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DEPARTMENT OF MEDICINE AND HEALTH SCIENCES

DEGREE IN MEDICINE

BEHAVIORAL AND MOLECULAR RESPONSES GENERATED IN RAT BRAIN STRUCTURES UPON A FEEDING BEHAVIOR PARADIGM

B A C H E L O R T H E S I S

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APPROVAL SHEET

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DEDICATION

My thesis is dedicated to my parents, Etiberio and Irma, who are my definition of perseverance. Both have provided me with a lifetime of love and words of encouragement which ring in my ears and inspire me to push myself every day to accomplish my goals. I am eternally grateful for the beautiful life you have paved for me.

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ABSTRACT

Classic non-homeostatic structures involved in food intake regulation are reciprocally influenced by metabolic signals. Orexigenic peptides expressed in the olfactory bulb (OB) and hippocampus (HP) modulate olfactory processing and memory, respectively. Hypothalamic circuits also modulate feeding behavior by activating and releasing AgRP in response to peripheral orexigenic signals. Adequate feeding in response to fasting states requires the expression of $p75^{NTR}$ in AgRP neurons. Given the close relation between different feeding structures, we questioned whether there may be a similar role for $p75^{NTR}$ and AgRP in the OB and HP on the consummatory ingestive behavior of fed and fasted rats. Female rats were divided as: 1) control group (n = 5) fed ad libitum (ALD), and 2) an experimental fasted group (FG) (n = 5) undergoing 4 h of food deprivation. Rats were confronted with a T-maze containing a chow pellet (CP) and an avocado extract-coated pellet (AVO) and allowed to explore and consume either pellet of choice. OB and HP were dissected for histology analysis and $p75^{NTR}$ and AgRP gene expression was analyzed by means of RT-PCR. We found that FG rats were significantly faster when performing feeding tasks compared to ALD rats (median latencies: 46.6” vs 2’ 46”, p= 0.032). Both, FG and ALD rats consumed higher portions of AVO in comparison to CP (total consumption: FG, 10.4 ± 0.7 g AVO vs 6.5 ± 0.8 g CP; ALD, 8.7 ± 0.8 g AVO vs 3.1 ± 0.3 g CP), however these results did not show statistical significance. AgRP RNA was not expressed in the HP of neither FG nor ALD rats, however, was expressed in the OB of both groups. $p75^{NTR}$ RNA was expressed in the HP and OB of FG rats. In conclusion, metabolic states motivate different behavioral feeding responses in rats as suggested by shorter latencies to feed in the FG group, and induce the expression of $p75^{NTR}$ and AgRP in brain structures associated with non-homeostatic food intake regulation.

Keywords: Food intake regulation, appetitive behavior, $p75^{NTR}$, AgRP, ¨Hass¨ avocado.

RESUMEN

Las estructuras clásicas no homeostáticas involucradas en la regulación de la ingesta de alimentos están influenciadas recíprocamente por señales metabólicas. Los péptidos orexigénicos expresados en el bulbo olfatorio (OB) y el hipocampo (HP) modulan el procesamiento y la memoria olfativa, respectivamente. Los circuitos hipotalámicos también modulan el comportamiento de alimentación mediante la activación y liberación de AgRP en respuesta a señales orexigénicas periféricas. Una alimentación adecuada en respuesta a estados de ayuno requiere la expresión de $p75^{NTR}$ en neuronas AgRP. Dada la estrecha relación entre las diferentes estructuras de alimentación, nos preguntamos si existe un papel similar para $p75^{NTR}$ y AgRP dentro del OB y HP en el comportamiento de la ingesta consumatoria de ratas saciadas y en ayuno. Ratas hembras se dividieron en: 1) grupo de control (n = 5) alimentadas ad libitum (ALD) y 2) un grupo experimental en ayuno (FG) (n = 5) sometido a 4 h de privación de alimentos. Las ratas se enfrentaron a un laberinto en T conteniendo un pellet de comida (CP) y un pellet recubierto de extracto de aguacate (AVO) y se les permitió explorar y consumir cualquier estímulo apetitivo de su elección. Se diseccionaron los OB y HP para análisis histológico y se evalúo la expresión de los genes $p75^{NTR}$ y AgRP mediante RT-PCR. Encontramos que las ratas FG realizaron las tareas conductuales a una velocidad significativamente más rápida en comparación con las ratas ALD (medianas de latencias: 46.6” vs 2’ 46”, p= 0.032). Tanto las ratas FG como ALD consumieron porciones más altas de AVO en comparación con CP (total consumido: FG, 10,4 ± 0,7 g AVO frente a 6,5 ± 0,8 g CP; ALD, 8,7 ± 0,8 g AVO frente a 3,1 ± 0,3 g CP), sin embargo, estos resultados no mostraron significancia estadística. El ARN de AgRP no se expresó en el HP de las ratas FG ni ALD, sin embargo, si se expresó en el OB de ambos grupos. El ARN de $p75^{NTR}$ se expresó en el HP y OB de ratas FG. En conclusión, los estados metabólicos motivan distintas respuestas conductuales en la alimentación, como

lo sugieren las latencias para alimentarse más cortas en el grupo FG, e inducen la expresión de $p75^{NTR}$ y AgRP en estructuras cerebrales asociadas con la regulación de la ingesta de alimentos no-homeostática.

Palabras clave: Regulación de la ingesta alimenticia, $p75^{NTR}$, AgRP, aguacate ¨Hass¨.

OBJECTIVES

Overall Objective

To determine the consummatory ingestive behavior and molecular responses in the olfactory bulb and hippocampus of fasted rats undergoing a decision-making environment.

Specific Objectives

1. To study the appetitive decisions of fed and fasted rats.
2. To determine the expression of $p75^{NTR}$ and AgRP transcripts in the olfactory bulb and hippocampus of fed and fastest rats.

INTRODUCTION

Over hundreds of thousands of years, food intake in humans has evolved into a sophisticated and complex system that has allowed us to fulfill qualitive and quantitative nutritional demands for survival (Berthoud et al., 2017; Zucoloto, 2011). Most species have similar nutritional needs compared to humans; however, vast differences can be found in regard to their feeding behavior (Zucoloto, 2011). Even amongst humans, this variation is enormous (Zucoloto, 2011).

Food intake is influenced by many factors that ultimately determine an individual's state of hunger, satiety, and appetite. Considering that these concepts are closely intertwined, it is important to establish their definitions and roles in energy intake. Hunger is defined as a physiological demand for nutritional replenishment generated by metabolic processing, which in turn stimulates a drive for food intake (Smith and Ferguson, 2008). In contrast, satiety is the sensation of feeling full after resolution of caloric deficiency (Andermann and Lowell, 2017; Smith and Ferguson, 2008). Appetite, in turn, compromises a psychological desire to eat and involves diverse cognitive and socio-environmental signals (Jain and Singh, 2018).

An adequate regulation of food intake requires the interaction of homeostatic and non-homeostatic factors (Figure 1) (Begg and Woods, 2013; Liu and Kanoski, 2018). Homeostatic pathways regulate food intake in response to metabolic needs (i.e., energy deficiency) (Liu and Kanoski, 2018). Stimulation of central hypothalamic feeding centers and the subsequent consumption of food depend on numerous orexigenic and anorexigenic peptides (particularly AgRP/NPY and POMC/CART, respectively) that are integrated to reflect energy status, alternating between hunger and satiety states. More particularly, hypothalamic AgRP neurons require the expression of the neurotrophin receptor p75 for stimulation of homeostatic feeding during fasting conditions (Podyma et al., 2020b).

Non-homeostatic pathways, also referred to as hedonic eating, are driven by cognitive, emotional, environmental, and executive factors (Berthoud et al., 2017). During a feeding event, multimodal sensory input contributes to the hedonic characteristics of food associated to the meso-cortico-limbic reward system (Hernández Ruiz de Eguilaz et al., 2018). Neural representations based off learned associations are essential for determining future food choices, especially when exposed to conditioned stimuli, such as olfactory clues.

Research studies indicate that homeostatic and hedonic eating share many neural pathways (Andermann and Lowell, 2017; Liu and Kanoski, 2018) and both may be activated in different degrees by intestinal hormones and other metabolic feedback (Hernández Ruiz de Eguilaz et al., 2018; Rossi and Stuber, 2018). Studies conducted under fasting states have reported that the reward system focuses on the attainment of high calorie dense foods rather that low energy content (Hernández Ruiz de Eguilaz et al., 2018). The influence of such pathways and their overlapping substrates are still a subject of research. To better understand the role of homeostatic influence on hedonic choices, we aimed to study the appetitive behavior of rodents placed in a T-maze and faced with two distinct food alternatives (normal pellet vs. avocado extract-coated pellet) following controlled food-restrictive conditions. An additional aim of the present study is to determine if $p75^{NTR}$ and AgRP are expressed in the olfactory bulb (OB) and hippocampus (HP) of fasted rats and challenge its role on food intake.

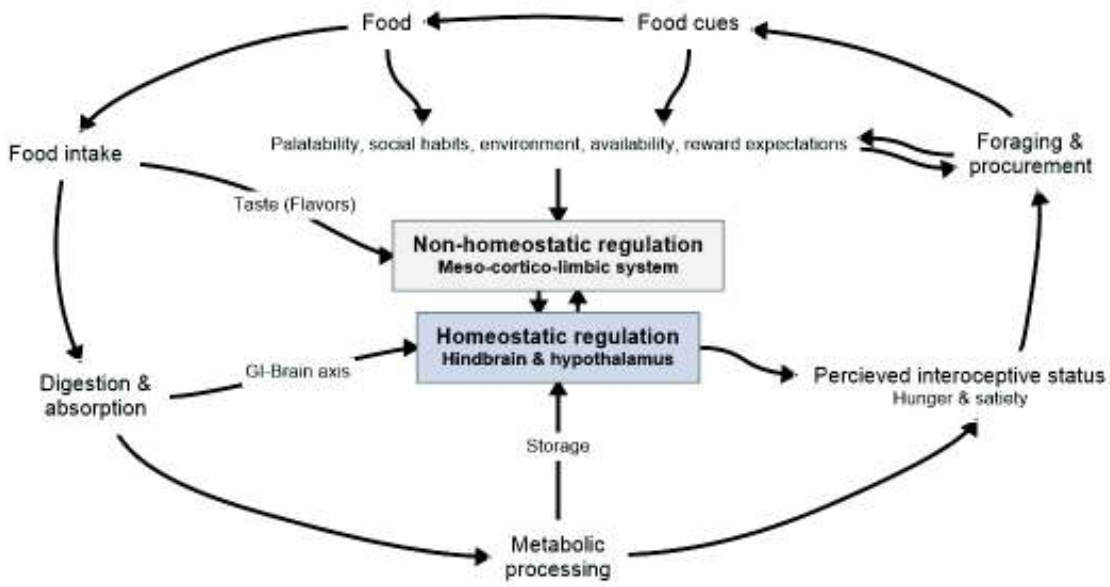

Figure 1. Influences upon the homeostatic and non-homeostatic systems. Food intake requires the interaction between the homeostatic system (receives and integrates nutritional information from periphery signals before, during, and after food consumption) and the non-homeostatic system (integrates emotional, cognitive, and executive functions based on memorial representations and environmental food-related cues) (modified from Berthoud et al., 2017).

THEORETICAL FRAMEWORK

Homeostatic Regulation of Food Intake

Hunger, satiety, and energy balance are controlled by an integrated neuro-endocrine system (Hernández Ruiz de Eguilaz et al., 2018). Energy homeostasis requires the central nervous system (CNS) to detect and interpret metabolic status signals in order to generate behavioral responses that ensure metabolic requirements are met (Begg and Woods, 2013; Ferrario et al., 2016; Smith and Ferguson, 2008). Unique taste receptors, mechanoreceptors, and chemoreceptors distributed in the gut detect and communicate the volume and nutritional content of food to the brain via two main mechanisms: by direct gastrointestinal vagal afferent stimulation, and via other peripheral signals including the production of neuropeptides, neurotransmitters, and hormones (Table I) (Berthoud et al., 2017; Ferrario et al., 2016; Smith and Ferguson, 2008; Williams and Elmquist, 2012).

Table I. Peripheral signals reflecting nutritional status.

Anorexigenic signals	**Orexigenic signals**
Cholecystokinin	Migrating motor complex
Glucagon-like peptide-1	Motilin
Peptide YY	Ghrelin
Pancreatic polypeptide	Orexin A & B
Somatostatin	
Leptin	
Glucose	
Insulin	
Amylin	

(data taken from Hernández Ruiz de Eguilaz et al., 2018)

Peripheral Signals

When an individual is in a fasting or intraprandial state, the stomach and small intestine go through a cycle of quiescence and contractions known as the migrating motor complex (MMC), largely associated with gut hormone release (Al-Missri and Jialal, 2020; Sanger et al., 2011). This system varies between species, for instance, phase III, described as “hunger contractions”, in rats occurs at a faster rate and lacks a functional motilin system, in opposition to humans (Sanger et al., 2011). The release of motilin, an oligopeptide periodically secreted by Microfold cells (M cells) in the small intestine and increased during fasting, is directly proportionate to increases in MMC peristalsis and is considered a primary orexigenic hormone (Al-Missri and Jialal, 2020; Zhao et al., 2018). It’s speculated that the orexigenic effect of motilin is conveyed via vagal afferents originated from phase III contractions (Zhao et al., 2018). Motilin may also indirectly promote hunger by inducing the release of ghrelin (Sanger et al., 2011).

