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The role of Alpha Beta Hydrolase 6 in the neuronal control of body weight, exercise and anxio-depressive behaviors

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Résumé

L'obésité a beaucoup attiré l'attention ces dernières années en raison de son expansion mondiale et de son association à plusieurs maladies. Malgré les énormes efforts déployés par les scientifiques pour comprendre les mécanismes moléculaires et physiologiques de l'obésité, il reste nécessaire de découvrir un traitement efficace. Plusieurs études indiquent que le système corticolimbique est un modulateur potentiel de l'appétit, de l'activité physique et de l'obésité. Le système corticolimbique comprend le noyau accumbens (NAc), une région du cerveau impliquée dans la régulation de la récompense alimentaire, de l'humeur et de l'activité physique. Le NAc est principalement composé de neurones épineux à médiation GABAergique (MSN) qui jouent un rôle important dans le contrôle du bilan énergétique. Les manipulations de MSN par les endocannabinoïdes (eCB) ont une incidence sur la consommation de nourriture et l'humeur, mais des recherches supplémentaires sont nécessaires pour comprendre l'impact de la CBE sur l'activité physique et ses effets gratifiants. Le 2-arachidonoyl-glycérol (2-AG) est l'ebc le plus abondant dans le cerveau. Il est synthétisé dans le neurone postsynaptique afin d'activer le récepteur de cannabinoïde de type 1 (CB1R) situé de manière présynaptique. La modulation de l'accumulation de 2-AG est réalisée par deux enzymes: (1) la monoacyglycérol lipase (MAGL) située au niveau du neurone présynaptique afin de dégrader la masse de la 2-AG, et (2) le domaine α / β-hydrolase de la sérine hydrolase. 6 (ABHD6) localisé au neurone postsynaptique agissant en tant que contrôleur d'accès de 2-AG. Il a été démontré que la suppression du gène ABHD6 protège de l'obésité d'origine alimentaire chez la souris; Cependant, le mécanisme exact de la façon dont ABHD6 exerce cette action reste à élucider. Des travaux antérieurs de notre laboratoire ont révélé que l'inactivation virale de ABHD6 dans les neurones NAc augmente les niveaux de 2-AG, réduit la transmission des entrées neuronales de l'acide gamma-aminobutyrique (GABA), supprime la prise alimentaire et prévient l'obésité induite par l'alimentation. Bien que les interventions CB1R aient montré une efficacité dans la perte de poids, elles étaient notoirement associées à des troubles émotionnels tels que des pensées suicidaires. Par conséquent, l'inhibition de l'ABHD6 pourrait constituer une nouvelle approche thérapeutique de l'obésité sans affecter l'humeur.

Le but de cette étude est d'identifier l'impact de l'inactivation du gène NAc ABHD6 sur le poids corporel, l'alimentation, la course à la roue et les comportements anxio-dépressifs. Le knock-out conditionnel des neurones ABHD6 NAc (ABHD6$^{NAc\ KO}$) de souris adultes a été réalisé en utilisant une approche virale Cre-LoxP. Les souris ABHD6$^{NAc\ KO}$ ont été générées par des microinjections bilatérales de NAc délivrant une recombinase CRE ou une GFP témoin de manière spécifique

aux neurones. Après cela, les souris ABHD6$^{NAc\ KO}$ et les souris témoins ont été soumises à un régime riche en graisses ou à une alimentation Control CHOW pendant 12 semaines. Nous avons mesuré le gain de poids corporel, l'apport alimentaire et les changements métaboliques à l'aide de cages métaboliques CLAMS. En conséquence, nous avons donné 5 semaines d'accès aux roues dans leurs cages pour assister aux performances de course sur roues volontaires entre les groupes. Enfin, nous avons évalué les altérations du comportement avec le test de labyrinthe surélevé et plus, le champ ouvert et la nage forcée chez des souris et des contrôles ABHD6$^{NAc\ KO}$.

Nos résultats suggèrent que l'épuisement neuronal de l'ABHD6 dans l'NAc réduit le gain de poids corporel évoqué par un régime riche en graisses sans différence significative de consommation de nourriture entre les groupes. De plus, nous démontrons que la perte de fonction neuronale ABHD6 augmentait la dépense énergétique et empêchait l'inactivité physique volontaire de l'alimenter par une alimentation riche en graisses. En outre, nous avons également montré que les souris ABHD6$^{NAc\ KO}$ ne présentaient pas de comportement anxio-dépressif après une exposition prolongée à un régime alimentaire riche en graisses. Pris ensemble, ces résultats démontrent que l'inhibition neuronale focalisée de l'enzyme ABHD6 dans le NAc joue un rôle important dans la régulation de l'équilibre énergétique et de l'humeur et peut donc potentiellement fournir une nouvelle stratégie de traitement de l'obésité.

Mots clés: endocannabinoïde, domaine sérine hydrolase α / β-hydrolase 6, 2-arachidonoyl-glycérol, récepteur cannabinoïde de type 1, noyau accumbens, obésité, rouage, comportements anxio-dépressifs.

Abstract

Obesity caught a lot of attention in the last years because of its expansion worldwide and its association with several diseases. Despite the enormous efforts of scientists trying to understand the molecular and physiological mechanisms of obesity, there is still the need to discover an effective treatment. Several studies point to the corticolimbic system as a potential modulator in appetite, physical activity and obesity. The corticolimbic system incorporates the nucleus accumbens (NAc) a brain region involved in the regulation of food reward, mood and physical activity. The NAc is mostly composed by GABAergic medium spiny neurons (MSNs) that are important for the control of energy balance. Manipulations of NAc MSNs by endocannabinoids (eCB) affects food intake and mood however additional research is necessary to understand the impact of eCB on physical activity and its rewarding effects. 2-arachidonoyl-glycerol (2-AG) is the most abundant eCB in the brain, it is synthesized in the postsynaptic neuron to activate the cannabinoid receptor type 1 (CB1R) located presynaptically. Modulation of the 2-AG accumulation is done by two enzymes: (1) monoacyglycerol lipase (MAGL) which is located at the presynaptic neuron to degrade the bulk of 2-AG, and (2) the serine hydrolase α/β-hydrolase domain 6 (ABHD6) localized at the postsynaptic neuron acting as a gatekeeper of 2-AG. ABHD6 gene deletion has been demonstrated to be protective from diet-induced obesity in mice; however, the exact mechanism of how ABHD6 exerts this action is still to be elucidated. Earlier findings from our lab reveal that viral-mediated knockout of ABHD6 selectively in NAc neurons increases 2-AG levels, reduces gamma-Aminobutyric acid (GABA) neuronal input transmission, suppresses food intake and prevents diet-induced obesity. While CB1R interventions showed efficacy for weight loss, they were notoriously associated with emotional disturbances such as suicidal thoughts. Therefore, ABHD6 inhibition could give a novel therapeutic approach for obesity without affecting mood.

The aim of the current study is to identify the impact of NAc ABHD6 gene knockout on body weight, feeding, wheel running and anxio-depressive behaviors. Conditional knockout of ABHD6 NAc neurons ($ABHD6^{NAc\ KO}$) of adult mice was accomplished using a viral Cre-LoxP approach. $ABHD6^{NAc\ KO}$ mice were generated by bilateral NAc microinjections delivering CRE recombinase or control GFP in a neuron-specific manner. After, the $ABHD6^{NAc\ KO}$ and control mice were subjected to either high fat diet or Control CHOW feeding for 12 weeks. We measured body weight gain, food intake and metabolic changes using CLAMS metabolic cages. Consequently, we gave 5 weeks running wheel access in their cages to see voluntary wheel running performance

between the groups. Finally, we evaluated behavioural impairments with the elevated plus maze, open field and forced swim test in ABHD6$^{NAc\ KO}$ mice and controls.

Our results suggest that neuronal depletion of ABHD6 in the NAc diminishes body weight gain evoked by high fat diet with no significant differences in food consumption between the groups. Furthermore, we demonstrate that ABHD6 neuronal loss of function increased energy expenditure and prevented from voluntary physical inactivity induce by high fat diet feeding. In addition, we also showed that ABHD6$^{NAc\ KO}$ mice fail to show anxio-depressive behaviors after a prolong exposure to high fat diet. Taking together, these results demonstrate that focal neuronal inhibition of the ABHD6 enzyme in the NAc plays an important role in the regulation of energy balance and mood and thus potentially can provide a new treatment strategy for obesity.

Key words: endocannabinoid, serine hydrolase α/β-hydrolase domain 6, 2-arachidonoyl-glycerol, cannabinoid receptor type 1, nucleus accumbens, obesity, running wheel, anxio-depressive behaviors.

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List of abbreviations

BMI: Body Mass Index
BMR: Basal Metabolic Rate
WAT: White Adipose Tissue
BAT: Brown Adipose Tissue
CNS: Central Nervous System
VMH: Ventromedial Hypothalamic
ARC: Arcuate Nucleus
PVN: Paraventricular
MC4R: Melanocortin Receptor 4
POMC: Proopiomelanocortin
MSH: Melanocyte Stimulating Hormones
AgRP: Agouti Related Peptide
POA: Preoptic Area
SNc: Substantia Nigra pars compacta
VTA: Ventral Tegmental Area
NAc: Nucleus Accumbens
MSNs: Medium Spiny Neurons
HFD: High-Fat Diet
HPA: Hypothalamo-Pitutary-Adrenal
THC: Δ9-tetrahydrocannabinol
ECS: Endocannabinoid System
eCB: Endocannabinoids
CB1: Cannabinoid receptor type 1
CB2: Cannabinoid receptor type 2
GPCRs: G-Protein-Coupled Receptors
cAMP: Cyclic Adenosine Monophosphate
2-AG: 2-Arachidonolyl Glycerol
FAAH: Fatty Acid Amide Hydrolase
MAGL: Monoacyglycerol Lipase
ABHD6: Alpha Beta Hydrolase Domain 6
DSI: Depolarization-induce Suppression of Inhibition
DSE: Depolarization-induced Suppression of Excitation
VGCCs: Voltage-Gated Calcium Channels
TRPV1: Vanilloid Receptor Type 1
μ: Mu
CLAMS: Comprehensive Lab Animal Monitoring System
EPM: Elevated Plus Maze
OFT: Open Field Test
FST: Forced Swim Test
ABHD6$^{NAc\ KO}$: Alpha Beta Hydrolase Domain 6 nucleus accumbens knockout
LC/MS: Liquid Chromatography-Mass Spectrometry
GFP: Green Fluorescent Protein
NeuN: Neuronal Nuclear protein

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Thank You,

Anna Kristyna Franco Flores

1. Chapter 1: Introduction

1.1 Obesity

1.1.1 Impact

Obesity has become one of the most important health problems in today's age and has been characterized as a global epidemic [5]. Obesity is caused by genetic, social and environmental factors and can affect people of all ages, sex and ethnicity. Obesity increases the probability to develop chronic diseases such as type 2 diabetes, cardiovascular disease, some types of cancer, mood disorders (depression, anxiety), sleep and respiratory disorders and, in worst cases, death [6], [7].

An overweight person is considered to have a body mass index (BMI) between 25 and 29.9, while an obese person has a BMI of 30 or higher [8]. BMI is a value obtained by dividing a person's weight (kg) by the square of his height (m) [9]. Approximately half a billion of the world`s population is now considered to be overweight or obese [10]. Statistical projections estimate that 20% of the world's population will be obese and 38% will be overweight by 2030 [11]. It was initially believed that this problem only affected developed countries; however, the problem has also emerged in developing countries in Central and South America [12].

With the rise in prevalence of these metabolic diseases (obesity, diabetes, etc), there is an augmentation in health costs related to preventative measures for obesity and to treat its comorbidities. Although there is a lower incidence of people with obesity in Canada compared to the U.S. there has been a significant increase during the last decade [13]. In 2010, $6 billion – 4.1 % of Canada's total health care budget – was spent towards obesity-related issues [14]. Some psychiatric disorders such as depression can predispose individuals and influences the development of obesity. It is thought that this could be a counter-balancing effect induced by the negative emotions caused by overeating [6].

There is an urgent need to find a treatment that can decrease obesity and its comorbidities. Currently, there are dietary interventions or surgical procedures that reduce around 60 to 80 percent of weight [15]; however, it becomes difficult to maintain long-term results from these approaches despite the efforts of both patients and physicians. Even more, repeated experiences of failure to lose weight can trigger psychological distress in patients, which further contributes to the problem. The common treatment consists in changing bad food habits (diet) and increase

physical activity. This can also be accompanied by medications that help to decrease food consumption or decrease fat absorption. If those strategies do not work, physicians may recommend surgical options such as gastric balloon or bariatric surgery [16]. These procedures reduce the stomach volume or length of the intestines, leading to feel early satiety. Multiple side effects can emerge during and after these surgical interventions, which is why we need to investigate other potential new treatments to stop or minimize the “globesity” expansion [17].

In this chapter we are going to explain the etiology of obesity along with a literature review of the brain areas implicated in the regulation of the energy homeostasis and the consequences of disruptions of these brain pathways. Finally, we are going to explain the importance of the endocannabinoid system and its impact on energy balance and body weight.

1.1.2 Etiology

As discussed above, obesity is defined as an augmentation or excess of adipose tissue in the body. This increase of fat mass can affect body homeostasis by altering metabolism. Obesity has a complex etiology arising from social, cultural, environmental, hormonal and genetic factors. Many genetic markers have been implicated in the development of obesity [18], [19], for example rare monogenic mutation such as the leptin gene [20]. There is a clear genetic susceptibility between populations that can determine which are more prone to become obese despite the environmental circumstances [6], [21]. Genes linked to obesity mainly affect metabolism, body fat composition, food intake and energy expenditure. Although, there are few sporadic mutations that result in the development of obesity, predisposition appears to be inherited from parents.

There are also gender differences related to body weight gain. In the third national health and nutrition examination survey from the United States (1988-94), found a higher prevalence of obesity in women (25% U.S. women) compared to men (20% U.S. men) [21]. It is now known that women have more trouble losing weight compared to men, although the mechanism underlying this is not well understood [22][21]. Moreover, maternal obesity can give a predisposition to the baby to develop the disease. Some interesting data from *Udall et al;* suggests an interaction between maternal and neonatal obesity. Weight gain during pregnancy has been associated with an increase in subcutaneous fat in neonatal humans [23]. *Broadney et al.,* also highlights the importance of parental health, specifically in the mother, where maternal obesity influences comorbidities such as inflammation and alterations in immune responses in children [24].

The two most recognized causes of the massive increase of obesity around the world are: high caloric intake and the lack of physical activity (sedentary lifestyle). This can be translated as an imbalance or disruption in energy balance between calories consumed and calories expended. In developed countries there is a relationship between low levels of physical activity and obesity. For example, a study in the United Kingdom showed no correlation between energy intake and the prevalence of obesity, although they did find a tight relationship between the amount of immobility (due to T.V. viewing and lack of walking) and weight gain [26]. In addition, gut flora has been shown to differ between lean and obese humans which can affect energy balance [25] . Therefore, obesity is the result of many environmental and biological factors, with their overall contribution accounting for the dramatic increase of obesity worldwide.

1.1.2.1 Regulation of energy balance

To understand the mechanisms behind obesity we must first understand the concept of energy balance. Energy balance is the equilibrium between the amount of food ingested and energy expended. There are multiple molecules from the gut and both, the periphery and the central nervous systems that contribute to maintain energy balance (and imbalance) will be discussed in the following sections.

1.1.2.1.1 Food intake

The world has increase diversity in food supplies. Nowadays, supermarkets offer a variety of palatable foods with high content of sugars, salts and fat. The 24H accessibility to a variety of palatable food can be a strong contributor to the increase of fat storage seen in the world. Additionally, reward and emotional processes can interfere with these needs by both increasing or decreasing appetite and physical activity beyond homeostatic mechanisms. The experience of eating palatable food produces positive or negative feelings and can influence our willingness to approach or avoid those foods again. Positive, rewarding, or pleasurable effects, also known as hedonic effects, is one of the major causes of overeating and the development of obesity [27]. Palatable food stimulates appetite and delay satiety which increase energy intake [28]. Food macronutrients can play an important role in satiety; fat for example does not have a good satiety time period contrary to proteins that have a better impact in our body. Surprisingly, satiety does not come from the food portion or meal size but from the nutritional content that is consumed [29].

Many studies have focused on high fat diets as a possible cause of obesity. Indeed, *Golay et al.,* showed that fat induces overconsumption and weight gain through its low satiety properties and high caloric density, thereby increasing fat storage [29]. In some cases, socioeconomic status can influence the choice for low cost, high calorie foods. Therefore, it is important to establish a balance diet that provides us the necessary nutrients to feel satisfied at each meal.

Moreover, TV advertisement can strongly influence meal preference including sweetened cereals, sweetened beverages and salty snacks; which leads to an energy imbalances that enhance weight gain and consequently obesity [30]. The accessibility of high caloric food gives reasonable foundations to blame obesity expansion. However, the prevalence of obesity and the global epidemic seen in this century cannot be only attributed to one factor, but several such as environmental, social, genetic, cultural and sedentary lifestyle.

2.1.2.1.1 Energy expenditure

Organisms need locomotor activity to function and provide necessities like food, reproduction and protection from predators that involve interactions with the environment [31]. Energy expenditure is the amount of energy that a single person needs to perform actions such as breathing, walking, eating and others. Total energy expenditure will be the total number of calories burned each day [32]. Currently, one strategy for treating obesity is to increase energy expenditure which is dependent on internal (e.g., temperature, metabolism, circadian rhythms, hormones) and external factors (e.g., weather, exercise, etc.). Energy expenditure is distinguished by three key components: basal metabolic rate (BMR), adaptive thermogenesis and physical activity. BMR is the minimal energy required to maintain bodily functions; i.e. energy under normal conditions when there is no extra demand for defensive adaptations such as cold-warm exposures, after meal digestions or in rest [33]. Adaptive thermogenesis is activated during chronic cold or heat exposures. At the cellular level, mitochondrial proton uncoupling from ATP production will be needed to release that energy as heat [34].

As a major component of energy expenditure, physical activity contributes considerably to whole-body energy balance. It can be divided into spontaneous and voluntary physical activity. Spontaneous physical activity is a low intensity and unplanned physical activity (e.g., standing, ambulating, talking and fidgeting). In rodents spontaneous physical activity can be measured by the activity in their home cage after the acclimation phase [35]. Voluntary physical activity in

contrast is defined by the willingness to increase locomotor activity and can vary in intensity and duration (e.g., sports). In humans, motivation to engage in voluntary exercise can be attributed to many internal or external factors. Physical activity can provide a rewarding effect and it can even become addictive [31]. In rodents the most effective way to measure voluntary physical activity is by the introduction of the wheel running. There are a number of studies using wheel running to assess the rewarding effect of voluntary physical activity in rodents and the underlying neurobiologically [36][37]. Moreover, running wheels are a useful tool to examine the association of physical activity to energy balance and obesity, however there are still a few challenges to fully understand the neuronal mechanisms affecting voluntary physical activity and its beneficial impact on diabetes and obesity.

The means by which the body maintains and achieves homeostasis are key to understanding for developing therapeutic approaches for reducing obesity. While some strategies appear to be promising in theory, there is no effective treatment to stall the increasing yearly rates of this epidemic.

1.1.3 Brain controls of food intake and energy expenditure

The brain integrates different circulating signals such as nutrients, gut-derived hormones and adiposity signals to regulate food intake and energy expenditure. In 1953, *Kennedy et al.* proposed a model of energy homeostasis where inhibitory signals generated by body fat stores act in the brain to reduce food intake [38]. If body fat decreases, peripheral signals are decreased and thus a demand for food intake is increased. Being among the first to describe a potential mechanism whereby periphery signals could control energy balance via their actions in the central nervous system (CNS), it had many shortcomings especially an explanation on how individual meals modulate inhibitory or excitatory signals to the brain. Fortunately, Gibbs and Smith proposed two decades later that meals can generate signals from the gastrointestinal tract which can act on the brain giving a feeling of satiety and to indicate the meal is finished [39]–[41]. Now we know that it is more complicated than that; metabolism implies neuronal control by the interaction with other organs such as gastrointestinal tract, liver, pancreas, white adipose tissue (WAT) and brown adipose tissue (BAT). Even more, the brain controls specific functions of these organs via the autonomic nervous system (responsible for the control of unconscious body functions like breathing) and endocrine mobilization [33].

Insulin was the first hormonal signal discovered to be implicated in the modulation of body weight at the CNS [42]. Studies using transgenic ob/ob mice (which had a mutation on the gene encoding leptin, a hormone secreted by adipocytes) gave insight that a hormone can be implicated in hyperphagia and obesity and could be mostly implicated in adipocyte signalling. Circulating leptin levels are in correlation with the amount of body fat content [43]. In the CNS, there are receptors for both leptin and insulin in hypothalamic neurons and they are known to be important in modulation of energy intake. Insulin and leptin act on two different groups of neurons in the hypothalamic arcuate nucleus (ARC) to give negative feedback for food intake and energy balance. More precisely, insulin and leptin act on proopiomelanocortin (POMC) neurons by stimulating the secretion of anorexic neuropeptide and on neuropeptide Y/ agouti gene-related protein (NPY/AGRP) neuron, therby decreasing the expression of NPY [44]. Some studies have found that leptin can have a greater impact than insulin in the CNS control of energy homeostasis [45]. In this section we are not going to give much detail about it, however, a good example is that lack of leptin causes severe obesity with hyperphagia that persists despite high insulin levels. Contrarily, insulin deficiency does not cause obesity having an impact in different critical roles on metabolism [33]. Thus, both leptin and insulin participate in the CNS control over energy homeostasis and disruption of these mechanisms in the development of obesity are focal points of research.

Energy expenditure is the other important aspect that regulates body weight and maintains a healthy life. Information regarding energy stores is sent to specialized neurons in the hypothalamus and in the brainstem [1]. Kennedy, among others, observed that rats that had ventromedial hypothalamic (VMH) lesions showed an increase in body weight and adiposity tissue even though they were food restricted, making clear that hypothalamus controls both energy intake and energy expenditure [46]. More specifically, a hypothalamic region called the arcuate nucleus (ARC) plays an important role in mediating leptin's effect on locomotor activity. In one study, leptin receptor null mice showed normalized locomotor activity after restoration of leptin signalling in ARC POMC neurons. Therefore leptin signaling is an important modulator to increase locomotor activity [47]. Thermogenesis is another important component in energy expenditure, it is dependent on BAT and it maintains body temperature created by internal factors. More information about thermogenesis and its regulation will be discussing in the following section.

