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# Perinatal malnutrition stimulates motivation through reward and enhances $drd_{1a}$ receptor expression in the ventral striatum of adult mice

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ABSTRACT

*Aim:* The aim of this study was to analyze the effects of protein perinatal malnutrition on the function of dopamine DRD1 and DRD2 receptors in regards to motivation and food consumption in adult mice. The study also analyzed the effect of protein perinatal malnutrition on the gene expression of these receptors in the ventral striatum.

*Methods:* Wistar lineage mice were divided into two groups according to maternal diet: control (17% casein), n = 30 and low protein (8% casein), n = 30. Between 30 and 120 days of life, the following factors were measured: body weight; the effect of dopamine D1 and D2 agonists on the ingestion of palatable food; the motivational aspect under the action of the D1 (SKF 38393) and D2 Quinpirole dopaminergic agonists; and the gene expression of DRD1 and DRD2 receptors in the ventral striatum.

*Results:* The body weights of the malnourished animals remained significantly lower than those of the control group from 30 to 120 days of life. Malnourished animals ingested a greater quantity of palatable food. There was a decrease in palatable diet consumption in both the control and malnourished groups after the application of D1 and D2 agonists; however, the anorexic effect of the D1 agonist was understated in malnourished animals. Perinatal malnutrition increases the motivational behavior of the animal when food reward is used. There was an increase in gene expression of the DRD1a receptor in the ventral striatum of malnourished animals, and there were no significant changes concerning the DRD2 receptor.

*Conclusions:* Perinatal protein malnutrition stimulates hedonic control of eating behavior by promoting increased intake of palatable foods, possibly due to increased expression of dopamine receptor DRD1a in the ventral striatum.



## 1. Introduction

During the developmental phase, the brain is highly vulnerable to different types of insults (Morgane et al., 2002). The deleterious effects of insults in the early phases of life are demonstrated through different experimental paradigms, such as prenatal stress (Vallee et al., 1997), maternal separation (Lehmann et al., 1999), overnutrition (Alsiö et al., 2010) and malnutrition (Lopes de Souza et al., 2008). Malnutrition is a worldwide issue that affects newborns and children during the most vulnerable stages of brain development, changing brain maturation events and leading to behavior changes, changes in cognition functions and learning and memory disorders (Morgane et al., 2002). Developing societies that are experiencing fast and intense transformations in their economic growth and demographic structure patterns are experiencing a decrease in malnutrition and an increase in obesity; this characterizes the nutritional transition in developing societies (Popkin, 1994). The etiology of obesity is multifactorial and is becoming a public health problem due to its prevalent increase and the repercussions of its comorbidities (Von Diemen et al., 2006).

The effect of malnutrition on encephalic mechanisms of food ingestion regulation and its relevance in obesity etiology is evident. However, the molecular, cellular and behavioral mechanisms underlying this phenomenon are still poorly understood, and understanding

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the extent of the damage to health of this phenomenon requires intensive scientific research (Zheng et al., 2009). The access to palatable food is currently considered as one of the signs of metabolic disorders, including obesity (Berridge and Kringelbach, 2008). The preference for this type of food is associated with hedonic responses, such as motivation, palatability and pleasure (Yamamoto, 2006; Berridge and Kringelbach, 2008). Dopamine participates in these mechanisms through the action of dopamine D1 and D2 receptors (Wang and Xu, 2007; Beaulieu and Gainetdinov, 2011). According to some authors, the dopamine D1 receptor can contribute to appetitive motivation and plays an important role in increasing the incentive for acquiring natural rewards, such as palatable food. They also suggest that the action of dopamine D2 receptors is particularly focused in satiety rather than motivation; therefore, these receptors mainly inhibit the consumption of food, controlling the size, duration and frequency of meals (Robinson et al., 2005; Wang and Xu, 2007; Volkow et al., 2008; Vucetic and Reyes, 2010; Beaulieu and Gainetdinov, 2011). Even in the absence of hunger, pleasure and the reward sensation associated with food can stimulate consumption (Kelley and Berridge, 2002). The nucleus accumbens is critically involved to behaviors linked to specific objectives, such as the search for natural and artificial reinforcements, such as food and abusive drugs (Kelley and Berridge, 2002). It is believed that the release of dopamine in the nucleus accumbens is associated with food ingestion, especially palatable food, such as chocolate and cookies (Beaulieu and Gainetdinov, 2011).

Studies on humans using tomography through the emission of positrons (PET scan) showed that food consumption is associated with dopamine release in the dorsal striatum and that the quantity of dopamine released is correlated with the level of pleasure associated with feeding (Small et al., 2003). This suggests that the hedonic mechanisms of food consumption regulation may be more complex than previously thought.

Although several studies have already stated the role of dopamine D1 and D2 receptors on feeding behavior, there are still few reports concerning the effect of perinatal malnutrition on the function of these receptors. Perinatal malnutrition may be involved in one of the neural mechanisms that are related to the etiology of obesity in the current worldwide population. Based on this, the present study analyzed the effect of protein perinatal malnutrition on the functions of dopamine DRD1a and DRD2 in regards to feeding motivation in adult mice. The study also analyzed the gene expression of these receptors in the ventral striatum, which includes the nucleus accumbens region in addition to the ventral portion of striatum body, of adult mice.

