the MBH's proximity to the median eminence, a circumventricular organ, it would have been conceivable that this region exhibits higher degrees of *FTHA incorporation. As discussed in the Introduction chapter, modulating FA entry in hypothalamic neurons has been shown to have an effect on food intake. It could be posited that the median eminence and MBH only reflect circulating levels of FA in non-obese mice to fulfil a simpler homeostatic role, and that their systemic and behavioral effect are solely reflective of FA chain length and saturation. Assessing the relative activity of various ARC neuron populations following systemic injection of varying concentrations and types of FA (short, medium, and LCFA) could help to explain these findings.

The data reported here reflects the uptake of *FTHA into tissues, and unlikely to be a reflection of its incorporation into the choroid plexus, which generates cerebrospinal fluid. Indeed, the lipid content of cerebrospinal fluid is low, approximately 10-13µg/mL, or about 0.2% of total serum lipid content.[275] Moreover, in mice, its production occurs at around 350nL/min, and to reach a total turnover of the 40µL would take about 114.5 minutes.[276] Taken together, it is unlikely for the choroid plexus, located around the 3rd ventricle, to contribute significantly to changes in *FTHA detection in the MBH or other brain regions.

Additionally, we have been able to determine that, with our method, 15 minutes post *FTHA injection is the optimal time for detection, i.e. how long mice should be left with *FTHA in circulation prior to sacrifice. After that peak, the signal intensity decreased in all studied areas.

This is rather unlike other studies which report metabolic trapping and gradual increase of *FTHA signal over time. [106,107,111] Rather, we observed a significant decrease in *FTHA signal between 15 and 30 minutes. The decrease we see between the 15- and 30-minute incubation period may reflect that we reached saturation in the mouse brain at 15 minutes, followed by a high turn-over rate. Others have reported relative reduction in *FTHA activity after longer delays between injection and detection, and have suggested this may be due to FA clearance due to turnover of lipid structures, loss of lipophilic metabolites, or variance in tissue workload.[105,109] Another possibility has to do with insulin levels. Indeed, inhaled anesthesia has been shown to suppress basal insulin levels and its response to glucose.[277] Importantly, insulin postprandially induces FA uptake into cells,[278] and hyperinsulinemia has been shown to promote *FTHA uptake.[109] As such, it is possible the longer duration of isoflurane anesthesia could dampen insulin's ability to promote FA uptake into the various tissues observed here.

Interestingly, our data also suggests that the transport of *FTHA into the VTA and DS occurs more rapidly than into the other regions we sampled. They were the only ones to have significantly greater radiotracer accumulation compared to plasma at the 5-minute post-injection timepoint. To our knowledge, this has never been reported. This finding is interesting to note given the role the VTA and DS have in motivation and food-seeking. The degree and rapidity at which these regions incorporate peripheral FAs, relative to the other areas assessed, could reflect the strength of the rewarding properties of dietary lipids. As we know, the release of DA in the mesolimbic system becomes elevated with rewarding substances such as drugs of abuse and palatable food. We also know that DA neurons in the VTA can incorporate LCFAs and that oleate exposure dampens their firing rate and food-motivated behavior.[260] Thus, the incorporation of *FTHA in these within these areas in the first 5 minutes of it entering

circulation may indicate that, similarly, postprandial FAs also reach mesolimbic regions to elicit a rapid and positively reinforcing response to food intake. Indeed, dietary TGs have been shown to be metabolized by neurons in the mesocorticolimbic system and have reinforcing effects of food-motivated behaviors by acting on D2R.[256] It remains to be seen whether this rapid accumulation is unique to the VTA and DS by measuring *FTHA in more brain regions, and whether it translates to immediate FA metabolism in these areas and changes in neural activity. It would be interesting to assess whether the dampened mesolimbic DA tone in conditions of obesity is similarly reflected in rapid FA uptake in the VTA and DS.

Strengths and Limitations

Our approach to detect *FTHA in the brain appears appropriate. Tail vein injection is a recognized method to deliver substances into systemic circulation of rodents. We ensured that most of the radioactive substance was actually delivered and measured the remaining radioactivity in the syringe to determine the actual injected dose. To increase the accuracy of our findings and to enable between-subject comparisons, radioactivity data was controlled for actual injected dose, sample weight, and circulating concentration. The data pertaining to detected *FTHA is a measure of proportion, but these measures allowed us to calculate the relative *FTHA accumulation and to perform comparisons between regions. Other studies have employed PET imaging to visualize *FTHA, which has the advantage of working with live animals in real time.[107,109,111] However, a comparison of FA radiotracer accumulation in tissues (%ID/g) using PET/CT scanner or ex vivo gamma counter has shown that the values of each method are highly correlated: $r^2 = 0.959$, slope = 0.634, $P < 0.0001$.[279] We therefore consider our data to be a valid representation of LCFA uptake and comparable to PET/CT studies. Our

rigorous procedures have allowed us to show the short-term entry of circulating LCFAs into selected brain regions at specific time points.

While our methodology is robust in terms of *FTHA detection, a more physiological approach could be of benefit. The tail-vein injection bypasses all digestion and absorption processes. Therefore, to improve the ecological validity of our findings, the administration of *FTHA could be done by gavage. In humans, oral administration of *FTHA appears to mimic normal lipid digestion and tissue uptake, with over 90% of it reaching circulation as TGs.[107] However, brain incorporation of *FTHA administered orally has yet to be studied. Assessing whether detection of *FTHA is altered depending on the method of administration would be important in assessing the validity of studies employing radiolabeled FAs.

Future Directions

This preliminary study is the first step for future experiments which will assess relative differences in brain regions of lean and obese mice. In these studies, measuring incorporation of *FTHA into visceral and subcutaneous fat would be of relevance as these tissues are relevant to lipid metabolism. Moreover, comparing lipid uptake in this compartment between lean and obese mice could provide a fuller picture of altered metabolism in this pathology.

Another method to assess FA entry into cells could be autoradiography. In this technique, coronal brain sections would be retrieved from mice post injection and covered with an x-ray film or phosphor screen. When left overnight, the screen “records” the emitted radioactivity. The advantage of this technique is that the brain slices can be used for subsequent staining or immunohistochemistry. Thus, the radioactivity images can be overlaid onto images

of various staining. These recreated brain images could allow for co-staining to evaluate transporter density, BBB, or cell type enrichment.

The role of oleate could also be further explored as it has been previously described as being involved both in the hypothalamic and mesolimbic control of feeding. Quantifying peripheral oleate uptake into specific brain tissues would be extremely relevant information to add to this field. In fact, a radiolabeled oleate analog, 18-[18F]fluoro-4-thia-oleate (FTO),[280] has been developed and is designed to be metabolically trapped in mitochondria in much the same way as *FTHA.[281] Analogs of stearic acid and palmitate have also been developed and tested with PET imaging to assess FA metabolism in the myocardium.[282] As the protocol and methodology have been established in our lab, these other ^{18}F-labeled FAs could also be used to assess their differential uptake and timing of uptake into different brain regions.

Conclusion

In summary, we were able to detect region-specific differences in relative *FTHA detection in the brain mice. We also determined that waiting 15 minutes before sacrifice was optimal for peak *FTHA detection within a gamma counter method. Other than the MBH, all sampled areas had greater detected *FTHA than plasma. The VTA and DS appeared in incorporate *FTHA faster, and in greater proportions relative to other sampled areas. This is a tentative support for the role of the mesolimbic system in the control of eating behavior, although further assessment to their participation and significance is required.

Experiment 2: Assessing the impact of fatty acid metabolism in the ventral tegmental area on the rewarding properties of food

Our first experiment highlighted the relevance of the VTA in FA uptake from periphery. As our lab has previously shown that oleate has a suppressive effect on motivation, and that inhibiting FA uptake at large with phloretin prevented this action,[260] the next objective of this Master was to evaluate whether more specific modulation of FA metabolism in the VTA would have similar effects on food-seeking behavior. To this end, we opted to inhibit the protein FATP1 as it has been shown to be relevant in periphery in conditions of DIO,[49] and is expressed in VTA DA neurons.[260] We employed FATP1 inhibitor 5k and performed a dose-response experiment to estimate the best concentration to use in vivo. Its inhibitory effect was confirmed in an *in vitro* model, where the high dose of 5k significantly reduced the amount of lipids, measured as LDs, incorporated into an immortalized hypothalamic neuronal cell line. This was expected, as FATP1 is involved in LCFA incorporation and intra-cellular transport. Equivalent area and signal intensity (excluding LD-like structures) indicates there was a similar number of cells in each image and equivalent background staining, or autofluorescence. This makes the data of different conditions comparable and adequate for interpretation. Therefore, the high dose was used for behavioral experiments.

In our behavioral model, a mouse model was favored as later studies would employ selective genetic knock down of fat transport proteins in the VTA. These genetic models are widely available in mice, but less so in rats. Therefore, mice were trained to lever press for high fat high sugar food pellets underwent stereotaxic surgery allowing intra-VTA injections to be performed. Contrary to what our lab previously found in rats,[260] oleate did not significantly reduce the breakpoint in this study. In fact, there was no significant difference in food-motivated

behavior after mice received intra-VTA oleate, FATP1 inhibitor 5k, both substances, or vehicle. Moreover, there was a lot of variability in breakpoint values between individual mice and conditions. There was a trend indicative of increased lever press rate during the first 10 minutes of the experiment when mice received any of the active substances. It is possible this trend reflects changes in local neural activity in response to the invasion of the area with fluid and foreign molecules, although this finding is likely non-specific.

The lack in significant findings in this paradigm may in part be explained by the fact that other transport proteins such as CD36, FATP-4, and FABP-5 are also involved in fat transportation in the brain.[283] Inhibition of FATP1 alone may not have been sufficient to produce an behavioral effect. As well, there may be a delayed functional outcome of TGs on VTA DA neurons. One study found that 10 minutes was insufficient to affect *in vivo* electrical activity of DA neurons in the VTA, although prolonged perfusions of 5-6 hours significantly decreased firing rate and burst activity.[256] This study also suggested that the reward-modulating effects of dietary fats are due to changes in excitability of striatal neurons.[256] Thus, it is possible that the local administration of oleate and FATP1 inhibitor to the VTA would require prolonged infusions. This group also suggested the delay in VTA changes to TG exposure would be related to modulating tyrosine hydroxylase.[256] Alternatively, it may be due to structural differences in mice and rat brains, which could have been impacted differently following cannula placement. Overall, these studies suggest that VTA-specific adaptations require medium- to long-term exposures to fatty diets.

Strengths & Limitations

The operant conditioning paradigm is a recognized method to assess food-motivated behavior. As well, the use of cannulation allows the precise delivery of drugs and substances to target regions. Moreover, delivering a pharmacological agent allows to assess short-term modulation of the FATP1 protein and the effect of oleate delivery in the VTA.