Ghrelin, a 28-amino acid peptide released from the stomach, is a key orexigenic hormone (Sanger et al., 2011; Zhao et al., 2018) that stimulates appetite via a growth hormone secretagogue receptor (GHSR) (Andermann and Lowell, 2017). Amongst its many actions, ghrelin promotes effortful food seeking, favoring primarily palatable food (Ferrario et al., 2016). In humans, plasmatic ghrelin concentration present a diurnal rhythm, displaying a nocturnal peak during fasting, and a decrease in response to rises in glucose and insulin, including once refed, individuals who consume chronic calorie-dense diets, and in obesity (Ibrahim Abdalla, 2015; Sanger et al., 2011). Although a minor role in appetite, increases in orexin A and B plasma levels have also been observed during fasting (Sanger et al., 2011).

Immediately during and after feeding, short-term homeostatic satiety signals are released in response to stretch receptors and chemoreceptors in the

gastrointestinal tract (Smith and Ferguson, 2008), many of which are proportional to the calories consumed, limiting meal sizes (Woods et al., 2006). These satiety signals are relayed via sensory nerves to the hindbrain or reach the hindbrain directly via circulation (Woods et al., 2006). Cholecystokinin (CCK) has been shown to influence the termination of food consumption by decreasing meal size and duration (Smith and Ferguson, 2008; Williams and Elmquist, 2012). The release of CCK activates stomach and duodenum vagal afferents sensitive to meal volume and composition which signal to the brain satiety and consequently suppresses food intake (Montiel-Herrera et al., 2018; Smith and Ferguson, 2008; Williams and Elmquist, 2012).

Glucagon-like peptide-1 (GLP-1) is an incretin mainly produced by the intestine and is co-released with peptide YY once a fasted individual is refed (Montiel-Herrera et al., 2018.; Williams and Elmquist, 2012). GLP-1 delays gastric emptying, inhibits the secretion of gastric acid, promotes the secretion of glucose-dependent insulin, and plays a role in the CNS by promoting satiety (Montiel-Herrera et al., 2018; Williams and Elmquist, 2012).

Additionally, pancreatic polypeptide and somatostatin, both pancreatic peptides, have shown to reduce food intake via vagus afferents in normal and obese mice when administered systemically (Woods et al., 2006).

Moreover, a second set of signals, ongoingly secreted in proportion to an individual's body fat, help determine long-term food intake (Woods et al., 2006). Adipose tissue secretes adipokines, metabolically active proteins involved in control of feeding, glucose and lipid metabolism, among other endocrine functions (Smith and Ferguson, 2008). Leptin, an adipokine circulating proportional to the amount of adipose tissue, has shown to provoke a downregulation of orexigenic neuropeptides in the hypothalamus, whereas an upregulation is produced on anorexigenic neuropeptides in said area (Smith and Ferguson, 2008). In other terms, when an individual gains weight, leptin levels rise and decrease food intake

(Smith and Ferguson, 2008). Leptin´s cytokine receptor is found in multiple of the hypothalamic and extrahypothalamic areas expressing ghrelin receptors, ultimately contributing to a counter-regulation amongst themselves (Williams and Elmquist, 2012).

Furthermore, insulin is an essential adiposity signal, wherein, its levels are parallel to the amount of white adipose (Woods et al., 2006). As a result, a negative feedback signal is conveyed by plasma insulin entering the CNS leading to both regulation of body fat and liver glucose secretion (Woods et al., 2006). Concurrently, overindulging to the point of body weight gain increases insulin levels in the CNS resulting in a decrease in food intake (Woods et al., 2006). Additionally, amylin, a 36-amino acid anorectic peptide, is co-secreted with insulin when an individual has initiated feeding, during the meal, and postprandially (Smith and Ferguson, 2008; Woods et al., 2006). Amylin functions as an adiposity signal and a satiety signal (Woods et al., 2006), playing a role on inhibition of feeding, possibly by reducing the expression of orexigenic neuropeptides in the lateral hypothalamic area (Smith and Ferguson, 2008) or by amplifying the brain´s sensitivity to satiety signals (Woods et al., 2006).

Central Feeding Centers

Peripheral signals regarding feeding converge primarily in the hindbrain via different nerve pathways (Figure 2): vagus nerve afferents and/or directly stimulating receptors (Begg and Woods, 2013). Hunger and satiety signals, including gastric stretch, relay onto the nucleus tractus solitarius (NTS) and/or stimulate receptors expressed on sensory circumventricular organs (CVOs), particularly the area postrema (AP) (Begg and Woods, 2013) and the subfornical organ (SFO) (*Figure 2*) (Ahima and Antwi, 2008; Smith and Ferguson, 2008). The NTS and CVOs relay these signals to several areas of the CNS, particularly

hypothalamic nuclei and forebrain areas (Begg and Woods, 2013; Liu and Kanoski, 2018; Smith and Ferguson, 2008). Although not considered a CVO, the arcuate nucleus can also detect circulating metabolic status signals (Smith and Ferguson, 2008).

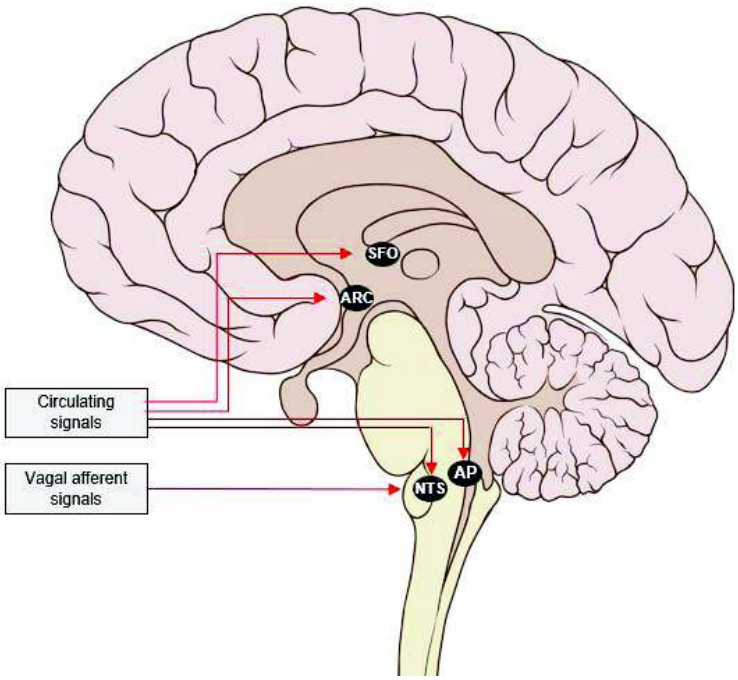

Figure 2. Detection of peripheral metabolic signals by sensory structures in the CNS. Homeostatic signals arising from the periphery are detected by structures in the central nervous system. Blood-borne signals cross the BBB and are detected by the ARC, SFO, NTS, and AP. Vagal afferents, such as gastric stretch, reflecting an individual´s nutritional status are relayed to the NTS. Abbreviations: ARC, arcuate nucleus; AP, area postrema; BBB, blood-brain barrier; CNS, central nervous system; NTS, nucleus of the solitary tract; SFO, subfornical organ (modified from Begg & Woods, 2013).

The NTS and AP are important relay centers for feeding behavior. Anatomically, the AP is positioned adjacent to the NTS, both having reciprocal projections and several peptidergic receptors (Smith and Ferguson, 2008). This unique placement grants the caudal brainstem an important role in the detection and signaling of energy status. Beyond relaying peripheral energy signals, individual AP and NTS neurons may also be stimulated by circulating CCK, orexin A, GLP-1, amylin (Rowland et al., 1997), insulin, and PYY (Smith and Ferguson, 2008). More particularly, the AP appears to also be responsive to ghrelin (Smith and Ferguson, 2008). The SFO, another CVO involved in the detection of peripheral feeding molecules, is also influenced by circulating ghrelin and amylin (Smith and Ferguson, 2008). Following the detection of peripheral status signals, the caudal brainstem and other CVO's project extensive connections to different brain regions.

The hypothalamus possesses the most important and crucial role in controlling food intake. The regulation of feeding involves well-defined hypothalamic nuclei that receive signals regarding nutritional status, along with non-homeostatic signals, and communicate them amongst themselves and to other CNS areas (Smith and Ferguson, 2008).

The arcuate nucleus (ARC) contains two distinct neuronal populations which are activated by either orexigenic or anorexigenic peptides (Smith and Ferguson, 2008). The stimulation of orexigenic neurons releases agouti gene-related peptide (AgRP) and neuropeptide Y (NPY), whereas, pro-opiomelanocortin (POMC) and cocaine-and amphetamine related transcript (CART) are released when anorexigenic neurons are activated (Smith and Ferguson, 2008; Williams and Elmquist, 2012). For instance, ghrelin signals the release of AgRP/NPY which consequently stimulates hunger (Gahagan, 2012). In contrast, the release of insulin, PYY, and leptin inhibit AgRP/NPY neurons, whereas POMC and CART neurons are stimulated, resulting in decreased feeding (Gahagan, 2012). In addition, AgRP neurons are one of many components of the food entrainable

oscillator (FEO), a circadian rhythm on food intake behavior, that seem to impact food anticipatory activity (FAA) (increased locomotor activity preceding feeding) in mice (Podyma et al., 2020b). In AgRP neuron ablated animals, FAA is diminished and along with FEO, both appear to be altered more prominently during daytime (Podyma et al., 2020b).

The ARC synapses to second-order neurons residing in the paraventricular nucleus (PVN), ventromedial nucleus (VMH), lateral hypothalamic area (LHA), and dorsomedial nucleus (DMN) which in turn convey signals to the dorsal vagal complex (DVC) (Smith and Ferguson, 2008). Research suggests that PVN neurons are wildly influenced by peripheral and central peptides associated with food intake and therefore has an enormous impact on feeding behavior (Smith and Ferguson, 2008). Potent stimulation on feeding by the PVN has been demonstrated as a result of direct microinjections of orexigenic peptides, whereas, a decrease in feeding appears when anorexigenic peptides, such as GLP-1, are directly introduced into the PVN (Smith and Ferguson, 2008). Moreover, in addition to receiving ARC projections, the PVN also receives input from LHA and NTS orexigenic neurons (Figure 3) (Smith and Ferguson, 2008).

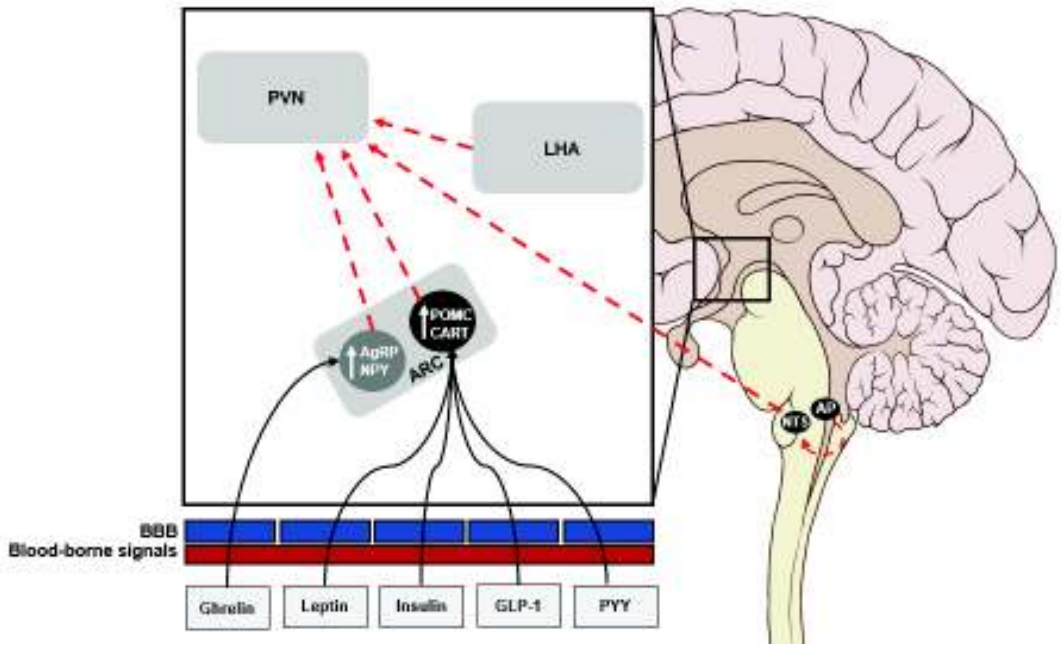

Figure 3. Anatomical projections to the paraventricular nucleus**.** Peripheral signals cross the blood-brain barrier and are transported to the ARC; leptin, insulin, GLP-1, and PYY stimulate POMC/CART neurons, whereas ghrelin stimulates AgRP/NPY cells. The PVN receives signals from the ARC, the LHA, and the NTS. The NTS contains NPY cells that relays metabolic information to the PVN. Abbreviations: AgRP, agouti-related peptide; AP, area postrema; ARC, arcuate nucleus; GLP-1, glucagon-like peptide 1; LHA, lateral hypothalamic area; NPY-neuropeptide Y; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin cells; PVN, paraventricular nucleus (modified from Begg & Woods, 2013)**.**

The stimulation of VMH neurons favors the recognition of satiety, leading to food intake termination. This nucleus contains CCK/leptin receptors and glucose-sensing neurons involved in short/long term satiety and glucose homeostasis regulation, respectively (Smith and Ferguson, 2008). Additionally, stomach distention, carried through vagus afferents, activates VMH neurons (Smith and Ferguson, 2008).