## 2. Material and methods

### 2.1. Animals

All experiments were approved by the Ethics Committee on Animal Experiments of the Center for Biological Sciences, Federal University of Pernambuco (processing number: 23076.034632/2010-79) and were performed in accordance with the recommendations of the Brazilian Committee for Animal Experimentation (COBEA). Virgin female Wistar rats (n = 10) weighing 250–300 g were obtained from their birth vivarium (Department of Nutrition, Federal University of Pernambuco) and were kept on a reversed 12-h light/dark cycle (lights on at 1800 h) under a controlled temperature (22 ± 2 °C) with water and a standard diet (Purina, Campinas, SP, Brazil S/A) (Table 1) provided *ad libitum*. After an adaptation period of 15 days, the rats were mated at a ratio of one male to one female. After confirmation of pregnancy through visualization of sperm in vaginal smears, females were moved to individual cages and fed either a normal-protein diet (17% of casein, n = 10) or a low-protein diet (8% of casein, n = 10) during pregnancy and lactation (Crnic and Chase, 1978; Wiener and Levine, 1983; Falcao-Tebas et al., 2012) (Table 1). The birth day was recorded as postnatal day zero (P0) for the pups. Sexing was performed at 24 h after birth, and the numbers of pups were adjusted to give 8 pups per mother with an equal ratio (4:4) of males and females. In this paper, female pups were used only to complete the litters to maintain the same male:female ratio. The experimental groups consisted of two male rats from each litter, and a total of 10 animals from the control and 10 animals from the low-protein restricted groups were used. After weaning, all animals were fed a standard diet (Labina®; Purina).

### 2.2. Agonists

The dopamine D1 and D2 agonists were obtained in crystallized form and dissolved in sterile water to a concentration of 5 mg/ml, according to the manufacturer's recommendation (Sigma-Aldrich®). In all experiments, the dopamine D1 agonist (SKF 38393) was administered in a 3 mg/kg body weight dose, and the dopamine D2 agonist (Quinpirole) was administered in a 0.3 mg/kg body weight dose; both were applied intraperitoneally (Cooper and Al-Naser, 2006).

### 2.3. Experimental procedures

#### 2.3.1. Body weight and feeding ingestion

Body weight was analyzed at 30, 60, 90 and 120 days of life. At 60 days of life, the animals were allocated to individual cages and were given between 100 and 150 grams of palatable food. The animals were allowed to adaptat to the cage and diet for three days; with the diet being offered to the animals for one hour a day. Food consumption was measured every 24 hours.

#### 2.3.2. Palatable food ingestion evaluation under stimuli of dopamine D1 and D2 agonists in adult rats

This experiment involved animals previously deprived of food for four hours. The subgroups were as follows: $C_{D1}$ (normonourished animals that received an acute dose of D1 agonists, n = 10), $LP_{D1}$ (malnourished animals that received an acute dose of dopamine D1 agonist, n = 10), $C_{D2}$ (normonourished animals that received an acute dose of D2 agonist, n = 10), $LP_{D2}$ (malnourished animals that received an acute dose of dopamine D2 agonist, n = 10), $C_w$ (normonourished animals that received distilled water, n = 10), and $LP_w$ (malnourished animals that received distilled water, n = 10). A half hour after the injections, palatable food was made available to the animals (30 g of chocolate cookies - Chocookies; Nabisco®, East Hanover, NJ, USA). After an hour, the food was removed and weighed to determine the consumption by subtracting the quantity of rejected food from the quantity of offered food (Cooper and Al-Naser, 2006).

#### 2.3.3. Runway task

At 70 days of age, rats in both the control and undernourished groups underwent a runway task incentive test (Silveira et al., 2010; da Silva et al., 2013). This test assesses the motivation of the animals

**Table 1**
Composition of macronutrients of the diet and period handling.

| DIETS | Protein (% kcal) | Carbohydrate (% kcal) | Lipids (% kcal) | (kcal/g) | Period handling |
|---|---|---|---|---|---|
| Normoprotein (AIN-93G) | 19.5 | 61.9 | 17.7 | 3.6 | Gestation and lactation |
| Low protein | 9.3 | 72.0 | 17.5 | 3.6 | Gestation and lactation |
| Chow (Labina Purina®)* | 26.0 | 63.0 | 11.0 | 3.6 | P36–P180 |

* Data of the standard diet are in agreement with those described in the manufacturer's packaging.

to obtain a food reward, evaluating the time necessary for the animal to get to the end of a center runway where a box containing the food reward is placed (Pecina et al., 2003). The runway task incentive is used as a tool to evaluate motivation for obtaining a food reward by training the animals to search for a specific target (reward). The food serves as a salient motivation incentive for the animals. As rats consume most of their food during the night, behavioral assessments were performed 6–8 h after the onset of the dark phase of the light/dark cycle. The apparatus used consisted of a tunnel with three compartments: a start box (19 cm × 14 cm × 30 cm), a center runway (150 cm × 14 cm × 30 cm) and a target box (19 cm × 14 cm × 30 cm). The boxes were made of transparent acrylic, and the center runway was made of opaque polypropylene. The images were captured with a video camera positioned in the center runway to allow viewing of the entire apparatus. The starting box could be moved along the tunnel to attain a 15–150 cm distance from the target box. The stimulus was placed inside the target box and consisted of 5 g of chocolate-flavored cookies (Chocookies; Nabisco®, East Hanover, NJ, USA). The test consisted of 11 training sessions of 5 min each held on alternating days for a total period of 22 days. The animals were deprived of food for 4 h before each training session. During the adaptation period (1–3 sessions), the animal was placed directly in the closed-target box and allowed to access the reward for 5 min. Starting with session 4, the starting box was placed 15 cm away from the target box, and the rats were placed in the start box for 30 s with the door closed. After 30 s, the door was opened, and the animal was allowed to enter the center runway. If the rat did not leave the start box within 3 min, it was gently pushed into the target box. For each subsequent session, the target box was moved further away from the start box (30 cm away for session 5; 60 cm for session 6; 75 cm for session 7; 90 cm for session 8; 120 cm for session 9, and 150 cm for sessions 10 and 11). The sessions were defined as previously described by Pecina et al. (2003): sessions 1–3 was considered as the adjustment phase in which the animal is exposed to a new environment and reward so that the natural neophobic behavior of the animal disappears; sessions 4–6 was considered as the pre-exposure phase in which the animal is exposed to the target box and execution center; and sessions 7–9 was considered as the learning-incentive or reinforcement phase in which the animal is encouraged by the stimulus of reward. In sessions 10 and 11, the control and low protein animals were subdivided into the following subgroups for drug application: $\mathbf{C_W}$ (control water): nourished animals that received distilled water (n = 10); $\mathbf{C_{D1}}$ (D1 control): nourished animals that received the D1 dopamine agonist (n = 10); $\mathbf{C_{D2}}$ (D2 control): nourished animals that received the dopamine D2 agonist (n = 10); $\mathbf{LP_W}$ (low protein water): undernourished animals that received distilled water (n = 10); $\mathbf{LP_{D1}}$ (low protein D1): undernourished animals that received the D1 dopamine agonist (n = 10); and $\mathbf{LP_{D2}}$ (low protein D2) undernourished animals that received the dopamine D2 agonist (n = 10). The speed of task completion for each session was calculated by dividing the latency time taken to reach the target box by the length of the track. The speed of searching for the reward indicates the motivation of the animal. The rat was considered to have left the starting box when all four limbs were out of the box, and the rat was considered to have entered the target box when all four limbs were within the target box. Once the rat entered the target box and started eating, it was allowed to consume the palatable food for 30 s before being removed. The following parameters were analyzed: (1) latency time before leaving the start box, (2) number of pauses on the track, (3) number of times the rat reversed direction away from the target (this behavior involved turning around toward the start box and was usually accompanied by sniffing), (4) latency before beginning intake of the palatable food, and (5) time taken to complete the task.