Despite this, the small sample number for our behavioral experiments (n=6 mice) may also restrain the interpretation of these findings. Other issues may have impacted these results. Firstly, the high mortality rate after the first set of cannulation surgeries indicates the approach may pose a significant challenge to mice. Rats (weighing 250-280g) in the previous study in our lab appeared to tolerate the surgery well, however, mice weigh only about a tenth of what a rat does (25-30g). Indeed, it is possible the 26-gauge bilateral cannulas (diameter = 0.4049mm) caused damage to a significant portion of their brains, and to a point which significantly altered function and survival. Alternative measures to modulate FAs metabolism in the VTA with a less invasive approach could include region- and/or neural-specific viral inhibition or genetic knock down. These types of stereotaxic surgeries are typically quite short, the injection needles used much smaller in diameter, inserted only once and removed thereafter. This would minimize any damage done to the brain and allow for repair as the injectors do not stay in place after the surgery is done. Secondly, all experiments were done every other day and without assessing whether food-seeking behavior had returned to baseline between conditions. It is also possible the repeated injections could have induced tissue damage, or that injected substances (oleate, 5k, both substances, or vehicle) had residual or carryover effects on subsequent conditions. Thirdly, while injection site was assessed during practice surgeries, the final location of the cannula was not evaluated on the experimental mice. Variability in injection site could also have contributed to the inconclusive behavioral data. Lastly, further validation of the FATP1 inhibitor

could also be warranted. Although it is known that DA neurons in the VTA express FATP1,[260] testing this compound *in vitro* in this specific cell population, or in an *ex-vivo* model, could further confirm its inhibitory effect. It is possible that the lack of findings is due to insufficient dosage, that it may have influenced other cell types in the VTA, or that a higher concentration is required to have a functionally significant effect *in vivo.* Optimization of 5k drug concentration for future behavioral studies would be warranted.

Future Directions

Future experiments utilizing this methodology would require more elaborate testing of the inhibitor's action, and for behavioral studies an optimization of dosage, larger sample, more time between experimental conditions, and histological analyses to confirm cannula placement. Protocol modification should also include re-assessing basal breakpoint ratio between conditions to limit carryover effects between conditions.

Conclusion

In summary, we demonstrated the ability of FATP1 inhibitor 5k to prevent FA incorporation in neurons in an *in vitro* model. Contrary to a previous study from our lab, *in vivo* intra-VTA injections of oleate did not alter food-motivated behavior, nor did FATP1 inhibitor or both substances combined. These findings may be due to the delayed changes in VTA neural activity in response to FAs, although important methodological limitations were highlighted.

Experiment 3: Assessing the impact of a 20-week high-fat diet on brain inflammation, lipid accumulation and blood brain barrier integrity

In the first two experiments, we addressed the impact of FAs on the brain over very acute time periods. Given the relevance of lipid metabolism and of the mesolimbic system in obesity, a long-term, chronic condition, we sought to evaluate the effect of a long-term HFD (20 weeks) on cerebral lipid accumulation, neuroinflammation, and BBB integrity and permeability. As expected, the mice placed on HFD rich in palm oil gained progressively more weight, consumed more food, had greater fat mass and reduced lean mass compared to those on an ingredient-matched control (soybean oil) diet. These are the typical findings of DIO studies, meaning experimental DIO conditions were met and that mice were followed appropriately. The metabolic profile of the HFD mice suggests a state of insulin resistance and compensation, as levels were almost tripled despite identical glucose levels. More comprehensive metabolic tests such as glucose and insulin tolerance tests would be useful to address this, but this was not the aim of this Master' study. Similar to other models of obesity,[116,284,285] there were also indications of an inflammatory state in the HFD mice, as cytokines TNFα and CRP values were raised. HFD mice did not display higher plasmatic TG or FFA concentrations, as has been previously reported in this mouse strain.[286] However, a separate study using this same diet similarly did not find elevated plasma FFA.[252] It has been suggested that this may be a compensatory effect of elevated leptin. Moreover, although sacrifice was timed to be at the end of the light cycle, they had not been fasted prior, which may also be impacting these findings.

We found no difference in basal locomotion between groups, although HFD mice expended more energy during the dark cycle, suggesting that increased metabolic activity is required to meet similar activity levels. As expected, the D1R agonist SKF-82958 induced a

significant increase in locomotion compared to saline, in both groups. However, the magnitude of this difference was the same between groups. In other words, both groups responded to the D1R agonist in the same way, which supposes there is no difference in D1R expression or sensitivity after 18 weeks on HFD. This is unlike a previous study in our lab which reported a dampening of mesolimbic D1R function in rats fed a diet rich in palm oil.[242] This may be explained by the low number of animals (n=4-5 per group) compared to 25 in the aforementioned article. However, we did not assess changes in D2R, which seems more involved in reward-related behavior in obesity models, a domain not affected by SKF. Indeed, more emphasis has been placed on the role and impact of D2R changes in mesolimbic circuits within the context of obesity.[287–289] The involvement of D1R in DIO is relatively unstudied, although there may be a differential effect of diet and obesity susceptibility on various DA receptor expression.[290] In addition, SKF-82958 has been shown to increase locomotor activity only among female rats under HFD, not in males.[291] This last study was published in 2020, after the completion of this study, and suggests a sex difference in D1R sensitivity in response to DIO. The authors also found no change in D1R expression under HFD.[291] As our studies were conducted in male mice of the same strain, it is possible our DIO model similarly did not develop changes in D1R, which would explain the absence if difference between our HFD and CTRL mice. Moreover, we did not further assess D1R or D2R expression in our study, so firm conclusions regarding these findings cannot be drawn.

Overall, our immunohistochemical analyses found few differences in the parameters assessed. Due to our small sample size (HFD=5, CTRL=4), and limitations of the semi-quantitative nature of the analysis, conclusions can only be drawn tentatively.

To begin, we sought to assess whether diet induced increased fat content in the brain, as is seen in periphery. We stained neutral lipids in the brains of our mice and found that the VTA contained more lipid than the NAC. Surprisingly, there was no difference between diet groups. This is suggestive of region-specific differences in fat content or perhaps intracellular fat requirements and metabolism. Alternatively, it could be due to variations in grey or white matter content of each region.[292,293] Another explanation for this difference may be drawn from results obtained in Experiment 1, where we saw proportionally higher uptake of *FTHA from periphery into the VTA in lean mice. It may thus be logical for it to express greater lipid levels in its parenchyma, in a manner independent of diet.

The link between obesity and peripheral inflammation is widely reported and established.[294] There is also increasing evidence linking obesity to neuroinflammation in multiple nuclei including the hypothalamus, hippocampus, cortex, brainstem, and amygdala.[188] Here, we have a tentative addition demonstrating a neuroinflammatory effect of DIO in the mesolimbic dopamine system. Specifically, we found the microglia of HFD mice showed a greater degree of activated phenotype, compared to the mice on the CTRL diet, in both the NAC and VTA. This adds to the growing amount of evidence linking obesity to neuroinflammation. Indeed, neuroinflammation marker in mesolimbic areas was shown to blunt neural response to reward anticipation, DA availability and release, and DA content in CSF, all of which correlated to reduced motivation in humans.[295] Therefore, it is possible that neuroinflammation secondary to obesity also contributes to blunted DA circuitry in obesity. The underlying mechanisms of this relationship should be explored. Moreover, we also found that the NAC contained a greater number of microglia as well as greater basal microglial activation than the VTA, independent of diet. Our lab had previously published findings in mice which robustly demonstrated

neuroinflammation in the NAC following HFD rich in saturated fats, and similarly found increased microglia activation phenotype despite no diet-induced change in their number.[252] A separate group has also demonstrated neural adaptions in the NAC in response to a high-calorie chocolate cafeteria diet was mediated by neuroinflammatory markers and microglia activation.[296] Some evidence appears to suggest inhibition of neuroinflammation would prevent these neurological and behavioral adaptions to HFD,[296] as well as reduce DA-dependent nicotine withdrawal symptoms.[297] Our findings could be another example of regional specificity or sensitivity to microgliosis,[298] whereby the NAC would be in some way predisposed to neuroinflammatory activation, but resistant to increased microglial number. Indeed, the NAC appears to consistently show elevation in microglial activity.[299] Nonetheless, our data extends the causal relationship between HFD and neuroinflammation, including for the first time the VTA.

As reviewed in the introduction, a proposed mechanism for the observed neuroinflammation in obesity is that this condition is associated with an impaired BBB. In the present study, we assessed the BBB permeability, which was estimated by co-staining the basal lamina with fibrinogen, a blood-bound protein which would not normally be found in cerebral parenchyma. We assessed BBB leakage by measuring the fibrinogen signal which went beyond the laminin signal. As laminin is a marker of blood vessel basement membrane, this excess fibrinogen is a reflection of endothelial impairment and protein leakage. Consistent with other studies, we found greater fibrinogen infiltration into the VTA of HFD mice compared to CTRL mice. This finding does not appear to be due to differences in TJ structural integrity as we showed no immunohistochemical difference in their expression.

To our knowledge, this is the first instance of reporting a DIO-induced increase in BBB permeability in the VTA. As we have seen in Experiment 1, the VTA appears to rapidly take up peripheral FAs compared to other regions in naïve mice. Although this relationship needs to be further explored, the effect of an increased leakiness in conditions of obesity could potentiate this postprandial uptake of FAs, and perhaps the effects of peripheral lipid signals on the reward system. Indeed, peripheral FAs and TGs are metabolized in the mesolimbic system, can increase the rewarding effect of food, facilitate food reinforcement learning, response to psychostimulants, and neural response to food cues.[256] Increased BBB permeability could allow TGs to more easily pass through and exert these effects in persons with obesity. This may be an underlying mechanism for neuroadaptations in the mesolimbic system, notably reduced D1R and D2R expression as well as DA tone.[242,249,255,300,301] It is currently unclear whether BBB permeability and infiltration in the VTA is related to neuroinflammation. More focused experiments would be required to obtain conclusive data in this regard. As the finding of increased leakiness did not extend to the NAC, the DIO-induced neuroinflammatory indicators seen here and as previously published,[252] may not be due to alterations in the BBB. Further evaluation of this relationship would be warranted before making firm conclusions.

Although our measurements were semi-quantitative, it is surprising to not find indication of changes in TJ expression in our study. Other studies using Western blot and immunofluorescence found reduced ZO-1 expression in hippocampal and hypothalamic BBB of mice fed a HFD compared to low-fat diet, although they reported no changes in claudin-5 expression.[302] That being said, one paper assessing the effects of a HFD on BBB in male C57BL/6J mice (the same strain used in this study) found significant downregulation in many endothelial proteins, but, as we found, no changes in TJ proteins or laminin.[303] Others reported

that altered BBB permeability and TJ downregulation has been identified in some areas, but did not extend similar changes in the striatum or PFC.[114,116] An alternative explanation is that BBB damages resulting from DIO appears to be proportional to the degree of weight gain,[112] and that DIO-resistant rats do not appear to develop BBB degradation.[113] Perhaps our findings may be a reflection of an insufficient degree of obesity development in our mice. However, there seems to be a mechanism underlying increased BBB permeability in DIO. A study has shown that increased region-specific permeability of the BBB (in the cortex, striatum, and hippocampus) was not due to tight junction disruption or pericyte loss, rather that changes in transcytosis underly these changes.[304] These support the idea of region specificities in terms of BBB alterations as a result of prolonged HFD, but suggest that changes in transporter expression may underly these findings. Thus, there is some evidence to support our results and that DIO does not impact the BBB in all cerebral areas.

