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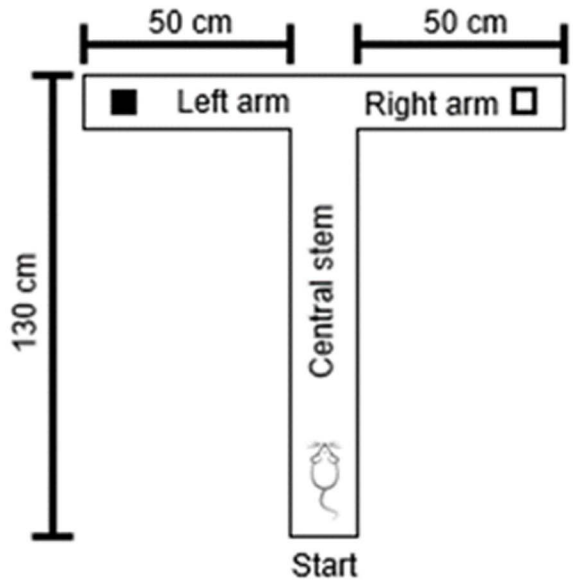

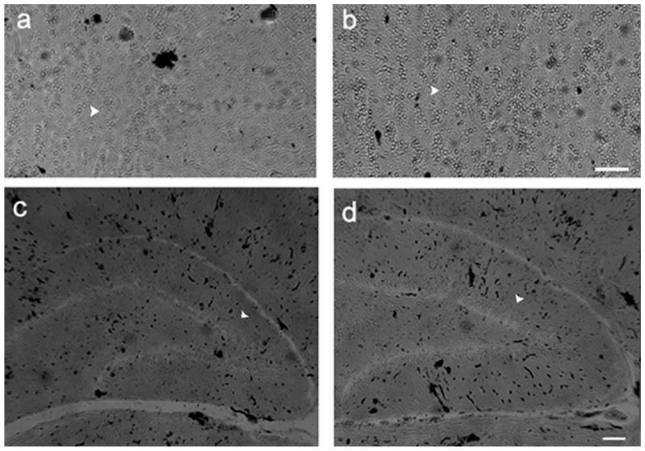

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

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A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

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RESEARCH ARTICLE

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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

avocado paste extract (AVO) on either end; their OB and HP were then analyzed with histological and molecular tools. FG rats had briefer feeding latencies, as compared to control rats fed *ad libitum*. They also had reduced cell counts in both brain structures, as compared to satiated rats. AgRP mRNA was not expressed in the HP of either group, however, it was found in the OB. p75^{NTR} mRNA was expressed in both brain structures of FG rats. These results suggest that contrasting metabolic states (fasted or satiated) motivate different feeding responses, which are influenced by p75^{NTR} and AgRP mRNA expression in non-homeostatic food intake brain structures.

Keywords: Food intake regulation, appetitive behavior, avocado, Agouti-related peptide, p75 neurotrophin receptor

Introduction

Food intake and cessation require tight regulation to ensure appropriate nutritional demands are met for survival (Berthoud et al., 2017; Goldstein et al., 2021; Zucoloto, 2011). This is accomplished by a complex system that is influenced by many factors that ultimately determine an individual's state of hunger, satiety, and appetite. An adequate regulation of food intake requires the interaction of homeostatic and non-homeostatic mechanisms (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, Agouti-related peptide/neuropeptide Y (AgRP/NPY) and proopiomelanocortin/cocaine and amphetamine regulated transcript (POMC/CART), respectively, whose effects are integrated to reflect energy status and to subsequently alternate between states of hunger and satiety. Post-ingestive mechanisms including duodenal vagal-dependent detection of fat, intestinal glucose uptake (sodium glucose cotransporter 1, SGLT1), and portal glucose detection, all of which inhibit AgRP activity, which leads to ceasing food consumption (Goldstein et al., 2021). Moreover, hypothalamic AgRP neurons require the expression of neurotrophin receptor p75 (p75^{NTR}) to stimulate homeostatic feeding during fasting conditions. This was demonstrated by Podyma et al. (2020a), who reported that p75^{NTR} was necessary to consume an adequate amount of energy after fasting, and to develop food anticipatory activity, 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 the hypothalamic arcuate nucleus (ARC) and dorsal medial nucleus, both regions required for detection of energy status (Podyma et al., 2020a, 2020b).