In opposition to VMH neurons, DMN stimulation plays an important role in increasing food intake. Neurons expressing NPY mRNA in the DMH are inhibited by CCK (Smith and Ferguson, 2008) and positively influenced by chronic food restriction or alternatively, in the absence of CCK (Smith and Ferguson, 2008). Interestingly, VMH is also involved in food entrainable rhythms, possibly by receiving inputs from the suprachiasmatic nucleus (Smith and Ferguson, 2008).

The LHA contains neuronal populations that affect short and long-term feeding (Smith and Ferguson, 2008). Alike the VMH, glucose-sensitive neurons in the LHA are stimulated by decreases in glucose, thus, contributing to glucose homeostasis (Smith and Ferguson, 2008). However, these neurons appear to be inhibited by leptin and activated by orexin (Smith and Ferguson, 2008). Neurons expressing orexin A are activated when an individual is in a hypoglycemic state or by starvation, resulting in the initiation of feeding, and are inhibited by satiety signals that are relayed onto the NTS and projected towards the LTA (Smith and Ferguson, 2008). Through ghrelin's stimulation, the LHA also appears to affect FAA by promoting wakefulness (Carneiro and Araujo, 2009). Furthermore, the LHA distributes orexigenic fibers to other areas of the CNS involved in the regulation of feeding (Smith and Ferguson, 2008). Increases in food intake may also be favored by LHA neurons expressing melanin-concentrating hormone, which may be modulated by glucose, insulin, and leptin (Smith and Ferguson, 2008).

Brain Derived Neurotrophic Factor and Neurotrophin Receptor p75

An exceptionally interesting homeostatic control is carried by the neurotrophin family, specifically the brain-derived neurotrophic factor (BDNF), which constitutes structurally related growth factors (Xu et al., 2003). BDNF receptor signaling, expressed in CNS and non-CNS regions (i.e. peripheral metabolic tissue), has been suggested to affect metabolic (Baeza-Raja et al., 2016) and circadian processes (Podyma et al., 2020b).

During development, target cells synthesize a BDNF precursor (pro-BDNF) that regulates its biological actions through a posttranslational mechanism known as proteolytic cleavage. Pro-BDNFs are cleaved into two fragments, a BDNF pro-domain, and a bioactive BDNF (mature fragment) (Chao, 2003; Kojima and Mizui, 2017), which produce opposing effects following receptor activation. BDNF fragments bind to two transmembrane receptors, tyrosine receptor kinase B (TrkB) and p75 neurotrophin receptor ($p75^{NTR}$), capable of signaling autonomously and modifying its coreceptors specificity and affinity (Figure 4) (Hasegawa et al., 2004; Ibáñez and Simi, 2012), and co-expressed in hypothalamic circuits controlling feeding (i.e. DMH, LH, and VMH) (Xu et al., 2003). Therefore, BDNFs' actions, such as cell survival, differentiation, and synaptic plasticity, are controlled by diverse factors including secretion levels, affinity to receptors, and duration/intensity of intracellular signaling (Chao, 2003).

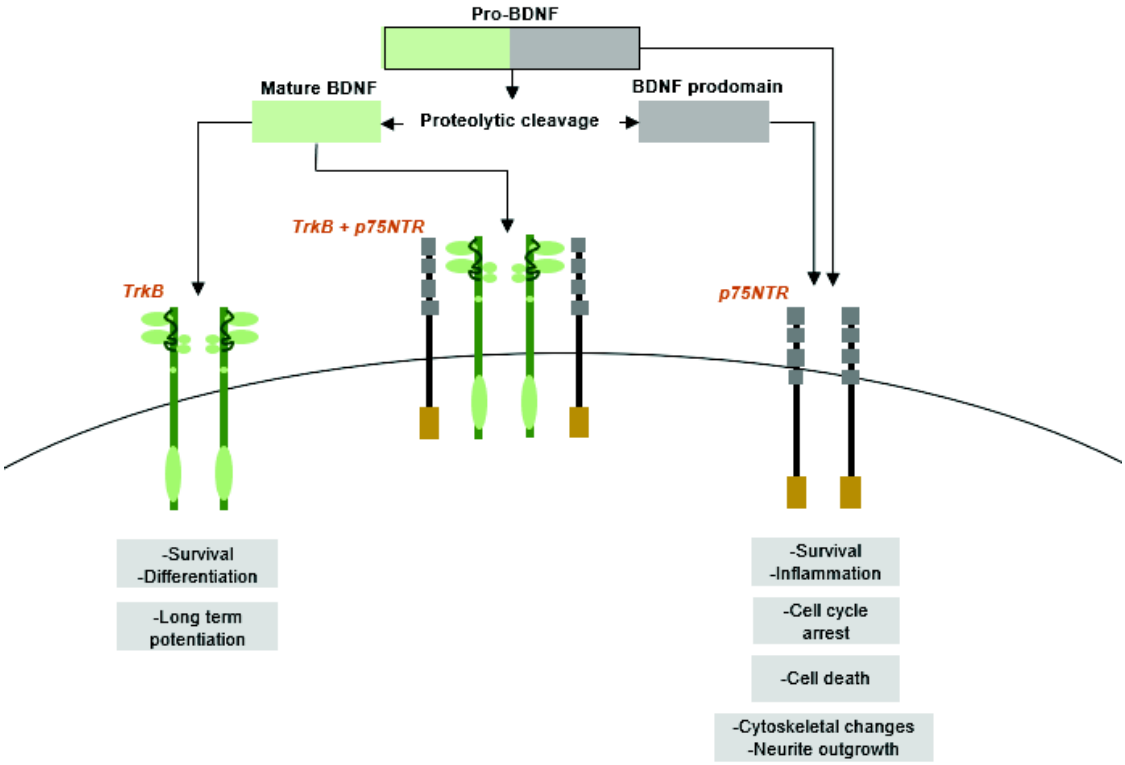

Figure 4. BDNF receptor complexes and biological actions. Mature BDNF ligands bind to TrkB receptors mediating cell survival and differentiation via diverse intracellular signaling pathways. Co-expression of $p75^{NTR}$ can modify TrkB receptor affinity and specificity to ligands. $p75^{NTR}$ complexes exerts cell death, survival, inflammation, cytoskeletal changes, neurite outgrowth, and cell death via intracellular adaptor proteins by binding (preferably) to pro-BDNF forms and the BDNF prodomain (modified from Chao, 2003; Hempstead, 2002).

Interestingly, BDNF receptor signaling in the CNS has shown to mediate certain behavioral and feeding abnormalities (Chao, 2003; Hashimoto et al., 2005). Mice lacking BDNF manifest increased aggressiveness, hyperactivity, and hyperphagia (Chao, 2003), thus correlating its activation to appetite suppression and reduction of body weight (Baeza-Raja et al., 2016).

Mice lacking BDNF show characteristic neuronal and behavioral anomalies, similar to those with null-mutations in TrkB genes (Hasegawa et al., 2004), including increased body weight, increased linear growth, hyperglycemia, hyperactivity, hyperleptinaemia, and hyperinsulinaemia, thereby constituting an obesity phenotype (Xu et al., 2003). Furthermore, selective deletion of TrkB in mice DMH neurons increases food intake exclusively during the day without affecting night food intake (Liao et al., 2019). In addition, mice presenting BDNF polymorphism exhibit diminished memory encoding and retrieval processing in the hippocampus (HP) (Chao, 2003; Hashimoto et al., 2005), analogous to mice expressing TrkB gene mutations during HP-dependent learning tasks (Chao, 2003). Thus, BDNF signaling via TrkB is responsible for facilitating hippocampal long-term potentiation and synaptic plasticity (Chao, 2003), perhaps playing a role in retrieving memorial representations of meals.

Although there are some scientific advances in the field, the molecular pathways involved in energy balance regulation remains unclear. It has been suggested that BDNFs' role in appetitive suppression may be the result of a downstream component via MC4R signaling to the VMH (Xu et al., 2003). Unique excitatory neurons in the VMH may transport the BDNF peptides to TrkB-expressing neurons receiving VMH projections (Xu et al., 2003). Furthermore, TrkB-expressing neurons located in the OB may also contribute to feeding by allowing the olfactory system to differentiate meals with low and moderate fat content, therefore promoting palatability (Xu et al., 2003).

In contrast to TrkB, $p75^{NTR}$ belongs to the tumor necrosis factor (TNF) receptor superfamily and possesses a low nanomolar affinity (Dechant and Barde, 2002) to BDNF of 10^{-9} M (Hasegawa et al., 2004). Structurally, $p75^{NTR}$ is a type I transmembrane protein that possesses a cysteine rich extracellular domain (Vilar, 2017). Rather than exhibiting intrinsic catalytic activity (Hasegawa et al., 2004; Vilar, 2017), short-term biological actions are produced via diverse intracellular pathways that may include different cytoplasmic interacting proteins, whereas long-term trophic effects are modified by gene regulation (Kojima and Mizui, 2017). Similar to BDNF, $p75^{NTR}$ can further modulate intracellular signaling by undergoing cleavage and receptor intramembrane proteolysis (Vilar, 2017).

Interacting protein molecules play key roles during developmental and pathological stages. $p75^{NTR}$´s most recognized function is to promote cell apoptosis, particularly during seizures and inflammation, via an intracellular death domain (Ibáñez and Simi, 2012). $p75^{NTR}$´s cell death function is preferentially activated by pro-BDNF, BDNF pro-domain (Chao, 2003), and other non-NT ligands, including the β-Amyloid precursor protein (APP) and the neurotoxic prion protein (PrP) fragment (Hasegawa et al., 2004). Furthermore, ligands binding to $p75^{NTR}$ in developing neurons promote axonal outgrowth via RhoA signaling (Hasegawa et al., 2004). In contrast, upon the interaction of $p75^{NTR}$ with the Nogo receptor, a receptor complex is formed for Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (Omgp), which in turn inhibits neuronal outgrowth (Chao, 2003; Hasegawa et al., 2004). Therefore, cell types and conditions along with the expression of specific adaptor proteins modulate $p75^{NTR}$ actions (Figure 5).

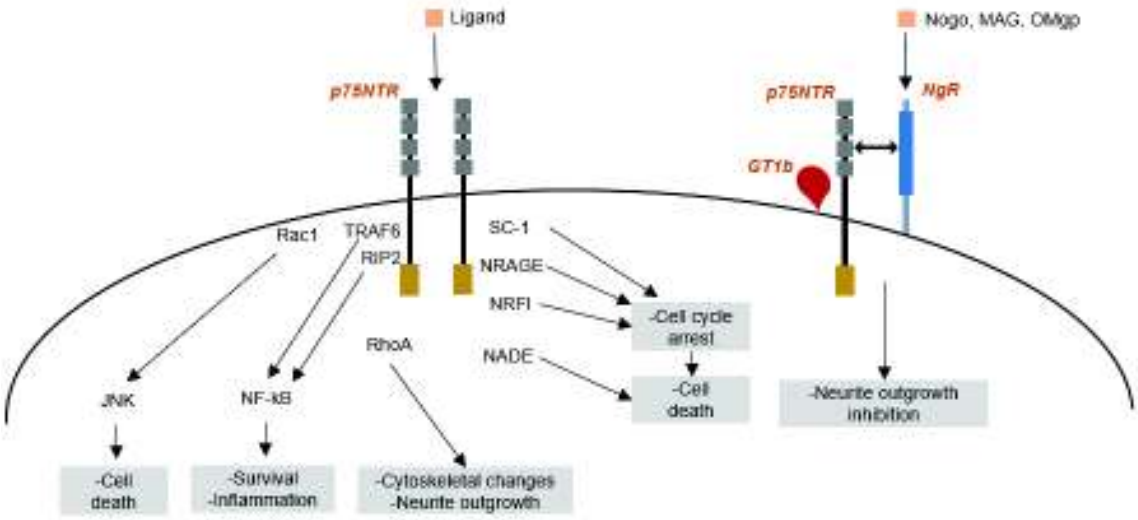

Figure 5. $p75^{NTR}$ signaling cascades. $p75^{NTR}$´s actions are mediated by specific adaptor proteins and receptor complexes. Adaptor proteins Rac1, JKN, and NADE mediate cell death, whereas TRAF6, RIP2, and NF-kB mediate survival and inflammation. RhoA signals cytoskeletal changes and neuronal outgrowth. Cell cycle arrest and subsequent cell death is signaled by SC-1, NGRAGE, and NRFI. The receptor complex formed upon the interaction of $p75^{NTR}$, the Nogo receptor (NgR), and ganglioside GT1b inhibits neuronal outgrowth in oligodendrocytes following the binding of ligands Nogo, MAG, and OMgp (modified from Hasegawa et al., 2004; Hempstead, 2002).