Strengths and Limitations

While many studies use a shorter duration of diet, we employed a 20-week HFD to model DIO. This long duration of diet is more reflective of diets and allows interpretation of long-term exposures to fatty diets. Body composition, energy expenditure and plasma analyses all yielded results consistent with other models of DIO.

As well, our neuroimaging technique were robustly designed to optimize the semi-quantitative data we generated. We analyzed many factors which had largely been studied alone in relation to obesity. By analyzing fat accumulation, neuroinflammation and BBB integrity, we could piece a more complete picture of the central effects of DIO. Moreover, we collected many slides of each brain area of interest, so future studies can expand on our findings.

A major limiting factor in our study was that of the small sample sizes. The final number of brains analyzed was of 5 HFD mice and 4 CTRL mice. Conclusions could be made only tentatively. Moreover, our immunohistochemical analyses were semiquantitative which limits the strength of these findings.

For this part of our work, we regret that 3 of the 9 brains suffered significant damage. This is possibly due to incomplete cryoprotection, although all samples underwent the same protocol. Upon slicing, it was observed that these brains (1 HFD, 2 CTRL) had visible holes in them. They were nonetheless used for immunohistochemical analyses. The images from these brains were obtained from viable tissue, but whether this damage could have influenced results is unclear. In addition, while immunohistochemistry is a powerful tool in visualizing differences in samples, it remains a qualitative approach. Our image analysis allowed semi-quantitative comparisons to be conducted, but these are limited by the nature of the data.

Future Directions

Regardless of these limitations, the proposed hypotheses are valuable. In future studies, measuring D1R and D2R expression (quantitative PCR or western blotting) in the mesolimbic system of male and female mice would be warranted to better understand the behavioral findings we observed. As well, assessing the tone of D2R with a specific D2R-modulating drug, for instance, using an operant training paradigm, could also provide some valuable insights to dopaminergic alterations in this DIO model. Another way to induce a DA surge could be to deliver L-dopa systemically. This would induce an increase in DA throughout the entire CNS but would act mainly of DA-rich areas such as the substantia nigra and VTA. This model could

be employed to assess both locomotor activity and reward-seeking behavior, although would not allow to differentiate whether findings are specific to either type of DA receptor.

Replicating this study design to either increase the sample size of both groups, and/or to allow for tissue collection directed at protein quantification analyses should yield more conclusive data. Indeed, the inclusion of quantitative approaches to support the immunohistochemistry data would increase the robustness of our findings. Moreover, alternative measures such as lipidomic analysis of brain tissue to assess central and peripheral fat content and FA breakdown could provide informative data to address the question of lipid accumulation in tissues. As well, conducting quantitative PCR for neuroinflammatory markers to complement the microglia count and morphology, and sodium fluorescein to visualize BBB leakage could be employed. Complementary approaches to measure the expression of these TJ proteins such as quantitative PCR and western blot could help clarify our findings. Additionally, BBB permeability could also be assessed by addressing levels of transporter expression, either by immunohistochemistry or by quantitative PCR, as it is possible that altered BBB permeability in DIO is due to an upregulation of transport or binding proteins in endothelial cells.[304]

The performed analyses in this work were indirect measures of structural integrity, and the degree to which molecules from periphery may enter brain parenchyma. Looking at different aspects of the BBB structure and other methods to assess leakiness would improve our ability to draw conclusions. Other future directions addressing the BBB structure and permeability should investigate the relationship between peripheral fat and cytokines, central lipid accumulation, and neuroinflammation. This could be accomplished by conducting regression analyses, or even structural equation modeling for multivariate analysis taking into account weight gained, percent body fat, peripheral inflammation markers, markers of BBB damage,

brain fat content and markers of neuroinflammation. Moreover, replicating these analyses and calculations in other ROIs such as the PFC, hippocampus, amygdala, and hypothalamus would give further insight into the differential effect of DIO on the brain. These data would, in turn, contribute to the growing knowledge on the central effects of obesity and associated neuropathogenesis depicted in the literature.

Conclusion

In this study we assessed the effect of a 20-week HFD on central fat accumulation, neuroinflammation and BBB integrity in the VTA and the NAC. Contrary to what we expected, there was no difference in lipid content in brain of HFD and CTRL mice. Despite relatively small size of our groups and some of the samples being damaged, we showed for the first time that DIO induced neuroinflammation and BBB permeability in the VTA. These findings may exacerbate or underlie the previously described behavioral changes and increased food-directed motivation in conditions of obesity.

CONCLUSIONS

The overarching goal of this work was to deepen our understanding on FA uptake and metabolism, its functional significance, and the effects of long term HFD in the mesolimbic system, specifically the VTA. Interestingly, we found that the prefrontal cortex and reward-related areas (DS, VTA and NAC), incorporate *FTHA from circulation. The VTA and DS also incorporated *FTHA compared to other brain regions. A 15-minute incubation time was determined optimal for *FTHA detection, although at 5-minutes post injection, the VTA and DS already showed significantly elevated uptake. This suggests that LCFA pass through BBB rapidly and accumulate in reward-related areas. Surprisingly, the MBH, a region traditionally associated with energy homeostasis and nutrient sensing, did not incorporate *FTHA above circulation levels. These findings provide some insight into how the diet can impact the brain and highlights the relevance of the VTA.

Surprisingly, we found no changes in food-motivated behavior when exposing the VTA to oleate or when modulating FA transport. Methodological limitations were discussed.

Lastly, we found an indicator of neuroinflammation in reward areas as well as increased BBB permeability in the VTA of mice under prolonged HFD. Microglia in HFD mice had a more activated phenotype, however, there was no impact of diet on their number. Contrary to our hypotheses, we did not find differences in global fat accumulation or BBB structural integrity. Interpretation of this experiment is limited by the small size of our samples and the more qualitative nature of our data.

Taken together, our data reveals the importance of the VTA in relation to FA uptake. The rapid and heightened incorporation of *FTHA suggests a rapid detection and potential mechanism for the rewarding response to food. As well, DIO-induced increase in BBB

permeability in this region could potentiate this rewarding effect, or underly the dampened dopaminergic neuroadaptations frequently observed in conditions of obesity.

REFERENCES

1. World Health Organisation. Obesity and Overweight Factsheet No.311. World Health Organisation Media Centre.
2. Anis AH, Zhang W, Bansback N, Guh DP, Amarsi Z, Birmingham CL. Obesity and overweight in Canada: An updated cost-of-illness study. *Obes Rev*. 2010. doi:10.1111/j.1467-789X.2009.00579.x
3. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet*. 2016. doi:10.1016/S0140-6736(16)30175-1
4. Kopelman PG. Obesity as a medical problem. *Nature*. 2000. doi:10.1038/35007508
5. Baker KD, Loughman A, Spencer SJ, Reichelt AC. The impact of obesity and hypercaloric diet consumption on anxiety and emotional behavior across the lifespan. *Neurosci Biobehav Rev*. 2017. doi:10.1016/j.neubiorev.2017.10.014
6. Gonzalez MC, Correia MITD, Heymsfield SB. A requiem for BMI in the clinical setting. *Curr Opin Clin Nutr Metab Care*. 2017. doi:10.1097/MCO.0000000000000395
7. Daniels SR. The use of BMI in the clinical setting. *Pediatrics*. 2009. doi:10.1542/peds.2008-3586F
8. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: An endocrine organ. *Arch Med Sci*. 2013. doi:10.5114/aoms.2013.33181
9. Karastergiou K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissues - The biology of pear shape. *Biol Sex Differ*. 2012. doi:10.1186/2042-6410-3-13
10. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011. doi:10.1038/nri2921
11. Luo L, Liu M. Adipose tissue in control of metabolism. *J Endocrinol*. 2016. doi:10.1530/joe-16-0211
12. Mittendorfer B. Origins of metabolic complications in obesity: Adipose tissue and free fatty acid trafficking. *Curr Opin Clin Nutr Metab Care*. 2011. doi:10.1097/MCO.0b013e32834ad8b6
13. Lara-Castro C, Garvey WT. Intracellular Lipid Accumulation in Liver and Muscle and the Insulin Resistance Syndrome. *Endocrinol Metab Clin North Am*. 2008. doi:10.1016/j.ecl.2008.09.002
14. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010. doi:10.1016/S0140-6736(10)60408-4
15. Parry SA, Hodson L. Influence of dietary macronutrients on liver fat accumulation and metabolism. *J Investig Med*. 2017. doi:10.1136/jim-2017-000524

16. Goodpaster BH, Theriault R, Watkins SC, Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism*. 2000. doi:10.1016/S0026-0495(00)80010-4

17. Ravikumar B, Carey PE, Snaar JEM, et al. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. *Am J Physiol - Endocrinol Metab*. 2005. doi:10.1152/ajpendo.00557.2004

18. Bhupathiraju SN, Hu FB. Epidemiology of obesity and diabetes and their cardiovascular complications. *Circ Res*. 2016. doi:10.1161/CIRCRESAHA.115.306825

19. Rønn PF, Andersen GS, Lauritzen T, et al. Ethnic differences in anthropometric measures and abdominal fat distribution: A cross-sectional pooled study in Inuit, Africans and Europeans. *J Epidemiol Community Health*. 2017. doi:10.1136/jech-2016-207813

20. Gérard P. Gut microbiota and obesity. *Cell Mol Life Sci*. 2016. doi:10.1007/s00018-015-2061-5

21. Li P, Tiwari HK, Lin WY, et al. Genetic association analysis of 30 genes related to obesity in a European American population. *Int J Obes*. 2014. doi:10.1038/ijo.2013.140

22. Locke A, Kahali B, Berndt S, Justice A, Pers T. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197-206. doi:10.1038/nature14177.Genetic

23. Schousboe K, Visscher PM, Erbas B, et al. Twin study of genetic and environmental influences on adult body size, shape, and composition. *Int J Obes*. 2004. doi:10.1038/sj.ijo.0802524

24. Stunkard AJ, Harris JR, Pedersen NL, McClearn GE. The Body-Mass Index of Twins Who Have Been Reared Apart. *N Engl J Med*. 1990. doi:10.1056/NEJM199005243222102

25. Silventoinen K, Rokholm B, Kaprio J, Sørensen TIA. The genetic and environmental influences on childhood obesity: A systematic review of twin and adoption studies. *Int J Obes*. 2010. doi:10.1038/ijo.2009.177

26. Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet*. 2010. doi:10.1038/ng.686

27. Devaux M, Sassi F. Social inequalities in obesity and overweight in 11 OECD countries. *Eur J Public Health*. 2013. doi:10.1093/eurpub/ckr058

28. Aitsi-Selmi A, Bell R, Shipley MJ, Marmot MG. Education modifies the association of wealth with obesity in women in middle-income but not low-income countries: An interaction study using seven national datasets, 2005-2010. *PLoS One*. 2014. doi:10.1371/journal.pone.0090403

29. Hasselbalch AL. Genetics of dietary habits and obesity - a twin study. *Dan Med Bull*.

2010.