Non-homeostatic pathways, also referred to as hedonic eating, are driven by cognitive, emotional and environmental factors (Berthoud et al., 2017). Neural representations based on learned associations are essential for determining future food choices, especially when exposed to conditioned stimuli, such as olfactory cues. The literature also indicates that homeostatic and hedonic eating share many neural pathways (Andermann and Lowell, 2017; Liu and Kanoski, 2018), and that both may be activated in varying degrees by metabolic feedback (Hernández Ruiz de Eguilaz et al., 2018; Rossi and Stuber, 2018). Studies in rats have suggested that the release of the hypothalamic neuropeptide orexin in the olfactory bulb (OB) increases olfactory sensitivity, and subsequently promotes the procurement of food, whereas leptin reduces olfactory sensitivity (Valladares Vega, 2015). Metabolic cues, previously learned experiences, and the visuospatial environment also influence episodic meal-related encoding in the hippocampus (HP) (Liu and Kanoski, 2018), although the influence of such pathways and their overlapping substrates are still a subject of research.

Central and peripheral communication via neuroendocrine pathways is influenced by dietary elements, in addition to physiological and environmental factors, which altogether determine hunger and satiety states and behaviors. Studies have shown that intake of distinct dietary compounds can modulate circulating concentrations and gene expression of orexigenic and anorexigenic hormones, which could be potentially used as an alternative to regulate appetite, and therefore weight gain. For instance, fruits and vegetables are rich in phenolic compounds, and their regular consumption can favor physiological benefits attributed mostly, but not entirely, to their antioxidant-related effects. These phenolic-rich foods can alter the plasma concentration of various hunger/satiety mediators. For example, *in vivo* and *in vitro* studies have demonstrated that different phenolic compounds like catechin, epicatechin, chlorogenic acid, gallic acid, and procyanidins are able to modulate hunger

and satiety mediators, including cholecystokinin, glucagon-like peptide-1, and leptin, which has made them subjects of study as anti-obesity agents (Singh et al., 2020). Moreover, bioactive phenolic compounds found in avocado paste (the industrial byproducts of its processing) include gallic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, quercetin, and kaempferol, among others (Zuñiga-Martínez et al., 2021). These have been shown to induce satiety effects and to decrease food intake in *in vivo* models, for example, Corella-Salazar et al., (2021) recently reported that consuming said phenolic-rich avocado paste extract induced glucagon-like peptide-1 and leptin-mediated satiety effects in a murine model. Nonetheless, there is no information about the possible effects that a phenolic-rich avocado paste extract has on the expression of p75^{NTR} and AgRP in the brain, and other components of rat feeding behavior.

The present study therefore aimed to analyze the feeding behavior of rodents under fed or fasted conditions when presented a different reward choice, and to determine differences in AgRP and p75^{NTR} mRNA expression and cell density in the OB and HP. Our data can help to better understand the role of homeostatic influence on hedonic choices.

Materials and methods

Subjects of study

Animals

All experiments were performed according to ethical policies for animal care and handling applicable in Mexico (NOM-062-ZOO-1999) and reviewed and approved by the Bioethics Committee of the Research Center for Food and Development (CE/014_1/2019). Female Wistar rats (n = 10; postnatal age 50-90 days; 173 ± 16 g) were obtained from the Department of Medicine and Health Sciences of the University of Sonora, Mexico and were collectively housed in a temperature-controlled room, under 12 h dark/light cycles.