*2.3.4. Ventral striatum isolation*

On the 120th day of life, the animals of both groups were sacrificed. The mice were weighed before sacrifice and were beheaded after sacrifice. After the beheading, a craniotomy was performed to remove the brain; then the ventral striatum, which includes the nucleus accumbens in addition to the ventral portion of the striatum body, was isolated and packaged in an Eppendorf® tube that was properly labelled. The ventral striatum was frozen in solid carbon dioxide (dry ice) and kept in a freezer at -80 ° C until the preparation of the samples for RT-PCR analysis.

*2.3.5. Total RNA extraction*

The total RNA of the ventral striatum was extracted using 1 ml of Trizol (Invitrogen®, Carlsbad CA USA) reagent according to the manufacturer's instructions, and its concentration was determined from the absorbance measure at 260 nm.

*2.3.6. cDNA synthesis (RT-PCR)*

cDNA synthesis was done using a QuantiTect® Reverse Transcription (Qiagen®, Hilden, Alemanha) kit. To eliminate genomic DNA, a reaction was prepared using 2 μl gDNAWipeout Buffer, 7×; RNA samples; and the amount of RNase-free water necessary to reach a final volume of 14 μl. The reverse transcription QuantiTect® reaction mix was prepared using 1 μl Quantiscript Reverse Transcriptase; 4 μl Quantiscript RT Buffer, 5×; 1 μl RT Primer Mix; and 14 μl of the elimination reaction, which contains the sample RNA. Then, an aliquot of each reverse transcription reaction, which contains a cDNA mix, was stored at -20°.

*2.3.7. PCR in real time*

The PCR reaction was done using the SYBR® Green PCR Master Mix (Qiagen®) Kit and was analyzed in real time through an automated system of sequence detection called the Rotor-Gene® TM RG 3000 (Corbett Life Science, Australia) to determine the mRNA expression of the samples. The total volume of the reaction was 25 μl and included 12.5 μl of the fluorescent compound SYBR Green PCR Master Mix (Qiagen®), 2 μl of cDNA (used as a mold to the reaction), 2.5 μl of the sense and antisense primers and 5.5 μl of RNase-free water. The reactions were incubated at 95 °C for 5 minutes to activate the DNA polymerase enzyme, followed by 40 cycles of 5 seconds at 95 °C for denaturation and 10 seconds at 60 °C, extension and collection of the fluorescent signal. The sequence of primers used for amplification were as follows: β-actin - forward, 5'- ACG GTC AGG TCA TCA CTA TCG-3′ and reverse, 5′- CAG CAC TGT GTT GGC ATA GAG-3′; DRD1a – forward, 5′ CTG GAG GAC ACC GAG GAT GAC-3′ and reverse, 5′- GTC GAT GAG GGA CGA TGA AAT GG -3′; DRD2 - forward, 5′- CAA CAA TAC AGG CAA ACC AGA ATG AG- 3′ and reverse, 5′- ACC AGC AGA GTG ACG ATG AAG G-3′. The primers were synthetized and purified by IDT – Integrated DNA Technologies (EUA). The relative expression levels of mRNA of the dopamine DRD1a and DRD2 receptors in the ventral striatum were calculated using the Ct (threshold cycle) comparative method (Livak and Schmittgen, 2001), with β-actin as a normalizing gene.

*2.4. Statistical analysis*

Data were expressed as averages ± standard error of the averages. To show the difference in the food ingestion by the experimental groups, the values were converted to calories relativized by the body weight of each animal, and multiplied by 100 [(g consumed × cal)/animal's weight × 100]. Furthermore, to compare the quantity of ingested food of the control group and low protein group in response to dopamine D1 and D2 agonists, data were expressed as percentages related to the food ingestion of the groups that received distilled water. To calculate significant differences, the Student's t test was used for food ingestion at 60 days of life, intergroup ingestion under the D1 agonist effect and gene expression of the DRD1a and DRD2 receptors. A two-way ANOVA, followed by a Bonferroni post-test, was used for the other factors analyzed (body weight and food ingestion in response to dopamine D1 and D2 agonists).

The significance level was considered $p \leq 0.05$. All data were analyzed using a GraphPad PRISM 5 version 7 (San Diego USA) program.

## 3. Results

### 3.1. Effect of perinatal malnutrition on body weight and palatable diet ingestion

The body weight of low protein animals remained significantly lower than the control animals from 30 to 120 days of life (Table 2) (Fig. 1). The animals exposed to the hypoproteic diet during the gestation period and lactation ingested a greater quantity of palatable food compared to the control group during the test period at 60 days of life (Table 3). The data were converted into calories and relativized to the body weight of each animal.