30. Townshend T, Lake A. Obesogenic environments: Current evidence of the built and food environments. *Perspect Public Health*. 2016. doi:10.1177/1757913916679860

31. Martini LA, Catania AS, Ferreira SRG. Role of vitamins and minerals in prevention and management of type 2 diabetes mellitus. *Nutr Rev*. 2010. doi:10.1111/j.1753-4887.2010.00296.x

32. Van Gammeren D. Vitamins and minerals. In: *Essentials of Sports Nutrition and Supplements*. ; 2008. doi:10.1007/978-1-59745-302-8_15

33. Huskisson E, Maggini S, Ruf M. The role of vitamins and minerals in energy metabolism and well-being. *J Int Med Res*. 2007. doi:10.1177/147323000703500301

34. Swinburn BA, Caterson I, Seidell JC, James WPT. Diet, nutrition and the prevention of excess weight gain and obesity. *Public Health Nutr*. 2004.

35. Hu S, Wang L, Yang D, et al. Dietary Fat, but Not Protein or Carbohydrate, Regulates Energy Intake and Causes Adiposity in Mice. *Cell Metab*. 2018. doi:10.1016/j.cmet.2018.06.010

36. Hall KD, Bemis T, Brychta R, et al. Calorie for calorie, dietary fat restriction results in more body fat loss than carbohydrate restriction in people with obesity. *Cell Metab*. 2015. doi:10.1016/j.cmet.2015.07.021

37. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. *Nutr Res Rev*. 2010. doi:10.1017/S0954422410000168

38. Bray GA, Popkin BM. Dietary fat intake does affect obesity. *Am J Clin Nutr*. 1998. doi:10.1093/ajcn/68.6.1157

39. Golay A, Bobbioni E. The role of dietary fat in obesity. In: *International Journal of Obesity*. ; 1997.

40. Astrup A. The role of dietary fat in obesity. *Semin Vasc Med*. 2005. doi:10.1055/s-2005-871740

41. Tvrzicka E, Kremmyda LS, Stankova B, Zak A. Fatty acids as biocompounds: Their role in human metabolism, health and disease - a review. part 1: Classification, dietary sources and biological functions. *Biomed Pap*. 2011. doi:10.5507/bp.2011.038

42. Calder PC. Functional Roles of Fatty Acids and Their Effects on Human Health. *J Parenter Enter Nutr*. 2015. doi:10.1177/0148607115595980

43. Calderon RO, Attema B, DeVries GH. Lipid Composition of Neuronal Cell Bodies and Neurites from Cultured Dorsal Root Ganglia. *J Neurochem*. 1995. doi:10.1046/j.1471-4159.1995.64010424.x

44. Phan CT. Intestinal lipid absorption and transport. *Front Biosci*. 2001. doi:10.2741/phan

45. Di Filippo M, Marçais C, Charrière S, et al. Post-heparin LPL activity measurement using VLDL as a substrate: a new robust method for routine assessment of plasma triglyceride lipolysis defects. *PLoS One*. 2014. doi:10.1371/journal.pone.0099721

46. Hussain MM, Kancha RK, Zhou Z, Luchoomun J, Zu H, Bakillah A. Chylomicron assembly and catabolism: Role of apolipoproteins and receptors. *Biochim Biophys Acta - Lipids Lipid Metab*. 1996. doi:10.1016/0005-2760(96)00041-0

47. Stahl A, Hirsch DJ, Gimeno RE, et al. Identification of the major intestinal fatty acid transport protein. *Mol Cell*. 1999. doi:10.1016/S1097-2765(00)80332-9

48. Masson CJ, Plat J, Mensink RP, et al. Fatty acid- and cholesterol transporter protein expression along the human intestinal tract. *PLoS One*. 2010. doi:10.1371/journal.pone.0010380

49. Wu Q, Ortegon AM, Tsang B, Doege H, Feingold KR, Stahl A. FATP1 Is an Insulin-Sensitive Fatty Acid Transporter Involved in Diet-Induced Obesity. *Mol Cell Biol*. 2006. doi:10.1128/mcb.26.9.3455-3467.2006

50. Guitart M, Osorio-Conles Ó, Pentinat T, et al. Fatty Acid Transport Protein 1 (FATP1) localizes in mitochondria in mouse skeletal muscle and regulates lipid and ketone body disposal. *PLoS One*. 2014. doi:10.1371/journal.pone.0098109

51. Michalski MC, Genot C, Gayet C, et al. Multiscale structures of lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism. *Prog Lipid Res*. 2013. doi:10.1016/j.plipres.2013.04.004

52. Goodman BE. Insights into digestion and absorption of major nutrients in humans. *Adv Physiol Educ*. 2010. doi:10.1152/advan.00094.2009

53. White B. Dietary fatty acids. *Am Fam Physician*. 2009.

54. Palomer X, Pizarro-Delgado J, Barroso E, Vázquez-Carrera M. Palmitic and Oleic Acid: The Yin and Yang of Fatty Acids in Type 2 Diabetes Mellitus. *Trends Endocrinol Metab*. 2018. doi:10.1016/j.tem.2017.11.009

55. Swanson D, Block R, Mousa SA. Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. *Adv Nutr*. 2012. doi:10.3945/an.111.000893

56. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. 2002. doi:10.1016/S0753-3322(02)00253-6

57. Nelson GJ, Schmidt PC, Kelley DS. Low-fat diets do not lower plasma cholesterol levels in healthy men compared to high-fat diets with similar fatty acid composition at constant caloric intake. *Lipids*. 1995. doi:10.1007/BF02536280

58. Silver HJ, Kang H, Keil CD, et al. Consuming a balanced high fat diet for 16 weeks improves body composition, inflammation and vascular function parameters in obese premenopausal women. *Metabolism*. 2014. doi:10.1016/j.metabol.2014.01.004

59. Crescenzo R, Bianco F, Mazzoli A, et al. Fat quality influences the obesogenic effect of

high fat diets. *Nutrients*. 2015. doi:10.3390/nu7115480

60. Demers G, Roy J, Machuca-Parra AI, et al. Fish oil supplementation alleviates metabolic and anxiodepressive effects of diet-induced obesity and associated changes in brain lipid composition in mice. *Int J Obes*. 2020. doi:10.1038/s41366-020-0623-6

61. Willett WC. Dietary fats and coronary heart disease. *J Intern Med*. 2012. doi:10.1111/j.1365-2796.2012.02553.x

62. Zock PL, Blom WAM, Nettleton JA, Hornstra G. Progressing Insights into the Role of Dietary Fats in the Prevention of Cardiovascular Disease. *Curr Cardiol Rep*. 2016. doi:10.1007/s11886-016-0793-y

63. Ahima RS, Antwi DA. Brain Regulation of Appetite and Satiety. *Endocrinol Metab Clin North Am*. 2008. doi:10.1016/j.ecl.2008.08.005

64. Stadelmann C, Timmler S, Barrantes-Freer A, Simons M. Myelin in the central nervous system: Structure, function, and pathology. *Physiol Rev*. 2019. doi:10.1152/physrev.00031.2018

65. Verkhratsky A, Parpura V. Astrocytes. In: *The Curated Reference Collection in Neuroscience and Biobehavioral Psychology*. ; 2016. doi:10.1016/B978-0-12-809324-5.02996-5

66. Le Foll C, Levin BE. Fatty acid-induced astrocyte ketone production and the control of food intake. *Am J Physiol Integr Comp Physiol*. 2016. doi:10.1152/ajpregu.00113.2016

67. Panov A, Orynbayeva Z, Vavilin V, Lyakhovich V. Fatty Acids in Energy Metabolism of the Central Nervous System. *Biomed Res Int*. 2014. doi:10.1155/2014/472459

68. Thevenet J, De Marchi U, Domingo JS, et al. Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte-neuron lactate and ketone body shuttle systems. *FASEB J*. 2016. doi:10.1096/fj.201500182

69. Gupta S, Knight AG, Gupta S, Keller JN, Bruce-Keller AJ. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. *J Neurochem*. 2012;120(6):1060-1071. doi:10.1111/j.1471-4159.2012.07660.x

70. Chung WS, Allen NJ, Eroglu C. Astrocytes control synapse formation, function, and elimination. *Cold Spring Harb Perspect Biol*. 2015. doi:10.1101/cshperspect.a020370

71. Daneman R. The blood-brain barrier in health and disease. *Ann Neurol*. 2012. doi:10.1002/ana.23648

72. Priego N, Valiente M. The potential of astrocytes as immune modulators in brain tumors. *Front Immunol*. 2019. doi:10.3389/fimmu.2019.01314

73. Norris GT, Kipnis J. Immune cells and CNS physiology: Microglia and beyond. *J Exp Med*. 2019. doi:10.1084/jem.20180199

74. Sarnat HB, Flores-Sarnat L. Nervous System, Neuroembryology of. In: *Encyclopedia of the Neurological Sciences*. ; 2014. doi:10.1016/B978-0-12-385157-4.01048-4

75. Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: Structural components and function under physiologic and pathologic conditions. In: *Journal of Neuroimmune Pharmacology*. ; 2006. doi:10.1007/s11481-006-9025-3

76. Fry M, Hoyda TD, Ferguson A V. Making sense of it: roles of the sensory circumventricular organs in feeding and regulation of energy homeostasis. *Exp Biol Med (Maywood)*. 2007.

77. Ganong WF. Circumventricular organs: Definition and role in the regulation of endocrine and autonomic function. In: *Clinical and Experimental Pharmacology and Physiology*. ; 2000. doi:10.1046/j.1440-1681.2000.03259.x

78. Thomsen MS, Routhe LJ, Moos T. The vascular basement membrane in the healthy and pathological brain. *J Cereb Blood Flow Metab*. 2017. doi:10.1177/0271678X17722436

79. Attwell D, Mishra A, Hall CN, O'Farrell FM, Dalkara T. What is a pericyte? *J Cereb Blood Flow Metab*. 2016. doi:10.1177/0271678X15610340

80. Haddad-Tóvolli R, Dragano NRV, Ramalho AFS, Velloso LA. Development and function of the blood-brain barrier in the context of metabolic control. *Front Neurosci*. 2017. doi:10.3389/fnins.2017.00224

81. Banks WA. Characteristics of compounds that cross the blood-brain barrier. In: *BMC Neurology*. ; 2009. doi:10.1186/1471-2377-9-S1-S3

82. Chassidim Y, Veksler R, Lublinsky S, Pell GS, Friedman A, Shelef I. Quantitative imaging assessment of blood-brain barrier permeability in humans. *Fluids Barriers CNS*. 2013. doi:10.1186/2045-8118-10-9

83. Zlokovic B V. The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders. *Neuron*. 2008. doi:10.1016/j.neuron.2008.01.003

84. Daneman R, Prat A. The Blood–Brain Barrier. *Cold Spring Harb Perspect Biol*. 2015;7:1-23. doi:10.1016/b978-0-12-802381-5.00010-5

85. Carrié I, Clément M, de Javel D, Francès H, Bourre JM. Specific phospholipid fatty acid composition of brain regions in mice: Effects of n-3 polyunsaturated fatty acid deficiency and phospholipid supplementation. *J Lipid Res*. 2000. doi:10.1111/wrr.12440

86. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. *J Lipid Res*. 1990.