Study groups and diets

All rats had *ad libitum* access to chow pellets from Laboratory Rodent Diet 5001 (CP) (full ingredient composition has been described before by Corella-Salazar et al., 2021) and water. They faced two appetitive stimuli, depending on the behavioral testing phase: 1) acclimation, a standard CP vs. no reinforcer; and 2) appetitive testing, a standard CP vs. avocado extract-coated pellet (AVO). AVO pellets consisted of an avocado phenolic-rich extract obtained from avocado paste that was used to coat standard CP, at a dose of 100 µL per 5 g pellet (Corella-Salazar et al., 2021). The phenolic composition of the extract has been previously reported but, in brief, it contained ferulic acid > protocatechuic acid > *p*-coumaric acid > quercetin > kaempferol > gallic acid (Corella-Salazar et al., 2021; Zuñiga-Martínez et al., 2021). The extract was obtained by homogenizing avocado paste at 25 °C with 80% ethanol in 50 mL tubes, in a 1:20 (w/v) ratio. Homogenate was sonicated for 30 min (Bransonic Ultrasonic), with controlled water temperature (<20 °C). The sample was centrifuged at 9,400 × g for 15 min at 4 °C; supernatant was recovered, and residue was subjected to 2 more extractions using a 1:10 (w/v) ratio of the extraction solution, under the same conditions. Supernatants were collected and filtered using Whatman No. 1 filter paper. Ethanol present in the extracts was eliminated using a rotary evaporator (Büchi B-490, Switzerland) at 35 °C, while water was removed by freeze-drying.

Behavioral experimentation

To study rat feeding behavior, we modified the novelty-suppressed feeding assay proposed by François et al. (Francois et al., 2022), utilizing free-election trials in a T-maze (Aguayo-Del Castillo et al., 2016) as follows:

Acclimation. In order to create an appetitively-motivated learning task, and for the rats to become accustomed to a decision-making environment, they were all (n = 10) subjected 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. Experimentation was performed at night during the animals' active hours (20:00 to 00:00 h) to avoid outside noise and other interfering stimuli. During each session, pairs of rats were transported in metal cages under dim red light, and individually transferred to a T-maze in an experimental room (same environmental conditions were maintained for all trials). The T-maze contained a single CP pellet that 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. All rats were allowed to freely explore the maze. The surfaces were swabbed with 70% ethanol between each subject trial to eliminate the effect of odors.

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, except the subject was taken out of the T-maze as soon as it 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 (s).

Appetitive testing

Following the acclimation phase, rats were divided into two groups: 1) a control group (n = 5) that continued to have *ad libitum* access to the standard CP diet before the experimental period (ALD), and 2) an experimental group (n = 5) that underwent 4 h of food deprivation (FG) prior to testing.

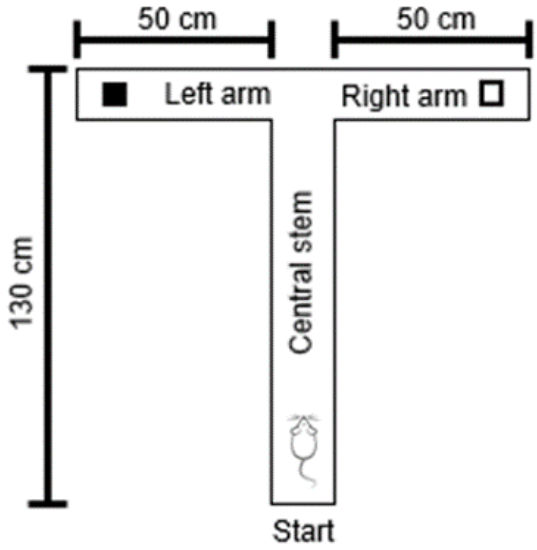

Figure 1. *Pellet placement during appetitive testing. A rat was placed at the start of the central stem of a T-maze containing a CP pellet (black square) and an AVO pellet (white square), each located at the end of an arm. Pellets were alternately situated in different arms at the start of a new trial.*

Testing sessions occurred for 2 weeks, 3 days per week, with 1 non-testing day in between. Each subject underwent 3 trials during each session. Subjects were confronted with a T-maze containing a CP pellet and an AVO pellet, which were alternatively placed at the end of each arm (**Figure 1**). They were initially allowed to freely explore the environment during a maximum period of 10 min. Time was recorded until the moment each subject had two consecutive bites of its chosen pellet. For the following 20 s, rats were free to either consume the pellet or continue exploring the maze. The maze was cleaned with 70% ethanol between each trial to remove odors. All food consumed was weighed and recorded the following day. Once the appetitive sessions concluded, standard CP pellets and water were returned to the FG rats for continuous consumption until the next appetitive test. Behavioral data is given as median ± SD, n = 180 trials.