### 3.2. Anorexic action of dopamine D1 and D2 agonists

The application of D1 and D2 agonists reduced the consumption of palatable food in both the control and malnourished group (Table 3). When comparing the percentage of consumption of the animals treated with saline, the D1 agonist inhibited 57% of the quantity of ingested food in the control group, and the same amount of the drug inhibited only 27% of the ingested food in the low protein group (Fig. 2). The DRD2 receptor agonist inhibited 85% of the food intake in both the control and malnourished groups (Fig. 2).

### 3.3. Runway task incentive test

#### 3.3.1. Evaluation of the number of pauses and reverses in direction, latency time to exit the initial box and latency time to react to the intended box

The malnourished animals showed fewer pauses in Sections 5 and 10 when compared to the control group (Table 4). In sessions 5, 6, 7 and 9, the low protein animals showed a smaller number of reverses in directions than the control group (Table 4). For the latency time to exit the initial box and reach the intended box, no statistically significant difference was found between the analyzed experimental groups.

#### 3.3.2. Evaluation of the latency time to consume the reward

In session 5, the pre-exposure phase, malnourished animals presented a shorter latency time to eat the reward when compared to the control group. In session 8, the learning period, the malnourished animals also showed a shorter latency time to eat the reward compared to the control group. During sessions 10 and 11, no statistically significant difference in latency time to consume the reward between the control and malnourished animals was observed.

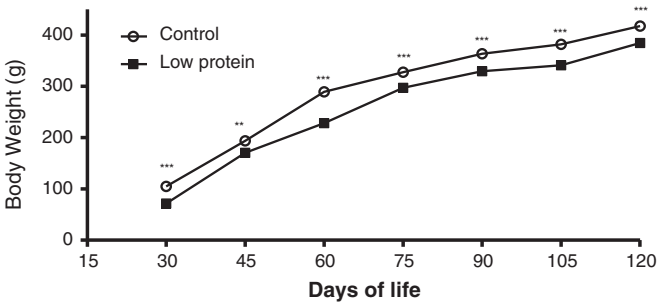


**Fig. 1.** Average bodyweight of male mice pups born from females fed with a normoproteic (casein 17%) or hypoproteic (casein 8%) diet during gestation and lactation. When weaning, the pups were fed with a standard vivarium diet. The data are averages ± SEM with n = 30 for control animals and n = 30 for malnourished animals. The analysis was performed between 30 and 120 days of life. The differences in body weight between control and malnourished animals are statistically significant with **P < 0.01 at 45 days and ***P < 0.001 at all other analyzed ages (two-way ANOVA to repeated measures by Bonferroni post-test). g = gram.

#### 3.3.3. Speed

In the pre-exposure period, the speed of the control and malnourished animals did not show any statistically significant difference. During the phase of encouragement of learning (sessions 7, 8, and 9), the malnourished animals showed a faster speed in all the analyzed sessions compared with the control group (Table 4). The malnourished animals maintained this difference after training, during sessions 10 ($LP_{s10}$) and 11 ($LP_{s11}$), when compared with the control group (Table 4) (Fig. 3).

#### 3.3.4. The effect of dopamine D1 and D2 agonists on the performance during sessions 10 and 11 of the runway task incentive test

In the motivational test, the application of dopamine D1 agonist enhanced the speed of the control and malnourished animals during sessions 10 and 11 when compared to the saline group (Table 5) (Fig. 4A and B). On the other hand, in the motivational test, the application of the dopamine D2 agonist slowed the speed of the control and malnourished animals during sessions 10 and 11 when compared with the saline group (Table 5) (Fig. 4A and B). When comparing control animals and malnourished animals that received the same dose of D1 agonist, the malnourished animals showed a faster speed in both sessions of the motivational test (Table 5) (Fig. 4A and B). No statistically significant difference in speed was found between the control and malnourished animals after the application of D2 agonists.

**Table 2**
Evolution of body weight of the malnourished animals and controls from 30 to 120 days.

| Variables | 30 days (g) | 45 days (g) | 60 days (g) | 75 days (g) | 90 days (g) | 105 days (g) | 120 days (g) |
|---|---|---|---|---|---|---|---|
| Group control | 104.81 ±1.85 | 193.72 ±2.48 | 289.17 ±3.44 | 327.42 ±4.82 | 363.57 ±3.99 | 382.00 ±5.23 | 417.47 ±9.84 |
| Group low protein | 71.46 ±0.58 | 170.55 ±3.74 | 228.02 ±5.30 | 297.20 ±4.43 | 329.59 ±5.22 | 341.11 ±2.50 | 384.57 ±3.97 |
| p | <0.001 | <0.01 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| t | 5.128 | 3.563 | 9.403 | 4.647 | 5.225 | 6.288 | 5.059 |
| N | 30 | 30 | 30 | 30 | 30 | 30 | 30 |

| Source of Variation | Df | p value | F |
|---|---|---|---|
| Interaction | 6 | 0.0025 | 3.441 |
| Animal group Factor | 1 | P < 0.0001 | 220.8 |
| Days Factor | 6 | P < 0.0001 | 1160 |
| Residual | 406 | | |

**Table 3**
Food intake of palatable diet of control and malnourished animals with and without pharmacological stimulation.

| Variables | 60 days (g) | Saline stimulation 100 days (g) | D1 agoist stimulation (g) | D2 agoist stimulation (g) |
|---|---|---|---|---|
| Group control | 26.80 ±0.30 | 20.3 ±0.65 | 8.8 ±1.18 | 3.0 ±0.45 |
| Group low protein | 40.70 ±0.30 | 28.9 ±1.13 | 21.1 ±0.19 | 4.8 ±0.45 |
| p | <0.0001 | <0.001 | <0.001 | <0.001 |
| N | 10 | 10 | 10 | 10 |

| ANOVA Table | Df |
|---|---|
| Treatment (between Animal group) | 2 |
| Residual (within Animal group) | 27 |
| Total | 29 |
| F | 115.3 |

### *3.4. Analysis of gene expression of DRD1a and DRD2 receptors in the ventral striatum*

The DRD1a and DRD2 gene amplification efficiency in relation to the normalizer gene β-actin, was systemically analyzed using cDNA samples from both the control and malnourished animals. A significant difference ($p < 0.05$) (an increase of 46.5%) was found in the levels of DRD1a gene expression in the malnourished animals (LP = 1.89 ± 0.45) in a comparison to the control animals (C = 1.29 ± 0.30). In regards to DRD2 gene expression, the average level of expression in the malnourished animals was LP = 1.29 ± 0.30 and that in the control animals was C = 1.07 ± 0.22, showing a 20.5% increase in the malnourished animals; however, this difference was not statistically significant. The relative mRNA expression level of the DRD1a receptor was increased in the ventral striatum of malnourished animals in relation to the control animals.