1.1.2.1 Hypothalamus and hindbrain

The hypothalamus is a brain region involved in homeostatic regulation (e.g. hunger, body temperature, sleep, etc), as well as energy intake and expenditure [48]. The hypothalamus participates in the organism's adaptation to environmental changes due to its high levels of plasticity. In the past decade, literature regarding the neuronal wiring and intercellular signalling of different peripheral hormones to the hypothalamus has grown exponentially [49]–[51]. For instance, lesions in the paraventicular nucleus (PVN), the ARC and the ventromedial nuclei of the hypothalamus lead to hyperphagia and obesity due to its neuronal integration that modulates energy balance [52]. In the next paragraphs we are going to explain with more detail some of the molecules involved in how the hypothalamus can regulate feeding and contributes to an important part of the increasing obesogenic population in the world.

A small section of the hypothalamus called ARC plays a critical role in the regulation of metabolism. Melanocortin receptors are found in the ARC, but it wasn't until some studies deleted the melanocortin receptor 4 (MC4R) in animals that they saw a significant difference in body weight [53]. Subsequently, researchers discovered that the melanocortin system is the neurocircuitry connecting appetite and neuroendocrine signalling to regulate metabolism. The generation of melanocortin ligands is done by proopiomelanocortin (POMC) neurons. In the CNS, POMC generates melanocyte stimulating hormones (MSH), α-MSH, β-MSH and γ –MSH that have high affinity (agonist) for the melanocortin receptors MC1R, MC3R, MC4R and MC5R [54]. They also found endogenous antagonists: Agouti and Agouti related peptide (AgRP) that interact with specific melanocortin receptors that interfere with MSH activity. Distribution of AgRP and POMC neurons can have similar communication to other brain areas, but it is believed that only POMC neurons have projections to the brainstem. These neuronal populations have opposite effects to their hypothalamic counterparts in the maintenance of energy homeostasis; which is important as POMC neurons (via MSH) inhibit food intake, while AgRP neurons increase food intake [52]. There are diverse new techniques (e.g. DREADD, KO mice, optogenetics) that facilitate specific neuronal control of POMC and AgRP activity, proving the importance of a balance between their connections. Long term AgRP neuronal loss results in severe aphagia and subsequient death [55] while mice POMC deficiency causes hyperphagia and obesity [56], [57].

Furthermore, POMC and AgRP neurons respond to leptin, insulin [58], ghrelin, serotonin and glucose. Their signalling results in the generation of melanocortin receptors to modulate food intake, glucose metabolism, energy expenditure and others (*Figure 1).*

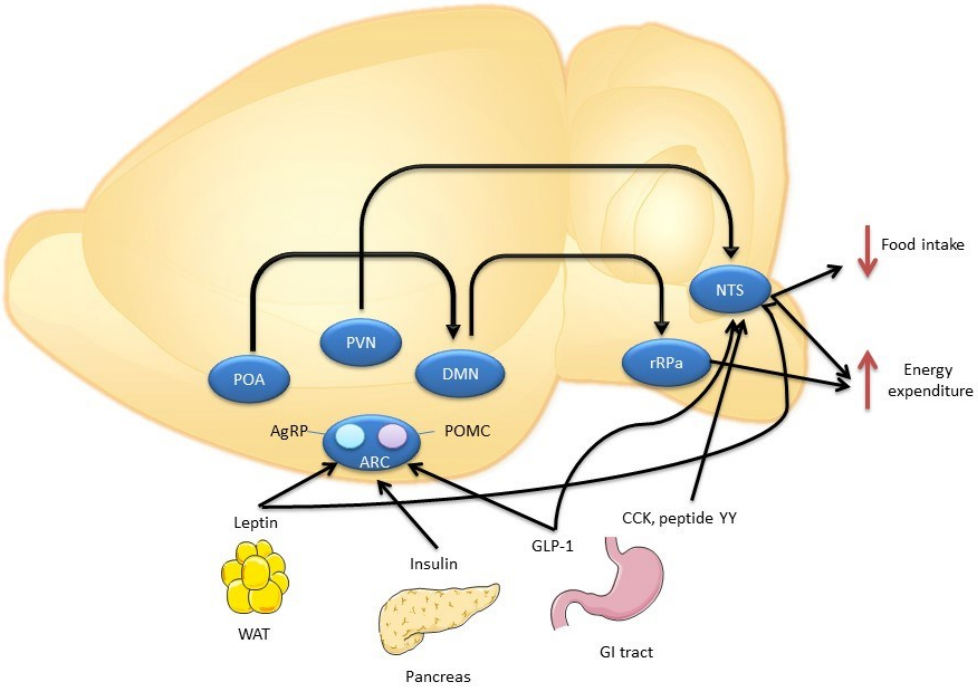


Figure 1: Hypothalamic neuronal circuits regulating food intake and energy expenditure. Proopiomelanocortin (POMC) and neuropeptide Y/agouti-related peptide (AgRP) neurons in the arcute nucleus (ARC) sense the body's energy and send inputs to other brain regions such as paraventricular nucleus (PVN) which will project to the nucleus of the solitary tract (NTS). The preoptic area (POA), dorsomedial hypothalamus (DMN), rostral raphe pallidus (rRPa), glucagon-like peptide-1 (GLP-1), cholecystokinin (CKK), withe adipose tissue (WAT), gastrointestinal (GI) [1].

Energy is expended during physical activity, basal metabolism and adaptive thermogenesis. The ARC is considered a key site for mediated locomotor activity induced by leptin signalling, but there are other brain areas that also contributes to locomotor behaviors. In a thermoregulatory perspective, heat must be generated to maintain body temperature or in response to the energy given by food intake. The preoptic area (POA) regulates the neuronal circuit of sympathetic BAT outflow by sending inputs to the premotor neurons in the rostral raphe pallidus. Moreover, administration of MC3R and MC4R agonists can stimulate BAT activity - meaning hypothalamic melanocortin system has a regulatory response in BAT thermogenesis [1]. In this regard,

endocrinal signalling by insulin, leptin, and GLP-1 can modulate BAT outflow: it has been shown that insulin and leptin act on POMC neurons to promote white adipose tissue browning and enhance energy expenditure. Since WAT is reduced during obesity, manipulation in the homeostatic mechanism to increase WAT could potentially prevent diet-induced obesity [59].

2.1.2.1 Extra-hypothalamic control of energy balance: corticolimbic circuits

There are extra-hypothalamic structures that play a very important role on the control of energy balance also known as the corticolimbic circuits. If we wonder why we prefer a cookie over broccoli, the answer lies in neural circuitry that is activated by these palatable foods. There are some foods that cause more pleasurable or rewarding effects triggering a higher release of the neurotransmitter called dopamine within the reward system. Dopamine's first precursor is tyrosine, which is converted to L-dopa to be catabolized as dopamine [60]. There are many physiological dopaminergic actions, which are mediated by G protein-coupled receptor subtypes. There are 5 different receptor subtypes: D1-like receptor (D1A-D and D5) that activates adenylyl cyclase by Gs protein and D2-like receptor (D2, D3 and D4) that inhibits adenylyl cyclase and activates K+ channels by inhibitory G protein. Both receptors' activity influence behavior, increasing or inhibiting reward depending on the brain area [2], [61]. Even though dopamine only accounts for 1% of the total neuronal population of the brain, it is involved in many brain areas associated with motor, memory, reward, and other functions. It has also been demonstrated that dopamine is involved in the hedonic component of reward [2], [62].

Reward is very important for survival and it is implicated in basic processes like eating, drinking, and sexual reproduction, as well as other pleasurable behaviors such as gambling. The reward system is activated in response to these activities and increases the chances of seeking these behaviors again. Reward can be defined as the willingness to work (spend time, energy and effort) for any goal or behavior from which we have previously derived pleasure [63]. Therefore, a rewarding situation has an impact on motivation. Because of these seeking-behaviors, scientist proposed a neuronal signalling pathway which processes reward such as the dopamine system. Studies revealed that dopaminergic neurons are localized in the mesencephalon, diencephalon and the olfactory bulb. As seen in *Figure 2*, dopamine neurons located in the midbrain structures substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) project to the striatum (composed by caudate nucleus, putamen, ventral striatum and nucleus accumbens (NAc)), and

the dorsal and ventral prefrontal cortex. There are additional projections, which influence reward, but they will not be discussed in this thesis.

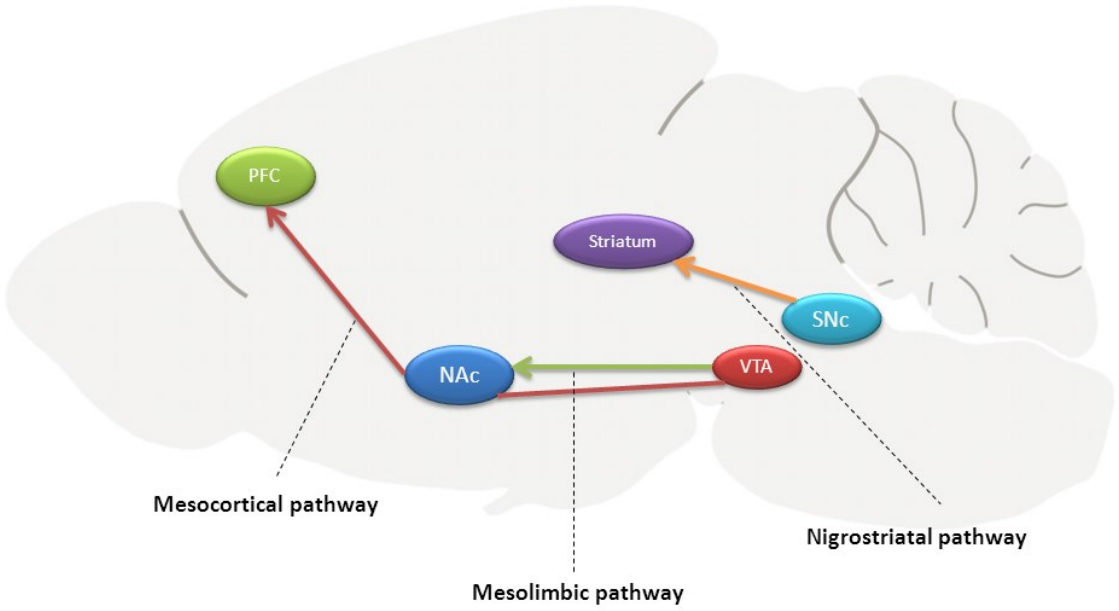


Figure 2: Overview of the reward system in rodent brain. Dopamine neurons are located in brain structures called substantia nigra pars compacta (SNc) and the ventral tagmental area (VTA). Nigrostriatal pathway arises from the SNc to striatum; the mesolimbic pathway projects from the VTA to the nucleus accumbens (NAc), and the mesocortical pathway arises from the VTA to the prefrontal cortex [2].

In this study, we are interested in the mesolimbic pathway that projects from the VTA to the NAc as it has been shown to be implicated in the motivations for natural rewards, locomotor activity and mood. The NAc is a large structure that has two sub-regions: the core and the shell. NAc core and shell execute different brain functions based on the inputs received [64]. The NAc shell has been well implicated in the activation of dopamine transmission during substance abuse such as cocaine, amphetamine, THC, MDMA and nicotine [65]. The NAc core, on the other hand, is involved in reward-related approaches such as the Pavlovian-instrumental transfer, as well as cognitive and motor functions [66]. Together the shell and core regulate, motivation, addiction,

reinforcement learning, mood, etc [64]. Approximately 95% of neurons in the NAc are GABAergic medium spiny neurons (MSNs) that express either D1-type or D2-type receptors. There are also cholinergic and GABAergic interneurons. From the NAc core, the GABAergic MSNs projects to other subcortical areas such as the globus palladus and the substantia nigra [67], [68]. A well - supported idea about the NAc dopamine system is that it could be an interface between motivation and action, meaning that it can play an important role in the motivation to work for palatable food but not necessarily in reward perception [69]. Also, dopamine is implicated in regulating voluntary physical activity. The difference between spontaneous and voluntary physical activity can be summarized that the second needs a purpose and motivation to do it while spontaneous activity is just an involuntary movement that the body creates.

In mice research evaluating the dopaminergic system in the control of voluntary physical activity, *Rhodes et al*. showed that administration of D1-like antagonist reduced wheel running compared to control mice. In contrast, D2-like antagonist had no effect between control and treated mice suggesting that D1-like receptor is a possible modulator of wheel running performance [70]. Studies have shown that increased dopamine activity can be dependent on physical activity, but it is not clear if dopamine is acting as an independent variable of physical activity levels or if physical activity is affecting dopamine functioning [71]. Therefore, it is important to investigate the implication of NAc dopamine system in the regulation of voluntary physical activity, especially D1-like receptor that has been shown to modulate these effects.

1.1.4 Brain control of mood

There are many neuronal networks implicated in emotional behaviors. The limbic system controls mood, memory, and others. The limbic system is located between the brainstem and the two hemispheres and involves the PFC, amygdala, hippocampus and the ventromedial parts of the basal ganglia. In fact, these areas provide some evidence of how their specific neuronal networks controls mood such as fear, anxiety, depression, etc. The amygdala, in particular, is responsible for modulating our perception and reactions to aggression and fear; functional magnetic resonance imaging studies in humans have found that fearful faces and fear conditioned cues activates the amygdala region [72]. Emotions and memory are very closely related; therefore, the hippocampus is an important region that stores information in a long- and short-term memory. The hippocampus is necessary to create new memories but also to store old memories. Moreover, disturbances in this brain region appears to have psychological consequences such as

schizophrenia and severe depression [73]. The mesolimbic dopamine areas such as NAc and VTA structures are important for reward and emotional states such as depression, anxiety and stress. Dysfunctions within these areas can induce disturbances on emotional and cognitive disorders such as depression and anxiety [74]. As we discussed in the previous section, the NAc is implicated in the control of food-motivated behaviors, in locomotor activity and mood disorders. NAc integrates inputs from diverse areas, making it one of the major controls of motivation, reward, stress, and mood. Deep brain stimulation in the NAc decreased behavioral responses of depression and anxiety in patients that were resistant to pharmacotherapy treatment, indicating a potential alternative therapy for mood disorders [75].

1.1.2.1 Anxio-depressive behaviors

Obesity is implicated in CNS impairments such as mood disorders. It has been shown that obesity increases the incidence of depressive symptoms and that depression could lead to the development of obesity and its associated complications; *Luppino et al.,* showed that the obese population has a 55% increased chance of developing depression and that individuals that suffer from diabetes have a doubled incidence of depression compared to healthy controls. This relationship could be due to poor food choices or lack of physical activity associated with depression and obesity [76]. Mood states like anxiety and depression can also be altered by food choices and energy metabolism. For example, people with negative emotional states tend to eat palatable foods (chocolate or ice cream) to alleviate these feelings. Palatable food consumption induces short-term positive emotions while chronic consumption of these foods will increase adipose tissue leading to anxio-depressive behaviors [77]. It is believed that there is a bidirectional relationship between mood disorders and obesity, however the nature of this association is still poorly understood. *Sharma et al*., showed that chronic high-fat fed mice resulted in molecular adaptations in NAc and expressed anxio-depressive behaviors in mice [78]. Additionally, Décarie-Spain et al., showed that saturated high-fat diet (HFD) fed mice produces obesity and hyperleptinemia (higher amounts of leptin in the bloodstream); triggered anxiety-like behaviors, peripheral inflammation and multiple pro-inflammatory signs in the NAc including gliosis (glial cell inflammation), enhance of cytokine expression and NFkB transcriptional activity. A saturated HFD also decreases in NAc dopaminergic tone and function as well as perturbations in hypothalamo-pitutary-adrenal (HPA) axis [79]. These data suggests that the NAc is important in anxio-depressive behaviors caused by saturated HFD [78]. Although more information is needed to

characterize the importance of the NAc in anxio-depressive behaviors, it appears the NAc could be a critical structure to investigate HFD-induced changes in metabolism, decrease of voluntary physical exercise, and comorbid anxio-depressive behaviors.

1.2 Endocannabinoid system

1.2.1 Generalities

For decades' cannabis or marijuana has been used by humans as a recreational drug. There is evidence that shows the use of cannabis in the second millennium BC in the Assyrians culture. Different cultures in the Middle East, Europe, Africa and others were engaged for the positive effects of cannabis such as euphoria, feelings of well-being, and its medical use. Its wide use in these countries has continued ever since and it has had a world impact that now is the longest recorded drug in history of human use [80]. Although in ancient cultures the use of cannabis not only was for recreational purposes but also medical (for example, as a pain killer, anxiety-relieving and sleeping disorders), little was known about the molecular actions of cannabis within the body. It was until 1964 when Dr. Mechoulam discovered the psychoactive product of cannabis (also known as marijuana) Δ9-tetrahydrocannabinol (THC) [81]. The discovery of THC brought the possibility to create synthetic cannabinoid-like compounds with different chemical structures or different arrangement. These compounds where divided in three classifications: classical, non-classical and aminoalkylindol. After all the research trying to understand how THC exerted its effect within the body among the different synthetic analogs (plant derivate and synthetic generated), researchers discovered endogenous cannabinoid-like compounds in our bodies called the endocannabinoid system (ECS).

ECS is widely distributed in the mammalian tissues and it is known for its properties regulating cardiovascular, nervous and immune system inside the cells to maintain body homeostasis [81], [82]. The endocannabinoid (eCB) mechanism of action was not known until the 19th century when Dr. Howlett published the first data indicating that eCBs inhibited the formation of adenylate cyclase, an enzyme important for the production of cyclic adenosine monophosphate (cAMP) within the cell. Not long time after the same laboratory showed the existence of binding sites in the brain [83] and shortly the first cloning of cannabinoid receptor was done [84]. Today, researchers have identified two cannabinoid receptors: cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2). The CB1 receptor is mostly expressed in the brain and it was

known to be the “brain receptor”, however, it can be also present in some peripheral organs. On the other hand, CB2 is abundant in immune cells, astrocytes and microglial [85]. Both receptors are 7-transmembrane domain macromolecules of the “G-protein-coupled” (GPCRs) [86].

The eCB’s, in the other hand, are lipophilic lipid compounds that are produced on-demand; the two major ligands are: N-arachidonoylethanolamide or anandamide and 2-arachidonolyl glycerol (2-AG). The chemical structures of the ligands are described in *figure 3* [87]. These two ligands have been investigated in great detail and showed not to be conventional neurotransmitters. Surprisingly, their mode of actions differ from standard neurotransmitters as they act mostly pre-synaptic instead of post-synaptic to inhibit neurotransmitter release [83]. The enzymes responsible of the brake-down of the two ligands are the fatty acid amide hydrolase (FAAH), the monoacyglycerol lipase (MAGL) and the alpha beta hydrolase domain 6 (ABHD6) who was been recently discovered [88]. **The purpose of this study is to focus more in the enzyme ABHD6 as it has been shown to play an important role in metabolism.**

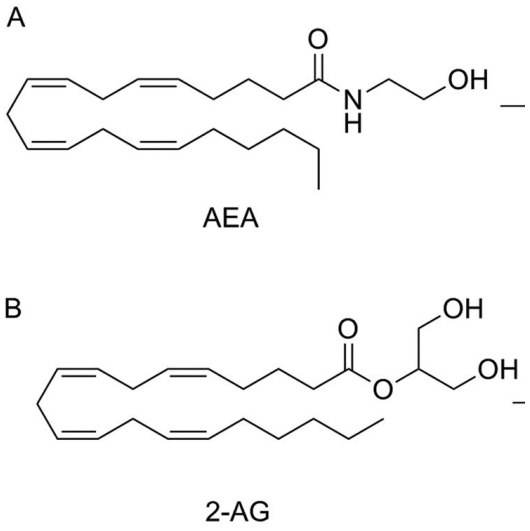


Figure 3: Chemical structures of endocannabinoids: a) Anandamide (AEA) and b) 2-arachidonoylglycerol (2-AG) [89].

In general, ECS are important for the body homeostasis. The ongoing research on the ECS has brought new therapeutic approaches in diseases like epilepsy, cancer, hypertension, diabetes, obesity and anxio-depressive behaviors. However, there is still lack of information regarding the molecular bias and mechanism of how ECS is helping the body to maintain its balance. In this section we are going to give evidence that point to the ECS as a mediator in the

brain and how it can be linked to obesity, physical activity and anxio-depressive behaviors, but more important, we are going to evidence how ECS could be thought as a potential therapeutic approach for obesity. The novel discovery of the ABHD6 enzyme (which constitute a part of the ECS) can be thought as a potential therapeutic approach for body weight loss, exercise and mood; important targets for decreasing obesity in the world without using an invasive procedure such as surgical interventions.

1.2.2 Endocannabinoid system in the brain

The central nervous system is composed by the brain and spinal cord [90], [91]. As discussed above, the endocannabinoid system is present in the CNS to act as a synaptic modulator. Although CB1 receptor was originally believed to be only expressed in the CNS, now we know that it is present in numerous peripheral organs. CB1 is the most abundant receptor in the brain and both anandamide and 2-AG have high affinity for this receptor [92]. There are several brain regions with high density in CB1 receptor such as basal ganglia, substantia nigra, globus pallidus, cerebellum and hippocampus [93], [94]. Because endocannabinoids are lipid-based compounds and the brain is surrounded by aqueous solution it is easy to have an interaction between intracellular environments for lipid messengers [95].

One well-known endocannabinoid mechanism is that they are synthesized “on demand”, which differentiate them from classical neurotransmitters that are constantly synthesized and then stored in synaptic vesicles [96]. In this regard, they modulate synaptic transmission by inhibiting the release of many excitatory and inhibitory neurotransmitters. Therefore, new definitions were implemented to describe that eCBs mediates short-term synaptic plasticity, also known as depolarization-induce suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). Not long time after scientist demonstrated that they also modulate long-term plasticity[95], [97]. On the other hand there has been some evidence that ECS has been implicated in synapse formation and neurogenesis [98]. Therefore, there is a clearly eCB synaptic modulation that can modify brain networks in certain circumstances.