Studies have suggested $p75^{NTR}$ to influence other higher-order functions, including learning (Chao, 2003) and the most novel finding, regulation of homeostatic feeding (Podyma et al., 2020b). A recent study, conducted by Podyma, et al. (2020), demonstrated that $p75^{NTR}$ depleted mice inadequately respond to energy deficits. Furthermore, $p75^{NTR}$ was reported to be necessary for (1) the consumption of adequate energy after fasting and (2) the development of FAA, both observed in a circadian phase dependent manner during daytime (Podyma et al., 2020b). The expression of $p75^{NTR}$ along with its fasting-induced refeeding effects was demonstrated in two hypothalamic nuclei required for detection of energy status, the ARC and DMH (Podyma et al., 2020a, 2020b). Daytime FAA induced by ARC AgRP activation was found to also require expression and signaling of $p75^{NTR}$ (Podyma et al., 2020b). These observations were suggested to be a result of AgRP dendritic remodeling in response to fasting, when $p75^{NTR}$ ARC neurons are adequately activated (Podyma et al., 2020b). Additionally, alterations in peripheral hunger signals were observed in $p75^{NTR}$-KO mice, such as a lack of diminished leptin levels during overnight fasting (Podyma et al., 2020b).

In opposition, Baeza-Raja, et al. (2016) demonstrated that mice lacking $p75^{NTR}$ did not present differences in appetitive behavior compared to control mice (Baeza-Raja et al., 2016). Despite this finding, the study reported that $p75^{NTR}$ promotes diet-induced obesity by inhibiting lipolysis and energy expenditure in peripheral fat (Baeza-Raja et al., 2016).

Hence, it is important to further study the possible role of $p75^{NTR}$ in other brain areas involved in food intake regulation, including non-homeostatic structures (i.e., the OB and HP).

Non-Homeostatic Regulation of Food Intake

In addition to metabolic feedback, feeding behavior involves complex neural mechanisms that allow individuals to adapt and coordinate food seeking in prevailing environments to satisfy metabolic needs (Zheng et al., 2009). Consequently, pursuing and consuming food is integrated by both homeostatic and non-homeostatic controls (Figure 6). Non-homeostatic controls provide reward expectancies and representations of food based on hedonic properties and previous learned experiences (Berthoud et al., 2011; Liu and Kanoski, 2018). In turn, all factors are influenced by our environment and are processed via the mesocorticolimbic system (Hernández Ruiz de Eguilaz et al., 2018).

From an evolutionary perspective, hedonic eating is arguably the result of an evolved system developed to motivate the attainment of calorie dense non-toxic foods in a scarce and hostile environment that would have facilitated survival. Through cognitive, reward, and emotional processes (Berthoud, 2011), all attributes of food engage in memorial representations that guide future behavioral responses (i.e., motivate or avoid consumption) (Berthoud et al., 2011; Shin et al., 2009).

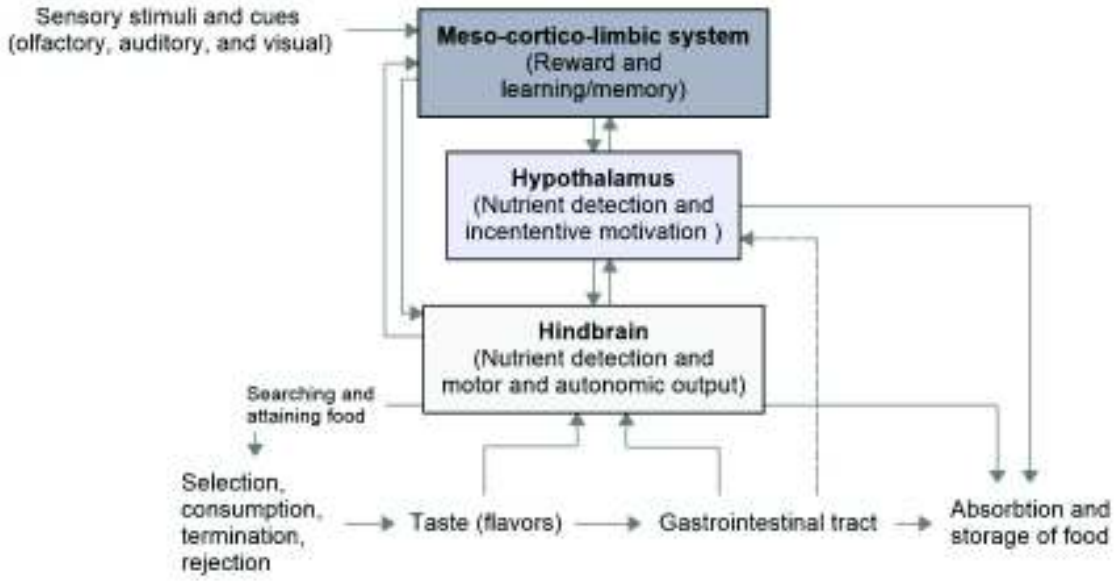

Figure 6. General overview of the neuroendocrine connections involved in food intake. The hindbrain detects sensory information via vagal afferents and circulating factors and controls motor output involved in the ingestion, digestion, and absorption of food. The hypothalamus can regulate peripheral signals by autonomic and endocrine outflow. The meso-cortico-limbic system is closely connected to the hypothalamus and the hindbrain and determines emotional, cognitive, and executive aspects of appetitive behavior (modified from Berthoud et al., 2017).

Hedonic and Rewarding Properties of Food Intake

Food palatability refers to the subjective feeling of pleasure upon meal consumption based on the full sensory impact of food (Berthoud, 2012; Hernández Ruiz de Eguilaz et al., 2018). This term generally refers to ¨liking¨ specific foods and flavors, whereas ¨wanting¨ refers to having appetitive disposition to eat (Havermans et al., 2009).

However, pleasantness derived from a specific food may decline upon prior consumption of that specific food until satiety. This phenomenon is known as sensory-specific satiety and is responsible for decreases in motivation to obtain a certain food reward, as demonstrated in experimental rat models (Havermans et al., 2009).

Multisensory inputs are integrated primarily in the amygdala, insular, and the orbitofrontal cortex (OFC) (implicated in encoding perceived pleasantness of taste and therefore, ¨liking¨). The OFC is responsible for olfactory-to-taste association learning giving place to the development of flavors (combination of taste, olfactory, and texture inputs) (Table II) (Rolls, 2015) and consequently determining a hedonic or predictive reward value (Liu and Kanoski, 2018). Such value may be modified by an individual´s metabolic state, where hunger increases perceived pleasantness and satiety (or sensory-specific satiety) promotes hedonically neutral experiences (Rolls, 2015).

Table II. Hedonic determinants**.**

Food property	Sensory input
Taste	Direct influence on taste perception. Generated by electrical changes in gustatory cells upon exposure of food to specific receptors.
Smell	Closely related to taste; smells can be perceived directly through the nostrils or by aromas released from food in the mouth. Generated by stimulation of olfactory sensory neurons by odorant molecules.
Texture	Mechanical, structural, and surface characteristics of food. Detected via sight, touch, and auditory signals. Important indicator of whether fat is present in a food.

(data taken from Hernández Ruiz de Eguilaz et al., 2018)

Input from the OFC following the consumption of highly palatable foods increases extracellular dopamine concentrations in the nucleus accumbens (ACB) via dopaminergic input from the ventral tegmental area (VTA) (Hernández Ruiz de Eguilaz et al., 2018; Liu and Kanoski, 2018). Dopamine release produces conditioned incentive motivation by associating conditioned cues to food reward and subsequently activating ACB dopamine release (Liu and Kanoski, 2018).

Along with non-homeostatic controls, intestinal hormones can also modulate cue induced dopaminergic signaling pathways (Hernández Ruiz de Eguilaz et al., 2018; Liu and Kanoski, 2018). Under food deprived conditions, leptin and GLP-1 inhibit dopamine signaling, whereas, ghrelin increases VTA dopamine release (Hernández Ruiz de Eguilaz et al., 2018). In rodents, ghrelin signaling in the VTA has demonstrated to promote incentive motivation and consumption of palatable food (Liu and Kanoski, 2018). Additionally, in response to conditioned stimuli, ghrelin also engages in phasic dopaminergic fluctuations (Liu and Kanoski, 2018). The ACB is thus part of a neural circuit integrated by the VTA, LHA, and orexin neurons (Figure 7), that is essential for communicating metabolic status signals and determining goal-directed incentive motivation (Berthoud, 2012).

Interestingly, sustained hyperphagia resulting from repeated exposure to palatable diets in Wistar rats led to an increased threshold of LHA electrical self-stimulation along with significantly reduced dorsal striatum dopamine D2-receptor expression in proportion to weight gain (Berthoud, 2012). These findings did not normalize after two weeks of abstinence and 10% body weight loss, indicating that high-fat diets may cause irreversible changes (Berthoud, 2012).

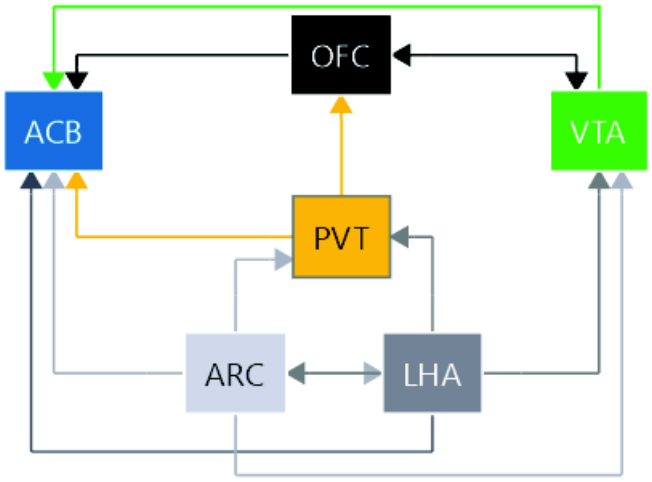

Figure 7. Major neural structures implicated in reward pathways. Dopamine signals from the VTA are projected to the OFC and the ACB. Hypothalamic nuclei, including ARC and LHA, and the PVT closely interact with the OFC, ACB, and VTA generating motivating behaviors upon rewarding stimuli. Abbreviations: ACB, nucleus accumbens; ARC, arcuate nucleus; LHA, lateral hypothalamic area; OFC, orbitofrontal cortex; PVT, paraventricular nucleus; VTA, ventral tegmental area (modified from Ferrario et al., 2016).

In addition to the VTA, changes in appetitive behavior have been observed in corticolimbic and thalamic afferents conveying GABA- and glutamate- information to the ACB. Studies have demonstrated that GABA agonists and glutamate antagonists increase food consumption in satiated animals (Liu and Kanoski, 2018). Consistent with these findings, mice consuming sodium-depleted diets have demonstrated to have elevated dopamine signaling in response to sodium, thus, mesolimbic reward pathways appear to be influenced by micronutrient status, along with energy status (Liu and Kanoski, 2018).

Memorial Representations of Food

When exposed to multiple food choices in an environment, an individual must consider its innate preferences and knowledge acquired through learned experiences to obtain the more pleasurable stimuli. Expectations initially develop when presented to novel food, associating the food's sensory characteristics with its perceived satiety effects (Fiszman and Tarrega, 2017). These neural representations are available even before physical exposure to meals by simply thinking about food or via food cues and can activate physiological responses (salivation, insulin secretion, and gastric acid) (Berthoud, 2007).

Multimodal sensory input, including auditory, visual, tactile, and olfactory sensations, serve as conditioned stimuli to recall pleasant or aversive food experiences (Berthoud, 2007). It is likely that these experiences are formed, stored, and recalled by specialized cortical areas via sensory channels (Berthoud, 2007). Along with olfactory-to-taste associative learning, studies indicate that the OFC also engages in visual-to taste associative learning (Rolls, 2015). Consistent with this, other neuroimaging studies have linked the prefrontal/orbitofrontal cortex to maintaining memorial representations along with updated incentive properties in response to these food-related cues (Berthoud, 2007).

Previously learned experiences along with the visuospatial environment, and internal metabolic cues influence episodic meal-related encoding in the HP (Liu and Kanoski, 2018). Furthermore, studies in rats indicate that the HP plays a role in establishing conditioned learned associations between environmental and interoceptive stimuli (Liu and Kanoski, 2018). Together, memorial representations guide an individual towards certain appetitive behaviors, including food and portion selection (Figure 8) (McCrickerd and Forde, 2016).