## 4. Discussion

The deficit of specific nutrients at the beginning of life can affect cognitive function in adult life. Experimental studies have highlighted the role of nutrition on neurodevelopment and have shown that premature nutritional disorders can permanently affect the function and structure of the brain (Pollitt et al., 1993). Studies suggest that nutritional intervention in the early periods of life may be associated with alterations in food behavior in adult life, particularly in regards to components of food reward behaviors (Vickers et al., 2000; Padoin et al., 2001). This study shows that animals that were subjected to protein perinatal malnutrition showed a body weight reduction in relation to control animals. These data are consistent with those of other studies (Remmers et al., 2008; Orozco-Solis et al., 2009; da Silva et al., 2013). The most frequent cause of body variation in weight in the early periods of life is the maternal nutritional contribution during the gestation and lactation periods (Page et al., 2009). In this research, the animals that had a low weight as newborns presented a high consumption of palatable food in adult life. The data corroborate experimental studies done with animals that were subjected to protein restriction during gestation and lactation, which resulted in hyperphagia (Bellinger et al., 2004; Desai et al., 2005) and a higher preference for food rich in sugar and fat (Bellinger and Langley-Evans, 2005; Bellinger et al., 2006). Previous literature describes that the consumption of food rich in sugar and fat provokes neurochemical modifications in neurotransmitter systems, such as the mesolimbic system, and this is related to food behavior and reward (Bellinger et al., 2006). Studies have also shown that food restriction increases reward value of palatable caloric food (Scheggi et al., 2013).

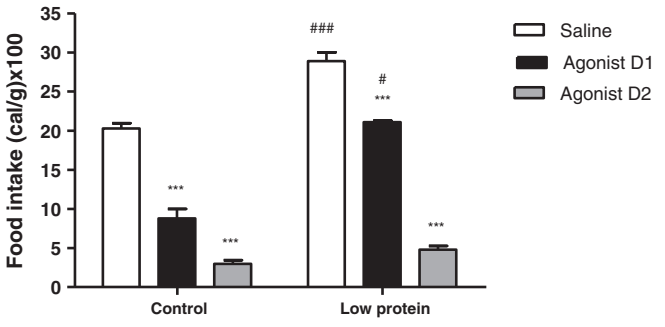


**Fig. 2.** Effects of D1 and D2 dopamine agonists over food intake of palatable diet in animals subjected to perinatal malnutrition. The hypophagic effect of D1 and D2 agonists was determined at 100 days of life. Ten animals for each analyzed experimental group were used, which received acute dose of D1 (3 mg/kg), D2 (0,3 mg/kg) agonists or saline (1 ml/kg). The data correspond to the amount of palatable diet consumed in 60 minutes of test, consumed by the animals that received saline from the same group. 1way ANOVA was used to compare intra-group followed by Bonferroni post-test. T Student test for inter-group comparison # $p < 0,05$ and the values compared among animals that received the same dose of D1 agonist. No statistically significant difference was found in speed between control and low protein animals after D2 agonist application.

Dopamine neurotransmission in the central nervous system (CNS) is associated with hedonic responses, such as palatability and pleasure when consuming food rich in energy (Erlanson-Albertsson, 2005). Several studies investigated the effects of dopamine agonists and their function on where the agonists to the D1 and D2 receptors, diminished the food ingestion, though they report the anorexic effect of the agonists of the D1 receptor (Kuo, 2002; Gambarana et al., 2003; Cooper et al., 2006; Goto and Grace, 2008).

In the present study, the intense administration of D1 and D2 receptor agonists caused a reduction in palatable food consumption regardless of the nutritional history of the evaluated animals. D1 receptor agonists reduced the feeding ingestion by decreasing the number of feeding episodes, while D2 agonists reduced the quantity of ingested food (Timmerman et al., 1989). The effects of D1 and D2 agonists on food ingestion were compared, and it was found that malnourished animals were less responsive to the anorexic action of D1 agonists and more motivated for palatable food consumption. These results are in agreement with those of another study, in which the D1 receptor agonist increased the preference of mice for highly palatable food (Terry and Katz, 1992) proving that perinatal malnutrition reduces the hypophagic action of the dopamine D1 receptor. However, the same effect was not observed after application of the D2 receptor agonist. Thus, D2 agonists seem to mitigate the preference for palatable food as a consequence of dopamine neurotransmission inhibition in the brain structures.

To better understand the neural circuits involved in feeding behavior and the possible changes that come from perinatal malnutrition in addition to changes in food consumption, the present research highlights the role of dopamine D1 and D2 receptors in different behavioral aspects, such as motivation and learning. Some studies have shown that perinatal malnutrition is linked to learning deficits in both mice