The eCB signalling is very complex system as they do not act as standard neurotransmitters; however, the retrograde signalling that many studies have found is that neuronal post-synaptic stimulation (induced by GABA or glutamate) will activate voltage-gated calcium channels (VGCCs) elevating intracellular calcium that will stimulates 2-AG synthesis. 2-AG will be release

to the synaptic clef to act in the CB1 located at the pre-synaptic neuron in a retrograde manner (showed in *Fig 4*). In contrast, studies have found a non-retrograde signalling in which neuronal modulation involves the transient receptor potential vanilloid receptor type 1 (TRPV1) or CB1 receptor located at the post-synaptic neuron [83].

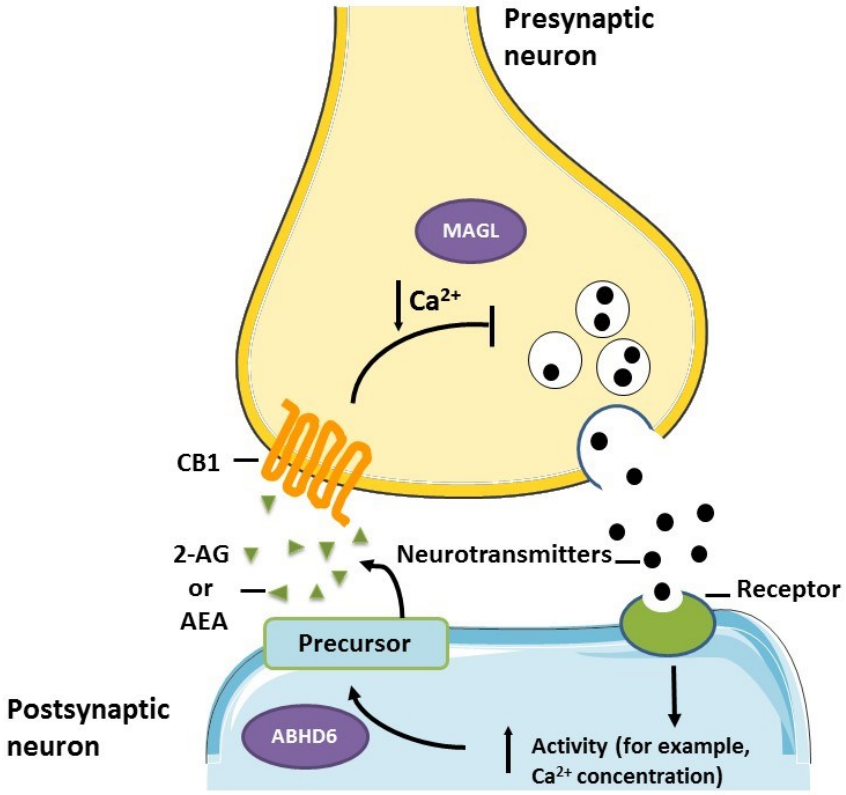


Figure 4: Endocannabinoid synaptic modulation. Pre-synaptic neurotransmitter release activates the post-synaptic neuron increasing calcium influx and stimulating the EC ligand synthesis acting in the pre-synaptic CB1 receptor. MAGL degrades 2-AG at the presynaptic neuron and ABHD6 catabolizes 2-AG at its site of synthesis. (figure modified from Velasco et al., [4]).

Moreover, eCB modulates synaptic strength, and depending on the brain region can induce neuronal changes in memory, locomotor activity, food related behaviors and pain [99]. The enzymes MAGL and ABHD6 play an important role in the modulation of synaptic strength. Many studies focus more on MAGL because it was known to be the “traditional” 2-AG breakdown but now growing evidence is pointing to ABHD6 as an important modulator of “fine tunes” in synaptic

plasticity. Therefore, in the next sections we are going to discuss about ABHD6 and the findings on synaptic plasticity and metabolism.

1.1.2.1 Alpha/beta hydrolase domain 6 (ABHD6)

As discussed above, there are many different enzymes important for the degradation of 2-AG. MAGL and FAAH were the first ECS enzymes to be studied in 2-AG degradation. In fact, there are plenty of pharmacological and genetic studies about these two enzymes, however; recent studies discovered new enzymes from the monoglycerol lipase family such as ABHD6 and ABHD12. We are interested in studying the enzyme ABHD6 because it is believed that it plays an important role in the pathogenesis of obesity. Some studies accomplish ABHD6 deletion in adipose tissue and saw a protective effect from metabolic syndrome and insulin resistance [100]. ABHD6 has been also detected in different tissues including liver, kidney, pancreatic islets and the brain [101]. However, the mechanisms of its physiological functions in the brain are still unknown.

ABHD6 is monoglycerol lipase that acts endogenously to decrease 2-AG accumulation and thereby signaling at CB1 receptor in the CNS. *Marrs et al.,* demonstrated that ABHD6 played an important role controlling the accumulation of 2-AG which was surprising because most of the monoglyceride lipase inhibition, exhibit a strong increase in 2-AG levels while ABHD6 inhibition caused minor changes in 2-AG levels [102].This minor activation of CB1 receptor caught a lot of attention in the recent years and some studies showed that pharmacological ABHD6 inhibition can have neuro-protective effects in several diseases such as traumatic brain injury, epilepsy, and cancer [103],[104]. The enzyme MAGL is responsible for approximately 85% of 2-AG hydrolysis and it is localized in the presynaptic axon terminal, same site as the CB1 receptor. In contrast ABHD6 hydrolyses the small quantity of approximately 4% of 2-AG in the brain and its localization is in the post-synaptic neuron [105]. Thus, ABHD6 acts to guard the accumulation of 2-AG and thus mediate CB1R signal transduction by reducing 2-AG. Furthermore, Marrs et al., demonstrated that ABHD6 was expressed in glutamatergic neurons, some GABAergic interneurons and astrocytes modulating synaptic plasticity but nor in microglia. Interestingly, ABHD6 was detected in mitochondrial fraction of microglial cell line BV-2 and its inhibition reduced 2-AG hydrolysis. Furthermore, ABHD6 acts as a modulator of synaptic plasticity and it has been shown that inhibition increased the induction of long-term synaptic depression by CB1 receptor but not short-term depression [105]. Inactivation of ABHD6 expression in neurons using the

molecular tool CRISPR/cas9 significantly increases excitatory neurotransmission while overexpression of ABHD6 reduces glutamate currents and even more. Wei et al., demonstrated that ABHD6 negatively regulates the synaptic function of AMPA receptors [101]. Thus, all this evidence expands our understanding of the importance of ABHD6 for many neuronal targets and integrates molecular mechanism necessary for new therapeutic approaches. In general ABHD6 looks to have an important action in the regulation of synaptic plasticity in different types of neurons, however, its specific actions in brain reward areas need to be defined.

2.1.2.1 Implications of ABHD6 on energy balance and mood

The eCB AEA and 2-AG have been implicated in energy balance and body composition. In humans, *Côté et al.,* demonstrated a relationship between eCB 2-AG and cardio-metabolic risk factors, showing a correlation in levels of 2-AG and BMI in obese men [106]. In the other hand, a few rodent studies showed that 2-AG levels increase after 18 hours of food deprivation and decrease right after food intake. 2-AG hypothalamic changes give the understanding that endocannabinoids can act as a pro-orexigenic mediator in this area. More studies showed similar effects when rodents are following a dietary restriction for a long period, reducing hypothalamic 2-AG levels while overweight rodents exhibit higher levels [107]. Interesting, the mice *of Matias et al*. were exposed to different high fat diets for different periods of time and then there was an evaluation of AEA or 2-AG where they measured their kidneys, hearts, skeletal muscle and thyroids. They found that obesity can cause dysregulation of the ECS in different organs important for endocrine functions and energy expenditure; these proposed important roles for the ECS in the control of metabolic, endocrine and cardiovascular functions [108]. **In addition, the 2-AG concentration levels can be controlled by ABHD6; *Fisette et al.,* 2016 showed that ABHD6 deletion in the VMH neurons increase 2-AG levels compared to the control group.** ABHD6 VMH KO mice exhibited disturbances in metabolic challenges such as impairment in feeding response to fasting, increased susceptibility to hypothermia, resistance to weight loss when transitioned from high fat diet to regular chow and higher prevalence in high fat diet-induced obesity (see *Figure 5*) [109]. Altogether, there is evidence to suggest that ABHD6 in the VMH plays an important role in energy metabolism.

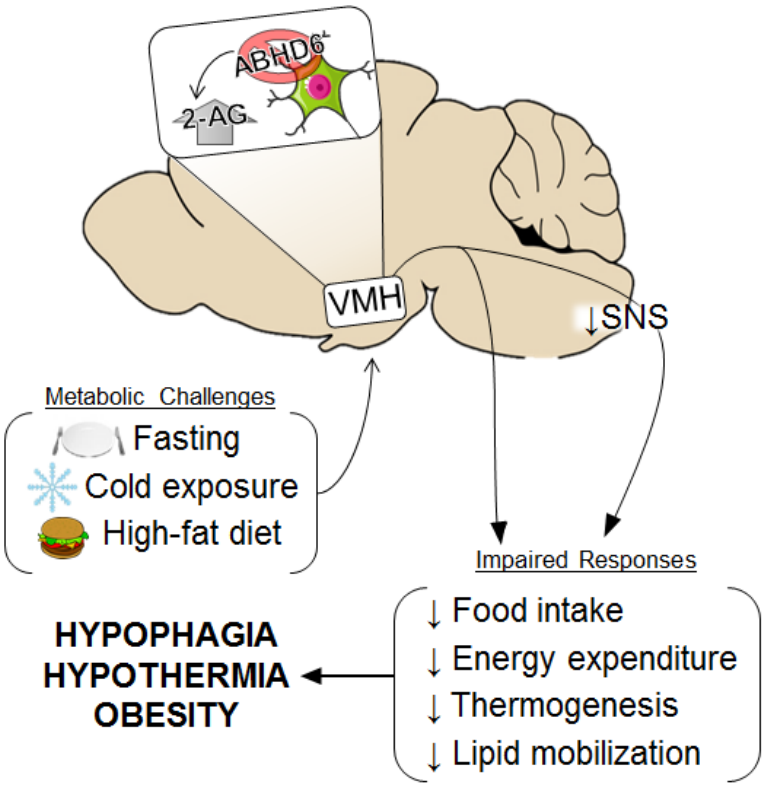


Figure 5: Modulation of ABHD6 KO in the VMH. ABHD6 KO in the ventromedial hypothalamus (VMH) regulates 2-AG accumulation and impairs energy metabolism when exposed to homeostatic challenges such as fasting, cold exposure and high-fat diet. SNS, sympathetic nervous system. (Taken from *Fisette et al.,* [3])

Physical activity has a great impact maintaining body homeostasis. In athletes scientific investigation showed that exercise gives states of consciousness and in the 1960s they discovered some psychological changes induced by prolonged physical activity. In the last decades these effects were better described as “runners high” which gather states of happiness, elation, feelings of peace, well-being and pain relief [110]. However, the lack of scientific information or evidence about the “runners high” lead to the question: Do these effects really exist? and Why haven’t all athletes experienced it? One of the hypotheses for the mechanism of the “runners high” goes to an opioid point of view. Opioids are well known to induce euphoria and analgesia thus some of the effects caused by physical activity could be related to opioid activation; however, the “endorphin-related hypothesis” didn’t last because it did not explain other problems

caused by the activation of μ (mu) receptor by beta endorphin such as severe respiratory depression accompanied by point-point pupils and constipation, effects that normally an athlete would not experience. Also, scientists were taking endorphin measurements of circulating blood and not directly from the central nervous system, thus those measurements cannot be considered to have a direct central effect, as they can also have to cross the blood brain barrier or just have a peripheral effect. Endogenous opiates could be involved in some effects of the runners high, but there is definitely another neurotransmitter that must be involved. Recent work in humans and some animal models indicate an important eCB role in the rewarding effect of exercise and a better neurobiological mechanism that could explain the runners high. Unlike endorphins, eCB can cross the blood brain barrier to have a central effect and when exercise is performed there is an activation during and after exercise that generates rewarding effects and similar psychological states as the runners high., for example, sedation, anxiolysis and sense of wellbeing [110]. In addition, eCB has a high expression in the brain, CB1 receptor is present in several brain regions; in this regard eCB could contribute to exercise-related changes in psychological state. Indeed, *Fuss et al.,* showed that wheel running increases endocannabinoid ligands and reduces anxiety and pain in mice through the activation of CB1 receptor on forebrain GABAergic neurons demonstrating for the first time that the actions of the runners high are dependent on cannabinoid receptor [111]. In contrast lack of CB1 receptor (by pharmacological or molecular approaches) reduces physical activity. Although reduction on physical activity can be due to many other factors, strong evidence suggests an eCB involvement in the motivation of voluntary exercise. In addition, Raichlen et al., used the antagonist rimonabant injections in rats to investigate operant responding to have access to a running wheel. Interestingly, rimonabant injections reduced operant responding supporting the hypothesis of an eCB role in the reinforcing properties of exercise [112].

Finally, *Zhao et al.,* showed that ABHD6–KO increased locomotor activity in a 24hr time period during the dark cycle and also showed enhanced energy expenditure on HFD fed mice, suggesting an increase in voluntary exercise due to ABHD6-KO. However, this approach was a whole-body deletion, thus the exact mechanism of how this enhancement is occurring still needs to be investigated [113]. The NAc plays a critical role in the motivation of voluntary running; in this regard deletion of ABHD6 in the NAc in a neuron-specific manner could be interesting to see if these results can be replicated and discover the physiological actions of this enzyme.

Growing evidence has pointed to the ECS to play an important role in behavioral responses such as mood and stress. The control of neurotransmitter release due to the eCB signalling in the limbic region seems to demonstrate a regulatory control over emotional behaviors [114]. Moreover, depressive and anxiety drugs are one of the top prescribed drugs. Just in the United States there are 40 million people that suffer from anxiety-like behaviors [93]. Cannabis was used as an anxiety-relief in the ancient cultures but how they provided these relaxation effects was not understood. Results from a new study suggested an increase of endocannabinoid signalling in the amygdala enhancing anxiety due to palatable food withdrawal [115], [116]. Disturbances of ECS by molecular and pharmaceutical approaches, is been shown to be involved in neuropsychiatric disorders such as depression and anxiety [83], [117]. Deficit in eCB signalling increase depressive and anxiogenic behaviors, while eCB signalling enhancement creates an opposite action [114] . CB1 antagonist receptor was developed to treat obesity as CB1 enhance appetite. This antagonist is rimonabant, and indeed was effective to decrease body weight as well as to block THC effects, however, some rimonabant-treated patients experienced anxiety problem that also lead to suicidal tendency [118], which was sufficient to withdrawal from the market.

Focusing on the enzyme ABHD6, *Zhao et al.,* demonstrated that a whole-body suppression of ABHD6 protects from diet-induced obesity in mice and reduces appetite and glucose intolerance, without any anxiety symptoms. They tested the mice in both elevated plus maze and open field tests to assets anxiety-like behaviors and no significant association was shown on treated ABHD6-KO mice compared to the control group. In addition, they wanted to prove that appetite suppression did not have any effect on depressive symptoms so they perform the Porsolt forced swim test showing again no significant difference in the KO and control groups [113].

In animal models there are plenty of studies trying to understand the eCB effects on anxiety and depression. Among other important function, the eCB in the NAc modulates mood behaviors and it has been shown that downregulation of eCB signalling in the NAc contribute to depressive-like behaviors elucidated by the chronic exposure to stressors [119]. The depletion of ABHD6 in the NAc has never been studied, therefore it will be very interesting to investigate the modulation of this enzyme in mood. More studies are needed to understand if ABHD6 invalidation in specific place such as the NAc could offer an anxiolytic effect in HFD fed mice.

The present study will focus on the role of ABHD6 in the regulation of body weight and food intake under a saturated HFD. Since the NAc is not only implicated in the control of food reward but also in the modulation of mood and exercise, we aim to determine whether

ABHD6 KO in the NAc (ABHD6$^{NAc\ KO}$) could attenuate diet induced-obesity, anxio-depressive behaviors, and voluntary physical activity, due to 2-AG increased levels in the GABA-ergic medium spiny neurons in the NAc that will inhibit the release of GABA (see *Figure 6*)

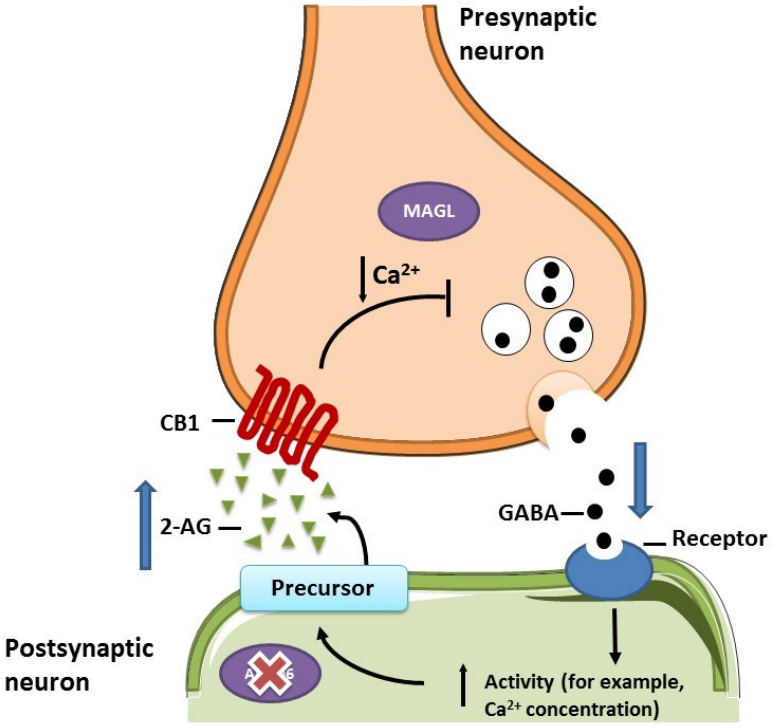


Figure 6: Mechanism of ABHD6$^{NAc\ KO}$ to increase 2-AG levels. ABHD6$^{NAc\ KO}$ enhance 2-AG synthesis that acts on CB1 receptor inhibiting GABA neurotransmission release (figure modified from Velasco et al., [4]).

1.3 OBJETIVE

The aims of this study are:

1. To identify the impact of NAc ABHD6 gene knockout on feeding, body weight, voluntary physical activity and mesolimbic dopamine functions in male mice.
2. To assess the function of NAc ABHD6 gene knockout in anxio-depressive behaviors in high fat fed mice.

1.4 HYPOTHESES

Taking together the discussed evidence, we hypothesize that NAc neuronal knockout of ABHD6 will:

- Have a protective effect against weight gain in mice fed with a high fat diet and possibly reduce food intake.
- Increase voluntary wheel running, increase in the amount of energy expenditure (heat production) and a decrease in fat mass compared to lean mass due to a higher locomotion activity.
- Have a positive effect alleviating anxiety-like and despair behaviors in elevated plus maze, open field test and forced swim test caused by HFD feeding.

2. Chapter 2: Methodology

2.1 Animals

ABHD6$^{lox/lox}$ mice were generated and backcrossed to C57BL/6N as previously described *Zhao et al., 2014* [120]. Adult male mice were selected for this study as differences in sexual dimorphism on metabolism were observed during pilot studies in our laboratory. A total of 76 adult male mice in between 12 and 16 weeks old were distributed on different experiments. Male mice were individually housed in order to measure food intake and body weight, and under reverse cycle (from 10 AM – 10 PM Dark cycle and from 10 PM – 10 AM light cycle). All cages were environmental controlled rooms (22ºC- 24ºC) with free access to control diet (chow) and water. All sacrifices were done during the dark cycle, under isoflurane anesthesia. Brain and plasma were collected and frozen under -80ºC for future analysis.

All protocols were approved by the CRCHUM animal care committee and were performed under the guidelines of the Canadian Council on Animal Care.

2.2 Stereotaxic surgery

ABHD6$^{lox/lox}$ mice in between 8-10 weeks old were placed in a mouse stereotaxic apparatus (Kopf, Tujunga, Ca, USA) and maintained on isoflurane (0.5 oxygen vs 1.5 isoflurane). None post-surgery treatment was required. We sought to have a flat skull based on bregma and lamba in a horizontal plane. Once we reached the flat skull we performed bilateral injections in the NAc using a neuroseringe (Hamilton company) and delivered 0.5microL of AAV2/1 vectors (AAV2/1.hSynap.HI.GFP.CreWPRE.SV40 or AAV2/1.hSynapsin.EGFP.bGH, 5.0 x 109 GC/uL, Penn Vector Core) encoding GFP or CRE recombinase (VMHKO or ARCKO, referred under the control of a neuron-specific synapsin promoter. The first injection was in the right NAc site (AP, +1.6 mm; ML, + 1.1 mm; DV, -4.1 mm) to delivered the correspondence virus, once the syringe was empty we waited 5 min to consolidate the neuron infection. The same procedure was done for the left NAc site (AP, +1.6 mm; ML, - 1.1 mm; DV, -4.1 mm), Figure 7. Two weeks recovery was given after surgery and body weight gain was measure to ensure complete recovery.