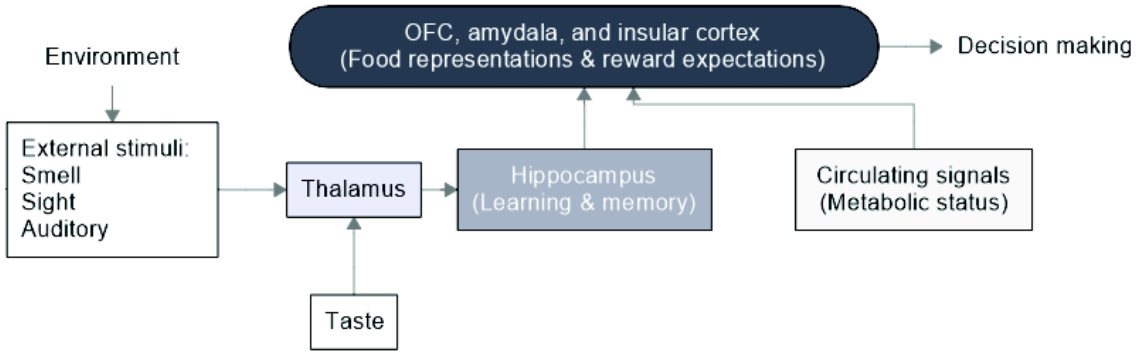

Figure 8. Factors influencing memorial representations. Sensory stimuli from the environment, including odors, visual input, and auditory cues, are relayed to the thalamus and form conditioned learned associations in the HP. The OFC, amygdala, and insular cortex maintain memorial representations along with updated incentive properties and are integrated with interoceptive metabolic status signals to influence food selection and consumption. Abbreviations: HP, hippocampus; OFC, orbitofrontal cortex (modified from Shin et al., 2009).

Expectations of Food Satiety

Learned associations of previous food experiences are attributed to intrinsic and extrinsic properties of food and establish expectations of satiety effects (Table III) (Fiszman and Tarrega, 2017). Thus, associations promote the consumption of specific foods and self-selected proportions.

Table III. Properties influencing food satiety expectations.

Property	Satiety expectations
Hedonic determinants (specifically texture)	Weaker appetite suppression is elicited by liquid foods, whereas solid or semi-solid foods have higher satiety values.
Oral processing behaviors	Orosensory exposure time and eating rate is positively related to expected satiation.
Macronutrient and energy content	Some studies show that fat content and energy dense foods are the best predictors of expected satiety, whereas other studies indicate high protein and fiber have higher satiating effects.
Familiarity	Novel foods elicit lower expected satiating effects until modified by experience.

(data taken from Fiszman & Tarrega, 2017)

Olfactory System

The upper segment of the nasal septum and the superior turbinates are covered by an olfactory epithelium containing Bowman's glands, support cells, and olfactory sensory neurons (OSNs). Each OSN contains a single odorant-receptor (OR) (Antunes and Simoes de Souza, 2016), responsible for detecting specific odorants. The OSN's axons group in filia which in turn form the olfactory nerves that continue through the olfactory foramina and reach the OB, located on the cribiform plate.

Researchers have found that rodents have between 1000–1500 functional ORs, whereas primates have around 350 (Zhang and Firestein, 2009). A particular OR family, guanine nucleotide G-protein coupled receptors (GPCRs), includes rhodopsin-like receptors and trace amine-associated receptors (TAARs), and can discriminate more than 1 trillion odorant stimuli (Zhang and Firestein, 2009). In rodents, TAARs have been presumably correlated with the detection of social cues, while odorant sensing of lipids may involve non-GPCR receptors (e.g. CD36) (Zhang and Firestein, 2009).

Studies in rats have suggested that the release of the hypothalamic neuropeptide orexin in the OB increases olfactory sensitivity and subsequently promotes the procurement of food, whereas leptin reduces olfactory sensitivity (Valladares Vega, 2015). Nutritional status therefore modulates olfactory sensitivity, where it has been documented that this increase is also seen in humans under fasted conditions (Valladares Vega, 2015). Additional studies in rats indicate that olfactory sensitivity follows rhythmic oscillations associated to the animal´s active phase and food deprivation alters OB oscillations from this normal pattern (Caba et al., 2014). Alternatively stated, the OB can be entrained by food, being periodic food the main cue for oscillations in the OB (Caba et al., 2014). This same study also suggested that the circadian clock in the OB regulates oscillations in the pyriform cortex

(structure responsible for identifying olfactory stimuli and intensity) (Caba et al., 2014; Rolls, 2015).

As mentioned, olfactory cues have demonstrated to stimulate the orbitofrontal cortex (in conjunction with the amygdala and insular cortex) (Berthoud, 2007) directly or via projections from the primary olfactory cortex (Rolls et al., 1996). Single neurons in the OFC receive the convergence of olfactory and taste inputs, providing associative learning by pairing (Rolls et al., 1996). Reduced activation in the OFC in response to olfactory cues and flavors (taste and olfactory components) has been observed in humans and monkeys when in a satiety state, further indicating that reward value/pleasantness is represented in the OFC (Rolls et al., 1996). Learned olfactory-to-taste association in the OFC allows remapping of original odor representations into more prominent representations associating odors to other stimuli (Rolls, 2015). The medial OFC tends to be activated primarily by pleasant odors, whereas unpleasant odors activate the lateral OFC (Rolls, 2015). In rat experimental trials, encoding associative olfactory cue memory requires a single brief training session (Bessières et al., 2017), whereas representations of flavors are learned at a slower rate (Rolls et al., 1996).

Avocados

Satiety is an important influence on inhibition of feeding following the postprandial period (Tremblay and Bellisle, 2015; Wien et al., 2013). In modern times, it is of special interest to study the satiating effects of foods capable of decreasing intake and therefore contributing to weight management in the obesogenic community (Haddad et al., 2018). In addition to the psychological mechanisms involved in promoting satiety, appetite control theories propose that energy density, macronutrient, micronutrient, and other non-nutrient constituents influence satiating effects of foods (Tremblay and Bellisle, 2015). Nutrient dense whole

foods, such as avocados, favor the release of postprandial biological factors that promote satiety (Sabaté & Wein, 2015).

Hass avocados are medium-energy dense foods and an excellent source of fiber (strongly linked to promoting satiety and modulating postprandial glucose and insulin levels) (Wien et al., 2013) and monounsaturated fatty acids (MUFAs) (linked to postprandial increases in GIP and PYY) (Haddad et al., 2018). Sabaté & Wein (2015) observed an acute postprandial increase in GLP-1 followed by a significant drop over the subsequent 2 hours in overweight adults fed meals containing ½ of a Hass avocado (Sabaté & Wein, 2015). Also documented were significant decreases in blood insulin 3 hours post-consumption, increased postprandial leptin levels, increased satisfaction, and reduced desire to eat following a 3-5 h post-ingestive period compared to control subjects (Sabaté & Wein, 2015). Consistent with clinical trials, research in rats fed diverse avocado extracts have also shown important changes in postprandial satiety factors. When studying the effect of avocado pulp on rats fed high-cholesterol diets, Monika and Geetha (2015) observed decreases in food intake, body weight, and total hepatic fat levels, concluding that avocado pulp has an appetite depressant effect and possibly interferes with the metabolism of hepatic fat (Tabeshpour et al., 2017). In parallel with these anti-obesogenic findings, several studies have proposed diverse mechanisms responsible for these effects, including, increased adiponectin and PPAR-γ mRNA expression, increased adipose tissue lipid catabolism, and reduced leptin levels in visceral adipose and subcutaneous tissue (Tabeshpour et al., 2017).

Animal Behavior Experimental Techniques

Diverse strategies and instruments have been employed to study cognitive processes and behavioral characteristics in animal models (Aguayo-Del Castillo et al., 2016). T-mazes have been applied for numerous behavioral paradigms in

rodents assessing cognitive functions such as learning, memory, stimuli discrimination, exploratory behavior, decision-making, and motivation (Sharma et al., 2010). T-mazes allow rodents to approach unknown or novel environmental stimuli by using two general protocols: free and forced trials (Aguayo-Del Castillo et al., 2016). Free election trials allow simultaneous access to both T-maze arms, whereas forced election blocks access to one of the two arms (Aguayo-Del Castillo et al., 2016).

Under standardized conditions, the measurement of satiation is accomplished by ad libitum intake of a specific experimental food, allowing subjects to terminate feeding when nutritionally satisfied (Fiszman and Tarrega, 2017). Additionally, the motivation to consume food is generally assessed by instrumental behavior to acquire food stimuli and typically measures licks and bites of voluntary feeding (Berridge, 2000; Havermans et al., 2009). This evaluation is referred to as consummatory ingestive behavior (Berridge, 2000), which should not be confused with appetitive behavior (occurs prior to attaining the goal of interest) (Berridge, 2000). Additionally, during the consummatory phase of food intake, food palatability (affective taste) is evaluated by observing hedonic/aversive taste reactivity patterns in subjects (Berridge, 2000; Havermans et al., 2009).

MATERIALS AND METHODS

Animals

All experiments were performed according to ethical policies for animal care and handling applicable in Mexico (NOM-062-ZOO-1999), and the study is being reviewed by the University of Sonora (Bioethics Committee). Initially, 12 female Wistar rats (P50-90; 173 ±16 g) from the Department of Medicine and Health Sciences at the University of Sonora were housed collectively in temperature-controlled polysulfonate cages on a 12 h dark/light cycles (lights out at 17:00 h), however, 2 rats were excluded from the study due to uncooperative behavior.

Diets

Rats had access to ad libitum rat chow (Labdiet Rodent 5001^{TM}) and water throughout testing, except where noted. Subjects were faced with distinct appetitive stimuli, depending on the behavioral testing phase: Acclimation, chow pellet (CP) vs. no reinforcer; appetitive testing, avocado extract-coated pellet (AVO) vs. CP pellet. AVO pellets consisted of an avocado phenolic extract elaborated from defatted Hass avocado pulp and used to coat pellets by the Food and Development Research Center A.C. (CIAD A.C.) in Hermosillo, Sonora (Velázquez-Jimenez, 2020).

Behavioral Experimentation

Acclimatation

In order to create an appetitively motivated learning task and for subjects to become accustomed to a decision-making environment along with determining arm preference, all rats (n = 10) were introduced to a 2-week acclimation phase prior to the appetitive testing period. Acclimation sessions occurred 3 days per week, with 1 non-testing day in between. All subjects followed an ad libitum CP diet throughout this phase resulting in satiation at the start of all sessions. During each session, pairs of rats were carried in metal cages in the dark under red light to a laboratory at 17:00 hours and were later individually transferred to a T-maze in a separate experimental conduct room (same environmental conditions were applied during the experimental phase).

The T-maze contained a single CP pellet which was alternatively baited in one of the two maze arms at the start of each trial (3 trials per session), resulting in a CP pellet-baited arm and a no-reinforcer arm (Figure 9). All rats were allowed to freely explore each aisle. Between each subject trial, 70% ethanol was swabbed on the T-maze to eliminate odors.

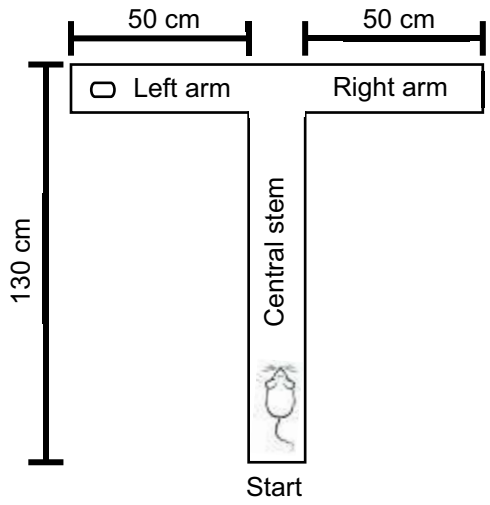

Figure 9. T-maze dimensions and reinforcer placement during the acclimatation phase. An individual rat was placed at the start of the central stem of a T-maze containing a single CP pellet at the end of an arm. The CP pellet was alternately placed in a different arm during each trial of the acclimatation phase. Abbreviations: CP, chow pellet.

During the first week, each session consisted of subjects exploring the maze for three 10 min trials with 55 min breaks in between. The number of visits per arm and order were recorded. Once habituated to the maze, researcher, and room environment, each rat conducted another set of trials (3 per session) the following week only this time subjects were taken out of the T-maze as soon as they entered the baited arm. Entries into the no-reinforcer arm were recorded as incorrect responses, whereas entries into the baited arm were recorded as correct responses. The time required to enter the baited arm was recorded as latency.

Appetitive Testing

Following the acclimation phase, rats were divided in two groups with different diets: 1) a control group (n = 5) that continued to have access to a normal CP ad libitum diet (ALD), and 2) an experimental group (n = 5) undergoing 4 h of food deprivation (FG) prior to testing.

Testing sessions occurred for 2 weeks, 3 days per week, with 1 non-testing day in between. Each rat underwent 3 trials during each session. Rats were confronted with a T-maze containing a CP pellet and an AVO pellet (Figure 10), which were alternatively placed at the end of each maze´s arms. All rats were allowed to freely explore all aisles during a maximum period of 10 min. The time was recorded until the moment each subject had two consecutive bites of their choice of pellet. For the following 20 s, rats were free to consume either pellet or continue exploring the maze. Between each trial, the maze was cleaned with 70% ethanol to remove odors. All food consumed was weighed and recorded the following day. Once the appetitive sessions concluded, normal CP pellet diets and water were returned to the FG rats for continuous consumption until the next appetitive test.