**Table 4**
Effect of perinatal malnutrition on the parameters of the 4th motivation test in the 11th session.

| Variables | | Group control | Group low protein | p | t | N | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of pauses | Sessão 4 | 0.70 ± 0.150 | 0.56 ± 0.180 | >0.05 | 0.5996 | 10 | Source of Variation | Df | p value | F |
| | Sessão 5 | 1.90 ± 0.100 | 1.00 ± 0.000 | <0.01** | 3.854 | 10 | Interaction | 7 | 0.5197 | 0.8854 |
| | Sessão 6 | 1.10 ± 0.180 | 0.67 ± 0.290 | >0.05 | 1.841 | 10 | Parameter Factor | 1 | p < 0.0001 | 40.83 |
| | Sessão 7 | 1.00 ± 0.210 | 0.44 ± 0.180 | >0.05 | 2.398 | 10 | Animal group Factor | 7 | p < 0.0001 | 8.901 |
| | Sessão 8 | 0.80 ± 0.130 | 0.33 ± 0.170 | >0.05 | 2.013 | 10 | Residual | 144 | | |
| | Sessão 9 | 0.80 ± 0.200 | 0.22 ± 0.150 | >0.05 | 2.484 | 10 | | | | |
| | Sessão 10 | 0.80 ± 0.130 | 0.11 ± 0.110 | <0.05* | 2.955 | 10 | | | | |
| | Sessão 11 | 0.56 ± 0.170 | 0.11 ± 0.110 | >0.05 | 1.927 | 10 | | | | |
| Number of reversals direction in the runway en route to the goal | Sessão 4 | 0.20 ± 0.130 | 0.33 ± 0.24 | >0.05 | 0.4251 | 10 | Source of Variation | Df | p value | F |
| | Sessão 5 | 1.00 ± 0.000 | 0.11 ± 0.11 | <0.05* | 2.910 | 10 | Interaction | 7 | 0.0024 | 3.362 |
| | Sessão 6 | 1.20 ± 0.200 | 0.22 ± 0.15 | <0.05* | 3.204 | 10 | Parameter Factor | 1 | p < 0.0001 | 23.57 |
| | Sessão 7 | 1.70 ± 0.540 | 0.33 ± 0.17 | <0.001 | 4.479 | 10 | Animal group | 7 | 0.0005 | 4.024 |
| | Sessão 8 | 0.60 ± 0.160 | 0.56 ± 0.18 | >0.05*** | 0.1308 | 10 | Residual | 144 | | |
| | Sessão 9 | 1.30 ± 0.300 | 0.44 ± 0.18 | <0.05 | 2.812 | 10 | | | | |
| | Sessão 10 | 0.30 ± 0.210 | 0.22 ± 0.15 | >0.05 | 0.2616 | 10 | | | | |
| | Sessão 11 | 0.22 ± 0.140 | 0.11 ± 0.11 | >0.05 | 0.3597 | 10 | | | | |
| Latency to leave the start box (s) | Sessão 4 | 5.10 ± 1.96 | 6.00 ± 2.28 | >0.05 | 0.1921 | 10 | Source of Variation | Df | p value | F |
| | Sessão 5 | 9.60 ± 3.78 | 15.11 ± 4.33 | >0.05 | 1.176 | 10 | Interaction 7 | 0.2225 | 1.370 | |
| | Sessão 6 | 13.80 ± 3.06 | 18.56 ± 5.63 | >0.05 | 1.016 | 10 | Parameter Factor | 1 | 0.2538 | 1.313 |
| | Sessão 7 | 12.80 ± 2.17 | 13.56 ± 4.44 | >0.05 | 0.1622 | 10 | Animal group | 7 | 0.0011 | 3.690 |
| | Sessão 8 | 16.30 ± 3.24 | 7.22 ± 0.92 | >0.05 | 1.938 | 10 | Residual | 144 | | |
| | Sessão 9 | 17.50 ± 3.81 | 10.00 ± 2.05 | >0.05 | 1.601 | 10 | | | | |
| | Sessão 10 | 17.80 ± 3.99 | 11.44 ± 2.33 | >0.05 | 1.358 | 10 | | | | |
| | Sessão 11 | 16.33 ± 3.06 | 13.56 ± 2.69 | >0.05 | 0.5913 | 10 | | | | |
| Latency to begin eating the reward once the mouse reached goal box (s) | Sessão 4 | 87.20 ± 0.90 | 108.11 ± 29.40 | >0.05 | 2.006 | 10 | Source of Variation | Df | p value | F |
| | Sessão 5 | 82.60 ± 0.74 | 51.00 ± 0.60 | <0.05* | 3.032 | 10 | Interaction | 7 | 0.0028 | 3.290 |
| | Sessão 6 | 60.30 ± 0.78 | 37.22 ± 0.67 | >0.05 | 2.215 | 10 | Parameter Factor | 1 | 0.0011 | 11.06 |
| | Sessão 7 | 48.00 ± 0.49 | 20.33 ± 0.49 | >0.05 | 2.655 | 10 | Animal group | 7 | p < 0.0001 | 8.901 |
| | Sessão 8 | 48.30 ± 0.65 | 17.44 ± 0.49 | <0.05* | 2.961 | 10 | Residual | 144 | | |
| | Sessão 9 | 18.20 ± 0.43 | 16.78 ± 0.52 | >0.05 | 0.1363 | 10 | | | | |
| | Sessão 10 | 8.30 ± 0.24 | 4.56 ± 0.24 | >0.05 | 0.3589 | 10 | | | | |
| | Sessão 11 | 5.00 ± 0.17 | 4.44 ± 0.25 | >0.05 | 0.05373 | 10 | Source of Variation | Df | p value | F |
| Speed (cm/s) | Sessão 4 | 5.01 ± 0.22 | 4.54 ± 0.18 | >0.05 | 0.9697 | 10 | Interaction | 7 | p < 0.0001 | 29.01 |
| | Sessão 5 | 6.95 ± 0.23 | 5.85 ± 0.27 | >0.05 | 2.270 | 10 | Parameter Factor | 1 | p < 0.0001 | 191.8 |
| | Sessão 6 | 7.13 ± 0.23 | 7.29 ± 0.28 | >0.05 | 0.3301 | 10 | Animal group | 7 | p < 0.0001 | 211.9 |
| | Sessão 7 | 8.03 ± 0.22 | 13.85 ± 0.39 | <0.001*** | 12.01 | 10 | Residual | 256 | | |
| | Sessão 8 | 8.23 ± 0.26 | 13.95 ± 0.23 | <0.001*** | 11.80 | 10 | | | | |
| | Sessão 9 | 11.58 ± 0.29 | 15.71 ± 0.30 | <0.001*** | 8.521 | 10 | | | | |
| | Sessão 10 | 12.66 ± 0.28 | 16.83 ± 0.30 | <0.001*** | 6.867 | 10 | | | | |
| | Sessão 11 | 12.87 ± 0.27 | 14.73 ± 0.30 | <0.05* | 3.063 | 10 | | | | |

and humans (Wang and Xu, 2007; Ranade et al., 2008). Nutritional insult in early life is harmful to hippocampus formation, and the hippocampus contains neurons that perform an essential role in learning and memory processes (Morgane et al., 2002; Matos et al., 2011) and motivational processes, particularly those related to food and feeding behavior control (Tracy et al., 2001).