Brain structure dissection

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

Histological analysis

Tissues were dehydrated, embedded into paraffin blocks, and 5 µm coronal sections (OB n>4; HP n>6) were obtained with a microtome (MICROM HM 355S, Thermo Fisher SCIENTIFIC, USA) and fixed onto slides. Slides were stained with eosin dye for 3 s, followed by a series of washes with increasing ethanol concentrations (70%, 80% 96%, and absolute alcohol) and xylol. Tissues were observed under an inverted Leica DM500 microscope (Leica Microsystems, USA) and images were obtained using an Electro-Retiga CCD camera (Teledyne Photometrics, USA) with 1376 x 1024 pixel resolution. Finite regions of interest (ROI) were selected with ImageJ FIJI software (NIH, USA) and masks were generated for cell count and distribution analysis. For OBs, a 10x objective lens was used and a ROI of an area of 360 mm^2 was selected, whereas HP images were obtained with a 4x objective lens and dorsal CA1 and CA3 areas of the dorsal hippocampus were analyzed. Data is given as mean ± SD, OB n = 9 ROIs and HP n= 16 ROIs.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from OB and HP tissues,

using Direct-zol RNA MiniPrep according to manufacturer's instructions (Zymo Research), and reverse transcribed into cDNA using the GoScript reverse transcription system (Promega). End-point PCR was performed in a C1000 thermalcycler (BioRad) to determine the expression of AgRP and p75^{NTR}, using β-actin as a reference gene. Amplicons were visualized through agarose gel electrophoresis, and all RT-PCRs were the result of at least three repetitions. Primer sequences and their resulting amplicon are listed on **Table 1.**

***Table 1**. Primer sequences and resulting amplicons of AgRP, p75^{NTR}, and β-actin genes.*

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

Table 1, Sequences were confirmed using BLAST (basic local alignments search tool) (www.pubmed.com).

Statistical analysis

Descriptive variable analysis 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 determine differences between the medians of latencies to feed, and amount of diet consumed. To correlate mean cell distribution of OB and HP tissues and latencies to feed, we used Person's correlation test for χ^2. In all cases, two-tailed hypotheses were tested, and p values <0.05 were considered statistically significant. Data was analyzed in XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France 2017).

Results

The ingestive behavior of FG and ALD rats tested in a T-maze containing two reinforcers, a CP and AVO pellet, revealed that all FG subjects consumed available diet (pellets), whereas consumption was only recorded in 55.5% of ALD subjects during the same period. FG rats were significantly faster at achieving the feeding task, as compared to ALD rats (median latencies: 55 vs 191 s, p = 0.032) (**Figure 2**), likely associated with an active motivation for feeding induced by the 4 h fasting period. Interestingly, FG and ALD subjects consumed statistically similar amounts (p>0.05, total consumption of AVO vs CP: FG, 10.4 ± 0.7 g vs 6.5 ± 0.8 g; and ALD, 8.7 ± 0.8 g vs 3.1 ± 0.3 g, respectively).

