Epigenetic dysregulation of dopaminergicsystem by maternal cafeteria dietduring early postnatal development

Authors: Rossetti, MF[a,b], Schumacher, R[a,b], Gastiazoro, MP[b,c],Lazzarino, GP[a,b], Andreoli, MF[d],Stoker C[a,b], Varayoud J[b,c], Ramos, JG[a,b].

[a] *Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina*

[b] *Instituto de Salud y Ambiente del Litoral (ISAL), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral-CONICET, Santa Fe, Argentina.*

[c] *Cátedra de Fisiología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.*

[d]*Laboratorio de Neurodesarrollo experimental, Instituto de Desarrollo e InvestigacionesPediátricas (IDIP), Hospital de niños de La Plata y Comisión de Investigacion Científicas de la provincia de Buenos Aires (CIC-PBA), La Plata, Argentina.*

Email addresses:
mfrossetti@fbcb.unl.edu.ar
rociosch09@gmail.com
paulagastiazoro@gmail.com
gplazzarino@fbcb.unl.edu.ar
mfandreoli@fbcb.unl.edu.ar
cstoker@fbcb.unl.edu.ar
varayoud@fbcb.unl.edu.ar
gramos@fbcb.unl.edu.ar

*Corresponding author: Address all correspondence and requests for reprints to Jorge Guillermo Ramos, PhD. Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina. Casilla de Correo 242, (3000) Santa Fe, Argentina. TEL/FAX: 54 342 4510283. E-mail: gramos@fbcb.unl.edu.ar

Keywords: EPIGENETIC, DOPAMINE-RELATED GENES,MATERNAL CAFETERIA DIET, RAT OFFSPRING.

Abstract:

Dopamine is a neurotransmitter crucial for motor, motivational, and reward-related functions. Our aim was to determine the effect of a palatable maternal diet on the transcriptional regulation of dopaminergic-related genes during perinatal development of the offspring. For that, female offspring from dams fed with a control (CON) or a cafeteria (CAF) diet were sacrificed on embryonic day 21 (E21) and postnatal day 10 (PND10). Using micropunch techniques, ventral tegmental area (VTA) and *nucleus accumbens*(NAc) were isolated from brain´s offspring. Bioinformatic analysis of the promoter regions, mRNA quantification and methylation studies were done. The increase in tyroxine hidroxylase (TH), dopamine receptor (DRD) 1 and ghrelin receptor (GHSR) expression in VTA and NAc from E21 to PND10 was correlated with changes in DNA methylation of their promoter regions. Maternal diet did not affect the expression patterns in E21.At PND10, maternal CAF diet decreased the transcription of TH, GHSR, DRD2 and dopamine transporter (DAT) in VTA. Interestingly, the changes in TH, DRD2 and DAT expression were related to the methylation status of their promoters. In NAc, maternal CAF diet reduced DRD1, DRD2 and DAT expression in the offspring at PND10, although alternations in the methylation patterns were only detectedin DAT promoter. These results show the importance of maternal nutrition and provide novel insights into the mechanisms through which maternal junk-food feeding can affect reward system during development and early postnatal life. Particularly important is the expression decline of DRD2 given its physiological implication in obesity and addiction.

Highlights

1. Maternal cafeteria diet decreased the transcription of TH, DRD2 and DAT in the ventral tegmental area of neonatal offspring.
2. In *nucleus accumbens*, maternal cafeteria diet reduced DRD1, DRD2 and DAT expression at postnatal day 10.
3. The methylation status of dopaminergic gene promoters was affected by maternal diet.

Abbreviations

ACT: activator

AP: activator protein

C/EBP: CCAAT/enhancer-binding protein

CAF: cafeteria

CRE: cAMP response element

CREB: cAMP response element-binding protein

DA: dopamine

DAT: dopamine transporter

DRD: dopamine receptor

E: embryonic day

GHSR: ghrelin receptor

GRE: glucocorticoid response element

HFD: high fat diet

INH: inhibitor

NAc: *nucleus accumbens*

NF-1: nuclear factor 1

NF-AT: nuclear factor of activated T cells

Sp1: selective promoter factor 1

TH:tyrosine hydroxylase

VTA: ventral tegmental area

Introduction

Obesity represents one of the major public health problems in the world and it is mainly caused by overeating and physical inactivity. Palatable, or high-fat, high-sugar, foods activate the dopaminergic signaling pathways within the mesolimbic reward system (Berthoud, 2006; Fulton, 2010). Dopamine (DA) is a neurotransmitter crucial for motor, motivational, and reward-related functions of the central nervous system(Cragg and Rice, 2004)and is also associated with the gratifying effects of sex and drugs of abuse (Nestler and Carlezon, 2006). This neurotransmitter is produced in the dopaminergic neurons of the ventral tegmental area (VTA)by the action of the tyrosine hydroxylase (TH) (Baik, 2013a,