The protocol used in this study allowed for the verification of animal motivation for the reward stimulus (Pecina et al., 2003; da Silva et al., 2013). Perinatal malnutrition did not show any effect on latency time for exiting the initial box or for the time to reach the intended box; nevertheless, it induced a longer time to start reward consumption during the adaptation sessions. This may indicate a learning deficit caused by malnutrition. Even so, when there was repeated activity by training, the deficit was minimized, as was noticed during the pre-exposure period (session 5) and the phase of learning incentive (session 8), where perinatal malnutrition contributed to a shorter latency time to consume the reward. This reduction in latency time to consume the reward in malnourished mice was also noticed by da Silva et al. (2013). Underweight newborns that showed a slight loss of cognitive capacity recovered from this deficit over time (Richards et al., 2001; Kar et al., 2008).

These results show that although malnutrition provokes changes in the nervous system, these changes can be reversed during life. On the other hand, this study presents data that opposes the results of related studies in the literature (Landon et al., 2007); those studies observed losses in the adaption capacities to reinforcement tasks for mice with a history of feeding restriction. These conflicting data might be explained by the type of malnutrition imposed in the experiments; for example, in studies by Landon et al. (2007), an energetic value restriction was performed, while in the current study, protein malnutrition was performed.

Another important observation of this experiment was that perinatal malnutrition increases motivational behavior for a food reward. When evaluating the time to complete the task, in other words, the time to roam the path and react to the reward, the malnourished animals had a

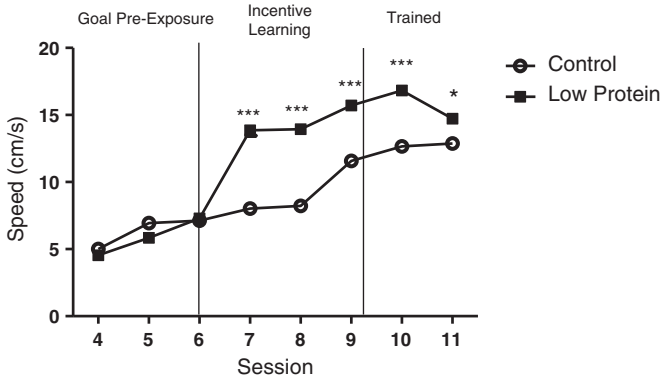


**Fig. 3.** Perinatal malnutrition effect on speed in the motivational test performed at 70 to 92 days of life in animals born from females fed with a normoproteic (casein 17%) or hypoproteic (casein 8%) diet during the gestation and lactation periods. The data are averages ± SEM. Two-way ANOVA to repeated measures by Bonferroni post-test. *** P < 0.0001, * P < 0.05.

**Table 5**
Effects of D1 and D2 dopamine agonists over speed during the sessions 10 and 11 of Runway Incentive Task Test.

| | Variables | Control D1 | Low protein D1 | p | t | N |
|---|---|---|---|---|---|---|
| Speed (cm/s) | Session 10 | 17.93 ± 0.22 | 19.01 ± 0.23 | <0.01** | 3.322 | 10 |
| | Session 11 | 14.73 ± 0.23 | 18.65 ± 0.25 | <0.001*** | 5.167 | 10 |
| | | Control D2 | Low protein D2 | p | t | N |
| | Session 10 | 5.00 ± 0.16 | 6.47 ± 0.11 | >0.05 | 2.092 | 10 |
| | Session 11 | 5.00 ± 0.17 | 5.41 ± 0.14 | >0.05 | 1.569 | 10 |

D1

| Source of Variation | Df | p value | F |
|---|---|---|---|
| Interaction | 7 | P < 0.0001 | 63.91 |
| Parameter Factor | 1 | P < 0.0001 | 47.18 |
| Animal group Factor | 7 | P < 0.0001 | 592.7 |
| Residual | 136 | | |

D2

| Source of Variation | Df | p value | F |
|---|---|---|---|
| Interaction | 7 | 0.0276 | 2.339 |
| Parameter Factor | 1 | 0.1235 | 2.401 |
| Animal group Factor | 7 | P < 0.0001 | 15.05 |
| Residual | 136 | | |

higher speed from the learning incentive phase (session 7) through the last performed session compared to the normonourished animals. The malnourished animals also showed a smaller number of pauses and reverses in direction, suggesting less distraction. These results are similar to those described by da Silva et al. (2013), who also performed motivational tests with malnourished animals.

When analyzing the motivation associated with dopamine agonist treatment, it was observed that the application of a D1 receptor agonist in the last sessions of the test resulted in a faster speed in the motivational test. Nevertheless, the effect of perinatal malnutrition potentiated the effect of the D1 agonist, exacerbating the speed and the motivation to acquire the reward. Some authors suggest that the dopamine D1 receptor contributes to appetitive motivation, performing an important role in the increase of incentive to acquire natural rewards, such as palatable food (Robinson et al., 2005; Wang and Xu, 2007; Volkow et al., 2008; Vucetic and Reyes, 2010). The function of the D1 receptor linked to the motivational aspect of feeding behavior seems to mediate neurons located, among other areas, in the nucleus accumbens, ventral pallidum, ventral tegmental area, prefrontal cortex, hippocampus and tonsils amígdala (Beaulieu and Gainetdinov, 2011). These regions form the food reward system (Patel and Srinivasan, 2010), which is predominantly located in the mesocortical and mesolimbic pathways and are particularly stimulated by the ingestion of food rich in fat and carbohydrates (Erlanson-Albertsson, 2005).