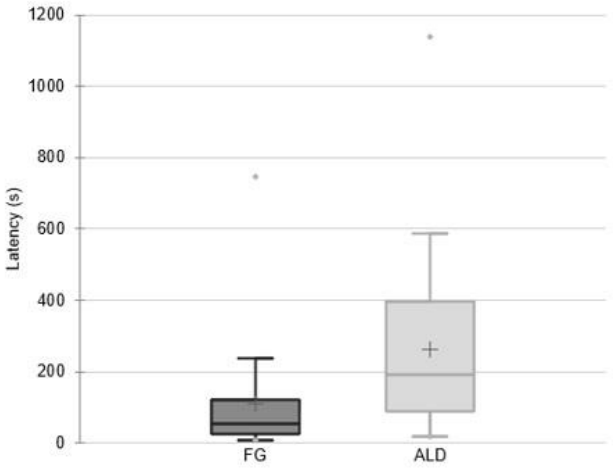

***Figure 2.** Median latency to complete feeding task by fasted (FG) and ad libitum diet (ALD) rats. Median (FG and ALD) 55 vs 191 s (minimum 7 vs 17 s; maximum 746 vs 1140 s); 111 ± 149 vs 263 ± 212 s, respectively. Data presented as median, and mean ± SD (n = 5 rats in each group).*

We then compared the mean latency to feed of both experimental groups, with their mean cell distribution in OB and HP tissue. ALD rats had a greater cell density in both brain tissues, as compared to FG rats [mean cell count (cells/area measured): OB, 1248 ± 148 vs 941 ± 139; HP, 1559 ± 665 vs 1469 ± 529, respectively, p <0.0001] (**Figures 3 and 4**).

End-point RT-PCR was then used to determine if mRNA expression of AgRP and p75^{NTR} in the OB and HP was altered by the decision-making paradigm with which they were confronted (**Figure 5**; full-length gels are included in a Supplementary Information file). We found that AgRP was expressed in the OB of FG and ALD rats, but not in the HP of either group. In contrast, p75^{NTR} was expressed in FG rat OB and HP, but was absent in ALD rats.

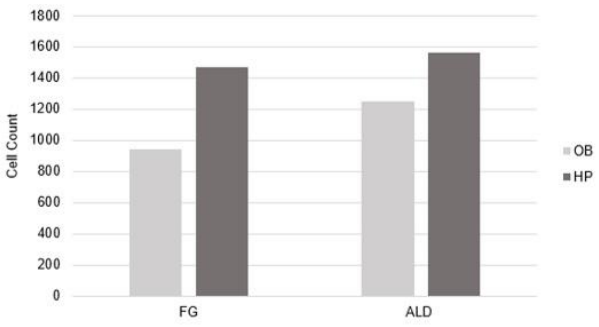

Figure 4. *Olfactory bulb (OB) and hippocampus (HP) cell count of fasted (FG) and ad libitum diet (ALD) rats. OB (FG) 941 ± 139 cells; OB (ALD) 1248 ± 148 cells; HP (FG) 1469 ± 529 cells; HP (ALD) 1559 ± 665 cells. Data presented as mean ± SD (n = 5 rats in each group).*