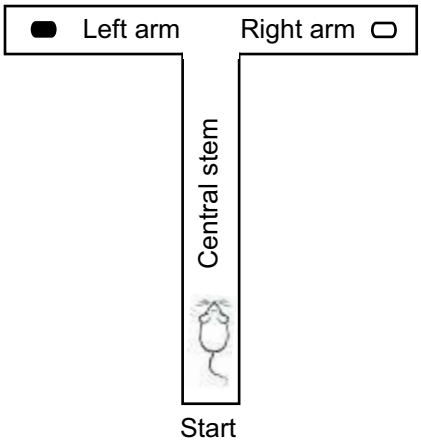

Figure 10. Reinforcer placement during the experimental phase. An individual rat was placed at the start of the central stem of a T-maze containing a CP pellet and an AVO pellet at the end of each arm. Pellets were alternately placed in different arms during each trial of the experimental phase. Abbreviations: AVO, avocado extract-coated pellet; CP, chow pellet.

Dissection of Rat Olfactory Bulb and Hippocampus

After both ALD and FG rats (n = 10) underwent behavioral experimentation, subjects were euthanized with an intraperitoneal overdose of sodium pentobarbital (250 mg/kg). Their OBs were dissected and immerged in cold (4-8°C) artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 3 KCl, 1.25 $NaH_2PO_4 \cdot H_2O$, 1 $MgCl_2$, 2 $CaCl_2$, 25 glucose; pH adjusted to 7.4.

Total RNA Isolation from Olfactory Bulb and Hippocampus Tissue

Total RNA was extracted, firstly, from the OB of ALD rats according to the procedure described by the provider Direct-zol RNA MiniPrep (Zymo Research) with sterile material. OB tissue was placed in separate Eppendorf tubes, containing 300 µL TRIzol (TRIzol Reagent, Invitrogen). OB cells were mechanically lysed by repeated suction with a micropipette. The suspensions were centrifuged in a microcentrifuge (Micromax fr) at 12,000 rpm for 1 min to remove cell debris. Then, 150 µL of ethanol (95-100%) was added to each tube and vigorously shaken by a vortex. The solutions were transferred to individual Zymo-Spin II C separation columns placed in collecting tubes. The samples were centrifuged again at 12,000 rpm for 1 min and the eluents were posteriorly removed. Then, 400 µL of Direct-zol RNA PreWash was added to each column and centrifuged at 12,000 rpm for 1 min. This step was repeated twice. Subsequently, 700 µL of RNA Wash Buffer was added to each column and centrifuged at 12,000 rpm for 2 min. tRNA was eluded with 50 µL of DNase/RNase free water, to then centrifuge again at 12,000 rpm for 2 min. Finally, the total RNA was frozen (-80°C) until use.

The above procedures were repeated to extract total RNA from the ALD and FG rats´ OB and HP.

Reverse Transcription of Olfactory Bulb and Hippocampus Total RNA

Complementary DNA (cDNA) was reverse transcribed (RT) from OB and HP total RNA as follows: samples were prepared in individual Eppendorf tubes containing 5.5 µL DNase/RNase free water, 1 µL random primers, 1 µL total RNA. Samples were heated at 65°C for 3 min and at 25°C 7.5 µL of an additional solution labeled as Stk (Stock) (containing 48 µL M-MLV RT Buffer, 24 µL DTT, 12 µL dNTP's, 6 µL Recombinant RNasin Ribonuclease Inhibitor) was added to each Eppendorf tube. Samples were then heated to 42°C. At this point, 1 µL M-MLV Reverse Transcriptase was added into each tube and the reaction ran for 2 h, then heated at 65°C during 25 min and cooled to 4°C. Total RNA concentration was measured using a Nanodrop apparatus (Thermo Scientific) and frozen (-80°C) until use. Data are described as mean ± SE ($n \geq 2$).

Polymerase Chain Reaction for $p75^{NTR}$ and AgRP

cDNAs were amplified using polymerase chain reaction (PCR). Each Eppendorf tube contained an initial solution of Taq Polymerase Buffer (2 µL), dNTP´s (100 µM), Taq Polymerase (0.1 µL), $MgCl_2$ (1.2 µL), 14.1 µL DNase/RNase free water. Next, 1 µL of cDNA from the previous RT was added to its corresponding tube along with both (100 µM) primers (Reverse and Forward sense) AgRP and $p75^{NTR}$. β-actin was used as a housekeeping gene. All endpoint PCRs were performed in a C1000 Thermal Cycler (Biorad) with the following conditions: 35 cycles of 40 s at 94°C, 60 s at 60°C and 40 s at 72°C. All data is the result of at least three replications. The sequences of all primers of interest and their resulting amplicon are depicted on Table IV.

Table IV. Oligonucleotide sequences and expected amplicons of AgRP and $p75^{NTR}$ genes.

Primer	Sequence	Amplicon	NCBI ID
$p75^{NTR}$ F	5'-AACCAAGGACTCCCACCCCA-3'	102	NM_012610.2
$p75^{NTR}$ R	5'-ACAGAGATATCTTGCTTTTC-3'	bp	
AgRP F	5'-CAGAGTTCTCAGGTCTAAGTC-3'	211	NM_033650.1
AgRP R	5'-TTGAAGAAGCGGCAGTAGCAC-3'	bp	
β-actin F	5'-TCGTGCGTGACATTAAAGAG-3'	198	NM_031144.3
β-actin R	5'-TGCCACAGGATTCCATAC-3'	bp	

Sequences were confirmed using BLAST (basic local alignments search tool) (www.pubmed.com). Abbreviations: AgRP, agouti gene-related peptide; β-actin, beta-actin; F, forward; ID, identification; NCBI, National Center for Biotechnology Information; $p75^{NTR}$, neurotrophin receptor p75; R, reverse.

Gel Electrophoresis

With the purpose of identifying sequence amplifications, electrophoresis agarose gel at 2% was prepared and used to separate PCR products and subsequently allowing qualitative analysis. Agarose gel was prepared by weighing and adding 300 mg Agarose (Promega, USA) to a solution of 1,500 µl 10X TBE buffer and 13,500 µl water Mili-Q. The solution was heated at 300°C on a hot plate (Fisher Scientific, Canada) until boiled and transparent. For staining 4.5 µl of SYBR Safe DNA Gel Stain (Invitrogen, USA) was added and placed on the hot plate until homogenized. The solution was evenly poured and distributed on a gel tray and a comb was placed in position. A 100 bp Ladder (Promega, USA) was used as a reference. Once PCR samples and the Ladder were placed in individual wells, the gel ran at 80 mV for 30 min. Results were visualized under UV light (Gel-Doc UV transilluminator system Bio-Rad) and captured on a digital camera.

Statistical Analysis

The descriptive analysis of the variables was carried out using measures of central tendency and dispersion. Given that a non-normal distribution was observed, the non-parametric Mann-Whitney U test was used to evaluate the differences between the medians of the following variables: latency to feed and grams consumed. In both cases, two-tailed hypotheses were tested, and p values <0.05 were considered statistically significant. For these tests, satistical analysis was carried out using the IBM SPSS Statistics for Windows, Version 25 (IBM SPSS Statistics for Windows, IBM Corporation, Armonk, NY).

RESULTS AND DISCUSSION

We investigated the effect of an avocado extract and metabolic states on motivated feeding responses and their association with the expression of $p75^{NTR}$ and AgRP genes in the rat OB and HP when confronted with a decision-making paradigm. First, we assessed the consummatory ingestive behavior of FG and ALD rats placed in a T-maze containing two distinct reinforcers, a CP and AVO pellet. All FG rats participated in food intake, whereas only 55.5% of ALD rats consumed food during the given period. As revised by previous literature, ALD rats may have had this outcome as a result of numerous influences on eating behavior including: food appeal, palatability, familiarity, availability, and increased satiety (Benelam, 2009; Fiszman and Tarrega, 2017). As hypothesized, FG rats were significantly faster when performing feeding tasks compared to ALD rats (median latencies: 46.6" vs 2' 46", $p = 0.032$) (Figure 11). Both, FG and ALD rats consumed higher portions of AVO in comparison to CP (total consumption: FG, 10.4 ± 0.7 g AVO vs 6.5 ± 0.8 g CP; ALD, 8.7 ± 0.8 g AVO vs 3.1 ± 0.3 g CP), however these results did not show statistical significance.

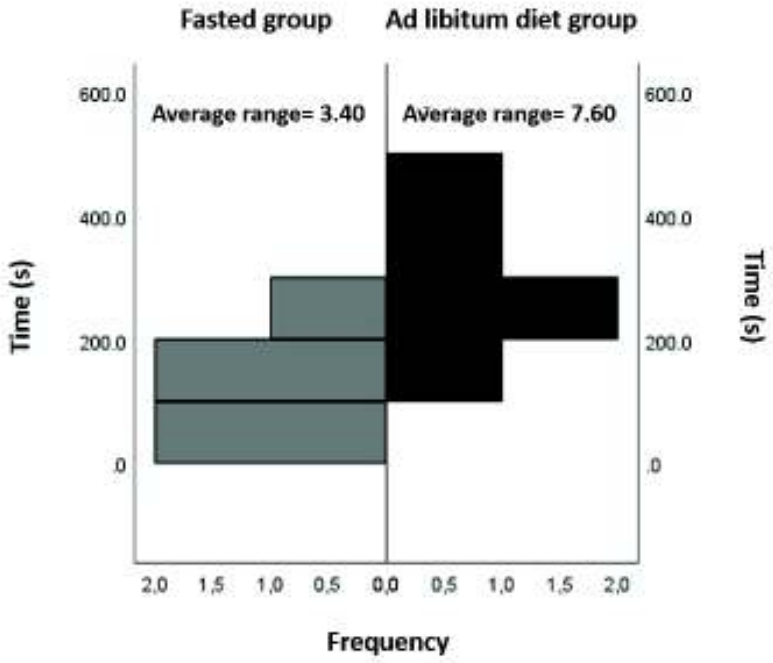

Figure 11. Mann-Whitney U test of median latency to feed by FG and ALD rats. Fasted rats initiated feeding at a faster speed compared to ALD rats (p=0.032; n = 5). Abbreviations: ALD, ad libitum diet group; FG, fasted group.

As demonstrated by the shorter latencies to feed, fasted conditions are powerful stimuli for motivated feeding behavior (Bake et al., 2019). The release of orexigenic hormones promote the consumption of food by acting on both homeostatic (hypothalamus and brainstem) and non-homeostatic pathways (nucleus accumbens via dopaminergic pathways from the ventral tegmental area) (Bake et al., 2019). In human subjects, acute fasting increases reward pathway activity in response to food when compared with fed conditions and has also been associated with enhancing food stimuli memory (Goldstone et al., 2009). Therefore, our finding that the FG group showed shorter latencies to feed in comparison to the ALD group may suggest that an increased motivation to carry out feeding decisions is associated to differential reward values between metabolic states.

Our study did not find a statistically significant difference in CP and AVO consumption by all rats (n = 10), however, previous studies have demonstrated that palatability is preferred over bland food, regardless of metabolic states. Imaging studies in humans have shown that skipping breakfast activates reward systems in response to palatable foods over low-calorie content, and at the same time, activation of the OFC shows direct correlation with food appeal and individual differences in reward sensitivity (Goldstone et al., 2009). Additionally, Hume et al. (2016) reported that rats with ad libitum access to standard bland food undergoing regular scheduled feeding of palatable food decreased bland food intake and consumed larger amounts of sweetened condensed milk (Hume et al., 2016). Danielli et al. (2010) demonstrated that satiated rats show an increase in extraneuronal dopamine in response to vanilla sugar in the nucleus accumbens and medial prefrontal cortex (Danielli et al., 2010). Furthermore, in ovariectomized rats receiving a high-polyunsaturated fatty acid (PUFA) diet, L-Dopa levels in the hippocampus were higher in comparison to rats receiving a standard chow diet (Dornellas et al., 2018). Although Hass avocado extract has previously been identified to have enhanced satiety effects and contain a rich source of PUFAs and

MUFAs, our study did not provide enough evidence to suggest that AVO pellets motivate increased consumption compared to normal CP pellets in neither FG nor ALD groups. Further studies with larger group populations are required to determine the effects of avocado extracts on food intake. In this context, it may be arguable that FG and ALD rats did consume higher portions of AVO in comparison to CP because of its palatability characteristics in addition to other unknown and not studied factors in this study.

Subsequently, we investigated through RT-PCR the expression of AgRP and $p75^{NTR}$ in the OB and HP of FG and ALD rats (Figure 12). Using our methodology, AgRP RNA was expressed in the OB of both groups, but not expressed in the HP of neither FG nor ALD rats. The $p75^{NTR}$ RNA was expressed only in FG rat HP and OB (Table V).