87. Fernandes MF, Mutch DM, Leri F. The relationship between fatty acids and different depression-related brain regions, and their potential role as biomarkers of response to antidepressants. *Nutrients*. 2017. doi:10.3390/nu9030298

88. Rodriguez-Navas C, Morselli E, Clegg DJ. Sexually dimorphic brain fatty acid composition in low and high fat diet-fed mice. *Mol Metab*. 2016. doi:10.1016/j.molmet.2016.06.014

89. Rapoport SI. In vivo fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. In: *Journal of Molecular Neuroscience*. ; 2001. doi:10.1385/JMN:16:2-3:243

90. Dhopeshwarkar GA, Mead JF. Fatty acid uptake by the brain. III. Incorporation of [I-14C]oleic acid into the adult ratbrain. *Biochim Biophys Acta (BBA)/Lipids Lipid Metab*. 1970. doi:10.1016/0005-2760(70)90169-4

91. Miller JC, Gnaedinger JM, Rapoport SI. Utilization of Plasma Fatty Acid in Rat Brain: Distribution of [14C]Palmitate Between Oxidative and Synthetic Pathways. *J Neurochem*. 1987. doi:10.1111/j.1471-4159.1987.tb01021.x

92. Rapoport SI. In vivo labeling of brain phospholipids by long-chain fatty acids: Relation to turnover and function. *Lipids*. 2007. doi:10.1007/bf02637059

93. Purdon D, Arai T, Rapoport S. No evidence for direct incorporation of esterified palmitic acid from plasma into brain lipids of awake adult rat. *J Lipid Res*. 1997.

94. Smith QR, Nagura H. Fatty acid uptake and incorporation in brain: Studies with the perfusion model. *J Mol Neurosci*. 2001. doi:10.1385/JMN:16:2-3:167

95. Kimes AS, Sweeney D, London ED, Rapoport SI. Palmitate incorporation into different brain regions in the awake rat. *Brain Res*. 1983. doi:10.1016/0006-8993(83)90707-2

96. Arai T, Wakabayashi S, Channing MA, et al. Incorporation of [1-carbon-11]palmitate in monkey brain using PET. *J Nucl Med*. 1995.

97. Spector R. Fatty Acid Transport Through the Blood-Brain Barrier. *J Neurochem*. 1988. doi:10.1111/j.1471-4159.1988.tb02958.x

98. Mitchell RW, On NH, Del Bigio MR, Miller DW, Hatch GM. Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *J Neurochem*. 2011;117(4):735-746. doi:10.1111/j.1471-4159.2011.07245.x

99. Chun HB, Scott M, Niessen S, et al. The proteome of mouse brain microvessel membranes and basal lamina. *J Cereb Blood Flow Metab*. 2011. doi:10.1038/jcbfm.2011.104

100. Mitchell RW, Edmundson CL, Miller DW, Hatch GM. On the mechanism of oleate transport across human brain microvessel endothelial cells. *J Neurochem*. 2009. doi:10.1111/j.1471-4159.2009.06199.x

101. Pan Y, Scanlon MJ, Owada Y, Yamamoto Y, Porter CJH, Nicolazzo JA. Fatty Acid-Binding Protein 5 Facilitates the Blood-Brain Barrier Transport of Docosahexaenoic Acid. *Mol Pharm*. 2015. doi:10.1021/acs.molpharmaceut.5b00580

102. Lee GS, Pan Y, Scanlon MJ, Porter CJH, Nicolazzo JA. Fatty Acid–Binding Protein 5 Mediates the Uptake of Fatty Acids, but not Drugs, Into Human Brain Endothelial Cells. *J Pharm Sci*. 2018. doi:10.1016/j.xphs.2017.11.024

103. Ochiai Y, Uchida Y, Ohtsuki S, Tachikawa M, Aizawa S, Terasaki T. The blood-brain barrier fatty acid transport protein 1 (FATP1/SLC27A1) supplies docosahexaenoic acid to the brain, and insulin facilitates transport. *J Neurochem*. 2017. doi:10.1111/jnc.13943

104. Peterson LR, Gropler RJ. Radionuclide imaging of myocardial metabolism. *Circ Cardiovasc Imaging*. 2010. doi:10.1161/CIRCIMAGING.109.860593

105. DeGrado TRTR, Coenen HHHH, Stocklin GG. 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. *J Nucl Med*. 1991.

106. Takala TO, Nuutila P, Pulkki K, et al. 14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. *Eur J Nucl Med*. 2002. doi:10.1007/s00259-002-0979-y

107. Labbé SM, Grenier-Larouche T, Croteau E, et al. Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. *Am J Physiol Metab*. 2011. doi:10.1152/ajpendo.00579.2010

108. Stone CK, Pooley RA, DeGrado TR, et al. Myocardial uptake of the fatty acid analog 14-fluorine-18-fluoro-6- thia-heptadecanoic acid in comparison to beta-oxidation rates by tritiated palmitate. *J Nucl Med*. 1998.

109. Guiducci L, Grönroos T, Järvisalo MJ, et al. Biodistribution of the fatty acid analogue 18F-FTHA: plasma and tissue partitioning between lipid pools during fasting and hyperinsulinemia. *J Nucl Med*. 2007.

110. Rhea EM, Salameh TS, Logsdon AF, Hanson AJ, Erickson MA, Banks WA. Blood-Brain Barriers in Obesity. *AAPS J*. 2017. doi:10.1208/s12248-017-0079-3

111. Karmi A, Iozzo P, Viljanen A, et al. Increased brain fatty acid uptake in metabolic syndrome. *Diabetes*. 2010. doi:10.2337/db09-0138

112. Cazettes F, Cohen JI, Yau PL, Talbot H, Convit A. Obesity-mediated inflammation may damage the brain circuit that regulates food intake. *Brain Res*. 2011. doi:10.1016/j.brainres.2010.12.008

113. Hargrave SL, Davidson TL, Zheng W, Kinzig KP. Western Diets Induce Blood-Brain Barrier Leakage and Alter Spatial Strategies in Rats. *Behav Neurosci*. 2016. doi:10.1037/bne0000110

114. Kanoski SE, Zhang Y, Zheng W, Davidson TL. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. *J Alzheimer's Dis*. 2010. doi:10.3233/JAD-2010-091414

115. Rijnsburger M, Unmehopa UA, Eggels L, Serlie MJ, la Fleur SE. One-week exposure to a free-choice high-fat high-sugar diet does not disrupt blood–brain barrier permeability in fed or overnight fasted rats. *Nutr Neurosci*. 2017. doi:10.1080/1028415X.2017.1418727

116. Tucsek Z, Toth P, Sosnowska D, et al. Obesity in aging exacerbates blood-brain barrier

disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: Effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2014. doi:10.1093/gerona/glt177

117. Hetherington AW, Ranson SW. The relation of various hypothalamic lesions to adiposity in the rat. *J Comp Neurol*. 1942. doi:10.1002/cne.900760308

118. Shor-Posner G, Azar AP, Insinga S, Leibowitz SF. Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. *Physiol Behav*. 1985. doi:10.1016/0031-9384(85)90255-0

119. Bellinger LL, Bernardis LL. The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation: Lessons learned from lesioning studies. In: *Physiology and Behavior*. ; 2002. doi:10.1016/S0031-9384(02)00756-4

120. Lytle LD, Campbell BA. Effects of lateral hypothalamic lesions on consummatory behavior in developing rats. *Physiol Behav*. 1975. doi:10.1016/0031-9384(75)90100-6

121. Gaur A, Pal GK, Ananthanarayanan PH, Pal P. Role of Ventromedial hypothalamus in high fat diet induced obesity in male rats: Association with lipid profile, thyroid profile and insulin resistance. *Ann Neurosci*. 2014. doi:10.5214/ans.0972.7531.210306

122. Zhang N, Yang L, Guo L, Bi S. Activation of dorsomedial hypothalamic neurons promotes physical activity and decreases food intake and body weight in zucker fatty rats. *Front Mol Neurosci*. 2018. doi:10.3389/fnmol.2018.00179

123. Chelsea L. Faber1, Jennifer D. Deem1, Bao Anh Phan1, Tammy P. Doan1, Kayoko Ogimoto1, 5 Zaman Mirzadeh2, Michael W. Schwartz1, Gregory J. Morton1Chelsea L. Faber1, Jennifer D. Deem1, Bao Anh Phan1, Tammy P. Doan1, Kayoko Ogimoto1, 5 Zaman Mirzadeh2, Micha GJM. Leptin-receptor neurons in the dorsomedial hypothalamus regulate the timing of circadian rhythms in feeding and metabolism in mice. *bioRxiv*. 2020.

124. Stuber GD, Wise RA. Lateral hypothalamic circuits for feeding and reward. *Nat Neurosci*. 2016. doi:10.1038/nn.4220

125. Hewson AK, Tung LYC, Connell DW, Tookman L, Dickson SL. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. *Diabetes*. 2002. doi:10.2337/diabetes.51.12.3412

126. Goodman T, Hajihosseini MK. Hypothalamic tanycytes-masters and servants of metabolic, neuroendocrine, and neurogenic functions. *Front Neurosci*. 2015. doi:10.3389/fnins.2015.00387

127. Millington GWM. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. *Nutr Metab*. 2007. doi:10.1186/1743-7075-4-18

128. Morton GJ, Schwartz MW. The NPY/AgRP neuron and energy homeostasis. *Int J Obes*. 2001. doi:10.1038/sj.ijo.0801915

129. Williams G, Bing C, Cai XJ, Harrold JA, King PJ, Liu XH. The hypothalamus and the

control of energy homeostasis: Different circuits, different purposes. In: *Physiology and Behavior*. ; 2001. doi:10.1016/S0031-9384(01)00612-6

130. Könner AC, Klöckener T, Brüning JC. Control of energy homeostasis by insulin and leptin: Targeting the arcuate nucleus and beyond. *Physiol Behav*. 2009. doi:10.1016/j.physbeh.2009.03.027

131. Roh E, Song DK, Kim MS. Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. *Exp Mol Med*. 2016. doi:10.1038/emm.2016.4

132. Betley JN, Cao ZFH, Ritola KD, Sternson SM. Parallel, redundant circuit organization for homeostatic control of feeding behavior. *Cell*. 2013. doi:10.1016/j.cell.2013.11.002

133. Stanley BG, Leibowitz SF. Neuroreptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci*. 1984. doi:10.1016/0024-3205(84)90032-8

134. Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide γ and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology*. 1984. doi:10.1210/endo-115-1-427

135. Hwa JJ, Witten MB, Williams P, et al. Activation of the NPY Y5 receptor regulates both feeding and energy expenditure. *Am J Physiol Integr Comp Physiol*. 2017. doi:10.1152/ajpregu.1999.277.5.r1428

136. Ollmann MM, Wilson BD, Yang YK, et al. Antagonism of Central Melanocortin receptors in vitro and in vivo by agouti-related protein. *Science (80-)*. 1997. doi:10.1126/science.278.5335.135

137. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*. 2001. doi:10.1038/35078085

138. Denis RGP, Joly-Amado A, Webber E, et al. Palatability can drive feeding independent of AgRP neurons. *Cell Metab*. 2015. doi:10.1016/j.cmet.2015.07.011

139. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech*. 2017. doi:10.1242/dmm.026609

140. Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, De Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*. 2007. doi:10.1038/nature06310

141. Sakurai T. Orexins and orexin receptors: Implication in feeding behavior. *Regul Pept*. 1999. doi:10.1016/S0167-0115(99)00076-2

142. Haynes AC, Chapman H, Taylor C, et al. Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept*. 2002. doi:10.1016/S0167-0115(01)00358-5

143. Ludwig DS, Tritos NA, Mastaitis JW, et al. Melanin-concentrating hormone

overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest*. 2001. doi:10.1172/JCI10660

144. Qu D, Ludwig DS, Gammeltoft S, et al. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature*. 1996. doi:10.1038/380243a0

145. Alon T, Friedman JM. Late-onset leanness in mice with targeted ablation of melanin concentrating hormone neurons. *J Neurosci*. 2006. doi:10.1523/JNEUROSCI.1203-05.2006

146. Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature*. 1998. doi:10.1038/25341

147. Cooke JH, Patterson M, Patel SR, et al. Peripheral and central administration of xenin and neurotensin suppress food intake in rodents. *Obesity*. 2009. doi:10.1038/oby.2008.652

148. Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature*. 2005. doi:10.1038/nature04071

149. Leinninger GM, Opland DM, Jo YH, et al. Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell Metab*. 2011. doi:10.1016/j.cmet.2011.06.016

150. Berthoud HR. Multiple neural systems controlling food intake and body weight. *Neurosci Biobehav Rev*. 2002. doi:10.1016/S0149-7634(02)00014-3

151. Mccrickerd K, Forde CG. Sensory influences on food intake control: Moving beyond palatability. *Obes Rev*. 2016. doi:10.1111/obr.12340

152. Gluck ME, Viswanath P, Stinson EJ. Obesity, Appetite, and the Prefrontal Cortex. *Curr Obes Rep*. 2017. doi:10.1007/s13679-017-0289-0

153. Lowe CJ, Reichelt AC, Hall PA. The Prefrontal Cortex and Obesity: A Health Neuroscience Perspective. *Trends Cogn Sci*. 2019. doi:10.1016/j.tics.2019.01.005

154. Stevenson RJ, Francis HM. The hippocampus and the regulation of human food intake. *Psychol Bull*. 2017. doi:10.1037/bul0000109

155. Higgs S, Spetter MS. Cognitive Control of Eating: the Role of Memory in Appetite and Weight Gain. *Curr Obes Rep*. 2018. doi:10.1007/s13679-018-0296-9

156. Voigt JP, Fink H. Serotonin controlling feeding and satiety. *Behav Brain Res*. 2015. doi:10.1016/j.bbr.2014.08.065

157. Yohn SE, Galbraith J, Calipari ES, Conn PJ. Shared Behavioral and Neurocircuitry Disruptions in Drug Addiction, Obesity, and Binge Eating Disorder: Focus on Group I mGluRs in the Mesolimbic Dopamine Pathway. *ACS Chem Neurosci*. 2019. doi:10.1021/acschemneuro.8b00601

158. Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L. Central administration of oleic acid inhibits glucose production and food intake. *Diabetes*. 2002.

doi:10.2337/diabetes.51.2.271

159. Schwinkendorf DR, Tsatsos NG, Gosnell BA, Mashek DG. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. *Int J Obes*. 2011. doi:10.1038/ijo.2010.159

160. Cheng L, Yu Y, Szabo A, et al. Palmitic acid induces central leptin resistance and impairs hepatic glucose and lipid metabolism in male mice. *J Nutr Biochem*. 2015. doi:10.1016/j.jnutbio.2014.12.011

161. Posey KA, Clegg DJ, Printz RL, et al. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *Am J Physiol Metab*. 2008. doi:10.1152/ajpendo.90377.2008

162. Benoit SC, Kemp CJ, Elias CF, et al. Palmitic acid mediates hypothalamic insulin resistance by altering PKC-θ subcellular localization in rodents. *J Clin Invest*. 2009. doi:10.1172/JCI36714

163. Tse EK, Belsham DD. Palmitate induces neuroinflammation, ER stress, and Pomc mRNA expression in hypothalamic mHypoA-POMC/GFP neurons through novel mechanisms that are prevented by oleate. *Mol Cell Endocrinol*. 2018. doi:10.1016/j.mce.2017.11.017

164. Ross RA, Rossetti L, Lam TKT, Schwartz GJ. Differential effects of hypothalamic long-chain fatty acid infusions on suppression of hepatic glucose production. *Am J Physiol Metab*. 2010. doi:10.1152/ajpendo.00190.2010

165. Dalvi PS, Chalmers JA, Luo V, et al. High fat induces acute and chronic inflammation in the hypothalamus: Effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. *Int J Obes*. 2017. doi:10.1038/ijo.2016.183

166. Orr SK, Palumbo S, Bosetti F, et al. Unesterified docosahexaenoic acid is protective in neuroinflammation. *J Neurochem*. 2013. doi:10.1111/jnc.12392

167. Oomura Y, Nakamura T, Sugimori M, Yamada Y. Effect of free fatty acid on the rat lateral hypothalamic neurons. *Physiol Behav*. 1975. doi:10.1016/0031-9384(75)90015-3

168. Wang R, Cruciani-Guglielmacci C, Migrenne S, Magnan C, Cotero VE, Routh VH. Effects of Oleic Acid on Distinct Populations of Neurons in the Hypothalamic Arcuate Nucleus Are Dependent on Extracellular Glucose Levels. *J Neurophysiol*. 2006. doi:10.1152/jn.00697.2005

169. Le Foll C, Irani BG, Magnan C, Dunn-Meynell A a, Levin BE. Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing. *Am J Physiol*. 2009;297(June 2009):R655-R664. doi:10.1152/ajpregu.00223.2009

170. Jo Y-H, Su Y, Gutierrez-Juarez R, Chua S. Oleic Acid Directly Regulates POMC Neuron Excitability in the Hypothalamus. *J Neurophysiol*. 2008. doi:10.1152/jn.91294.2008

171. Akrouh A, Halcomb SE, Nichols CG, Sala-Rabanal M. Molecular biology of KATP

channels and implications for health and disease. *IUBMB Life*. 2009. doi:10.1002/iub.246

172. Moullé VS, Le Foll C, Philippe E, et al. Fatty Acid Transporter CD36 Mediates Hypothalamic Effect of Fatty Acids on Food Intake in Rats. *PLoS One*. 2013. doi:10.1371/journal.pone.0074021

173. Pugazhenthi S, Qin L, Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta - Mol Basis Dis*. 2017. doi:10.1016/j.bbadis.2016.04.017

174. Morselli E, Fuente-Martin E, Finan B, et al. Hypothalamic PGC-1α protects against high-fat diet exposure by regulating ERα. *Cell Rep*. 2014. doi:10.1016/j.celrep.2014.09.025

175. Kaushik S, Rodriguez-Navarro JA, Arias E, et al. Autophagy in hypothalamic agrp neurons regulates food intake and energy balance. *Cell Metab*. 2011. doi:10.1016/j.cmet.2011.06.008

176. Maya-Monteiro CM, Corrêa-da-Silva F, Hofmann SS, Hesselink MKC, la Fleur SE, Yi C-X. Lipid Droplets accumulate in the hypothalamus of mice and humans with and without metabolic diseases. *Neuroendocrinology*. 2020. doi:10.1159/000508735

177. Camandola S. Astrocytes, emerging stars of energy homeostasis. *Cell Stress*. 2018. doi:10.15698/cst2018.10.157

178. Kwon YH, Kim J, Kim CS, et al. Hypothalamic lipid-laden astrocytes induce microglia migration and activation. *FEBS Lett*. 2017. doi:10.1002/1873-3468.12691

179. Angelova PR, Abramov AY. Role of mitochondrial ROS in the brain: from physiology to neurodegeneration. *FEBS Lett*. 2018. doi:10.1002/1873-3468.12964

180. Ioannou MS, Jackson J, Sheu SH, et al. Neuron-Astrocyte Metabolic Coupling Protects against Activity-Induced Fatty Acid Toxicity. *Cell*. 2019. doi:10.1016/j.cell.2019.04.001

181. Gao Y, Layritz C, Legutko B, et al. Disruption of lipid uptake in astroglia exacerbates diet-induced obesity. *Diabetes*. 2017. doi:10.2337/db16-1278

182. Douglass JD, Dorfman MD, Fasnacht R, Shaffer LD, Thaler JP. Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation. *Mol Metab*. 2017. doi:10.1016/j.molmet.2017.01.010

183. Kim SM, McIlwraith EK, Chalmers JA, Belsham DD. Palmitate induces an anti-inflammatory response in immortalized microglial BV-2 and IMG cell lines that decreases TNFα Levels in mHypoE-46 hypothalamic neurons in co-culture. *Neuroendocrinology*. 2019. doi:10.1159/000494759

184. Valdearcos M, Douglass JD, Robblee MM, et al. Microglial Inflammatory Signaling Orchestrates the Hypothalamic Immune Response to Dietary Excess and Mediates Obesity Susceptibility. *Cell Metab*. 2017. doi:10.1016/j.cmet.2017.05.015

185. Douglass JD, Dorfman MD, Thaler JP. Glia: silent partners in energy homeostasis and obesity pathogenesis. *Diabetologia*. 2017. doi:10.1007/s00125-016-4181-3

186. André C, Quevedo OG, Rey C, et al. Inhibiting microglia expansion prevents diet-induced hypothalamic and peripheral inflammation. *Diabetes*. 2017. doi:10.2337/db16-0586

187. Miller AA, Spencer SJ. Obesity and neuroinflammation: A pathway to cognitive impairment. *Brain Behav Immun*. 2014. doi:10.1016/j.bbi.2014.04.001

188. Guillemot-Legris O, Muccioli GG. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. *Trends Neurosci*. 2017. doi:10.1016/j.tins.2017.02.005

189. Arias-Carrián O, Stamelou M, Murillo-Rodríguez E, Menéndez-Gonzlez M, Pöppel E. Dopaminergic reward system: A short integrative review. *Int Arch Med*. 2010. doi:10.1186/1755-7682-3-24

190. Ungless MA, Magill PJ, Bolam JP. Uniform Inhibition of Dopamine Neurons in the Ventral Tegmental Area by Aversive Stimuli. *Science (80-)*. 2004. doi:10.1126/science.1093360

191. Karakaya S, Kipp M, Beyer C. Oestrogen regulates the expression and function of dopamine transporters in astrocytes of the nigrostriatal system. *J Neuroendocrinol*. 2007. doi:10.1111/j.1365-2826.2007.01575.x

192. Khan ZU, Gutiérrez A, Martín R, Peafiel A, Rivera A, De La Calle A. Dopamine D5 receptors of rat and human brain. *Neuroscience*. 2000. doi:10.1016/S0306-4522(00)00274-8

193. Martel JC, Gatti McArthur S. Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia. *Front Pharmacol*. 2020. doi:10.3389/fphar.2020.01003

194. Missale C, Russel Nash S, Robinson SW, Jaber M, Caron MG. Dopamine receptors: From structure to function. *Physiol Rev*. 1998. doi:10.1152/physrev.1998.78.1.189