Studies on the effect of malnutrition on dopamine receptors are scarce. However, high levels of brain dopamine in malnourished animals (Cooper et al., 2006) as well as changes in the sensitivity of the dopamine agonist receptors due to food restriction have been reported (Baladi and France, 2010). Therefore, in malnourished animals, a reduction in the sensitivity of the dopamine D1 receptor may occur.

The results of the present study show that the relative expression of the mRNA levels of the DRD1a receptor increased in the nucleus accumbens and striatum in malnourished animals when compared to controls. There were no significant changes in the mRNA levels of the DRD2 receptor. Available evidence has shown that dopamine D1-like receptors are important in reward-related learning, including instrumental learning (Ranaldi and Beninger, 1995; Sutton and Beninger, 1999; Trevitt et al., 2001) and translation of motivation into action (Fibiger, 1993). Systemic and intra-accumbal infusions of dopamine D1-like receptor antagonists have been shown to attenuate food-reinforced lever pressing and to blunt the rewarding effects of palatable food (Beninger et al., 1987; McDougall et al., 1991; Cousins et al., 1994; Hodge et al., 1996; Aberman et al., 1998; Koch et al., 2000). D1 mutant mice have bene shown to take longer to learn to discriminate between two levers and had significantly lower operant responding to sucrose pellets and sucrose solution than wild-type and heterozygous mice under all schedules of reinforcement (El-Ghundi et al., 2003). This may indicate a deficit in reward-related learning. Indeed, the dopamine D1 receptor has been shown to play a role in incentive learning (Beninger, 1983) and may be important at the initial stage of instrumental learning, when reward stimuli are novel and unpredictable (Schultz, 1998).

Alsiö et al., 2010 suggested a reduction in the expression of D1 dopamine receptors in the nucleus accumbens in rats exposed to a chronic palatable diet, and they concluded that exposure to HFHS diets has lasting consequences for the NAcc dopamine system, perhaps modifying the motivation to search for food reward. Some studies have shown that sugary food ingestion is associated with an increase in dopamine release in the nucleus accumbens (Carr et al., 2001; Hajnal et al., 2004). The consumption of palatable food induces the activation of the reward system (Spiller et al., 2008). It has been observed that mice exposed to sugary food show a greater dopamine response indicated by increased dopamine in the nucleus accumbens in the medial prefrontal cortex (Cooper et al., 1990).

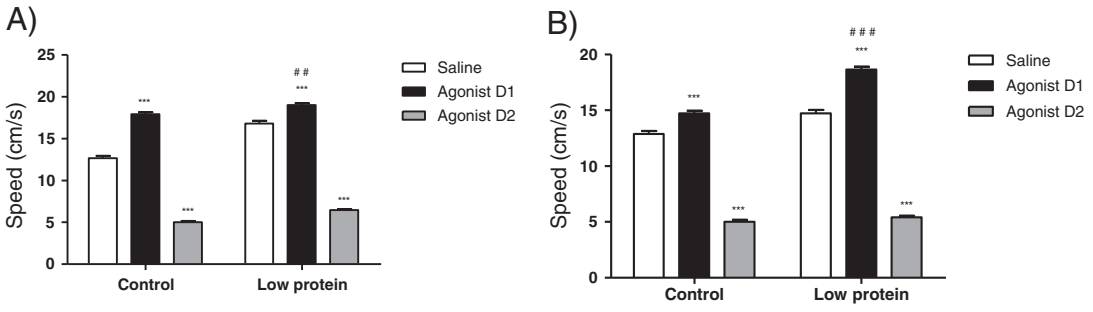


**Fig. 4.** A-B. Effects of D1 and D2 dopamine agonists over motivation in control and malnourished animals during Runway Task Incentive Test. The test was performed from 70 to 92 days of life. (A) Speed- Control and malnourished animals, during the 10th session after application of dopamine D1 (3 mg/kg, n = 10), D2 (0,3 mg/kg, n = 10) agonists and saline (1 ml/kg, n = 10). (B) Speed- Control and malnourished animals, during 11th session after application of dopamine D1 (3 mg/kg, n = 10), D2 (0,3 mg/kg, n = 10) agonists and saline (1 ml/kg, n = 10). The data are averages ± SEM.. Two-way ANOVA to repeated measures by Bonferroni post-test was used to compare intra-group. T Student test to intergroup comparison $^{###}P < 0{,}001$ and the values compared between animals that received the same acute dose of D1 agonist. No statistically significant difference in speed was found between control and malnourished animals after D2 agonist application.

Manuel-Apolinar et al. (2014) demonstrated that animals subjected to nutritional restriction during the prenatal period presented, after 90 days of life, with hyperphagia, and they attributed this result to an increase in the expression of D1 receptors in the arcuate nucleus of the hypothalamus, a region that is directly linked to energy metabolism. Food restriction is associated with a raise in the reward value of abusive drugs (Cabeza de Vaca and Carr, 1998) and palatable food (Scheggi et al., 2013). This is most likely through the improvement of the functional activity of dopamine receptors (Carr et al., 2001) induced by changing dopamine receptor numbers or affinity, or through transduction mechanisms that might be responsible for these changes in manipulated and non-manipulated animals (Silveira et al., 2010). Nevertheless, the exact mechanisms by which the nucleus accumbens develops these functions are not completely understood (Valdomero et al., 2007). More studies are needed to assess and provide solid foundations on the role of dopamine receptors in the motivational aspects of food reward in organisms nutritionally programmed during the perinatal period.

## 5. Conclusions

Perinatal protein malnutrition stimulates hedonic control of eating behavior by promoting increased intake of palatable foods, possibly due to increased expression of dopamine receptor DRD1a in the ventral striatum.

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