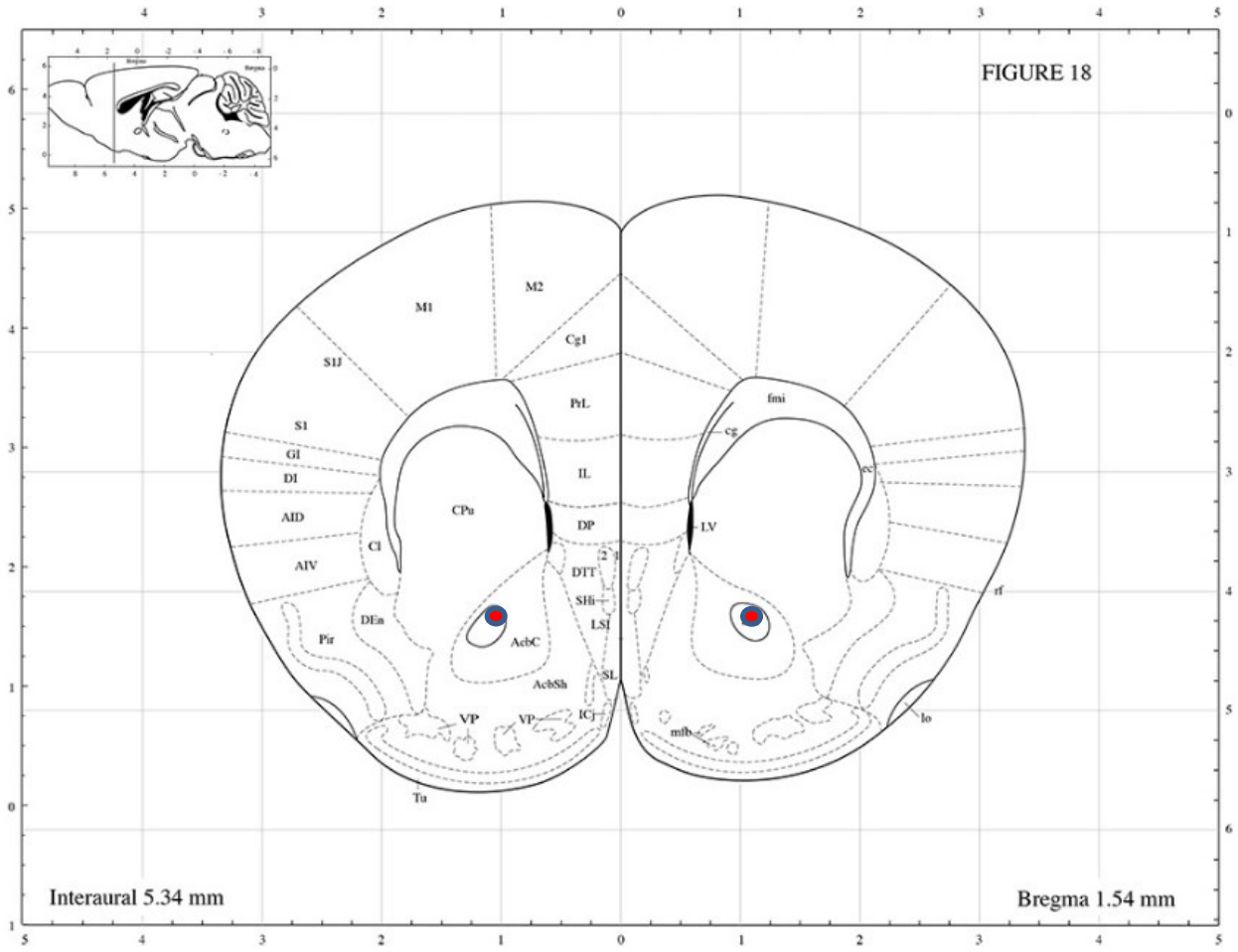


Figure 7: Mouse Brain atlas. NAc viral infection coordinates (red) for the ABHD6 Loss-of Function in neurons.

2.3 Diet composition

Animals were subjected to either 30 grams of palm oil saturated high-fat diet (PALM, see *Table1*) or control diet (chow, provided by the CRCHUM animal facility) each week for 10-12 weeks. Palm oil was chosen for the HFD due to its use in many processed foods. Food consumption and body weight were measured every week.

Table I. PALM diet composition (edited from *Decarie et al*) [78]

	Control	Palm
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
Vitamine 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

N/A: data not available

2.4 Metabolic assessment

2.4.1 Comprehensive Lab Animal Monitoring System ("CLAMS")

"CLAMS" is a system that facilitates measurements for feeding, drinking, body core temperature, energy expenditure (RER) and locomotor activity. Mice were placed in metabolic cages for a

period of 48 hours with free access to water and food (Accuscan Instruments Inc., Columbus, OH, USA). For the purpose of this study we only took measurements of body core temperature, RER and spontaneous locomotor activity. Distance travelled (horizontal activity) was measured by nearby computer-controlled software. The CLAMS is placed in the CRCHUM Rodent Metabolic Phenotyping.

2.4.2 ECHO-MRI

After 12 weeks on HFD we looked at the body composition of the animals (n= 10 per group) with the Echo-MRI located at Metabolic Phenotyping Core and Metabolomics Core of CRCHUM. This analysis consisted in measuring whole body fat and lean mass.

2.5 Voluntary Wheel Running

Each mouse had access to their own wheel in their home cages. Voluntary wheel running was measured in ABHD6 KO mice and controls. As it was discussed before, all animals were individual housed, therefore, we added a running wheel in their home cages. All running wheels used a wireless system to transmit or collect the data to the hub as long as they are connected to battery power and in the same channel (ENV-044, MED associates, INC). The sensors transmit data approximately every 30 seconds or 0.5 minutes. The messages from the running wheels contain the total number of wheel rotations (revolutions). See *Figure 8* for specific dimensions.

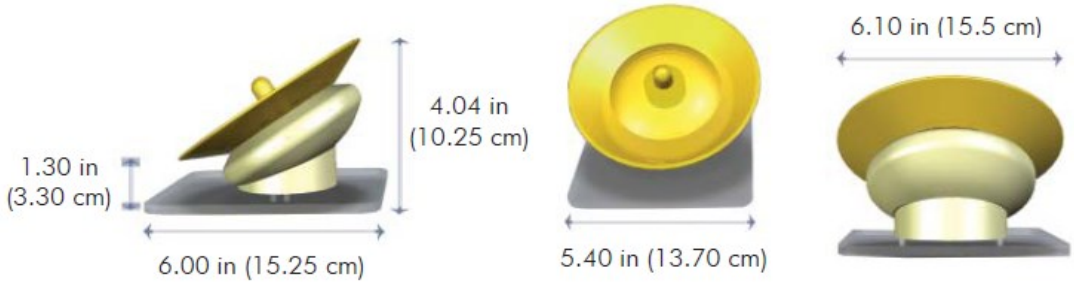


Figure 8: running wheel dimensions [121]

2.6 Locomotor activity measurements

2.6.1 Amphetamine locomotor sensitization

Amphetamine sensitization was performed as previously described in our laboratory [58] in order to compare the effects of different dosage amphetamine exposure on sensitivity and evaluate changes in locomotor activity induced by the increase of dopamine release. All mice (n= 10 per group) were naïve-drug mice that were first habituated to the metabolic chambers for 12 hours. After the habituation time, each mouse received a total of three IP injections with a separation of 2 days. More precisely, the first injection was 1 mg/kg of amphetamine (low dose) following 2 days of rest, then the second injection was 4 mg/kg of amphetamine (high dose) following of 2 day of rest and finally third injection 1 mg/kg amphetamine (repeat low dose). D-amphetamine (Sigma-Aldrich, Dorset, UK) was dissolved in 0.9% saline (vehicle of control group). Locomotor activity was recorded and analyzed for 2 hours after each injection. Area under the curve was calculated and used to compare locomotion differences between groups.

2.6.2 D1 receptor-induced locomotor stimulation

The same mice that were used for the amphetamine sensitization were used to follow with this experiment. We gave 2 weeks of recovery after amphetamine sensitization to continue with D1R agonist injection. Again, mice were habituated to CLAMS for 12 hours before collecting the final data. Treated and control mice (n=10 per group) were administrated 0.1mg/kg intraperitoneal (IP) injection of the D1R agonist SKF82958 during the light phase and then they were placed back in the metabolic chambers. Locomotor activity analysis was measured for the first 3 hours after IP injection by computer-controlled software. Dependent variable: locomotor activity (beam breaks). D1R agonist SKF82958 solution was freshly prepared and dissolved in 0.9% saline [122].

2.7 Anxio-depressive behaviors

2.7.1 Elevated plus maze test

Elevated Plus Maze (EPM) is a test widely used to test anxiety behaviors in laboratory animals. The model is based on the natural aversion to high and open spaces and their exploratory behaviors [123]. It has a cross form composed by two open arms and two closed arms (see *Figure 9* for more details). Anxiogenic animals will spend more time in the closed arms rather than the open arms.

Anxiety-like behavior was assessed in mice after 8-12 weeks on high fat diet, using an EPM at 330 LUX in the center of the maze. A video camera was used to recorded mice movement in the maze for 5 min and the analysis was made using a tracking software (Ethovision XT, Med Associates Inc., St. Albans, VT, USA) as is has been described by Sharma et al., [124]. Time spent in open arm, closed arm and in the center portion of the maze was converted into % to compare the groups. All behavior tests were performed during the dark cycle (active phase).

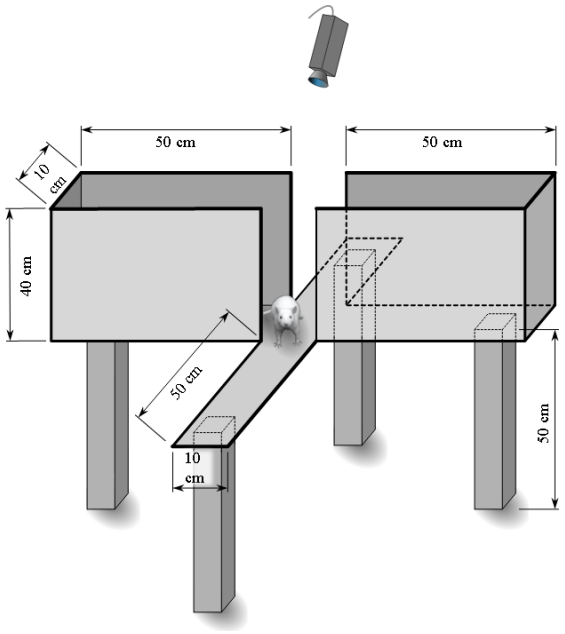


Figure 9: Elevated plus maze scheme and dimensions

2.7.2 Open Field Test

The Open Field Test (OFT) is another well-known test that measure anxiety-like behaviors and locomotor activity in rodents by the willingness to explore a novel place [125] (91). It consists in a rectangular box (see *Figure 10*) with dimensions of 50 x 50x 30 cm that is placed in a bright room. Each mouse is placed in the corner of the box and then is allowed to explore the field for 5 min. The less anxious animals will tend to go to the middle of the box instead of staying in the periphery. Animal exploration was recorded and tracked by a video camera and analyzed by Ethovision XT software (Med Associates, Inc., St Albans, VT, USA). Dependent variables: entries to center and time spent in center.

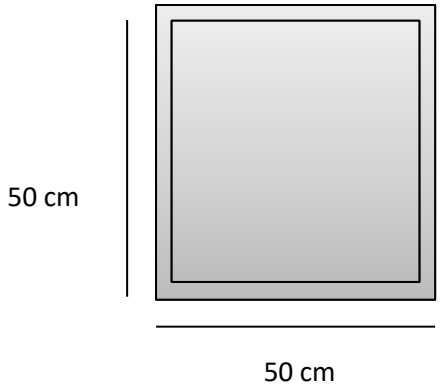


Figure 10: Open Field Test scheme and dimensions

2.7.3 Forced Swim Test

The Forced Swim Test (FST) is one of the most used assessments to screen or validate antidepressants. The protocol consists in a transparent tank (height, 15 cm; diameter, 12 cm) filled with water (23 °C) in which mice are forced to swim. This test measures "behavioral despair" which is indicated by an augmentation of immobility and demotivation to escape from the tank. Mice were placed in the tank for 6 min; the first 2 min were considered as habituation time and the last 4 min were used to calculate immobility and velocity as indicators of behavioral despair. All trials were recorded with a video camera an analyzed with the EthoVision software (Med Associates, Inc., St Albans, VT, USA). Dependent variables: immobile time and velocity.

2.8 Molecular assessments

2.8.1 Real-Time quantitative PCR (q-PCR) for ABHD6 depletion

To validate ABHD6 KO q-PCR were performed in all experimental animals (n=76) by first cutting 300 µM of frozen brain sections to obtain specific biopsy punches (1.5mm diameter) at the NAc.

Collected punches were processed to mRNA extraction which consists in homogenize the punches with TRIzol method (Invitrogen). Afterwards, we quantified RNA concentrations using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). We next proceeded to synthetized cDNA from 1000ng of total RNA using M-MLV Reverse Transcriptase (Invitrogen) and random

hexamers. Then, we diluted the cDNA 1:10 to perform q-PCR using SYBR Green PCR kit (Qiagen) according to the specific guideline on the Corbett Rotor-Gene.

Specific primers sequences were used to performed q-PCR to quantify and analyze the percentage of ABHD6 invalidation.

Table II. Primer sequences used for qPCR

Gene	Sequences
ABHD6	F: AGACCAGGTGCTTGATGT R: CTCTCCATCACTACCGAAT
β-Actin	F :TTCTTGGGTATGGAATCCTGTGGCA R: ACCAGACAGCACTGTGTTGGCATA

Relative gene expression was calculated using the ΔΔCT method using actin as the housekeeping gene.

2.9 Statistical Analysis

Results are expressed as mean ± SEM. Groups were compared using either a one-way or two-way ANOVA with Bonferroni post hoc tests or t-tests as indicated, using Prism 5.0 software (GraphPad). Statistical significance was set as $p<0.05$, where *p <0.05, **p <0.01, and ***p <0.001. Outliers were checked in each graph using the same software (GraphPad) and taken out if *p>0.05.

3. Chapter 3: Results

Mouse intra-cranial surgeries for data shown in *Figure 12* were carried out by Alexandre Fisette. I contributed to the measures of body weight and food intake and we collaborated and worked together to evaluate metabolic rates in those animals in the CLAMS and Echo MRI. I carried out surgeries from results shown from *Figure 14 to Figure 19*.

3.1 Relative mRNA expression of ABHD6 in the NAc

Previous results from our laboratory showed the specificity of the neuronal infection (*Appendix A*). In addition, lipidomic LC/MS (liquid chromatography-mass spectrometry) done by the metabolomics platform at the CRCHUM showed that ABHD6 KO in the NAc ($ABHD6^{NAc\ KO}$) increase 2-AG levels (*Appendix B*). In *Figure 11A* position of stereotaxic injection it is shown from three different animals to demonstrate the NAc viral infection. Moreover, in *Figure 11B* it is shown the percentage of mRNA ABHD6 depletion in CRE HFD mice (40%) compared to their controls GFP HFD and GFP CHOW ($p=0,0177$; $F_{2,21}=4,922$) of the present experiments. Based upon the overall data we choose to eliminate mice that had less than 20% of ABHD6 KO as considered that those mice had an un-successful surgery that did not target the correct coordinates or the virus did not work.

3.2 Neuronal ABHD6 depletion in the NAc protects against diet-induced obesity and metabolic changes resulting from high fat diet.

We found in *Figure 12A* that neuron-specific ABHD6 invalidation in the nucleus accumbens (CRE) produced a significant difference ($P=0,0011$; $F_{1,11}=19,17$) in body weight gain being lower compared to control group (GFP) after 8 weeks on HFD. This result was not caused by a difference in food consumption (*Figure 12B*), as no significant difference ($P=0,3843$; $F_{1,11}=0,4745$) in food consumption was seen between the CRE and GFP groups during the 8 weeks. Taking these results, we wanted to investigate what was the cause of the body weight difference on these groups Therefore, we measured metabolic rates in the CLAMS metabolic chambers for 3 days. Interestingly, we found a higher energy expenditure within the CRE group compared to GFP

(P=0,0468; t=2.267 df=10) in *Figure 12C*; however, this difference was seen only during the dark phase. These measurements were also accompanied by the amount of ambulatory activity described in *Figure 12D,* where again a significant increase in ambulatory activity was seen in the CRE group during the dark phase (P=0,0194; t=2.781 df=10), accompanied by a slightly higher, but not significant, tendency during the light phase compared with the GFP group. Finally, we used Echo MRI to address changes in body composition caused by NAc ABHD6 depletion. There was no significant difference (P=0,2687; t=1.165 df=11) in fat mass between the groups (*Figure 12E*), in contrast, there was a significant decrease in lean mass in the CRE group compared to the GFP group (*Figure 12F*) (P=0,0375; t=2.365 df=11).

3.3 Neuronal ABHD6 depletion in the NAc rescues voluntary physical inactivity phenotype elicited by HFD feeding.

After Echo MRI measurements, we introduced running wheels on individual housed mice for 5 weeks to see differences on wheel running performance between both groups. As shown in *Figure 13A*, there was a striking difference in voluntary wheel running in the CRE group compared to the GFP group (P=0,0353; $F_{1,11}$=5,754). This relationship can similarly be appreciated from *Figure 13B,* where total running rotations during the 5 weeks is shown (P=0.0353; t=2.399; df=11). Differences on body weight during running performance are seen (*Figure 13C*) on CRE mice due to a higher running performance (P=0, 0016; $F_{1,11}$=10,24). In contrast, GFP mice did not show the same decrease in body weight. No differences on food consumption were seen during wheel running access time (*Figure 13D*) (P=0,2111; $F_{1,11}$=1,028).

3.4 Neuronal ABHD6$^{NAc\ KO}$ does not impact voluntary wheel running on chow diet, however, neuronal ABHD6 depletion in the NAc prevents rebound caused by HFD feeding.

Because of the surprising running difference between CRE and GFP mice shown in *Figure 13A and B*, we were wondering if the effect was due to the ABHD6 KO viral infection or due to high-fat inactivity related factor. In this regard, we did surgery in a new cohort of mice (CRE=9, GFP=9) to evaluate voluntary wheel running on chow fed conditions (instead of HFD) during three weeks. In three weeks running wheel Access, no significant difference was shown between both groups on running performance (*Figure 14A&B*) (P=0,9357; $F_{1,36}$= 7,735), body weight changes (*Figure 14C*) (P= 0,5764; $F_{1,12}$= 0,5021) and food intake (*Figure 14D*) (P= 0,4698; F 1,12= 0,9527).

Therefore, we decided to withdrawn the running wheels from their home cages and give high-fat diet to provoke diet-induced body weight gain for 3 weeks, and then give again access to the wheel running to see if we could replicate the running wheel activity effects seen in *Figure 13*. In *Figure 15*A it is shown how during the 3 weeks on HFD and without the running wheels the GFP and CRE groups increase in body weight, however this increase is higher in the GFP group, giving a significant increase in body weight compared to CRE group at three weeks on HFD (P=0, 0063; $F_{1,12}$=1,231). Moreover, this increase on body weight was stopped in the fourth week by the re-exposure of wheel running in their individual home cages, dropping and normalizing their body weight in both groups, despite continued access to high-fat diet. No difference in food consumption (P=0,3639; $F_{1,12}$=0,01569) during HFD feeding was seen between the groups (*Figure 15B*) and no significant difference (P= 0,7088; $F_{1,12}$= 0,03145) was shown on running wheel rotation HFD fed mice (*Figure 15 C&D*).

3.5 Neuronal ABHD6$^{NAc\ KO}$ increased locomotor-stimulating effects caused by amphetamine; however, these effects are not related to a higher activation on dopamine D1 receptor.

The next step was to measure the response of mesolimbic dopamine functions with ABHD6 KO. In *Figure 16A* it's shown that, as expected, the higher dose injection of amphetamine resulted in an increased locomotor response. CRE group resulted in higher locomotor sensitization (P=0, 0455; t=2.212; df=13) in response to amphetamine compared to control GFP group (*Figure 16B&C*). These results were analyzed by taking the second area under the curve (AUC) low dose data and subtracting the first AUC low dose data. We next assessed the locomotor-stimulating effects of dopamine D1 receptor agonist but they did not observe any significant effect in either group (*Figure 16D&E*) (P= 0,2638; t=1.156 df=13).

3.6 Depletion of ABHD6 from neurons in the NAc attenuates HFD-induced anxio-depressive behaviors.

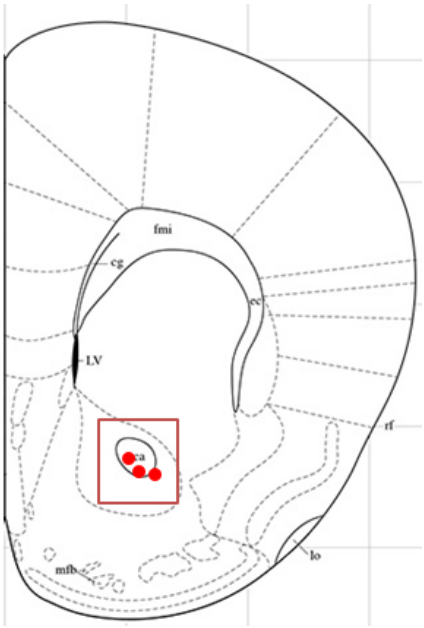
We wanted to assess if ABHD6 KO in the NAc could prevent from anxio-depressive behaviors provoked by HFD. After 2 weeks of surgical recovery, we gave free HFD access for 12 weeks to treated CRE and GFP mice, and also, we add another GFP group but on chow feeding for a control group. Percentage of body weight gain was measured each week (*Figure 17A*), where a

significant difference was seen between the HFD groups and the control CHOW (P=0,0079; $F_{48,24}$=1.603), but no interaction between the GFP HFD and CRE HFD. Cumulative food intake was also measured (*Figure 17B*) and converted to kcal (P=0,39; $F_{48,24}$=7.657).

Depletion of ABHD6 had an impact on the animal's behavior after 12 weeks on HFD. We started with the elevated plus maze (EPM) test to evaluate differences in anxiety-like behaviors. In the EPM test, less anxious animal will tend to spend more time in the open arms of the maze rather than the closed arms. In *Figure 18A* shows the total distance travelled in the EPM test, with no significant difference between the groups (P=0.9645). *Figure 18B* illustrates that the GFP HFD group had a significantly increased frequency of entries (P= 0, 0092; $f_{2,21}$=5.911) and time spent in the open arms of the EPM compared to the GFP CHOW (control) and CRE HFD (P=0.0282; $f_{2,21}$=4.248). In addition, CRE HFD showed a significantly decrease in the amount of time spent in the open arms compared to GFP CHOW and GFP HFD. Later the same day mice were tested on the open field test (OFT) to further examine anxiety-like behavior, where less anxious mice will tend to explore more and spend more time in the center rather than in the periphery of the box. As shown in *Figure 18D*, no significant difference was seen in the distance travelled comparing the three groups in the OFT (P=0.3310; $F_{2,21}$=1.66). In the OFT no significant difference was seen in the total entries to the center (P=0,3604; $f_{2,20}$=1.074) or the time spent in the center of the field (P= 0,3612; $F_{2,21}$=1.069) between the control group and the GFP and CRE HFD mice (*Figure 18E&F*).