195. Lindgren N, Xu ZQD, Herrera-Marschitz M, Haycock J, Hökfelt T, Fisone G. Dopamine D2 receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. *Eur J Neurosci*. 2001. doi:10.1046/j.0953-816X.2000.01443.x

196. Benoit-Marand M, Borrelli E, Gonon F. Inhibition of dopamine release via presynaptic D2 receptors: Time course and functional characteristics in vivo. *J Neurosci*. 2001. doi:10.1523/jneurosci.21-23-09134.2001

197. Miyazaki I, Asanuma M, Diaz-Corrales FJ, Miyoshi K, Ogawa N. Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. *Brain Res*. 2004. doi:10.1016/j.brainres.2004.09.014

198. Grace AA, Floresco SB, Goto Y, Lodge DJ. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci*. 2007. doi:10.1016/j.tins.2007.03.003

199. Wanat MJ, Willuhn I, Clark JJ, Phillips PEM. Phasic dopamine release in appetitive behaviors and drug addiction. *Curr Drug Abuse Rev*. 2009. doi:10.2174/1874473710902020195

200. Overton PG, Clark D. Burst firing in midbrain dopaminergic neurons. *Brain Res Rev*. 1997. doi:10.1016/S0165-0173(97)00039-8

201. Baik J-H. Dopamine Signaling in reward-related behaviors. *Front Neural Circuits*. 2013. doi:10.3389/fncir.2013.00152

202. Björklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. *Trends Neurosci*. 2007. doi:10.1016/j.tins.2007.03.006

203. Luo SX, Huang EJ. Dopaminergic neurons and brain reward pathways: From neurogenesis to circuit assembly. *Am J Pathol*. 2016. doi:10.1016/j.ajpath.2015.09.023

204. Galaj E, Ranaldi R. The strength of reward-related learning depends on the degree of activation of ventral tegmental area dopamine neurons. *Behav Brain Res*. 2018. doi:10.1016/j.bbr.2018.04.012

205. Di Chiara G. Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behav Brain Res*. 2002. doi:10.1016/S0166-4328(02)00286-3

206. West EA, Carelli RM. Nucleus accumbens core and shell differentially encode Reward-Associated cues after reinforcer devaluation. *J Neurosci*. 2016. doi:10.1523/JNEUROSCI.2976-15.2016

207. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A*. 1988;85(14):5274-5278. http://www.ncbi.nlm.nih.gov/pubmed/2899326. Accessed November 2, 2017.

208. Imperato A, Mulas A, Di Chiara G. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur J Pharmacol*. 1986. doi:10.1016/0014-2999(86)90629-1

209. Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the “shell” as compared with the “core” of the rat nucleus accumbens. *Proc Natl Acad Sci*. 2006. doi:10.1073/pnas.92.26.12304

210. Ikemoto S. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev*. 2007. doi:10.1016/j.brainresrev.2007.05.004

211. Lammel S, Hetzel A, Häckel O, Jones I, Liss B, Roeper J. Unique Properties of Mesoprefrontal Neurons within a Dual Mesocorticolimbic Dopamine System. *Neuron*. 2008. doi:10.1016/j.neuron.2008.01.022

212. Kravitz A V., Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*. 2012. doi:10.1038/nn.3100

213. Tai LH, Lee AM, Benavidez N, Bonci A, Wilbrecht L. Transient stimulation of distinct subpopulations of striatal neurons mimics changes in action value. *Nat Neurosci*. 2012. doi:10.1038/nn.3188

214. Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi Shigetada S. Distinct Roles of Synaptic Transmission in Direct and Indirect Striatal Pathways to Reward and Aversive Behavior. *Neuron*. 2010. doi:10.1016/j.neuron.2010.05.011

215. Soares-Cunha C, Coimbra B, David-Pereira A, et al. Activation of D2 dopamine receptor-expressing neurons in the nucleus accumbens increases motivation. *Nat Commun*. 2016. doi:10.1038/ncomms11829

216. Natsubori A, Tsutsui-Kimura I, Nishida H, et al. Ventrolateral striatal medium spiny neurons positively regulate food-incentive, goal-directed behavior independently of D1 and D2 selectivity. *J Neurosci*. 2017. doi:10.1523/JNEUROSCI.3377-16.2017

217. Kenny PJ. Common cellular and molecular mechanisms in obesity and drug addiction. *Nat Rev Neurosci*. 2011. doi:10.1038/nrn3105

218. Sweet LH, Hassenstab JJ, McCaffery JM, et al. Brain response to food stimulation in obese, normal weight, and successful weight loss maintainers. *Obesity*. 2012. doi:10.1038/oby.2012.125

219. Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM. Relation of Reward From Food Intake and Anticipated Food Intake to Obesity: A Functional Magnetic Resonance Imaging Study. *J Abnorm Psychol*. 2008. doi:10.1037/a0013600

220. Berthoud HR, Münzberg H, Morrison CD. Blaming the Brain for Obesity: Integration of Hedonic and Homeostatic Mechanisms. *Gastroenterology*. 2017. doi:10.1053/j.gastro.2016.12.050

221. Del Parigi A, Gautier J-F, Chen K, et al. Neuroimaging and obesity: mapping the brain responses to hunger and satiation in humans using positron emission tomography. *Ann N Y Acad Sci*. 2002. doi:10.1111/j.1749-6632.2002.tb04294.x

222. Gautier JF, Chen K, Salbe AD, et al. Differential brain responses to satiation in obese and lean men. *Diabetes*. 2000. doi:10.2337/diabetes.49.5.838

223. Gautier JF, Del Parigi A, Chen K, et al. Effect of satiation on brain activity in obese and lean women. *Obes Res*. 2001. doi:10.1038/oby.2001.92

224. Hernandez L, Hoebel BG. Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci*. 1988. doi:10.1016/0024-3205(88)90036-7

225. Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience*. 2005. doi:10.1016/j.neuroscience.2005.04.043

226. Hajnal A, Smith GP, Norgren R. Oral sucrose stimulation increases accumbens dopamine in the rat. *Am J Physiol Integr Comp Physiol*. 2004. doi:10.1152/ajpregu.00282.2003

227. Rada P, Avena NM, Barson JR, Hoebel BG, Leibowitz SF. A high-fat meal, or intraperitoneal administration of a fat emulsion, increases extracellular dopamine in the nucleus accumbens. *Brain Sci*. 2012. doi:10.3390/brainsci2020242

228. Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M. Changes in brain activity related to eating chocolate. *Brain*. 2001. doi:10.1093/brain/124.9.1720

229. Small DM, Jones-Gotman M, Dagher A. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage*. 2003. doi:10.1016/S1053-8119(03)00253-2

230. Zhou QY, Palmiter RD. Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell*. 1995. doi:10.1016/0092-8674(95)90145-0

231. Szczypka MS, Rainey MA, Kim DS, et al. Feeding behavior in dopamine-deficient mice. *Proc Natl Acad Sci U S A*. 1999. doi:10.1073/pnas.96.21.12138

232. Décarie-Spain L, Hryhorczuk C, Fulton S. Dopamine signalling adaptations by prolonged high-fat feeding. *Curr Opin Behav Sci*. 2016. doi:10.1016/j.cobeha.2016.03.010

233. Stice E, Burger KS, Yokum S. Reward Region Responsivity Predicts Future Weight Gain and Moderating Effects of the TaqIA Allele. *J Neurosci*. 2015. doi:10.1523/jneurosci.3607-14.2015

234. Stice E, Yokum S, Bohon C, Marti N, Smolen A. Reward circuitry responsivity to food predicts future increases in body mass: Moderating effects of DRD2 and DRD4. *Neuroimage*. 2010. doi:10.1016/j.neuroimage.2010.01.081

235. Oginsky MF, Maust JD, Corthell JT, Ferrario CR. Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity. *Psychopharmacology (Berl)*. 2016. doi:10.1007/s00213-015-4157-x

236. Oginsky MF, Ferrario CR. Eating "junk food" has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible versus -resistant rats. *J Neurophysiol*. 2019. doi:10.1152/jn.00361.2019

237. Xu M, Hu XT, Cooper DC, et al. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. *Cell*. 1994. doi:10.1016/0092-8674(94)90026-4

238. Drago J, Gerfen CR, Lachowicz JE, et al. Altered striatal function in a mutant mouse lacking D1A dopamine receptors. *Proc Natl Acad Sci U S A*. 1994. doi:10.1073/pnas.91.26.12564

239. Durst M, Könczöl K, Balázsa T, Eyre MD, Tóth ZE. Reward-representing D1-type neurons in the medial shell of the accumbens nucleus regulate palatable food intake. *Int J Obes*. 2019. doi:10.1038/s41366-018-0133-y

240. Grippo RM, Tang Q, Zhang Q, et al. Dopamine Signaling in the Suprachiasmatic

Nucleus Enables Weight Gain Associated with Hedonic Feeding. *Curr Biol*. 2020. doi:10.1016/j.cub.2019.11.029

241. Cansell C, Castel J, Denis RGP, et al. Dietary triglycerides act on mesolimbic structures to regulate the rewarding and motivational aspects of feeding. *Mol Psychiatry*. 2014. doi:10.1038/mp.2014.31

242. Hryhorczuk C, Florea M, Rodaros D, et al. Dampened mesolimbic dopamine function and signaling by saturated but not monounsaturated dietary lipids. *Neuropsychopharmacology*. 2016;41(3):811-821. doi:10.1038/npp.2015.207

243. Baldo BA, Sadeghian K, Basso AM, Kelley AE. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behav Brain Res*. 2002. doi:10.1016/S0166-4328(02)00293-0

244. Baldo BA, Kelley AE. Discrete neurochemical coding of distinguishable motivational processes: Insights from nucleus accumbens control of feeding. *Psychopharmacology (Berl)*. 2007. doi:10.1007/s00213-007-0741-z

245. Soto PL, Hiranita T, Xu M, Hursh SR, Grandy DK, Katz JL. Dopamine D2-Like Receptors and Behavioral Economics of Food Reinforcement. *Neuropsychopharmacology*. 2016. doi:10.1038/npp.2015.223

246. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. *Lancet*. 2001. doi:10.1016/S0140-6736(00)03643-6

247. Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci*. 2010. doi:10.1038/nn.2519

248. Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience*. 2009. doi:10.1016/j.neuroscience.2009.02.007

249. Cook JB, Hendrickson LM, Garwood GM, Toungate KM, Nania C V., Morikawa H. Junk food diet-induced obesity increases D2 receptor autoinhibition in the ventral tegmental area and reduces ethanol drinking. *PLoS One*. 2017. doi:10.1371/journal.pone.0183685

250. Koyama S, Kawaharada M, Terai H, et al. Obesity decreases excitability of putative ventral tegmental area GABAergic neurons. *Physiol Rep*. 2013. doi:10.1002/phy2.126

251. Huang XF, Zavitsanou K, Huang X, et al. Dopamine transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-induced obesity. *Behav Brain Res*. 2006. doi:10.1016/j.bbr.2006.08.034

252. Décarie-Spain L, Sharma S, Hryhorczuk C, et al. Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat. *Mol Metab*. 2018. doi:10.1016/j.molmet.2018.01.018