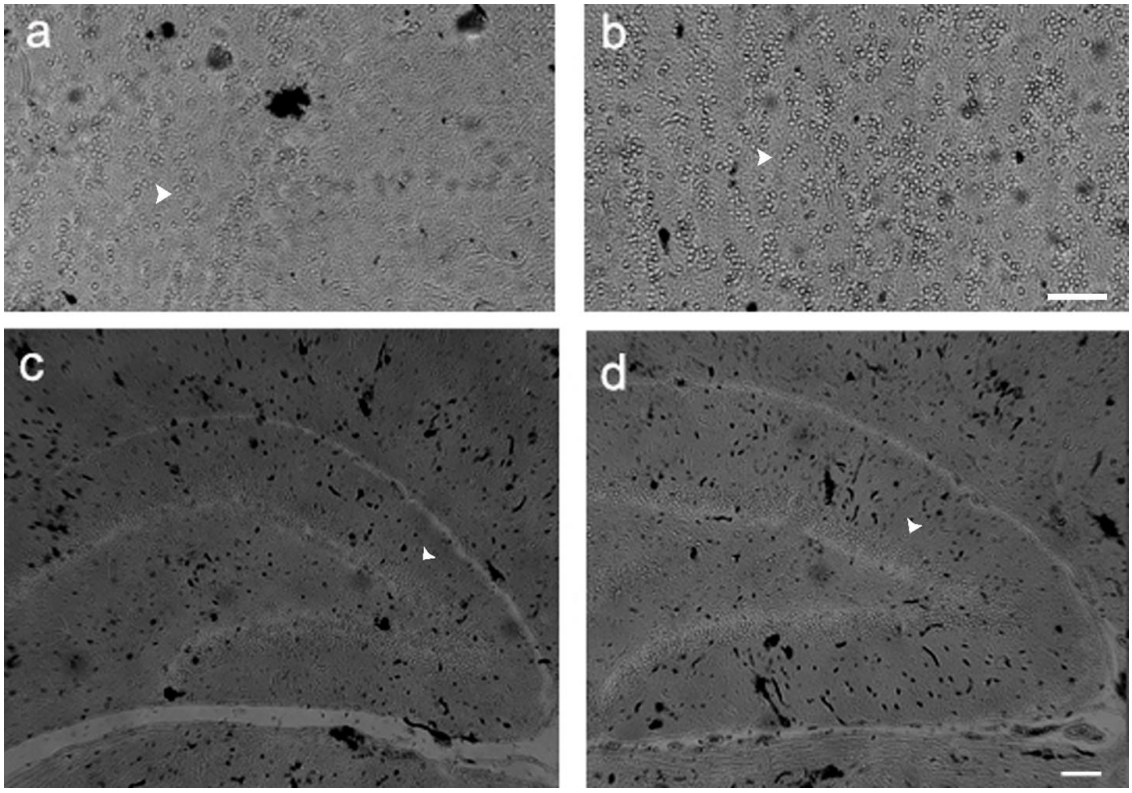

Figure 3. *Cell distribution in olfactory bulbs (10x; a and b) and hippocampi (4x; c and d) tissue of fasted (FG) (a and c) and ad libitum diet (ALD) (b and d) rats. Arrowheads indicate cell nuclei. Scale bars, 10 µm.*

Discussion

We investiated the effect of contrasting metabolic states (fasted or satiated) and an avocado-derived phenolic rich extract on motivated feeding responses, and their association with the expression of p75^{NTR} and AgRP genes in rat OB and HP, when confronted with a decision-making paradigm. A rat's eating behavior is known to be influenced by food appeal, palatability, familiarity, availability, and hunger/satiety (Benelam, 2009; Fiszman and Tarrega, 2017). The release of orexigenic hormones promotes 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). For example, acute fasting in humans increases reward pathway activity in response to food, as compared to a fed state (Goldstone et al., 2009). It was therefore expected that the FG group showed briefer latencies to feeding, as compared to ALD group. This may suggest that experimental rats increased their motivation to carry out feeding decisions, associated to differential reward values according to their metabolic states. Others have demonstrated that palatability is preferred over bland food, regardless of metabolic states, for example, Hume et al. (2016) reported that rats with *ad libitum* access to standard bland food undergoing regular scheduled feeding of palatable food (sweetened condensed milk), decreased bland food intake and consumed larger amounts of the palatable one. Several brain structures participate in decision making when it comes to food consumption. 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. In ovariectomized rats receiving a high-polyunsaturated fatty acid (PUFA) diet, L-dopa levels in the HP were higher, as compared to rats fed a standard chow diet (Dornellas et al., 2018). Although 'Hass' avocado paste extract has been previously demonstrated to induce satiety effects (and is a rich source of PUFAs and MUFAs) (Corella-Salazar et al., 2021), the present work suggests that AVO pellets motivated increased feeding, as compared to standard CP pellets in FG and ALD groups, likely attributed to the higher palatability and/or novelty imparted by its presence on the regular pellets.

Metabolic states have also been shown to regulate neurogenesis in adult mice through hypothalamic connections, as discussed by Paul et al. (2017). Specifically, fasting was shown to decrease OB deep granule interneurons, by regulating the ventricular-subventricular zone via hypothalamic influence. Likewise, adult mice and human hippocampal dentate gyrus neurogenesis is also influenced by the ventricular-subventricular zone (Zuloaga and Temple, 2017). The literature has also described how chronic stressors, such as food deprivation, stimulate glucocorticoid release via the hypothalamic-pituitary-adrenocortical axis, which negatively impacts mammalian and avian hippocampal neurogenesis (Robertson et al., 2017). These findings may explain the difference in brain cell density found in fasted and satiated rats reported herein.