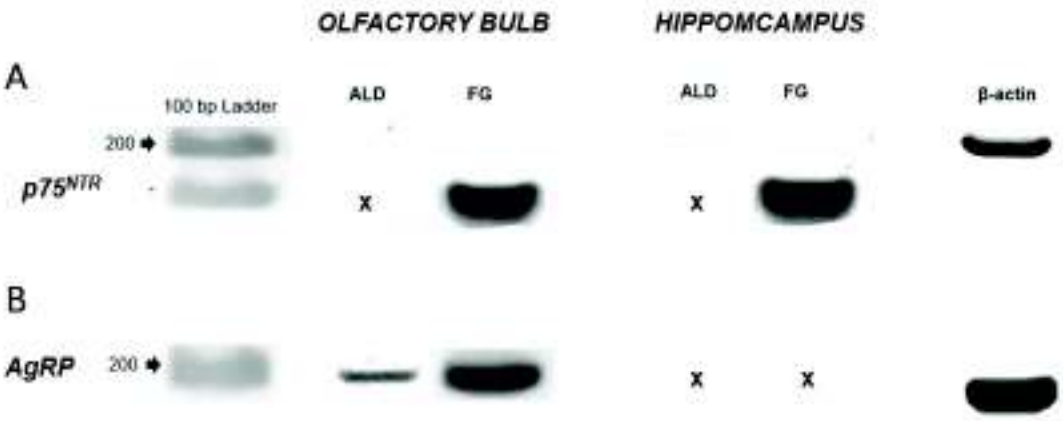

Figure 12. RT-PCR of $p75^{NTR}$ (A), AgRP (B), and β-actin (A & B) in the OB and HP of ALD and FG rats visualized on agarose gel. Abbreviations: ALD, ad libitum diet group; AgRP, agouti gene-related peptide (211 bp); β-actin, beta-actin (198 bp); FG, fasted group; p75NTR, neurotrophin receptor p75 (102 bp); PCR, polymerase chain reaction; RT, reverse transcription.

Table V. Transcription expression of $p75^{NTR}$ and AgRP in the OB and HP of fasted and fed rats.

	ALD		FG	
	OB	**HP**	**OB**	**HP**
$p75^{NTR}$	-	-	+	+
AgRP	+	-	+	-
β-actin	+	+	+	+

β-actin was used as a housekeeping gene. Abbreviations: ALD, ad libitum diet group; AgRP, agouti gene-related peptide; FG, fasted group; HP, hippocampus; OB, olfactory bulb; p75NTR, neurotrophin receptor p75.

Similar to our findings, investigations using in situ hybridization have studied the expression of AgRP in animal HP and OB. Boswell et al. (2002) assessed the distribution of orexigenic mRNA in different brain areas of Japanese quail. It was reported that AgRP mRNA was not expressed in the hippocampus of fed birds (Boswell et al., 2002). Haskell-Luevano et al. (1999) reported that AgRP did not express in the hippocampus of rats with ad libitum access to food (Haskell-Luevano et al., 1999). Similarly, Broberger et al. (1998) did not detect AgRP mRNA in the OB nor HP of mice, although it was not clear whether these findings were a result of normal, anorectic, or monosodium glutamate-treated mice (Broberger et al., 1998). In all studies, including ours, expression of AgRP in the hippocampus was absent. However, unlike Broberger et al. (1998), we observed a clear expression of AgRP in the OB of fasted and satiated rats, suggesting a local production of this orexigenic peptide by OB neurons, despite metabolic states.

Furthermore, the consumption of omega-3 PUFA diets in mice has shown to influence the hippocampus by increasing its volume, expression of BDNF, neurogenesis, and serotonergic activity (Dornellas et al., 2018). Several studies have demonstrated that serotonin increases the activation of POMC/CART neurons in the hypothalamus and decreases the expression of AgRP, as demonstrated by ovariectomized rats receiving a high-fat fish oil diet (Dornellas et al., 2018). These results were not observed in ovariectomized rats receiving a saturated fatty acid diet (Dornellas et al., 2018). In accordance with these findings, PUFAs and MUFAs of avocado extract-coated pellets may have similar effects on AgRP expression, however further studies are required to confirm this hypothesis.

Recently, several studies have been done on $p75^{NTR}$ in the OB and HP regarding its role on neurogenesis, however, not many studies have looked at the receptor´s effects on food intake. Catts el at. (2008) found that $p75^{NTR}$ deficient mice had a longer latency to feed in a novelty-suppressed feeding test, similar to BDNF deficiency phenotypes (Catts et al., 2008). In addition to this altered behavior, decreased hippocampal neurogenesis was found and no difference was shown in

food consumed between genotypes ($p75^{NTR\ -/-}$ and $p75^{NTR\ +/+}$) (Catts et al., 2008). These results may have simulated a satiated state among mice, similar to our ALD rats, and therefore created a decreased motivated behavior for food consumption, as demonstrated by Catts et al. (2008) and our results in this study in relation to longer feeding latencies. Additionally, Duan at al. (2000) and Smiljanic et al. (2015) reported that dietary restriction stimulates the production of BDNF in the hippocampus compared to rats fed ad libitum (Duan et al. 2000; Smiljanic et al., 2015). These results may be related to food memory and motivation to feed (Smiljanic et al., 2015) and perhaps explains the correlation between motivated feeding behaviors and metabolic states observed in our study. Despite these similarities, lower $p75^{NTR}$ levels were reported (Smiljanic et al., 2015), in opposition to our results which demonstrated a clear expression of hippocampal $p75^{NTR}$ mRNA in FG rats in contrast to the ALD group. Regarding the OB, $p75^{NTR}$ has not been studied in the context of food intake. Given our results, and that the other BDNF receptor, TrkB, in the OB has previously been suggested to contribute to feeding (Xu et al., 2003), we hypothesize that an upregulation of $p75^{NTR}$ expression in the OB may play a role in food attainment when faced with an energy depleted metabolic state.

CONCLUSION

Non-homeostatic structures involved in food intake regulation have shown to be reciprocally influenced by homeostatic mechanisms. Given these closely intertwined feeding systems, we questioned whether $p75^{NTR}$ and AgRP play a role in the OB and HP on consummatory ingestive behavior of fed and fasted rats. Consistent with previous research, we found: 1) Metabolic states motivate behavioral feeding responses in rats as suggested by shorter latencies to feed when in fasted conditions; 2) Food deprivation also induced the expression of $p75^{NTR}$ in the OB and HP, perhaps participating in olfaction sensitivity and food memory, and subsequently creating motivated states to attain food. Additionally, AgRP transcripts were expressed in the OB of both fed and fasted rats. Understanding the interactive pathways and neuro-behavioral changes leading to food intake may contribute to better comprehend the etiology of motivated behavioral feeding responses.

PERSPECTIVES

Further research is required to explore how our findings could be applied in health and medical fields where eating disorders account for a dysregulation of optimal energy intake. Therefore, along with a larger population, same age subjects, and recording the amount of CP consumed in rat cages during rest days, further parameters must be implemented to assess a broader spectrum of factors influencing food intake regulation.

Perhaps measuring body fat before and after implementing an AVO diet in both ALD and FG rats could orientate our understanding on the beneficial effects of avocado pulp on fat and further explore its role on satiety. Along the same lines, further research will need to assess blood samples throughout the experimental period in search of changes in circulating anorexigenic and orexigenic peptides, such as glucose, ghrelin, and leptin levels. Blood analysis will allow a better correlation between the precise metabolic state of rats, AVO effects, and subsequent $p75^{NTR}$ and AgRP expression in brain structures.

Moreover, we propose including 2 additional subgroups of fed and fasted rats that do not undergo neither T-maze behavioral tests nor AVO diets. The implementation of these groups will allow us to determine if our molecular biology results were a direct effect of metabolic states or if AVO content influenced $p75^{NTR}$ and AgRP expression.

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APPENDIX

Histological Analysis

In addition to behavoiral and molecular biology experiments, we assessed histological procedures by means of Golgi-Cox and HE staining of the OB and HP of fed and fasted rats.

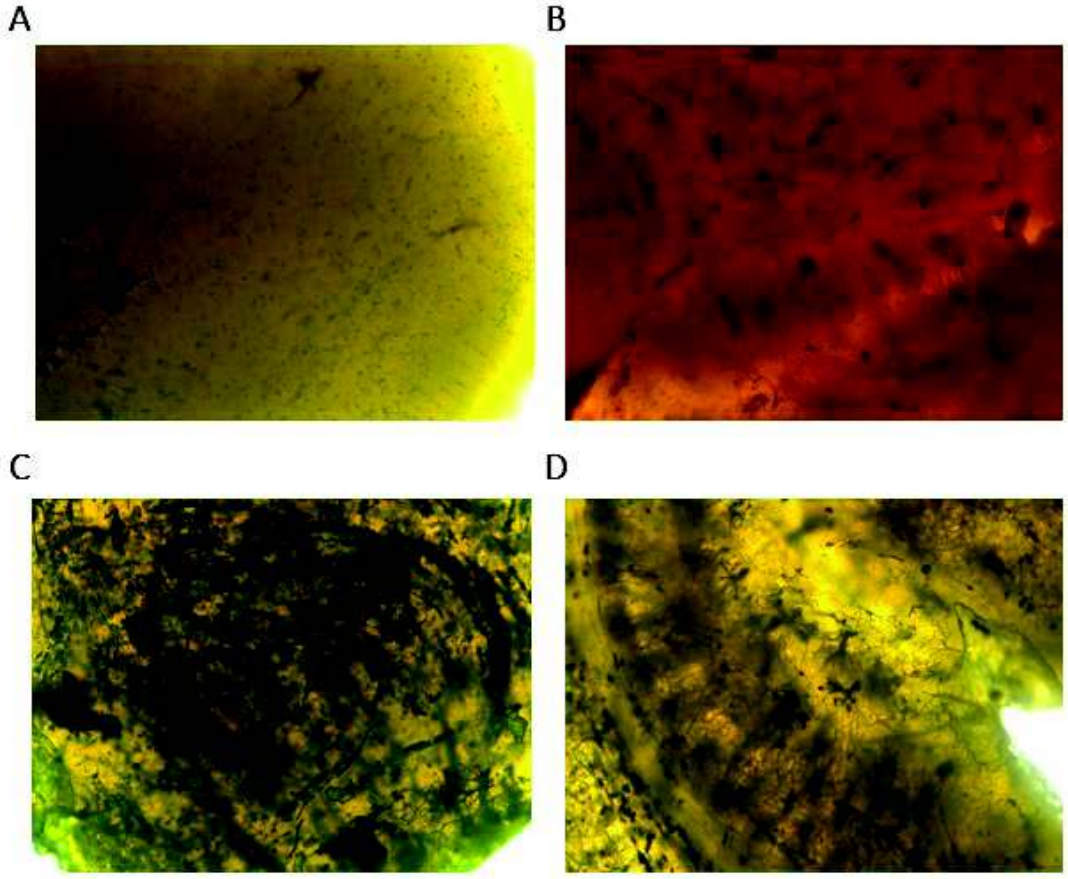
Materials and Methods

Golgi-Cox staining. A Golgi-Cox solution was prepared (5% potassium dichromate, 5%mercuric chloride, and 5% potassium chromate) and stored in a glass bottle in the dark. The Golgi-Cox solution was transferred to plastic bottles containing brain tissue previously dissected from ALD and FG rats. After 10 days of Golgi-Cox staining, brain tissues were washed with distilled water and transferred to clean bottles containing a protective solution. This was repeated following 24 h. After 5 days, tissues were processed into paraffin blocks and were sectioned using a microtome. 80 μm sagittal OB and HP sections were placed on slides and observed under inverted microscopy.

Hematoxylin-Eosin Staining. OB and HP slides were flooded with Hematoxylin-Eosin (HE) dye for 1 min and rinsed with distilled water. Slides were observed under inverted microscopy.

Results

OB and HP histological sections were analyzed trough an inverted microscope. As shown in Figure 13, OB histological slides from ALD rats stained by Golgi-Cox showed 248 neurons/quadrant (4x magnification), whereas 329 neurons/quadrant were identified in FG rat OB. ALD and FG rat HPs presented 233 and 179 neurons/quadrant, respectively. HE-stained OB slides showed 717 and 869 nuclei/quadrant for ALD and FG rats, respectively. Finally, 398 nuclei/ quadrant were found in ALD rat HP, whereas FG rats showed 402 nuclei/quadrant.