Furthermore, we were interested in evaluating whether the lack of ABHD6 in the NAc could have a positive effect on depressive-like behaviors resulting from HFD feeding. The day after the anxiety test, we performed the forced swim test (FST) on the same groups of mice. The FST lasted 6 min in total, however, analysis only included the last 4 min, as the first 2 min are for habituation. As shown in *Figure 19A*, the time spent immobile is significantly increased in the GFP HFD compared to GFP CHOW (P=0,0106; $F_{2,21}$=5.685). This suggests that CRE HFD behaves similar to control GFP CHOW in the time spent immobile during the FST. *Figure 19B* shows no difference in swim velocity during the test between the groups (P=0,2858; $F_{2,21}$= =1,330).

A)



B)

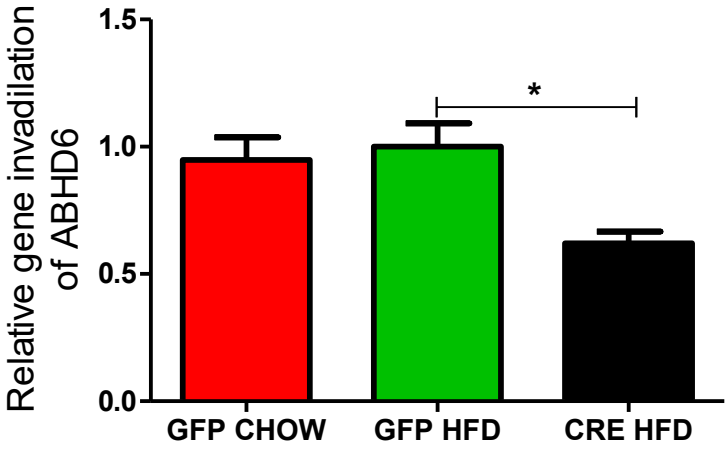


Figure 11: Validation of ABHD6 knockout in the NAc. A) Analysis of proper injections after surgeries, the three injection coordinates were obtained from three different animals. B) Amplification of ABHD6 mRNA of the three groups of mice was done by q-PCR in real time. The CRE HFD-treated mice showed a significant 40% depletion compared to the GFP CHOW and GFP HFD control groups (n=6-9). Group mean ± SEM; one-way analysis of variance, Bonferroni post hoc *<0.01

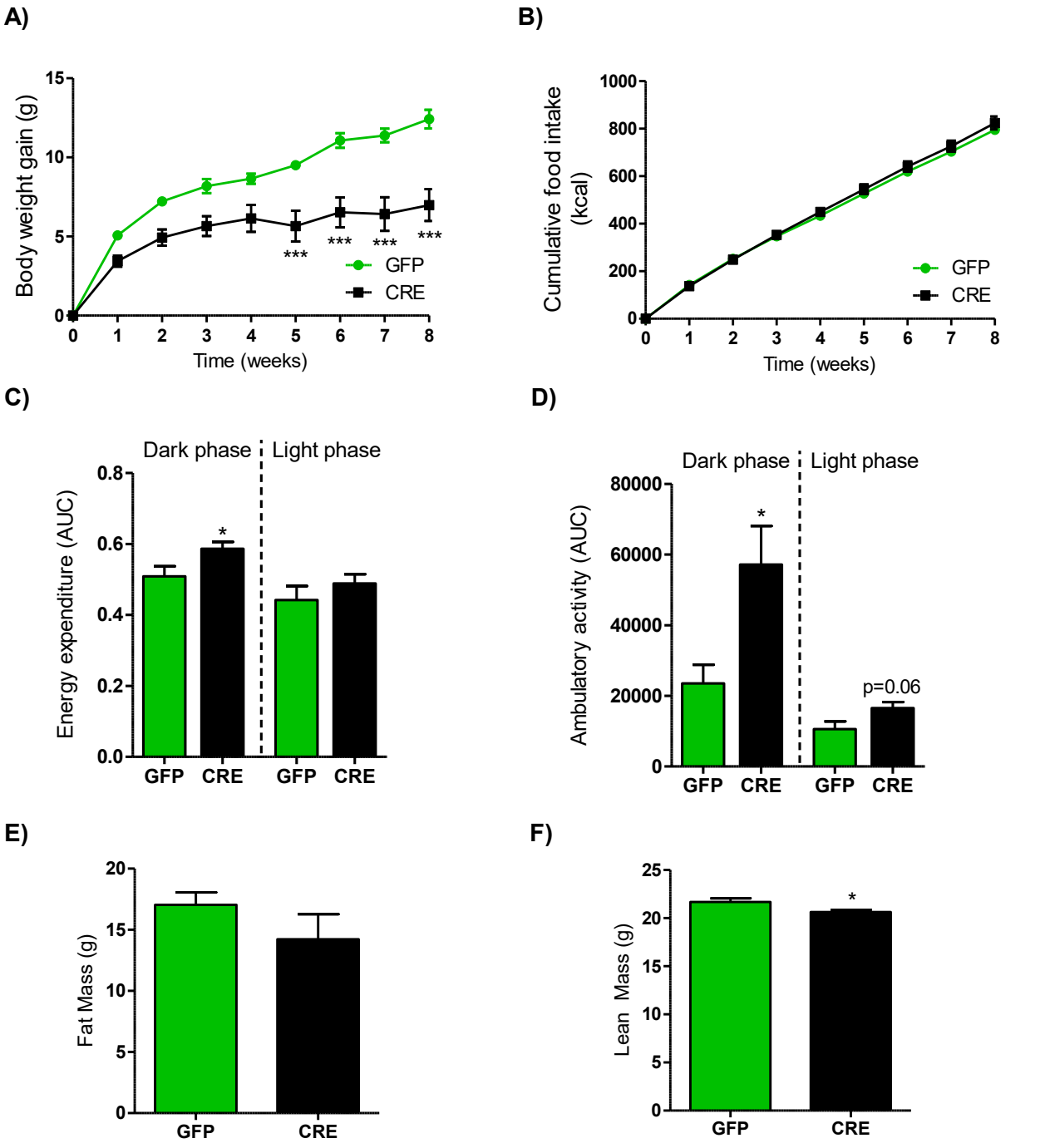


Figure 12: ABHD6$^{NAc\ KO}$ diminishes diet-induced obesity and metabolic changes resulting from high fat diet. A) Treated mice CRE (n=7) showed a reduction in body weight gain after 8 weeks on HFD compared to the control GFP (n=6) and B) have no difference on food consumption. CRE mice C) have an increase in energy expenditure during the dark phase and D) an increase in ambulatory activity. E&F) Echo MRI measurements showing no significant difference between groups in fat mass, however there is a significant difference in lean mass on CRE mice compared to GFP. Group mean ± SEM, P*<0.05, P***<0.001

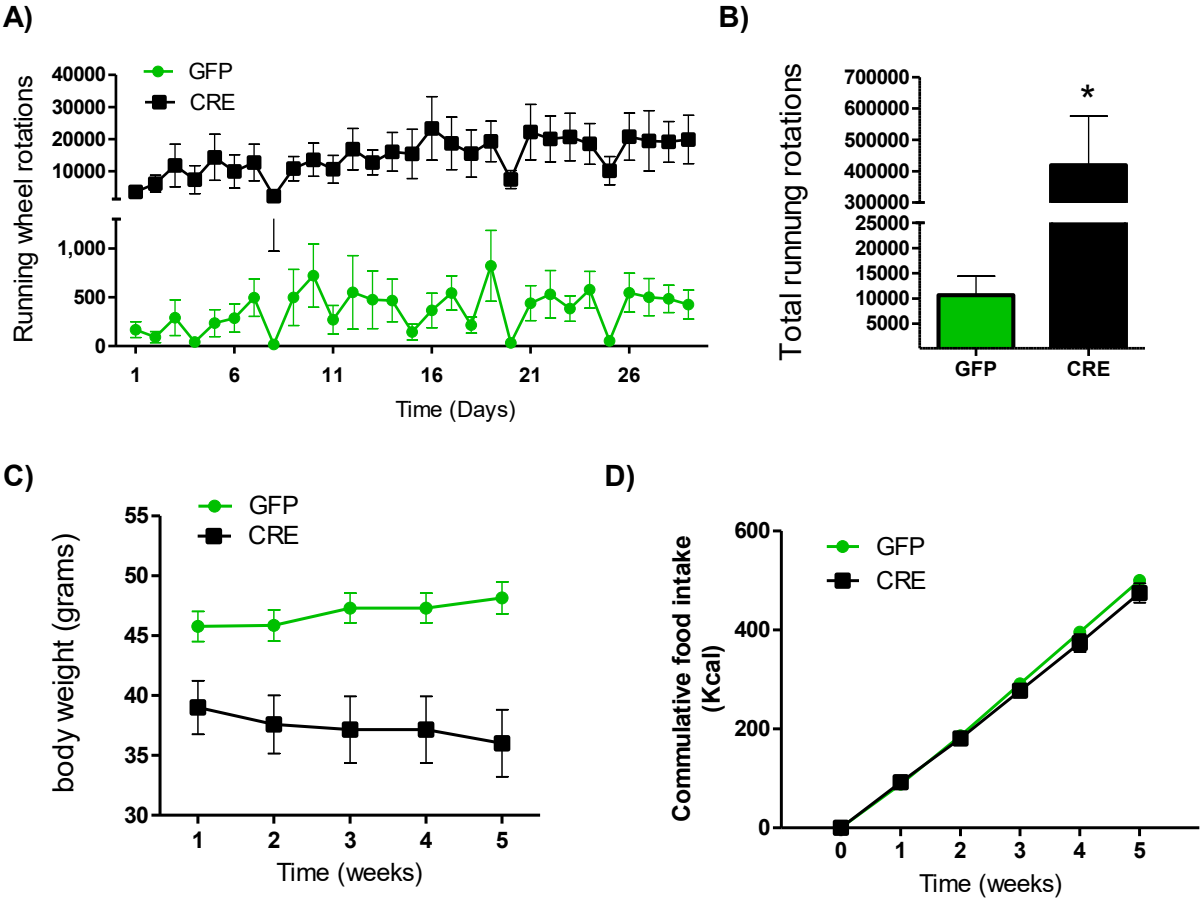


Figure 13: ABHD6$^{NAc\ KO}$ rescues voluntary physical inactivity elicited by HFD feeding. A) Running wheel performance during 5 weeks B) Significant increase in total running rotations during 5 weeks of voluntary physical activity on CRE (n=7) compared to GFP (n=6). C&D) body weight gain and food consumption during the running performance on HFD. Group mean ± SEM; two-way analysis of variance, Bonferroni post hoc P*<0.05

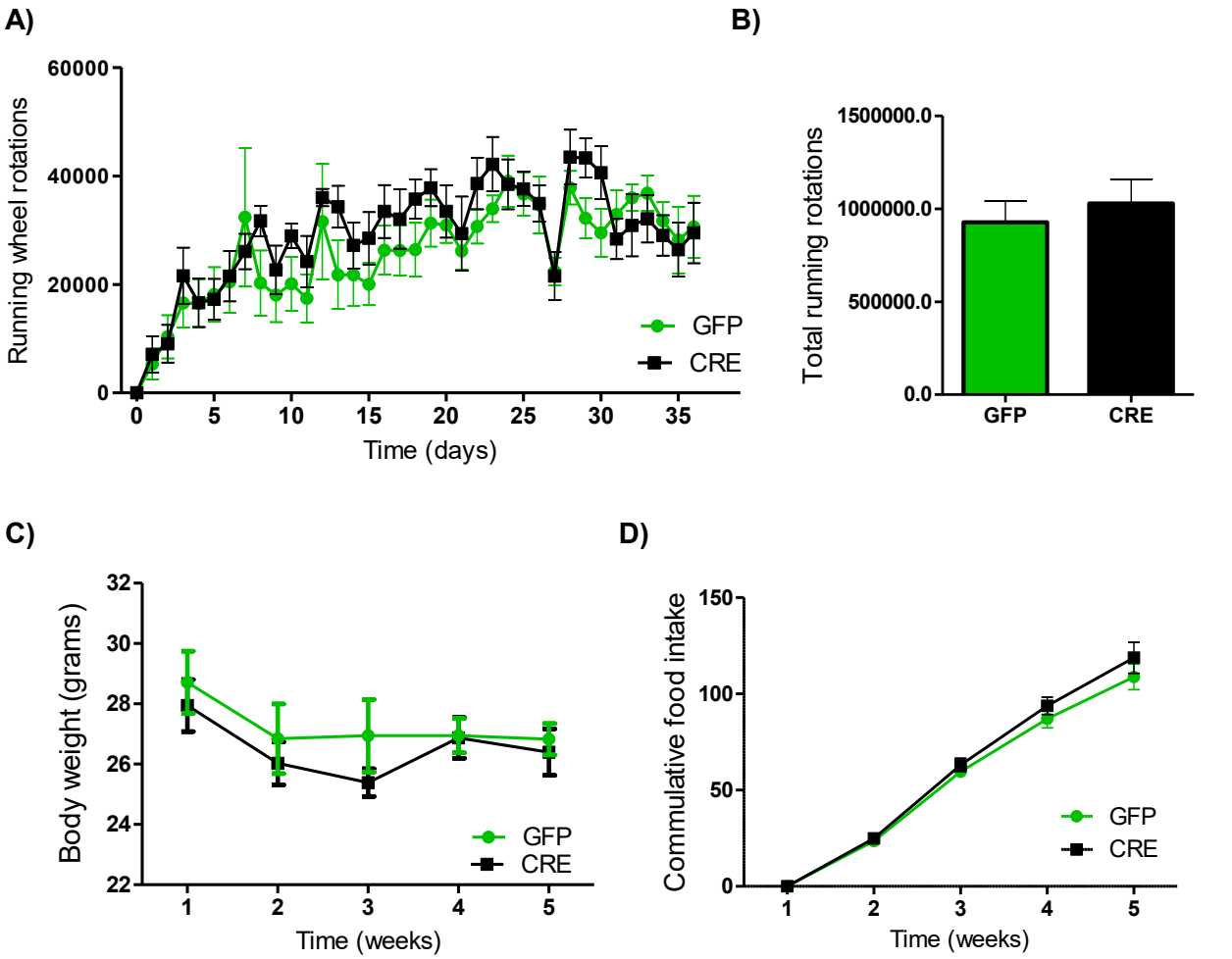


Figure 14. Neuronal ABHD6$^{NAc\ KO}$ does not increase running performance on chow fed conditions compared to GFP mice. A) Running wheel rotation during 5 weeks on chow feeding (control diet) CRE (n=8) vs. GFP (n=8) B) Total cumulative running rotations, C) body weight gain during wheel running and D) cumulative food intake. No significant difference between the groups in any measurement.

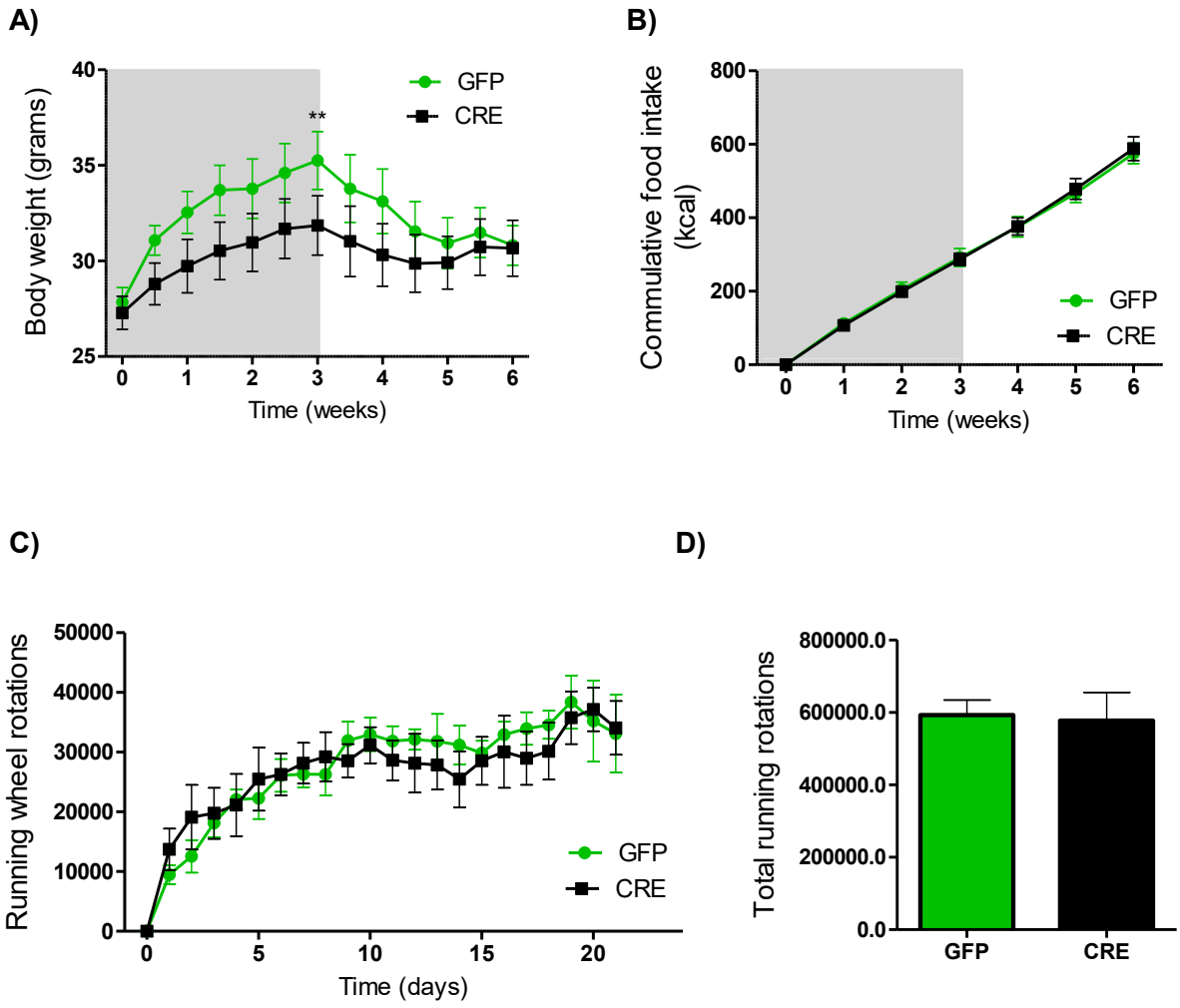


Figure 15: ABHD6$^{NAC\ KO}$ prevents from rebound of HFD feeding but no voluntary wheel running difference was seen during the 3 weeks of wheel access on HFD feeding. A) Body weight gain CRE (n=8) vs. GFP (n=8) on HFD following withdrawal of running wheels (grey block) and re-introduction of running wheel access (white block) while on HFD. B) HFD food intake during the same period. C) Running wheel re-exposure on HFD feeding D) Total running rotations. Group mean ± SEM; two-way ANOVA analysis of variance P**<0.01

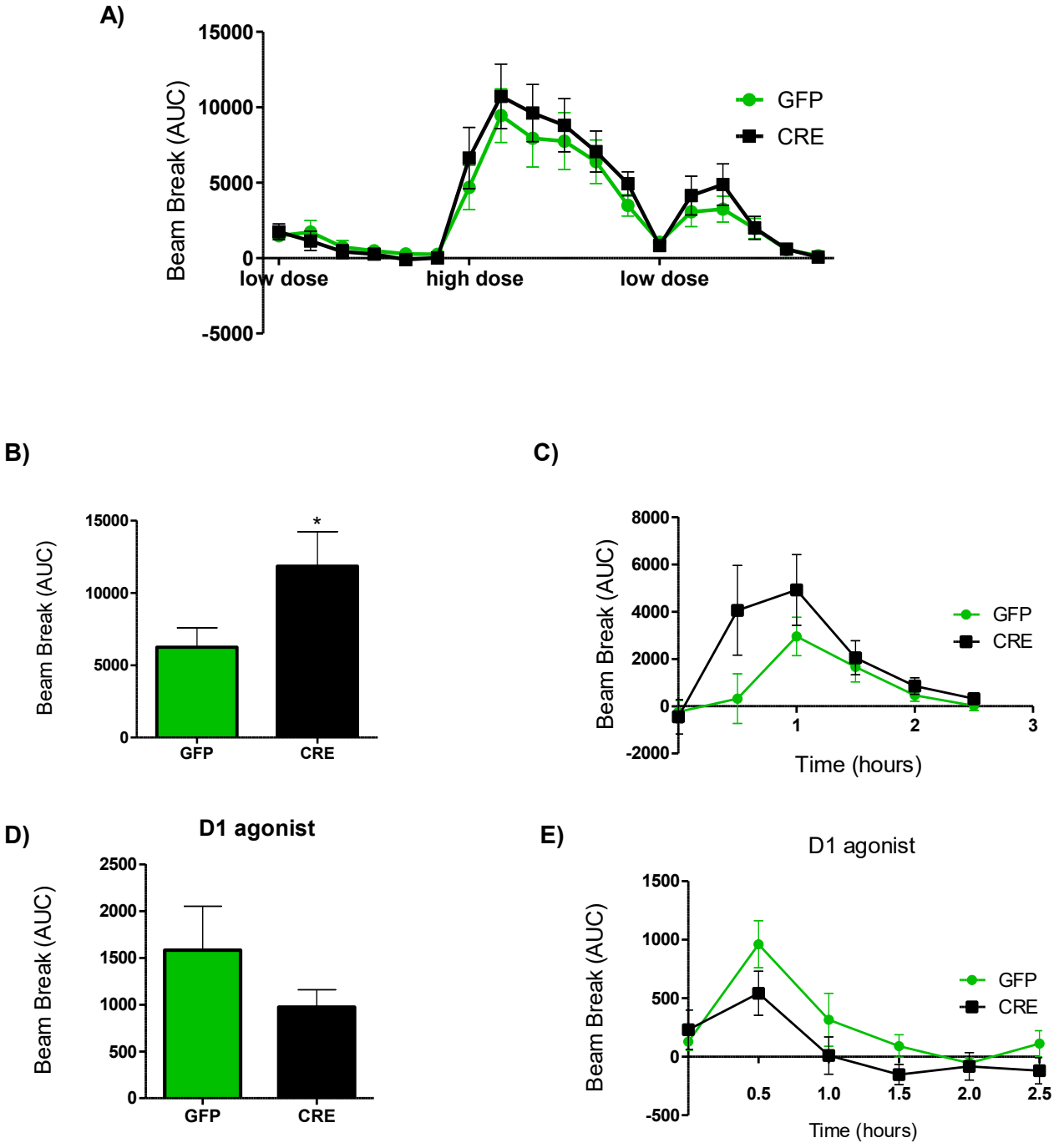


Figure 16. Neuronal ABHD6$^{NAc\ KO}$ increased locomotor-stimulating effects of amphetamine but not D1R-dependent locomotion. A) Overall amphetamine sensitization induction. B) Total Locomotion activity obtained by the subtraction of the second low dose from the first low dose CRE vs. GFP (n=6-9) C) Beam brakes data obtained during the effect time of the amphetamine injection D) Total locomotor activity measured in beam breaks from D1 agonist injection and E) Locomotor activity during the D1 effect. Group mean ± SEM, unpaired t test *<0.05