AgRP/NPY neuronal activation in the ARC leads to increased food intake, whereas ablation of these neurons decreases feeding (Bunner et al., 2020). One study demonstrated that the loss of fragile X mental retardation protein (FMRP) increased MAP1B protein expression in the ARC, whereas ARC AgRP levels dropped. Furthermore, MAP1B-KO mice showed a reduction in feeding and body weight, that correlated with AgRP levels, suggesting that the reduction in feeding behavior seen in FMRP-KO mice may be the result of diminished ARC AgRP mRNA, via upregulation of MAP1B (Long et al., 2020). Another study showed that a single bout of treadmill exercise leads to an acute increase in ARC AgRP neuronal activation in mice, subsequently causing an immediate increase in post-exercise food consumption (Bunner et al., 2020). Importantly, AgRP expression has also been shown by *in situ* hybridization in the OB and HP of different species. For example, Boswell et al. (2002) assessed the distribution of orexigenic mRNA in different brain areas of Japanese quail, and reported that AgRP mRNA was not expressed in the HP of fed birds. Haskell-Luevano et al. (1999) reported that AgRP was not expressed in the HP of rats with *ad libitum* access to food, whereas Broberger et al. (1998) did not detect AgRP mRNA in neither the OB or HP of mice, although it was not clear whether these findings were a result of normal, anorectic, or monosodium glutamate-treated mice. In all studies, including the present one, expression of AgRP in the HP was absent. However, unlike Broberger et al. (1998), we observed a clear expression of AgRP in the OB of FG and ALD rats, suggesting a local production of this neuropeptide by OB cells, despite metabolic states.

Brain-derived neurotrophic factor (BDNF) plays a critical role on the eating behaviors of both humans and

animals. Female patients with anorexia nervosa or bulimia nervosa were found to have decreased BDNF levels, as compared to a control (Hashimoto et al., 2005). Accordingly, heterozygous BDNF-KO mice present hyperphagia, weight gain, increased linear growth, and elevated plasma levels of leptin, glucose, insulin, and cholesterol (Kernie et al., 2000; Lyons et al., 1999; Rios et al., 2001). The literature also suggests that pro-BDNF is preferentially activated by p75^{NTR}, while tropomyosin receptor kinase B (TrkB) binds the mature form (Chao, 2003). Mice lacking TrkB genes show characteristic neuronal and behavioral anomalies similar to BDNF-KO mice (Hasegawa et al., 2004), including increased body weight and linear growth, hyperglycemia, hyperactivity, hyperleptinemia, and hyperinsulinemia, thereby constituting an obesity phenotype (Xu et al., 2003). TrkB receptors are widely distributed in the mammalian central nervous system (CNS), whereas in zebrafish, this receptor is confined to reticulospinal neurons in the HP, suggesting the role of BDNF is restricted to this area (Abbate et al., 2014).

On the other hand, several studies have investigated the role of p75^{NTR} on neurogenesis in the OB and HP, however, not many studies have looked at its effects on food intake. Catts and colleagues (Catts et al., 2008) found that p75^{NTR}-deficient mice had a longer latency to feed in a novelty-suppressed feeding test, similar to BDNF-deficient phenotypes. In addition to this altered behavior, decreased hippocampal neurogenesis was found, with no difference shown in food consumption between genotypes (p75$^{NTR\ -/-}$ and p75$^{NTR\ +/+}$). These results may have simulated a satiated state among mice, similar to the ALD rats of the present study, as well as decreasing motivation for food consumption, according to the longer feeding latencies recorded. Additionally, Smiljanic et al. (2015) reported that dietary restriction increased the production of BDNF in the HP, as compared to rats fed *ad libitum*, perhaps suggesting a relationship between food memory and motivation to feed. Despite these similarities, lower p75^{NTR} levels were reported (Smiljanic et al., 2015), in contrast to our results, where a clear expression of hippocampal p75^{NTR} mRNA was reported in FG, but not ALD rats. It is possible that this may be attributed to the different ages of rats used on each study. Regarding the OB, p75^{NTR} has not been studied in the context of food intake. Given our results, and that TrkB expression in the OB has been previously suggested to contribute to feeding (Xu et al., 2003), it is possible that an upregulation of p75^{NTR} expression in the OB may play a role in food attainment when faced with a fasting state.