253. Bromberg-Martin ES, Matsumoto M, Hikosaka O. Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron*. 2010. doi:10.1016/j.neuron.2010.11.022

254. Palmiter RD. Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci*. 2007. doi:10.1016/j.tins.2007.06.004

255. Fulton S. Appetite and reward. *Front Neuroendocrinol*. 2010;31(1):85-103. doi:10.1016/j.yfrne.2009.10.003

256. Berland C, Montalban E, Perrin E, et al. Circulating Triglycerides Gate Dopamine-Associated Behaviors through DRD2-Expressing Neurons. *Cell Metab*. 2020. doi:10.1016/j.cmet.2020.02.010

257. Barnes CN, Wallace CW, Jacobowitz BS, Fordahl SC. Reduced phasic dopamine release and slowed dopamine uptake occur in the nucleus accumbens after a diet high in saturated but not unsaturated fat. *Nutr Neurosci*. 2020. doi:10.1080/1028415X.2019.1707421

258. Valdivia S, Patrone A, Reynaldo M, Perello M. Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model. *PLoS One*. 2014. doi:10.1371/journal.pone.0087478

259. Peterschmitt Y, Abdoul-Azize S, Murtaza B, et al. Fatty acid lingual application activates gustatory and reward brain circuits in the mouse. *Nutrients*. 2018. doi:10.3390/nu10091246

260. Hryhorczuk C, Sheng Z, Decarie-Spain L, et al. Oleic Acid in the Ventral Tegmental Area Inhibits Feeding, Food Reward, and Dopamine Tone. *Neuropsychopharmacology*. 2017. doi:10.1038/npp.2017.203

261. Drozd K, Ahmadi A, Deng Y, et al. Effects of an endothelin receptor antagonist, Macitentan, on right ventricular substrate utilization and function in a Sugen 5416/hypoxia rat model of severe pulmonary arterial hypertension. *J Nucl Cardiol*. 2017. doi:10.1007/s12350-016-0663-4

262. Ohira H, deKemp R, Pena E, et al. Shifts in myocardial fatty acid and glucose metabolism in pulmonary arterial hypertension: a potential mechanism for a maladaptive right ventricular response. *Eur Heart J Cardiovasc Imaging*. 2016. doi:10.1093/ehjci/jev136

263. Paxinos G, Franklin KBJ. *Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates*.; 2001. doi:10.1364/OE.20.020998

264. Matsufuji T, Ikeda M, Naito A, et al. Arylpiperazines as fatty acid transport protein 1 (FATP1) inhibitors with improved potency and pharmacokinetic properties. *Bioorganic Med Chem Lett*. 2013. doi:10.1016/j.bmcl.2013.02.116

265. Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016. doi:10.4103/0976-0105.177703

266. Sharma S, Hryhorczuk C, Fulton S. Progressive-ratio Responding for Palatable High-fat and High-sugar Food in Mice. *J Vis Exp*. 2012. doi:10.3791/3754

267. Richardson NR, Roberts DCS. Progressive ratio schedules in drug self-administration

studies in rats: A method to evaluate reinforcing efficacy. *J Neurosci Methods*. 1996. doi:10.1016/0165-0270(95)00153-0

268. Cordain L, Eaton SB, Sebastian A, et al. Origins and evolution of the Western diet: Health implications for the 21st century. *Am J Clin Nutr*. 2005. doi:10.1093/ajcn.81.2.341

269. Mehlem A, Hagberg CE, Muhl L, Eriksson U, Falkevall A. Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease. *Nat Protoc*. 2013. doi:10.1038/nprot.2013.055

270. Reis A, Rudnitskaya A, Blackburn GJ, Fauzi NM, Pitt AR, Spickett CM. A comparison of five lipid extraction solvent systems for lipidomic studies of human LDL. *J Lipid Res*. 2013. doi:10.1194/jlr.M034330

271. Fukumoto S, Fujimoto T. Deformation of lipid droplets in fixed samples. *Histochem Cell Biol*. 2002. doi:10.1007/s00418-002-0462-7

272. Huber JS, Hernandez AM, Janabi M, et al. Longitudinal Evaluation of Myocardial Fatty Acid and Glucose Metabolism in Fasted and Nonfasted Spontaneously Hypertensive Rats Using MicroPET/CT. *Mol Imaging*. 2017. doi:10.1177/1536012117724558

273. Ci X, Frisch F, Lavoie F, et al. The effect of insulin on the intracellular distribution of 14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid in rats. *Mol Imaging Biol*. 2006. doi:10.1007/s11307-006-0042-7

274. Honkala SM, Johansson J, Motiani KK, et al. Short-term interval training alters brain glucose metabolism in subjects with insulin resistance. *J Cereb Blood Flow Metab*. 2018. doi:10.1177/0271678X17734998

275. Seyer A, Boudah S, Broudin S, Junot C, Colsch B. Annotation of the human cerebrospinal fluid lipidome using high resolution mass spectrometry and a dedicated data processing workflow. *Metabolomics*. 2016. doi:10.1007/s11306-016-1023-8

276. Simon MJ, Iliff JJ. Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. *Biochim Biophys Acta - Mol Basis Dis*. 2016. doi:10.1016/j.bbadis.2015.10.014

277. Halter JB, Pflug AE. Effects of anesthesia and surgical stress on insulin secretion in man. *Metabolism*. 1980. doi:10.1016/0026-0495(80)90021-9

278. Stahl A, Evans JG, Pattel S, Hirsch D, Lodish HF. Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev Cell*. 2002. doi:10.1016/S1534-5807(02)00143-0

279. Wang YY, Wang K, Xu ZY, et al. High-resolution dynamic imaging and quantitative analysis of lung cancer xenografts in nude mice using clinical PET/CT. *Oncotarget*. 2017. doi:10.18632/oncotarget.17263

280. DeGrado TR, Bhattacharyya F, Pandey MK, Belanger AP, Wang S. Synthesis and Preliminary Evaluation of 18-18F-Fluoro-4-Thia-Oleate as a PET Probe of Fatty Acid

Oxidation. *J Nucl Med*. 2010. doi:10.2967/jnumed.109.074245

281. Pandey MK, Bansal A, Degrado TR. *Fluorine-18 Labeled Thia Fatty Acids for PET Imaging of Fatty Acid Oxidation in Heart and Cancer*.; 2011.

282. Knust EJ, Kupfernagel C, Stoecklin G. Long-chain F-18 fatty acids for the study of regional metabolism in heart and liver; odd-even effects of metabolism in mice. *J Nucl Med*. 1979.

283. Zhang W, Chen R, Yang T, et al. Fatty acid transporting proteins: Roles in brain development, aging, and stroke. *Prostaglandins Leukot Essent Fat Acids*. 2018;136. doi:10.1016/j.plefa.2017.04.004

284. Schmidt FM, Weschenfelder J, Sander C, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical Activity. *PLoS One*. 2015. doi:10.1371/journal.pone.0121971

285. Wang T, He C. Pro-inflammatory cytokines: The link between obesity and osteoarthritis. *Cytokine Growth Factor Rev*. 2018. doi:10.1016/j.cytogfr.2018.10.002

286. Uchida A, Whitsitt MC, Eustaquio T, et al. Reduced triglyceride secretion in response to an acute dietary fat challenge in obese compared to lean mice. *Front Physiol*. 2012. doi:10.3389/fphys.2012.00026

287. Kenny PJ, Voren G, Johnson PM. Dopamine D2 receptors and striatopallidal transmission in addiction and obesity. *Curr Opin Neurobiol*. 2013. doi:10.1016/j.conb.2013.04.012

288. Benton D, Young HA. A meta-analysis of the relationship between brain dopamine receptors and obesity: A matter of changes in behavior rather than food addiction. *Int J Obes*. 2016. doi:10.1038/ijo.2016.9

289. DiFeliceantonio AG, Small DM. Dopamine and diet-induced obesity. *Nat Neurosci*. 2019. doi:10.1038/s41593-018-0304-0

290. Valenza M, Steardo L, Cottone P, Sabino V. Diet-induced obesity and diet-resistant rats: Differences in the rewarding and anorectic effects of d-amphetamine. *Psychopharmacology (Berl)*. 2015. doi:10.1007/s00213-015-3981-3

291. Ramos J, Hardin EJ, Grant AH, et al. The Effects of Eating a High Fat Diet on Sensitivity of Male and Female Rats to Methamphetamine and Dopamine D1 Receptor Agonist SKF 82958. *J Pharmacol Exp Ther*. 2020. doi:10.1124/jpet.119.263293

292. Dawson G. Measuring brain lipids. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2015. doi:10.1016/j.bbalip.2015.02.007

293. Chavko M, Nemoto EM, Melick JA. Regional lipid composition in the rat brain. *Mol Chem Neuropathol*. 1993. doi:10.1007/BF03160026

294. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity & inflammation: The linking mechanism & the complications. *Arch Med Sci*. 2017.

doi:10.5114/aoms.2016.58928

295. Felger JC, Treadway MT. Inflammation Effects on Motivation and Motor Activity: Role of Dopamine. *Neuropsychopharmacology*. 2017. doi:10.1038/npp.2016.143

296. Gutiérrez-Martos M, Girard B, Mendonça-Netto S, et al. Cafeteria diet induces neuroplastic modifications in the nucleus accumbens mediated by microglia activation. *Addict Biol*. 2018. doi:10.1111/adb.12541

297. Adeluyi A, Guerin L, Fisher ML, et al. Microglia morphology and proinflammatory signaling in the nucleus accumbens during nicotine withdrawal. *Sci Adv*. 2019. doi:10.1126/sciadv.aax7031

298. Liraz-Zaltsman S, Alexandrovich AG, Trembovler V, et al. Regional sensitivity to neuroinflammation: In vivo and in vitro studies. *Synapse*. 2011. doi:10.1002/syn.20889

299. Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD. Stress and neuroinflammation: A systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology (Berl)*. 2016. doi:10.1007/s00213-016-4218-9

300. Blum K, Thanos PK, Gold MS. Dopamine and glucose, obesity and reward deficiency syndrome. *Front Psychol*. 2014. doi:10.3389/fpsyg.2014.00919

301. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. *Lancet*. 2001. doi:10.1016/S0140-6736(00)03643-6

302. Salameh TS, Mortell WG, Logsdon AF, Butterfield DA, Banks WA. Disruption of the hippocampal and hypothalamic blood-brain barrier in a diet-induced obese model of type II diabetes: Prevention and treatment by the mitochondrial carbonic anhydrase inhibitor, topiramate. *Fluids Barriers CNS*. 2019. doi:10.1186/s12987-018-0121-6

303. Ouyang S, Hsuchou H, Kastin AJ, Wang Y, Yu C, Pan W. Diet-induced obesity suppresses expression of many proteins at the blood-brain barrier. *J Cereb Blood Flow Metab*. 2014. doi:10.1038/jcbfm.2013.166

304. Villaseñor R, Kuennecke B, Ozmen L, et al. Region-specific permeability of the blood–brain barrier upon pericyte loss. *J Cereb Blood Flow Metab*. 2017. doi:10.1177/0271678X17697340