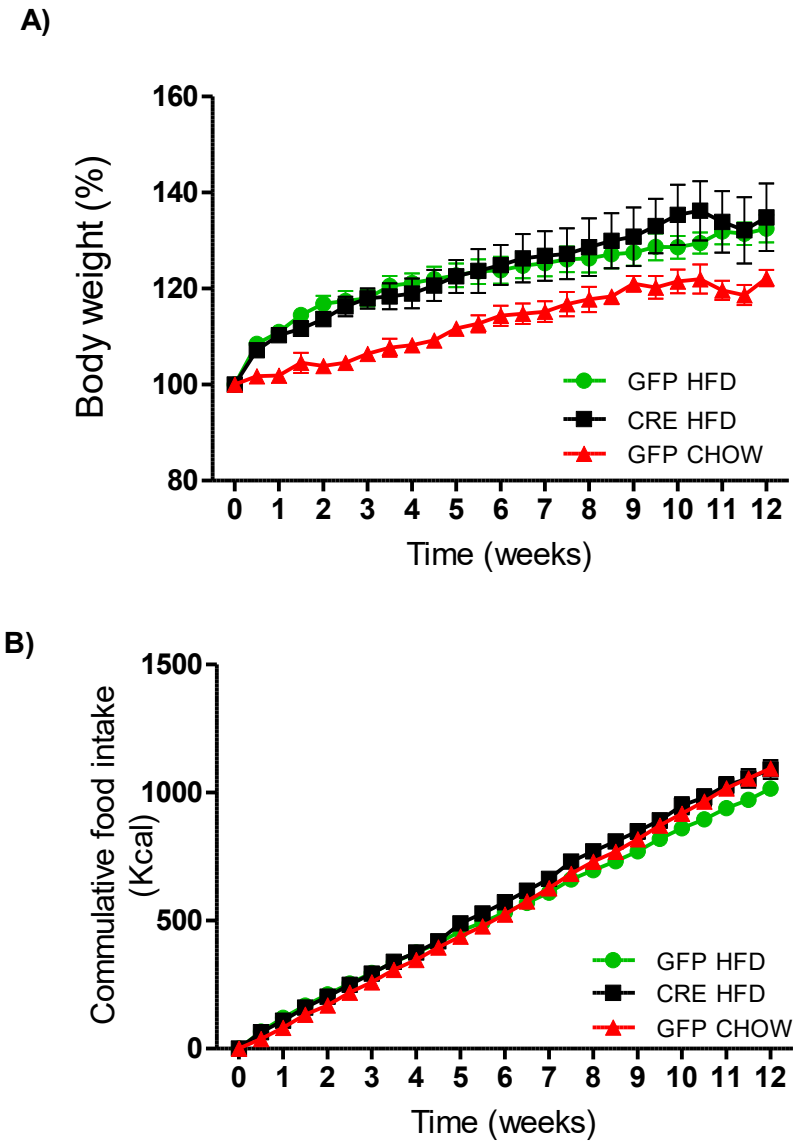


Figure 17. Impact of ABHD6$^{NAc\ KO}$ in neurons on body weight and food intake on a high-fat diet. A) Percentage of body weight gain during 12 weeks on HFD or CHOW, B) Cumulative food intake during 12 weeks on HFD or CHOW. Group mean ± SEM; two-way analysis of variance, Bonferroni post hoc (n=6-9).

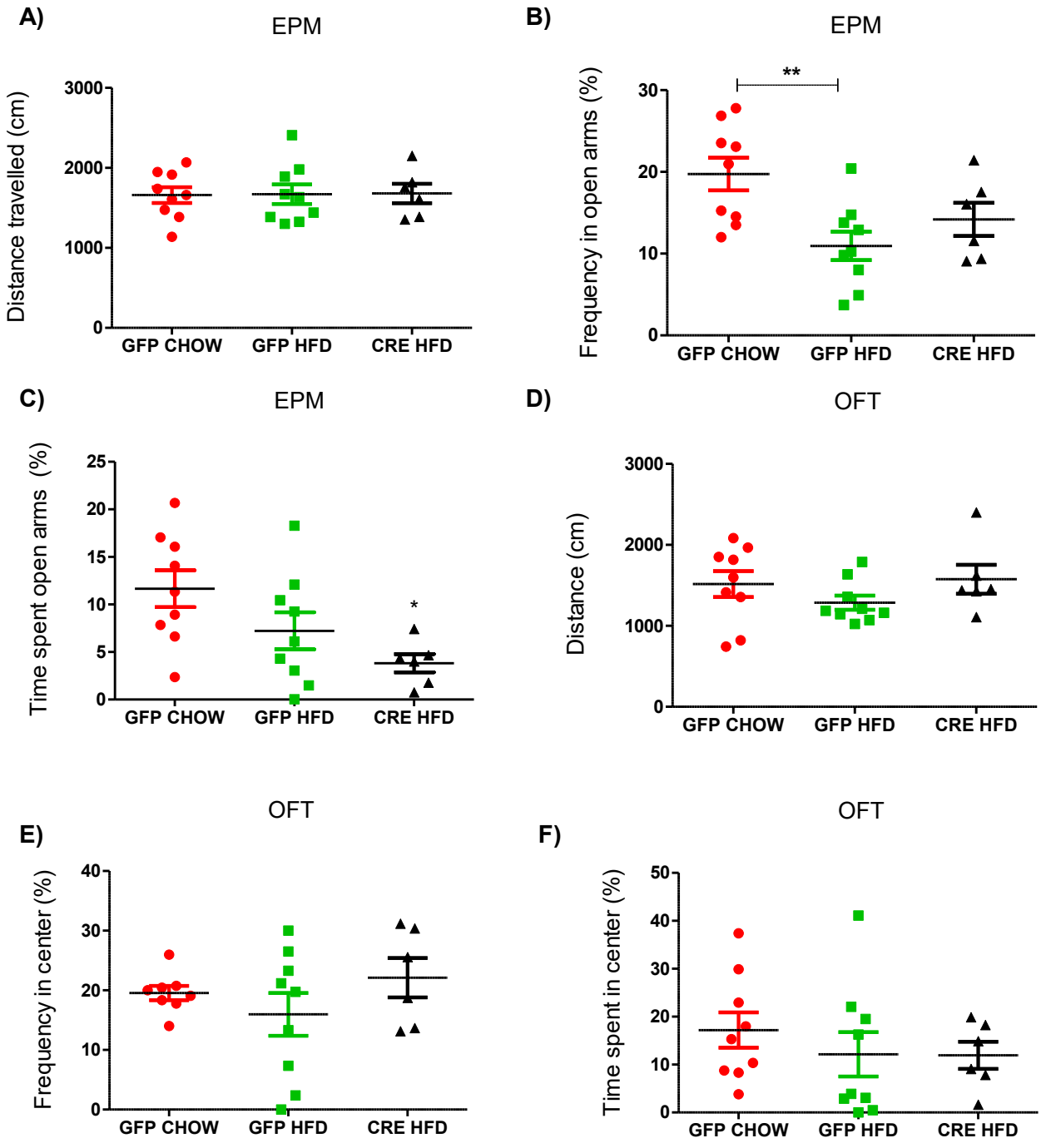


Figure 18. ABHD6$^{NAc\ KO}$ does not increase anxiety-like behaviors caused by HFD feeding.
A) Total distance travelled during the EPM test. B&C) Number of entries and time spent in the elevated plus maze (n= 6-9). D) Total distance travelled during the OFT. E&F) Number of entries and time spent in the center of the open field test. Group mean ± SEM; one-way analysis of variance, Bonferroni post hoc *<0.05 **<0.01

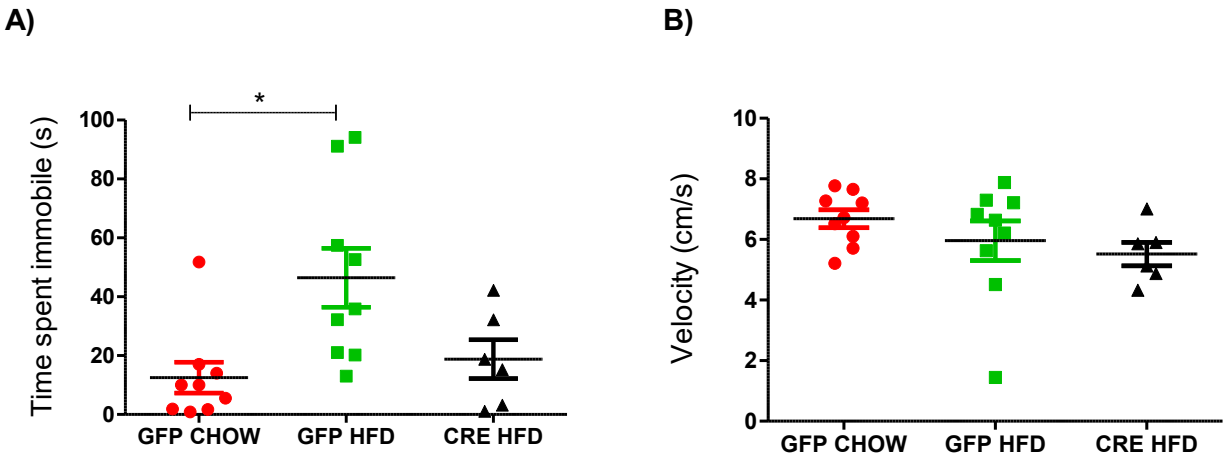


Figure 19. Depletion of neuronal ABHD6 in the NAc does not affect depressive-like behaviors elicited by HFD feeding. A) Time spent immobile during the last 4 min in the forced swim test (n=6-9) GFP CHOW vs. GFP HFD and B) Swim velocity from each group since the test started. Group mean ± SEM; one-way analysis of variance, Bonferroni post hoc. P*<0.05

4. Chapter 4: Discussion

4.1 Effects of ABHD6 loss-of-function within the corticolimbic system on metabolism and physical activity

Obesity is one of the biggest problems in the world that scientists have been trying to decrease in the last years. Dietary and sedentary lifestyle are key influencers modulating energy balance; in addition, recent data show changes in brain function that can contribute to the obese population. The endocannabinoid system has been shown to have an important impact on regulating body homeostasis [113], [126], [127] and there are more studies investigating potential pharmaceutical targets to reduce obesity and the associated health risks every year. This study shows that specific deletion of ABHD6 in a particular brain region, such as the nucleus accumbens (NAc), has a positive impact on body weight and running endurance on HFD fed mice.

Several brain regions are implicated in the regulation of feeding and physical activity. Among them, the NAc has been studied in the control of these factors since it has excitatory inputs that comes from VTA dopamine neurons and some cortical and limbic structures [128], [129]. Chronic consumption of palatable food modifies DA signaling within the reward system along an up-regulation of orexigenic signals and miscommunication to important satiety signals such as leptin and cholecystokinin [130]. Small signaling changes such as the deletion of ABHD6 enzyme could restore the signaling output to other brain areas, and modify the metabolic actions. Endocannabinoid modulation in GABAergic medium spiny neurons (MSNs) of the NAc involves 2-AG. 2-AG levels and its accumulation are modulated principally by two enzymes: MAGL, which is localized in the presynaptic neuron to degrade 2-AG, and ABHD6, localized postsynaptically to act as a gatekeeper of 2-AG levels at the site of 2-AG synthesis. In this regard, ABHD6 regulates neuronal production of 2-AG to modulate its signaling in pathophysiological conditions such as multiples sclerosis, chronic pain, epilepsy, etc [131]. ABHD6 loss-of-function can fine-tune 2-AG accumulation and, consequently, influence signaling actions of 2-AG at presynaptic CB1Rs to reduce GABA transmission, having a positive impact in catabolic actions.

The present study evaluated the ABHD6 neuronal loss of function in the NAc in control of body weight, food intake, locomotor activity and anxio-depressive behaviors. Treated CRE mice showed 40% of ABHD6 genomic depletion in the NAc compared to our control GFP mice; in fact, our study accomplished a larger ABHD6 invalidation (~15% more) than a similar study were the

KO was done on the VMH [3]. In addition, we have demonstrated that ABHD6$^{NAC\ KO}$ increases 2-AG levels (*Appendix B*), therefore the phenotype seen in this study can be confirmed by the depletion of ABHD6 gene expression in the NAc.

In the first cohort of mice, partial deletion of neuronal ABHD6 in the NAc resulted in a reduced body weight gain after 8 weeks on HFD compared to the GFP control mice, and was not related to the amount of food intake during the same period. *Soria-Gómez et al.,* showed that 2-AG enhancement in the NAc increases food intake [132], however, they sought to target the shell of the NAc structure, whereas in this study we aim to target the NAc core. Although there is a possibility to spill a little bit of the virus to the shell; differences in phenotype can be explained by the target structure core vs shell. However, it is hard to speculate this assumption as we did not have histological sections to prove the viral infection boundaries in the NAc. Even more, as we did not see differences in food intake, these data suggested metabolic differences between the groups, therefore we evaluated other important factors such as energy expenditure and ambulatory activity. The evaluation of energy expenditure and ambulatory activity in the CLAMS metabolic cages resulted in higher energy expenditure and a higher ambulatory activity during the dark cycle in the CRE mice compared to GFP mice. These results not only showed that depletion of ABHD6 in the NAc prevents from HFD induced-obesity but also contributes to modulation on locomotor activity. Energy expenditure and thermogenesis augmentation have been suggested to be an effective mechanism to decrease obesity and the metabolic disorders [133]; therefore ABHD6$^{NAc\ KO}$ appears to have a beneficial effect decreasing negative association with HFD consumption and, therefore, reducing obesity.

Together, these results suggest that increased energy expenditure and ambulatory activity in the CRE group may underlie the reduced body weight gain seen in this group compared to the control GFP mice. In accordance with Zhao et al., ABHD6 whole body deletion mice increased locomotor activity and energy expenditure as it is also shown in this study [101]. Interestingly, female mice were more responsive than males. Further investigation will be needed to investigate the role of ABHD6 on sexual dimorphism.

Results from several experimental studies demonstrate that ECS modulates some of the physical activity effect within the CNS: first, CB1 is the most abundant in the brain; second, the endocannabinoid can cross the blood brain barrier [134] and third, some studies demonstrate that eCB plasma levels increase with exercise [135], [136]. Nevertheless, in our knowledge, none of these studies had evaluated the central inhibition of the enzyme ABHD6 as a positive regulator

of voluntary physical activity. Here we demonstrate that ABHD6$^{NAc\ KO}$ prevents from voluntary physical inactivity, caused by consumption of high caloric density diets such as palm diet. ABHD6$^{NAc\ KO}$ increases locomotor activity and diminishes body weight gain on HFD, which allows performing exercise in a more comfortable way compared to control mice that were bigger at the moment to introduce the running wheels. Perhaps, there is a lack of physical activity motivation due to the over consumption of palatable food (in this case HFD); mesocorticolimbic DA circuit plays a key role in the rewarding effects to biological relevant stimuli [137], but more studies will need to be done to confirm this hypothesis.

Moving forward with the study, we wanted to see whether ABHD6$^{NAc\ KO}$ in chow feeding could also increase locomotor activity measured with voluntary wheel running. Surgery was performed in a second cohort and after two weeks we introduced the running wheels and recorded their performance for 5 weeks. We also kept track of food intake and body weight. We did not see any differences in voluntary wheel running, body weight and food consumption between CRE and GFP control mice on chow diet. Therefore, we next examined the possibility of ABHD6$^{NAc\ KO}$ preventing voluntary physical inactivity due to HFD feeding in the same mice but with a shorter exposure to HFD. We withdrew the running wheels from their home cage for three weeks and we introduce HFD. Then, we return the running wheels in addition to the HFD for an additional 3 weeks. From week 1, CRE mice showed a lower body weight than the GFP control group despite similar amounts of food intake. Once we returned the running wheels, we saw a normalization on the GFP control with the CRE mice body weight (*Figure 15A*). Surprisingly, both groups CRE and GFP showed a high running wheel performance. Thus, pre-exposure of running wheel may reinforce motivation to engage in voluntary physical activity while having a prolonged sedentary lifestyle can aggravate DIO.

The eCB tone strongly plays a role in physical activity and growing evidence is supporting these findings; in the brain the positive modulation by the ABHD6$^{NAc\ KO}$ in locomotor activity can improve other brain processes such as cognitive function and neurogenesis. Experimental data showed that eCB mediates motor activity through the CB1 receptor: cannabinoid agonist in large doses can produce a motor inhibition while a competitive antagonist of CB1 can be reverse [110]. In addition, in lower doses, the agonist can produce hyperactivity [138]–[140], a possible explanation of the phenotype that we saw in this study.

We wanted to evaluate the potential of ABHD6$^{NAc\ KO}$ to induce a tonic increase of DA activity in the mesolimbic circuitry; therefore, we decided to use the amphetamine sensitization protocol.

Repeated amphetamine administration causes hypersensitivity to the psychoactive effect of the drug, therefore a lower dose can provoke the effect of the higher dose. Amphetamine exerts its central effects of increasing DA release in the synaptic cleft by blocking the dopamine transporter, and potentiating the action potentials in the neurons [141]. From a locomotor perspective, amphetamine produces an increase in locomotor activity due to an increase in dopamine release [142]. Psychostimulant drugs enhance dopamine release in the NAc an effect that is stronger in animals that were pre-exposed to the drug than in naïve animals; this difference is seen in the core but not in the shell subregion of the NAc *(Di Chiara, 2002)* [143]. Indeed, our study suggested an augmentation in dopamine transmission at the ABHD6$^{NAc\ KO}$ mice that is associated with the behavioral increase of locomotor activity. The increase of 2-AG tone at the postsynaptic neuron, enhance DA-ergic transmission mediated by CB1 receptors in GABAergic neurons; electrophysiological studies such as patch clamp will be needed to confirm these hypotheses.

The ECS exerts an interaction with the dopaminergic system to control motor activity since they co-localize with D1R and D2R [144]. The respective roles of D1 and D2 neuronal receptor subtypes on locomotor activity is controversial. D1R-KO demonstrate to reduced locomotor activity in the NAc [145] and in Parkinson models reduce D2-R expression showed a significant decrease on locomotor activity [146]. In this regard we wanted to investigate the specific contribution of D1 MSN neurons in mediating locomotor activity in ABHD6$^{NAc\ KO}$ mice. Although D1-like receptor has been associated with the regulation of physical activity [129], [145], in our study, we did not see a difference on locomotor activity, after a single systemic D1R-agonist injection (i.p. administration) between CRE and GFP mice. The precise significance of this results is still unclear due to the complexity of the NAc inputs and outputs surrounding the structure, therefore more studies will need to be done to understand the precise mechanism of ABHD6 KO in the NAc.

4.2 Lack of ABHD6 in the NAc positive modulates mood behaviors on HFD

Numerous lines of evidence demonstrate that the NAc has deep effects on behavior, especially in reward, anxiety, drugs and depression [68], [78], [147]. Excessive HFD intake leads to obesity accompanied by anxio-depressive behaviors that also provoke molecular adaptations within the NAc [148], [149]. With the intent to decrease obesity, a potent body weight loss pill was produced known as rimonabant; however, psychiatric side effects were enough to remove it from the market [115], [118].

In this study we investigate the impact of ABHD6 deletion in the NAc to determine the effect on anxio-depressive behaviors on CRE and control GFP mice that were subjected to isocaloric HFD or control diet CHOW for 12 weeks. Both, CRE and GFP mice subjected to HFD feeding showed a significant increase in body weight gain compared to GFP CHOW fed mice. Moreover, in this study we did not find a significant difference in body weight between CRE HFD and GFP HFD control mice, which was different from our study (*Figure 12)* where we saw a significant difference in body weight between these groups. One explanation about the behavioral differences on body weight gain can be attributed to different surgical manipulations accomplished by two different persons. Therefore, placement differences where the ABHD6 KO virus was injected, could create a least robust effect in body weight. This shows the importance of accuracy while performing surgical interventions to have the same effect in all the mice. Regardless differences in surgical placements, food consumption results were consistent within all mice cohorts in this thesis. CRE HFD mice did not show a significant effect in food consumption compared to the control GFP HFD and GFP CHOW groups. Therefore, ABHD6$^{NAc\ KO}$ does not affect food intake in mice subjected to diets rich in saturated fats, contrary to our initial hypothesis. In this regard, ABHD6$^{NAc\ KO}$ shows to play an important role in the catabolic actions to diminish diet-induced obesity, which is consistent with a few studies [3], [113], [150].