Finally, the consumption of a diet rich in omega-3 PUFAs in mice has shown to influence the HP by increasing its volume, expression of BDNF, neurogenesis, and serotonergic activity (Dornellas et al., 2018). In adipocytes, p75^{NTR} has been found to be upregulated following a high-fat diet, directly promoting lipolysis, thermogenesis, and fat oxidation (Baeza-Raja et al., 2016). Additionally, several studies have demonstrated that serotonin increases the activation of POMC/CART neurons in the hypothalamus, while decreasing the expression of AgRP, as demonstrated in ovariectomized rats that were fed a high-fat fish oil diet, but not in those fed a saturated fatty acid diet (Dornellas et al., 2018). In accordance with these findings, MUFAs and PUFAs of avocado extract-coated pellets may have similar effects on p75^{NTR} and AgRP expression, however further studies are required to confirm this hypothesis.

In conclusion, we found that 1) metabolic states motivate behavioral feeding responses in rats, as suggested by briefer latencies to feed when under fasted conditions; 2) food deprivation reduced cell density in the OB and HP; 3) fasting 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. These findings further demonstrate how non-homeostatic structures involved in food intake regulation are reciprocally influenced by homeostatic mechanisms. Understanding the interactive pathways and neuro-behavioral changes leading to food intake may contribute to a better comprehension on the etiology of motivated behavioral feeding responses.

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Supplementary information file
Description:

1) First gel: AgRP positive samples represent two different experimental OB rats

2) Second gel: AgRP represents a sample from an experimental ALD rat. Notice AgRP is not expressed by hippocampus.

3) Third gel: two positive samples of p75 in OB from two different experimental rats; and three positive samples of p75 in hippocampi from three different experimental rats.

In all experiments, beta-actin was used as a housekeeping gene.

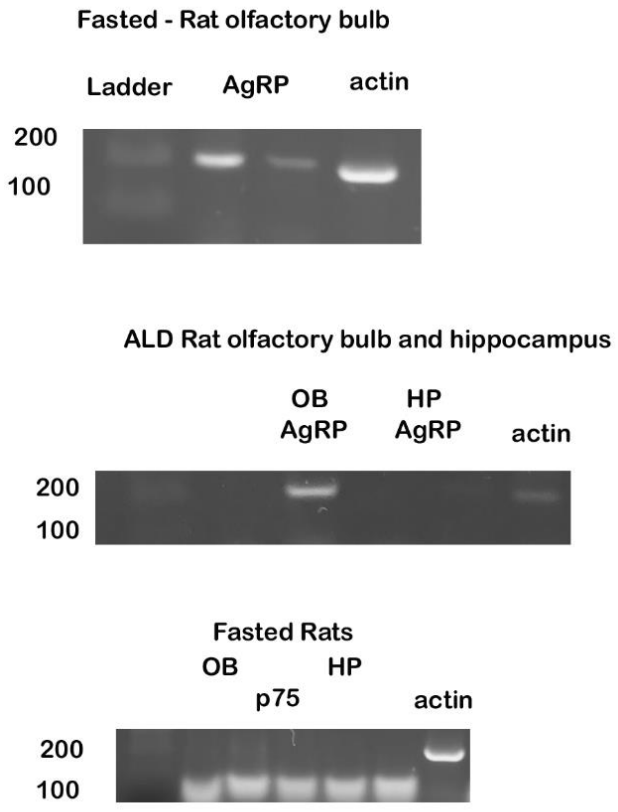

Figure 5. *Endpoint RT-PCR amplicons of AgRP and p75NTR in the olfactory bulb (OB) (a) and hippocampus (HP) (b) of fasted (FG) and ad libitum diet (ALD) rats visualized on an agarose gel.*