A
B
C
D

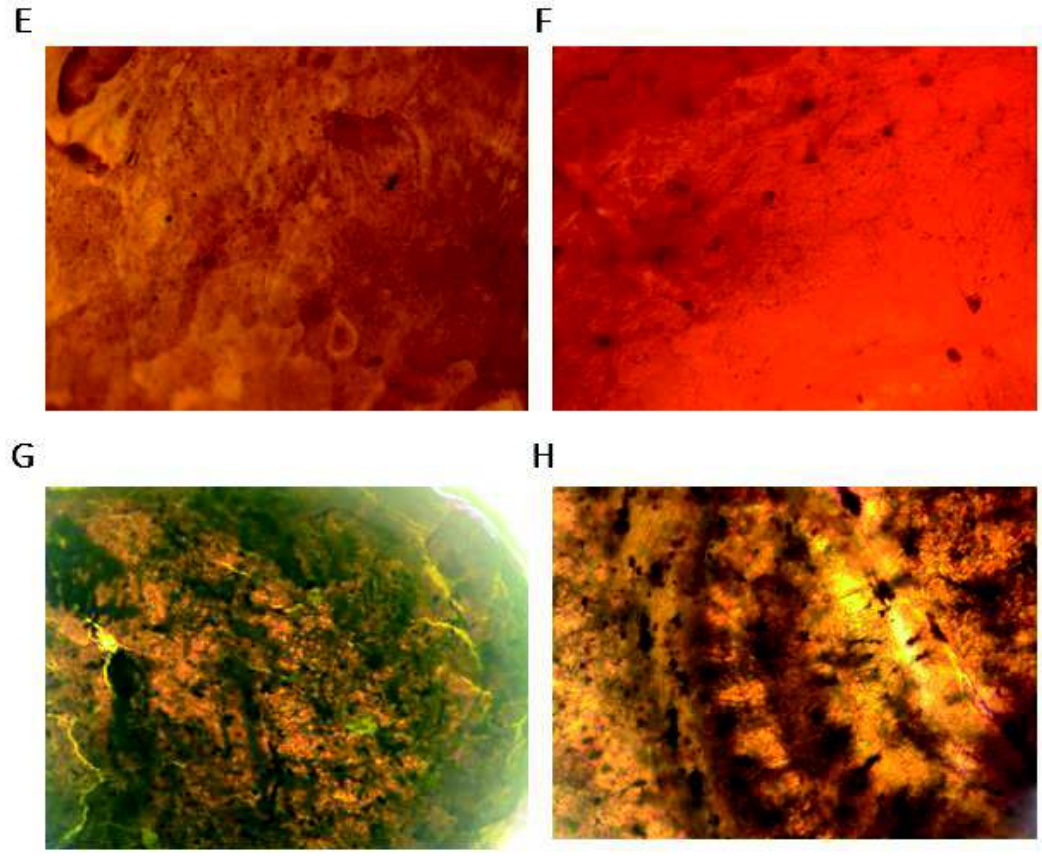


Figure 13. Histological micrographs of the OB and HP of rodents (Golgi-Cox and HE staining). OB and HP tissue slides from ALD rats stained with Golgi-Cox (A and B, respectively) and HE (E and F, respectively) were observed using inverted microscopy. OB and HP tissues from FG rats were also stained with Golgi-Cox (C and D, respectively) and HE (G and H, respectively). Abbreviations: ALD, ad libitum diet group; FG, fasted group; HE, hematoxylin-eosin; HP, hippocampus; OB, olfactory bulb.

Statistical Analysis

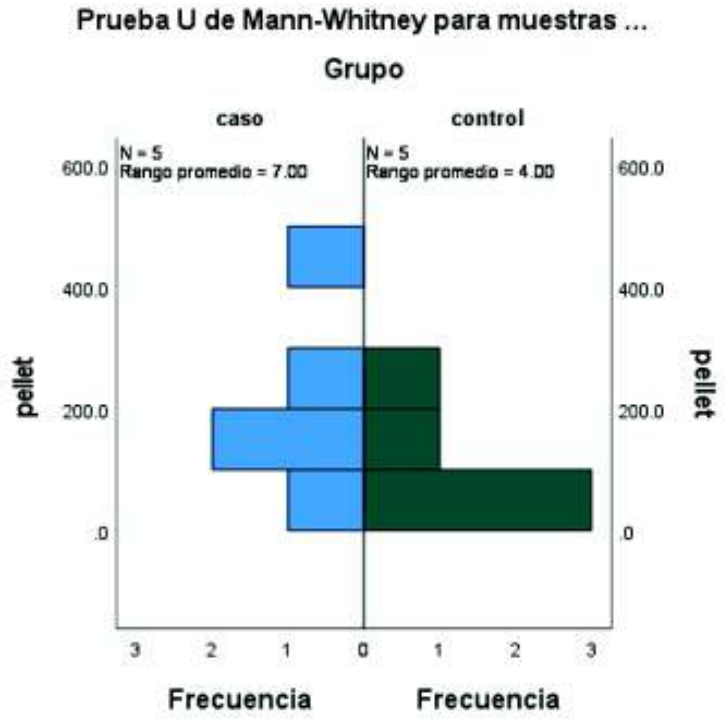


Figure 14. Mann-Whitney U test of mean quantity of CP pellets consumed (mg) by FG and ALD rats. Abbreviations: ALD, ad libitum diet group; CP, chow pellets; FG, fasted group.

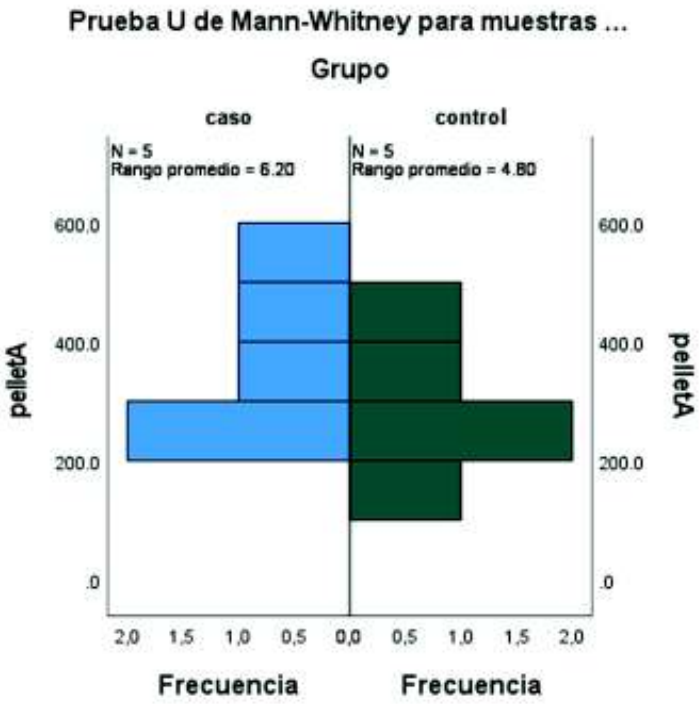


Figure 15. Mann-Whitney U test of mean quantity of AVO pellets consumed (mg) by FG and ALD rats. Abbreviations: ALD, ad libitum diet group; AVO, avocado extract-coated pellets; FG, fasted group.

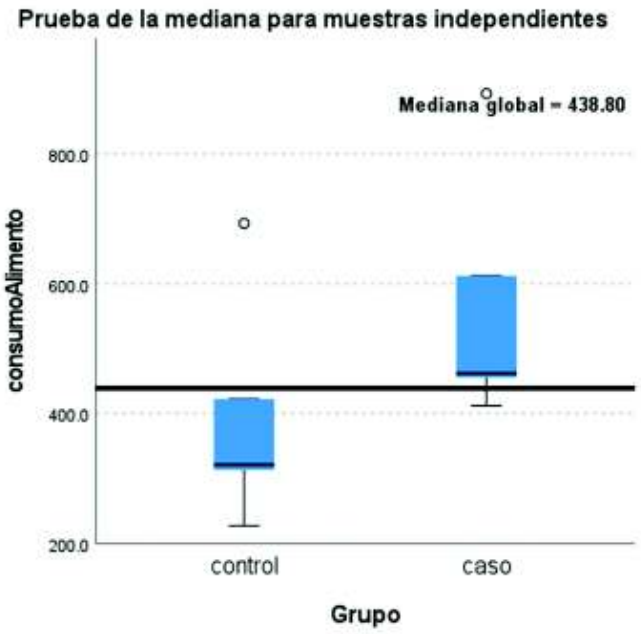


Figure 16. Median quantity of pellets (CP and AVO) consumed (mg) by ALD and FG rats. Abbreviations: ALD, ad libitum diet group; AVO, avocado extract-coated pellets; CP, chow pellets; FG, fasted group.

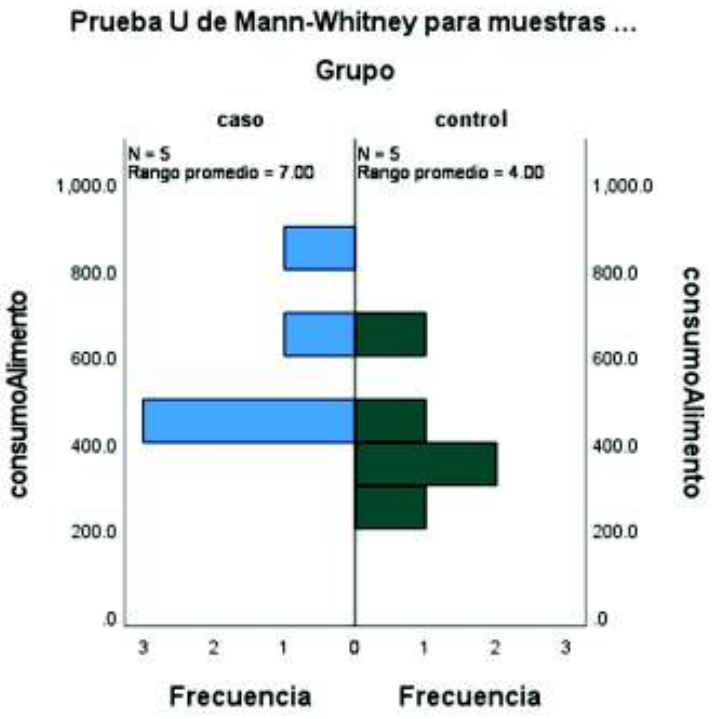


Figure 17. Mann-Whitney U test of mean quantity of pellets (CP and AVO) consumed (mg) by FG and ALD rats. Abbreviations: ALD, ad libitum diet group; AVO, avocado extract-coated pellets; CP, chow pellets; FG, fasted group.

Table VI. Chi-Square table of CP and AVO pellet preference by all rats.

Pruebas de chi-cuadrado

	Valor	gl	Significación asintótica (bilateral)
Chi-cuadrado de Pearson	12.566[a]	9	.183
Razón de verosimilitud	13.116	9	.157
Asociación lineal por lineal	2.962	1	.085
N de casos válidos	139		

a. 6 casillas (30,0%) han esperado un recuento menor que 5. El recuento mínimo esperado es 2,42.

Abbreviations: AVO, avocado extract-coated pellets; CP, chow pellets.

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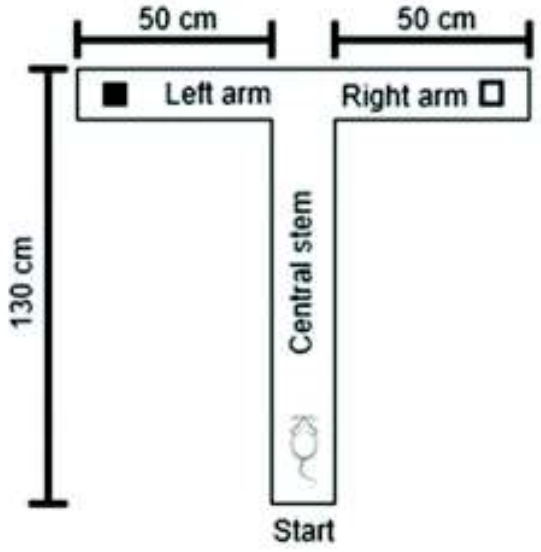

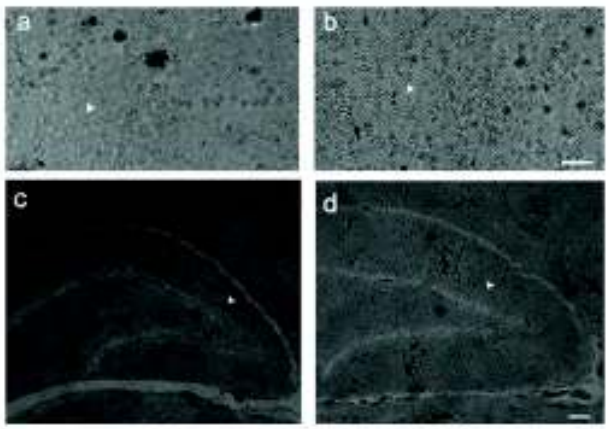

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Fasting alters p75^{NTR} and AgRP mRNA expressions in rat olfactory bulb and hippocampus

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List of Abbreviations;

***AgRP**, agouti-related peptide; **ALD**, ad libitum diet; **ARC**, arcuate nucleus; **AVO**, avocado group; **BDNF**, brain derived neurotrophic factor; **CP**, chow pellet; **FG**, fasted group; **HP**, hippocampus; **OB**, olfactory bulb; **p75^{NTR}**, p75 neurotrophin receptor; **ROI**, region of interest.*

Abstract

Classic non-homeostatic structures involved in food intake regulation are reciprocally influenced by metabolic signals. Orexigenic peptides expressed in the olfactory bulb (OB) and hippocampus (HP) modulate olfactory processing and memory, respectively. Hypothalamic circuits also modulate feeding behavior by activating and releasing Agouti-related peptide (AgRP) in response to orexigenic signals. An adequate response to fasting requires the expression of p75 neurotrophin receptor (p75^{NTR}) in AgRP neurons. The present study aimed to determine whether the expression of p75^{NTR} and AgRP differed in the OB and HP of fasted and satiated rats. A group of fasted rats (FG) was confronted with a decision-making paradigm in a T-maze containing a standard chow pellet (CP), and the same pellet coated with a phenolic-rich