Furthermore, we assessed anxiety- and depressive-like outcomes with three different tests. We started with the EPM, where no anxiety phenotype was found on the CRE HFD mice compared to control GFP CHOW, in contrast, control GFP HFD had a significant difference compared to control GFP CHOW in the entries to the open arms. An interesting result that we found is that the CRE HFD mice enter more to the open arms but they did not want to stay for a long time; as this is an exploratory test, they probably were curious to enter to these arms but not to stay in them. Therefore, we could suggest that ABHD6$^{NAc\ KO}$ has a partial-protection on the anxiety behaviors provoke by HFD feeding. Then, we used the OFT test, where no significant difference was found in the time spent or frequency in center and travelled distance between all mice groups. These findings suggest that ABHD6$^{NAc\ KO}$ prevents from anxiety-like behaviors associated with the overconsumption of HFD (palm diet) in the EPM test, but not in the OFT. Discrepancies between tests can be argue by how animals were handled prior testing and also because both tests were done on the same day (EMP first and after OFT). On the other hand*, Zhao et al.,* investigated whether deletion of ABHD6 in whole body could be associated with anxiety-like behaviors; in agreement with our findings*, Zhao et al.,* showed that ABHD6 KO did not produce any changes in EPM or OFT test [113]. Thus, focalization of neuronal invalidation of

ABHD6 NAc suggests to have a positive effect regulating anxiety-like behaviors in mice fed with high caloric food.

As rimonabant caused depression in the intent to decrease body weight, we wanted to see if neuronal inhibition of ABHD6 in the NAc could have a different result. Therefore, we assessed FST to look at despair behaviors in mice. In our results, CRE HFD mice did not show depressive-like behaviors caused by DIO, in contrast, GFP HFD mice showed an increase in depressive-like behavior, as measured by time spent immobile in the FST. There are some studies showing different results whereas higher doses of eCB can negative modulate anxiety among with depression [151] but other studies show the opposite effect [152]. In fact, heavy use of cannabinoid compounds (like marijuana) can increase depressive symptoms [153]. However, ABHD6 as it has been discussed in the previous chapters, fine-tunes endocannabinoid signaling in the brain, and these results suggest that its deletion does not induce anxio-depressive behaviors. Furthermore, *Decarie et al.,* showed that high caloric dense food cause inflammation in the NAc brain section which lead to depressive symptoms [154] and some studies have shown that ABHD6 KO high fat fed mice had an anti-inflammatory effects; as they found an increase of circulating FG and FGF21 factors (known to have anti-inflammatory properties) and a decrease of circulating cytokine [104]. In this regard, alleviation of depressive behaviors done by ABHD6$^{NAc\ KO}$ can be related not only for the synaptic modulation but also for a decrease in inflammatory markers provoke by HFD. More studies have to be done to validate this hypothesis.

Overall this study demonstrates that lack of neuronal ABHD6 depletion in the NAc does not affect body weight and food intake, however, it seems to have a positive effect on anxio-depressive behaviors in mice.

4.3 Conclusion

The biological understanding of how obesity increased dramatically during the last decades is essential for finding an effective therapeutic treatment. Its etiology arises mainly from the overconsumption of high caloric diets and the sedentary lifestyle; however, many other factors such as environmental, social, cultural among others also plays a role in the obese population. So far, the strategies to decrease overweight relies on healthier diets, an increase of physical activity and in some cases, subjects can have surgical interventions to decrease body weight. Surgical and pharmacological therapies can have a major impact, however, it is accompanied by

many side effects that can contribute to the relapse to increase body weight. In this regard it is urgent to find a competent treatment to reduce obesity in the world. The central nervous system incorporates neuronal signalling from many brain areas and peripheral organs that control body homeostasis. Several lines of evidence demonstrate that the NAc integrates inputs regarding eating behaviors, mood and physical activity. Moreover, the dopaminergic system has been also found to mediate these behaviors in addition to other signalling networks as the endocannabinoid system. Therefore, the NAc has been postulated as a potential target for obesity pharmacological treatment.

The ECS is a biological system that is present in the mammalians body. They are lipid-based molecules that act retrogradely to inhibit or enhance neurotransmitter release. They are only activated on demand to maintain body's homeostasis. The most known eCB ligands are AEA and 2-AG; they act in the CB2 in the periphery and CB1 in the CNS. Both are G protein-coupled receptors located strategically to mediate a variety of physiological processes such as memory, appetite and mood. During the last decade the endocannabinoid system research has increased enormously regarding its possible modulation in chronic diseases such as epilepsy, cancer, pain and obesity. Moreover, the enzymes in charge of synthesize or degrade the endogenous ligands are FAAH, MAGL, and more recently scientist discovered ABHD12 and ABHD6. The discovery of new monoglycerol lipase such as ABHD6 opened a new field to explore the eCB mechanism in the brain. In fact, a group of scientists demonstrated that ABHD6 controls the neuronal accumulation of 2-AG in the brain as it is located at the same site (post-synaptic neuron) of 2-AG hydrolysis [102].

ABHD6 only accounts for 4% of 2-AG hydrolysis while MAGL accounts for the 85%, therefore, ABHD6 only causes small changes in the neuronal signalling activating CB1 receptor [105]. Pharmacological inhibition of ABHD6 showed a neuroprotective effect in diseases such as traumatic brain injury, cancer and epilepsy. Interestingly, ABHD6 whole body deletion was demonstrated to prevent from the metabolic syndrome and insulin resistance, involving a strong modulation in energy balance. The function of ABHD6 is little known but it seems to have a strong involvement in energy balance. In this regard the purpose of this study was to identify the function of ABHD6 in the NAc and its involvement in body weight, physical activity, appetite and anxio-depressive behaviors.

This study demonstrated that 40% of focal neuronal deletion of ABHD6 in the NAc has an important role in energy balance as ABHD6$^{NAc\ KO}$ diminished DIO caused by the consumption of HFD. ABHD6$^{NAc\ KO}$ does not affect food consumption, which is contrary of our first hypothesis; however, a study in the VMH demonstrated the same effect in mice subjected to HFD feeding. ABHD6$^{NAc\ KO}$ modulates locomotor activity, increasing energy expenditure and voluntary wheel running despite the negative effect of HFD feeding. Moreover, ABHD6$^{NAc\ KO}$ shows to play a role in the corticolimbic locomotor stimulation induced by psychostimulant drug such as amphetamine. However, the exact mechanism is still unknown.

Neuronal depletion of ABHD6 in the NAc demonstrates to contribute positively in the control of mood disorders such as anxiety and depression, both caused by the consumption of food rich in saturated fats. These results lead us to consider the modulation of the ABHD6 enzyme as a potential treatment for emotional-type complications related to obesity. The ABHD6$^{NAc\ KO}$ suggests having a strong neuronal mechanism in the regulation of body's homeostasis, thus, ABHD6 signalling seems to be a strong therapeutically candidate to prevent obesity and its comorbidities.

4.4 Future directions

There are many things that can be studied, but I am only going to mention the ones that can have a major impact for future directions in obesity. Further investigations have to be done to evaluate differences of ABHD6 KO in the NAc between males and females, for example: metabolism, lipid accumulation and voluntary physical activity. Specially the last factor as Zhao et al., 2015 showed that females had a higher levels of voluntary wheel running compared to male mice [113].

In an inflammation aspect, it would be interesting to investigate if ABHD6$^{NAc\ KO}$ can prevent inflammation caused by DIO. As we discussed before, HFD consumption induce inflammation within the brain and for time reasons we couldn't evaluate if ABHD6$^{NAc\ KO}$ diminishes inflammation compared to control mice. Also, it would be important to evaluate long-term viral infection effect on neurons and microglia as there are some studies that showed neuronal damage caused by viruses for gene therapy [155], [156]. In case that viral infection does not cause long-term inflammation, we could increase the viral dose to increase the levels of KO.

Neurons interact with glial cells such as astrocytes, recent evidence point that astrocyte activation is required for DIO and hypothalamic inflammation, therefore it would be interesting to investigate the role of ABHD6 KO in astrocytes activation at the NAc and other brain areas.

Although here we demonstrate a neuronal modulation of ABHD6$^{NAc\ KO}$ in control of energy balance and mood, is unclear which specific neuronal pathways are involved. More molecular and electrophysiogical studies must be accomplished to understand the synaptic modulation between brain areas such as VTA, LH, SNs, etc. in the NAc and the ECS and its effect in biological functions such as reward, energy balance and mood.

The eCB are very promiscuous molecules; they not only bind to the cannabinoid receptors but can also activate other types of receptors. Therefore, is really hard to know the exact pathway they work on. In one way they help to decrease body weight but at the same time they affect mood. Therefore, there is still a lot to be investigated. The ECS could be a potent molecular and pharmacological tool to help to decrease obesity in the future.

References

[1] E. Roh and M. S. Kim, “Brain Regulation of Energy Metabolism.,” *Endocrinol. Metab. (Seoul, Korea)*, vol. 31, no. 4, pp. 519–524, Dec. 2016.

[2] O. Arias-Carrión, M. Stamelou, E. Murillo-Rodríguez, M. Menéndez-González, and E. Pöppel, “Dopaminergic reward system: a short integrative review,” *Int. Arch. Med.*, vol. 3, no. 1, p. 24, Oct. 2010.

[3] A. Fisette *et al.*, “α/β-Hydrolase Domain 6 in the Ventromedial Hypothalamus Controls Energy Metabolism Flexibility,” *Cell Rep.*, vol. 17, no. 5, pp. 1217–1226, Oct. 2016.

[4] G. Velasco, C. Sánchez, and M. Guzmán, “Towards the use of cannabinoids as antitumour agents,” *Nat. Rev. Cancer*, vol. 12, no. 6, pp. 436–444, Jun. 2012.

[5] margot shields, margaret carroll, and cynthia ogden, “Data from the Canadian Health Measures Survey, 2007-2009; the Canadian Heart Health Surveys, 1986-1992; and the U.S. National Health and Nutrition Examination Survey,” 2011.

[6] P. G. Kopelman, “Obesity as a medical problem,” *Nature*, vol. 404, no. 6778, pp. 635–643, Apr. 2000.

[7] C. Pozza and A. M. Isidori, “What’s Behind the Obesity Epidemic,” in *Imaging in Bariatric Surgery*, Cham: Springer International Publishing, 2018, pp. 1–8.

[8] “Adult Obesity | Obesity Prevention Source | Harvard T.H. Chan School of Public Health.” [Online]. Available: https://www.hsph.harvard.edu/obesity-prevention-source/obesity-trends/obesity-rates-worldwide/. [Accessed: 12-Mar-2018].

[9] “WHO | Obesity and overweight,” *WHO*, 2018.

[10] S. Rössner, “Obesity: the disease of the twenty-first century,” *Int. J. Obes.*, vol. 26, no. S4, pp. S2–S4, Dec. 2002.

[11] A. Hruby and F. B. Hu, “The Epidemiology of Obesity: A Big Picture.,” *Pharmacoeconomics*, vol. 33, no. 7, pp. 673–89, Jul. 2015.

[12] M. Ng *et al.*, “Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013,” *Lancet*, vol. 384, no. 9945, pp. 766–781, Aug. 2014.

[13] K. M. Flegal, M. D. Carroll, B. K. Kit, and C. L. Ogden, “Prevalence of Obesity and Trends in the Distribution of Body Mass Index Among US Adults, 1999-2010,” *JAMA*, vol. 307, no. 5, p. 491, Feb. 2012.

[14] “About Obesity in Canada - Canadian Obesity Network.” [Online]. Available: http://www.obesitynetwork.ca/obesity-in-canada. [Accessed: 12-Mar-2018].

[15] “Bariatric Surgery Procedures – ASMBS | American Society for Metabolic and Bariatric Surgery.” [Online]. Available: https://asmbs.org/patients/bariatric-surgery-procedures. [Accessed: 03-Nov-2018].

[16] C. M. Tate and A. Geliebter, “Intragastric Balloon Treatment for Obesity: Review of

Recent Studies," *Adv. Ther.*, vol. 34, no. 8, pp. 1859–1875, Aug. 2017.

[17] F. Lifshitz and J. Z. Lifshitz, "Globesity: the root causes of the obesity epidemic in the USA and now worldwide.," *Pediatr. Endocrinol. Rev.*, vol. 12, no. 1, pp. 17–34, Sep. 2014.

[18] P. Li *et al.*, "Genetic association analysis of 30 genes related to obesity in a European American population," *Int. J. Obes.*, vol. 38, no. 5, pp. 724–729, May 2014.

[19] A. E. Locke *et al.*, "Genetic studies of body mass index yield new insights for obesity biology," *Nature*, vol. 518, no. 7538, pp. 197–206, Feb. 2015.

[20] J.-B. Funcke *et al.*, "Monogenic forms of childhood obesity due to mutations in the leptin gene.," *Mol. Cell. Pediatr.*, vol. 1, no. 1, p. 3, Dec. 2014.

[21] S. N. Bhupathiraju and F. B. Hu, "Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications.," *Circ. Res.*, vol. 118, no. 11, pp. 1723–35, May 2016.

[22] K. Sahoo, B. Sahoo, A. K. Choudhury, N. Y. Sofi, R. Kumar, and A. S. Bhadoria, "Childhood obesity: causes and consequences.," *J. Fam. Med. Prim. care*, vol. 4, no. 2, pp. 187–92, 2015.

[23] J. N. Udall, G. G. Harrison, Y. Vaucher, P. D. Walson, and G. Morrow, "Interaction of maternal and neonatal obesity.," *Pediatrics*, vol. 62, no. 1, pp. 17–21, Jul. 1978.

[24] M. M. Broadney *et al.*, "Impact of parental obesity on neonatal markers of inflammation and immune response," *Int. J. Obes.*, vol. 41, no. 1, pp. 30–37, Jan. 2017.

[25] M. Aguirre, C. Bussolo de Souza, and K. Venema, "The Gut Microbiota from Lean and Obese Subjects Contribute Differently to the Fermentation of Arabinogalactan and Inulin.," *PLoS One*, vol. 11, no. 7, p. e0159236, 2016.

[26] S. L. Gortmaker, A. Must, A. M. Sobol, K. Peterson, G. A. Colditz, and W. H. Dietz, "Television viewing as a cause of increasing obesity among children in the United States, 1986-1990.," *Arch. Pediatr. Adolesc. Med.*, vol. 150, no. 4, pp. 356–62, Apr. 1996.

[27] M. Hopkins, J. Blundell, J. Halford, N. King, and G. Finlayson, *The Regulation of Food Intake in Humans*. MDText.com, Inc., 2000.

[28] F. Johnson and J. Wardle, "Variety, palatability, and obesity.," *Adv. Nutr.*, vol. 5, no. 6, pp. 851–9, Nov. 2014.

[29] A. Golay and E. Bobbioni, "The role of dietary fat in obesity.," *Int. J. Obes. Relat. Metab. Disord.*, vol. 21 Suppl 3, pp. S2-11, Jun. 1997.

[30] G. Cairns, K. Angus, G. Hastings, and M. Caraher, "Systematic reviews of the evidence on the nature, extent and effects of food marketing to children. A retrospective summary," *Appetite*, vol. 62, pp. 209–215, Mar. 2013.

[31] T. Garland *et al.*, "The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives.," *J. Exp. Biol.*, vol. 214, no. Pt 2, pp. 206–29, Jan. 2011.

[32] J. Heydenreich, B. Kayser, Y. Schutz, and K. Melzer, "Total Energy Expenditure, Energy

Intake, and Body Composition in Endurance Athletes Across the Training Season: A Systematic Review.," *Sport. Med. - open*, vol. 3, no. 1, p. 8, Dec. 2017.

[33] H. Münzberg, E. Qualls-Creekmore, H.-R. Berthoud, C. D. Morrison, and S. Yu, "Neural Control of Energy Expenditure.," *Handb. Exp. Pharmacol.*, vol. 233, pp. 173–94, 2016.

[34] L. A. Rowland, N. C. Bal, and M. Periasamy, "The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy," *Biol. Rev.*, vol. 90, no. 4, pp. 1279–1297, Nov. 2015.

[35] C. Perez-Leighton, M. R. Little, M. Grace, C. Billington, and C. M. Kotz, "Orexin signaling in rostral lateral hypothalamus and nucleus accumbens shell in the control of spontaneous physical activity in high- and low-activity rats.," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 312, no. 3, pp. R338–R346, 2017.

[36] C. M. Novak, P. R. Burghardt, and J. A. Levine, "The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward.," *Neurosci. Biobehav. Rev.*, vol. 36, no. 3, pp. 1001–1014, Mar. 2012.

[37] J. Goh and W. Ladiges, "Voluntary Wheel Running in Mice.," *Curr. Protoc. Mouse Biol.*, vol. 5, no. 4, pp. 283–90, Dec. 2015.

[38] M. W. Schwartz, "Central Nervous System Regulation of Food Intake," 2006.

[39] G. . Smith, *Satiation: from gut to brain*. New York, USA, 1998.

[40] D. E. Cummings and J. Overduin, "Gastrointestinal regulation of food intake.," *J. Clin. Invest.*, vol. 117, no. 1, pp. 13–23, Jan. 2007.

[41] J. Gibbs, R. C. Young, and G. P. Smith, "Cholecystokinin elicits satiety in rats with open gastric fistulas.," *Nature*, vol. 245, no. 5424, pp. 323–5, Oct. 1973.

[42] G. S. Tannenbaum, H. J. Guyda, and B. I. Posner, "Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain.," *Science*, vol. 220, no. 4592, pp. 77–9, Apr. 1983.

[43] D. G. Baskin, D. Figlewicz Lattemann, R. J. Seeley, S. C. Woods, D. Porte, and M. W. Schwartz, "Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight," *Brain Res.*, vol. 848, no. 1–2, pp. 114–123, Nov. 1999.

[44] Y. Benomar, A.-F. Roy, A. Aubourg, J. Djiane, and M. Taouis, "Cross down-regulation of leptin and insulin receptor expression and signalling in a human neuronal cell line.," *Biochem. J.*, vol. 388, no. Pt 3, pp. 929–39, Jun. 2005.

[45] J. M. Friedman and J. L. Halaas, "Leptin and the regulation of body weight in mammals," *Nature*, vol. 395, no. 6704, pp. 763–770, Oct. 1998.

[46] G. C. Kennedy, "The hypothalamus and obesity.," *Proc. R. Soc. Med.*, vol. 59, no. 12, pp. 1276–7, Dec. 1966.

[47] R. Coppari *et al.*, "The hypothalamic arcuate nucleus: A key site for mediating leptin's effects on glucose homeostasis and locomotor activity," *Cell Metab.*, vol. 1, no. 1, pp. 63–72, Jan. 2005.

[48] M. O. Dietrich and T. L. Horvath, “Hypothalamic control of energy balance: insights into the role of synaptic plasticity.,” *Trends Neurosci.*, vol. 36, no. 2, pp. 65–73, Feb. 2013.

[49] H. J. Grill, “Distributed Neural Control of Energy Balance: Contributions from Hindbrain and Hypothalamus,” *Obesity*, vol. 14, p. 216S–221S, Aug. 2006.

[50] S. G. Bouret, S. J. Draper, and R. B. Simerly, “Trophic Action of Leptin on Hypothalamic Neurons That Regulate Feeding,” *Science (80-.).*, vol. 304, no. 5667, pp. 108–110, Apr. 2004.

[51] G. Cavaliere *et al.*, “Long Feeding High-Fat Diet Induces Hypothalamic Oxidative Stress and Inflammation, and Prolonged Hypothalamic AMPK Activation in Rat Animal Model.,” *Front. Physiol.*, vol. 9, p. 818, 2018.

[52] C. Girardet and A. A. Butler, “Neural melanocortin receptors in obesity and related metabolic disorders.,” *Biochim. Biophys. Acta*, vol. 1842, no. 3, pp. 482–94, Mar. 2014.

[53] D. Huszar *et al.*, “Targeted disruption of the melanocortin-4 receptor results in obesity in mice.,” *Cell*, vol. 88, no. 1, pp. 131–41, Jan. 1997.

[54] Z. A. Abdel-Malek, “Melanocortin receptors: their functions and regulation by physiological agonists and antagonists,” *Cell. Mol. Life Sci.*, vol. 58, no. 3, pp. 434–441, Mar. 2001.

[55] G. A. Bewick *et al.*, “Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype,” *FASEB J.*, vol. 19, no. 12, pp. 1680–1682, Oct. 2005.

[56] M. Koch *et al.*, “Hypothalamic POMC neurons promote cannabinoid-induced feeding,” *Nature*, vol. 519, no. 7541, pp. 45–50, Mar. 2015.

[57] A. C. Shin *et al.*, “Insulin Receptor Signaling in POMC, but Not AgRP, Neurons Controls Adipose Tissue Insulin Action.,” *Diabetes*, vol. 66, no. 6, pp. 1560–1571, 2017.

[58] S. Fulton *et al.*, “Leptin Regulation of the Mesoaccumbens Dopamine Pathway,” *Neuron*, vol. 51, no. 6, pp. 811–822, Sep. 2006.

[59] G. T. Dodd *et al.*, “Leptin and Insulin Act on POMC Neurons to Promote the Browning of White Fat,” *Cell*, vol. 160, no. 1–2, pp. 88–104, Jan. 2015.

[60] O. Arias-Carrión and E. Pöppel, “Dopamine, learning, and reward-seeking behavior.,” *Acta Neurobiol. Exp. (Wars).*, vol. 67, no. 4, pp. 481–8, 2007.

[61] D. Vallone, R. Picetti, and E. Borrelli, “Structure and function of dopamine receptors.,” *Neurosci. Biobehav. Rev.*, vol. 24, no. 1, pp. 125–32, Jan. 2000.

[62] R. A. Wise, “Dopamine and reward: The anhedonia hypothesis 30 years on,” *Neurotox. Res.*, vol. 14, no. 2–3, pp. 169–183, Jun. 2008.

[63] W. Schultz, “Subjective neuronal coding of reward: temporal value discounting and risk,” *Eur. J. Neurosci.*, vol. 31, no. 12, pp. 2124–2135, May 2010.

[64] F. Ambroggi, A. Ghazizadeh, S. M. Nicola, and H. L. Fields, “Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition.,” *J. Neurosci.*, vol. 31, no. 18, pp. 6820–30, May 2011.

[65] V. Bassareo, F. Cucca, R. Frau, and G. Di Chiara, “Changes in Dopamine Transmission in the Nucleus Accumbens Shell and Core during Ethanol and Sucrose Self-Administration,” *Front. Behav. Neurosci.*, vol. 11, p. 71, May 2017.

[66] S. B. Floresco, “The Nucleus Accumbens: An Interface Between Cognition, Emotion, and Action,” *Annu. Rev. Psychol.*, vol. 66, no. 1, pp. 25–52, Jan. 2015.

[67] A. J. Robison and E. J. Nestler, “Transcriptional and epigenetic mechanisms of addiction.,” *Nat. Rev. Neurosci.*, vol. 12, no. 11, pp. 623–37, Oct. 2011.

[68] Y. Shirayama and S. Chaki, “Neurochemistry of the nucleus accumbens and its relevance to depression and antidepressant action in rodents.,” *Curr. Neuropharmacol.*, vol. 4, no. 4, pp. 277–91, Oct. 2006.

[69] K. C. Berridge, “‘Liking’ and ‘wanting’ food rewards: brain substrates and roles in eating disorders.,” *Physiol. Behav.*, vol. 97, no. 5, pp. 537–50, Jul. 2009.

[70] J. S. Rhodes and T. Garland, “Differential sensitivity to acute administration of Ritalin, apormorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior,” *Psychopharmacology (Berl).*, vol. 167, no. 3, pp. 242–250, May 2003.

[71] A. M. Knab and J. T. Lightfoot, “Does the difference between physically active and couch potato lie in the dopamine system?,” *Int. J. Biol. Sci.*, vol. 6, no. 2, pp. 133–50, Mar. 2010.

[72] K. J. Ressler, “Amygdala activity, fear, and anxiety: modulation by stress.,” *Biol. Psychiatry*, vol. 67, no. 12, pp. 1117–9, Jun. 2010.

[73] L. Nadel, “The hippocampus and space revisited,” *Hippocampus*, vol. 1, no. 3, pp. 221–229, Jul. 1991.

[74] W. C. Drevets, J. L. Price, and M. L. Furey, “Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression,” *Brain Struct. Funct.*, vol. 213, no. 1–2, pp. 93–118, Sep. 2008.

[75] B. H. Bewernick *et al.*, “Nucleus Accumbens Deep Brain Stimulation Decreases Ratings of Depression and Anxiety in Treatment-Resistant Depression,” *Biol. Psychiatry*, vol. 67, no. 2, pp. 110–116, Jan. 2010.

[76] F. S. Luppino *et al.*, “Overweight, Obesity, and Depression,” *Arch. Gen. Psychiatry*, vol. 67, no. 3, p. 220, Mar. 2010.

[77] C. Hryhorczuk, S. Sharma, and S. E. Fulton, “Metabolic disturbances connecting obesity and depression.,” *Front. Neurosci.*, vol. 7, p. 177, Oct. 2013.

[78] L. Décarie-Spain *et al.*, “Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat,” *Mol. Metab.*, vol. 10, pp. 1–13, Apr. 2018.

[79] C. Hryhorczuk *et al.*, “Dampened Mesolimbic Dopamine Function and Signaling by Saturated but not Monounsaturated Dietary Lipids,” *Neuropsychopharmacology*, vol. 41, no. 3, pp. 811–821, Feb. 2016.

[80] P. Pacher, S. Bátkai, and G. Kunos, “The endocannabinoid system as an emerging target

of pharmacotherapy.," *Pharmacol. Rev.*, vol. 58, no. 3, pp. 389–462, Sep. 2006.

[81] Y. Gaoni and R. Mechoulam, "Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish," *J. Am. Chem. Soc.*, vol. 86, no. 8, pp. 1646–1647, Apr. 1964.

[82] M. I. Khan, A. A. Sobocińska, A. M. Czarnecka, M. Król, B. Botta, and C. Szczylik, "The Therapeutic Aspects of the Endocannabinoid System (ECS) for Cancer and their Development: From Nature to Laboratory.," *Curr. Pharm. Des.*, vol. 22, no. 12, pp. 1756–66, 2016.

[83] R. Mechoulam and L. A. Parker, "The Endocannabinoid System and the Brain," *Annu. Rev. Psychol.*, vol. 64, no. 1, pp. 21–47, Jan. 2013.

[84] L. A. Matsuda, S. J. Lolait, M. J. Brownstein, A. C. Young, and T. I. Bonner, "Structure of a cannabinoid receptor and functional expression of the cloned cDNA," *Nature*, vol. 346, no. 6284, pp. 561–564, Aug. 1990.

[85] M. Guzmán, "Cannabinoids: potential anticancer agents," *Nat. Rev. Cancer*, vol. 3, no. 10, pp. 745–755, Oct. 2003.

[86] B. E. Alger, "Getting high on the endocannabinoid system.," *Cerebrum*, vol. 2013, p. 14, Nov. 2013.

[87] T. Sugiura *et al.*, "2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain.," *Biochem. Biophys. Res. Commun.*, vol. 215, no. 1, pp. 89–97, Oct. 1995.

[88] R. Mechoulam, L. O. Hanuš, R. Pertwee, and A. C. Howlett, "Early phytocannabinoid chemistry to endocannabinoids and beyond," *Nat. Rev. Neurosci.*, vol. 15, no. 11, pp. 757–764, Nov. 2014.

[89] M. L. Yates and E. L. Barker, "Inactivation and biotransformation of the endogenous cannabinoids anandamide and 2-arachidonoylglycerol.," *Mol. Pharmacol.*, vol. 76, no. 1, pp. 11–7, Jul. 2009.

[90] "Central nervous system | definition of Central nervous system by Medical dictionary." [Online]. Available: https://medical-dictionary.thefreedictionary.com/central+nervous+system. [Accessed: 30-Jul-2018].

[91] D. Purves, "Neuroscience, 3rd Edition."

[92] W. A. Devane *et al.*, "Isolation and structure of a brain constituent that binds to the cannabinoid receptor.," *Science*, vol. 258, no. 5090, pp. 1946–9, Dec. 1992.

[93] R. Mechoulam and L. A. Parker, "The Endocannabinoid System and the Brain," *Annu. Rev. Psychol.*, vol. 64, no. 1, pp. 21–47, Jan. 2013.

[94] H. Horn, B. Böhme, L. Dietrich, and M. Koch, "Endocannabinoids in Body Weight Control.," *Pharmaceuticals (Basel).*, vol. 11, no. 2, May 2018.

[95] T. Ohno-Shosaku, T. Maejima, and M. Kano, "Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals.," *Neuron*, vol. 29, no. 3, pp. 729–38, Mar. 2001.

[96] H.-C. Lu and K. Mackie, "An Introduction to the Endogenous Cannabinoid System.," *Biol.*

Psychiatry, vol. 79, no. 7, pp. 516–25, Apr. 2016.

[97] G. L. Gerdeman, J. Ronesi, and D. M. Lovinger, “Postsynaptic endocannabinoid release is critical to long-term depression in the striatum,” *Nat. Neurosci.*, vol. 5, no. 5, pp. 446–451, May 2002.

[98] T. Harkany, K. Mackie, and P. Doherty, “Wiring and firing neuronal networks: endocannabinoids take center stage,” *Curr. Opin. Neurobiol.*, vol. 18, no. 3, pp. 338–345, Jun. 2008.

[99] P. E. Castillo, T. J. Younts, A. E. Chávez, and Y. Hashimotodani, “Endocannabinoid signaling and synaptic function.,” *Neuron*, vol. 76, no. 1, pp. 70–81, Oct. 2012.

[100] G. Thomas *et al.*, “The Serine Hydrolase ABHD6 Is a Critical Regulator of the Metabolic Syndrome,” *Cell Rep.*, vol. 5, no. 2, pp. 508–520, Oct. 2013.

[101] M. Wei *et al.*, “α/β-Hydrolase domain-containing 6 (ABHD6) negatively regulates the surface delivery and synaptic function of AMPA receptors.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 113, no. 19, pp. E2695-704, May 2016.

[102] W. R. Marrs *et al.*, “The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors,” *Nat. Neurosci.*, vol. 13, no. 8, pp. 951–957, Aug. 2010.

[103] M. A. Pribasnig *et al.*, “α/β Hydrolase Domain-containing 6 (ABHD6) Degrades the Late Endosomal/Lysosomal Lipid Bis(monoacylglycero)phosphate.,” *J. Biol. Chem.*, vol. 290, no. 50, pp. 29869–81, Dec. 2015.

[104] P. Poursharifi, R. Murthy, M. Prentki, and S. R. Murthy Madiraju, “Monoacylglycerol signalling and ABHD6 in health and disease,” 2017.

[105] J. R. Savinainen, S. M. Saario, and J. T. Laitinen, “The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors,” *Acta Physiol.*, vol. 204, no. 2, pp. 267–276, Feb. 2012.

[106] M. Côté *et al.*, “Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men,” *Int. J. Obes.*, vol. 31, no. 4, pp. 692–699, Apr. 2007.

[107] V. Di Marzo, “Targeting the endocannabinoid system: to enhance or reduce?,” *Nat. Rev. Drug Discov.*, vol. 7, no. 5, pp. 438–455, May 2008.

[108] I. Matias, S. Petrosino, A. Racioppi, R. Capasso, A. A. Izzo, and V. Di Marzo, “Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: Effect of high fat diets,” *Mol. Cell. Endocrinol.*, vol. 286, no. 1–2, pp. S66–S78, Apr. 2008.

[109] A. Fisette *et al.*, “α/β-Hydrolase Domain 6 in the Ventromedial Hypothalamus Controls Energy Metabolism Flexibility,” *Cell Rep.*, vol. 17, no. 5, pp. 1217–1226, Oct. 2016.

[110] A. Dietrich and W. F. McDaniel, “Endocannabinoids and exercise,” *Br. J. Sports Med.*, vol. 38, no. 5, pp. 536–541, Oct. 2004.

[111] J. Fuss *et al.*, “A runner’s high depends on cannabinoid receptors in mice,” *Proc. Natl. Acad. Sci.*, vol. 112, no. 42, pp. 13105–13108, Oct. 2015.

[112] D. A. Raichlen, A. D. Foster, G. L. Gerdeman, A. Seillier, and A. Giuffrida, “Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the ‘runner’s high,’” *J. Exp. Biol.*, vol. 215, no. 8, pp. 1331–1336, Apr. 2012.

[113] S. Zhao *et al.*, “α/β-Hydrolase Domain 6 Deletion Induces Adipose Browning and Prevents Obesity and Type 2 Diabetes,” *Cell Rep.*, vol. 14, no. 12, pp. 2872–2888, Mar. 2016.

[114] M. Hill and B. Gorzalka, “The Endocannabinoid System and the Treatment of Mood and Anxiety Disorders,” *CNS Neurol. Disord. - Drug Targets*, vol. 8, no. 6, pp. 451–458, Dec. 2009.

[115] A. Blasio *et al.*, “Rimonabant Precipitates Anxiety in Rats Withdrawn from Palatable Food: Role of the Central Amygdala,” *Neuropsychopharmacology*, vol. 38, no. 12, pp. 2498–2507, Nov. 2013.

[116] B. B. Gorzalka and M. N. Hill, “Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants,” *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, vol. 35, no. 7, pp. 1575–1585, Aug. 2011.

[117] C. J. Hillard, K. M. Weinlander, and K. L. Stuhr, “Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence,” *Neuroscience*, vol. 204, pp. 207–229, Mar. 2012.

[118] R. Christensen, P. K. Kristensen, E. M. Bartels, H. Bliddal, and A. Astrup, “Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials,” *Lancet*, vol. 370, no. 9600, pp. 1706–1713, Nov. 2007.

[119] W. Wang *et al.*, “Deficiency in Endocannabinoid Signaling in the Nucleus Accumbens Induced by Chronic Unpredictable Stress,” *Neuropsychopharmacology*, vol. 35, no. 11, pp. 2249–2261, Oct. 2010.

[120] S. Zhao *et al.*, “α/β-Hydrolase Domain-6-Accessible Monoacylglycerol Controls Glucose-Stimulated Insulin Secretion,” *Cell Metab.*, vol. 19, no. 6, pp. 993–1007, Jun. 2014.

[121] “Med Associates Inc. Low-Profile Wireless Running Wheel for Mouse • Med Associates Inc.” [Online]. Available: https://www.med-associates.com/product/low-profile-wireless-running-wheel-for-mouse/. [Accessed: 15-Aug-2018].

[122] M. F. A. Fernandes *et al.*, “Leptin Suppresses the Rewarding Effects of Running via STAT3 Signaling in Dopamine Neurons.,” *Cell Metab.*, vol. 22, no. 4, pp. 741–9, Oct. 2015.

[123] A. A. Walf and C. A. Frye, “The use of the elevated plus maze as an assay of anxiety-related behavior in rodents,” *Nat. Protoc.*, vol. 2, no. 2, pp. 322–328, Feb. 2007.

[124] S. Sharma, M. F. Fernandes, and S. Fulton, “Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal,” *Int. J. Obes.*, vol. 37, no. 9, pp. 1183–1191, Sep. 2013.

[125] L. Prut and C. Belzung, “The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review,” *Eur. J. Pharmacol.*, vol. 463, no. 1–3, pp. 3–33, Feb.

2003.

[126] J. Komorowski and H. Stepień, “[The role of the endocannabinoid system in the regulation of endocrine function and in the control of energy balance in humans].,” *Postepy Hig. Med. Dosw. (Online)*, vol. 61, pp. 99–105, 2007.

[127] B. K. Lau, D. Cota, L. Cristino, and S. L. Borgland, “Endocannabinoid modulation of homeostatic and non-homeostatic feeding circuits,” *Neuropharmacology*, vol. 124, pp. 38–51, Sep. 2017.

[128] J. A. Beeler, R. P. Faust, S. Turkson, H. Ye, and X. Zhuang, “Low Dopamine D2 Receptor Increases Vulnerability to Obesity Via Reduced Physical Activity, Not Increased Appetitive Motivation.,” *Biol. Psychiatry*, vol. 79, no. 11, pp. 887–97, 2016.

[129] A. M. Knab, R. S. Bowen, A. T. Hamilton, A. A. Gulledge, and J. T. Lightfoot, “Altered dopaminergic profiles: Implications for the regulation of voluntary physical activity,” *Behav. Brain Res.*, vol. 204, no. 1, pp. 147–152, Dec. 2009.

[130] R. Coccurello and M. Maccarrone, “Hedonic Eating and the "Delicious Circle": From Lipid-Derived Mediators to Brain Dopamine and Back.,” *Front. Neurosci.*, vol. 12, p. 271, 2018.

[131] V. Di Marzo, “A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists,” *Trends Pharmacol. Sci.*, vol. 27, no. 3, pp. 134–140, Mar. 2006.

[132] E. Soria-Gómez *et al.*, “Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus.,” *Br. J. Pharmacol.*, vol. 151, no. 7, pp. 1109–16, Aug. 2007.

[133] K. L. Townsend and Y.-H. Tseng, “Brown fat fuel utilization and thermogenesis,” *Trends Endocrinol. Metab.*, vol. 25, no. 4, pp. 168–177, Apr. 2014.

[134] M. Maccarrone *et al.*, “Regulation by cannabinoid receptors of anandamide transport across the blood-brain barrier and through other endothelial cells.,” *Thromb. Haemost.*, vol. 95, no. 1, pp. 117–27, Jan. 2006.

[135] M. Tantimonaco *et al.*, “Physical activity and the endocannabinoid system: an overview,” *Cell. Mol. Life Sci.*, vol. 71, no. 14, pp. 2681–2698, Jul. 2014.

[136] P. B. Sparling, A. Giuffrida, D. Piomelli, L. Rosskopf, and A. Dietrich, “Exercise activates the endocannabinoid system.,” *Neuroreport*, vol. 14, no. 17, pp. 2209–11, Dec. 2003.

[137] A. E. Kelley and K. C. Berridge, “The neuroscience of natural rewards: relevance to addictive drugs.,” *J. Neurosci.*, vol. 22, no. 9, pp. 3306–11, May 2002.

[138] E. Sulcova, R. Mechoulam, and E. Fride, “Biphasic effects of anandamide.,” *Pharmacol. Biochem. Behav.*, vol. 59, no. 2, pp. 347–52, Feb. 1998.

[139] M. Arendt, R. Rosenberg, L. Foldager, G. Perto, and P. Munk-Jørgensen, “Cannabis-induced psychosis and subsequent schizophrenia-spectrum disorders: follow-up study of 535 incident cases,” *Br. J. Psychiatry*, vol. 187, no. 06, pp. 510–515, Dec. 2005.

[140] V. Di Marzo, L. De Petrocellis, F. Fezza, A. Ligresti, and T. Bisogno, “Anandamide

receptors," *Prostaglandins, Leukot. Essent. Fat. Acids*, vol. 66, no. 2–3, pp. 377–391, Feb. 2002.

[141] S. M. Berman, R. Kuczenski, J. T. McCracken, and E. D. London, "Potential adverse effects of amphetamine treatment on brain and behavior: a review," *Mol. Psychiatry*, vol. 14, no. 2, pp. 123–142, Feb. 2009.

[142] R. C. Pierce and P. W. Kalivas, "Amphetamine produces sensitized increases in locomotion and extracellular dopamine preferentially in the nucleus accumbens shell of rats administered repeated cocaine.," *J. Pharmacol. Exp. Ther.*, vol. 275, no. 2, 1995.

[143] G. Di Chiara, "Nucleus accumbens shell and core dopamine: differential role in behavior and addiction," *Behav. Brain Res.*, vol. 137, no. 1–2, pp. 75–114, Dec. 2002.

[144] R. G. Pertwee *et al.*, "International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: Beyond CB1 and CB2," *Pharmacol. Rev.*, vol. 62, no. 4, pp. 588–631, Dec. 2010.

[145] A. H. Tran *et al.*, "Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 99, no. 13, pp. 8986–91, Jun. 2002.

[146] I. Draper, P. T. Kurshan, E. McBride, F. R. Jackson, and A. S. Kopin, "Locomotor activity is regulated by D2-like receptors in Drosophila: An anatomic and functional analysis," *Dev. Neurobiol.*, vol. 67, no. 3, pp. 378–393, Feb. 2007.

[147] M.-R. Zarrindast and F. Khakpai, "The Modulatory Role of Dopamine in Anxiety-like Behavior.," *Arch. Iran. Med.*, vol. 18, no. 9, pp. 591–603, Sep. 2015.

[148] P. Willner, "Antidepressants and serotonergic neurotransmission: an integrative review.," *Psychopharmacology (Berl).*, vol. 85, no. 4, pp. 387–404, 1985.

[149] F. P. Moreira *et al.*, "Metabolic syndrome in subjects with bipolar disorder and major depressive disorder in a current depressive episode: Population-based study," *J. Psychiatr. Res.*, vol. 92, pp. 119–123, Sep. 2017.

[150] G. Thomas *et al.*, "The Serine Hydrolase ABHD6 Is a Critical Regulator of the Metabolic Syndrome," *Cell Rep.*, vol. 5, no. 2, pp. 508–520, Oct. 2013.

[151] P. Pacher, S. Bátkai, and G. Kunos, "The endocannabinoid system as an emerging target of pharmacotherapy.," *Pharmacol. Rev.*, vol. 58, no. 3, pp. 389–462, Sep. 2006.

[152] R. F. Genn, S. Tucci, E. M. Marco, M. P. Viveros, and S. E. File, "Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test," *Pharmacol. Biochem. Behav.*, vol. 77, no. 3, pp. 567–573, Mar. 2004.

[153] L. Degenhardt, W. Hall, and M. Lynskey, "Exploring the association between cannabis use and depression," *Addiction*, vol. 98, no. 11, pp. 1493–1504, Nov. 2003.

[154] L. Décarie-Spain *et al.*, "Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat."

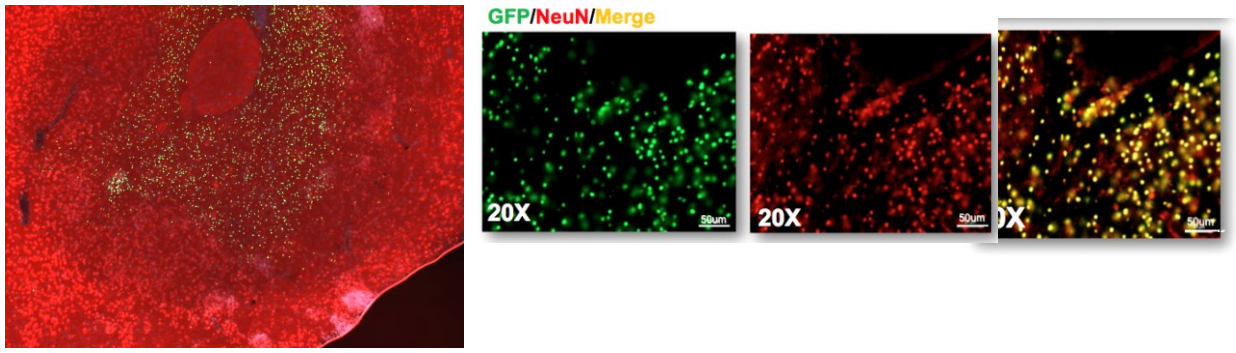
[155] C. E. Thomas, A. Ehrhardt, and M. A. Kay, "Progress and problems with the use of viral

vectors for gene therapy," *Nat. Rev. Genet.*, vol. 4, no. 5, pp. 346–358, May 2003.

[156] S. E. Raper *et al.*, "Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer," *Mol. Genet. Metab.*, vol. 80, no. 1–2, pp. 148–158, Sep. 2003.

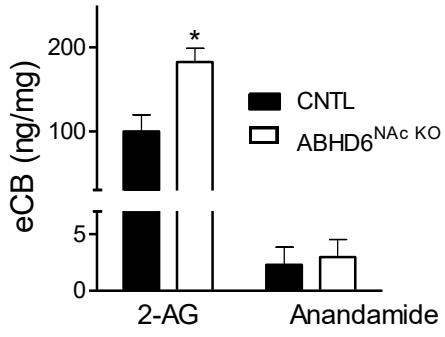
5. Appendix

5.1 Appendix A



Neuron specific ABHD6 deletion in nucleus accumbens of adult male mice. Images of neurons using the Neuronal Nuclear protein (NeuN) marker shown in red, Green Fluorescent Protein (GFP) in green, and merge (yellow) in floxed mice injected with AAV2/1h.Synap.HI.GFP.CreWPRES.SV40 at the NAc. (Tobin, Franco *et al.,* unpublished)

5.2 Appendix B



Liquid chromatography/mass spectrometry (LC/MS) results of the endocannabinoids 2-AG and anandamide in ABHD6$^{NAc\ KO}$. Group mean ± SEM, unpaired t test *<0.05 (Tobin, Franco *et al.,* unpublished)