2013b). Interestingly, it was shown that there is a high degree of co-expression of TH and ghrelin receptor (GHSR) in VTA in adults (Zigman et al., 2016a, 2016b). It is well known that ghrelin impairs VTA by inducing DA release and stimulating food intake (Fulton, 2010); thus, GHSR-TH coexistence suggests a possible coordinated regulation of DA levels. VTA dopaminergic neurons project to the *nucleus accumbens* (NAc), where DA is released and binds to specific DA receptors (DRD1 and DRD2). This nucleus receives sensitive information from various regions, and then projects to the hypothalamic and midbrain areas that contribute to the motor action of food (Valdivia et al., 2014). NAc mediates reward effects in response to natural stimuli and is where termination of DA signaling occurs through reuptake by the active DA transporter (DAT)(Cragg and Rice, 2004). Prolonged exposure to palatable food in adult rodents is associated with behavioral and neurophysiological adaptations comparable to those seen in drug addicts. In particular, desensitization of the central reward pathway, which then drives continued overconsumption(Ong and Muhlhausler, 2011).

Several epidemiological and experimental studies have demonstrated that susceptibility to obesity can have its origins early in life and can be influenced by the nutritional experience during critical periods of fetal and early postnatal development. In rodents, an experimental model used to reproduce the characteristics of the Western obesogenic food is the cafeteria diet (CAF) (Sampey et al., 2011).Numerous authors reported that exposure to a maternal diet dominated by palatable food and/or high-fat diet before and during pregnancy and throughout lactation, disturb glucose and lipid homeostasis, predispose to adiposity, modify food preference, alter the development of the central reward circuitry and modify the expression of brain genes such as DAT, DRD1 and DRD2 in the offspring after birth and later in life (Akyol et al., 2009; Bayol et al., 2007; Bayol et al., 2008; Chen et al., 2008; Ong et al., 2012; Ong and Muhlhausler, 2011; Sarker et al., 2018; Vucetic et al., 2010). However, there are no prior reports about the effects of these maternal diets on the dopaminergic reward system during perinatal periods.

Metabolic and eating disorders are associated with alterations in the DNA methylation pattern of particularly genes, such as TH and DAT(Vucetic et al., 2012). DNA methylation represents one of the most important epigenetic mechanisms for blocking gene expression and implicates the addition of methyl groups to CpG dinucleotides. A CpG

island is a DNA sequence generally greater than 250 bp that is rich in CpG sitesand, thus, ithas a key role in transcriptional control(Deaton and Bird, 2011).In this context, DNA methylation provides a mechanism by which maternal diet can modify the predispositionof the offspring to obesity-associated disordersor other pathologies.

In the present study, we hypothesized that maternal CAF diet is associated with alterations in the epigenetic control of dopaminergic system of the reward pathway in early postnatal development. Thus, the objective of our work was to analyzed the transcriptional regulation of dopamine-related genes in the brain of female offspring (F1),from dams fed with standard chow and CAF diet,at embryonic day 21 (E21) and postnatal day 10 (PND10). The study was performed in two discrete brain reward areas, NAc and VTA, using microdissection techniques. This experimental model allowed us to study the individual and combined effects of age (E21 vs. PND10) and maternal diet (CON vs. CAF) on the mRNA expression of dopaminergic genes and the DNA methylation mechanisms that are involved in their control.

Experimental Procedures

Animals an experimental design

Wistar female rats were obtained from the Department of Human Physiology of the School of Biochemistry and Sciences (UNL). All animals´ procedures were approved by the Ethical Committee of the School of Biochemistry and Biological Sciences (UNL, Santa Fe, Argentina) and designedin accordancewith the Guide for the Care and Use of Laboratory Animals issued by the U.S. National Academy of Sciences (Commission on Life Sciences, National Research Council, Institute of Laboratory Animal Resources, 1996).

Rats were housed two per cage,at 22 ± 2 °C and with a 12-h light–dark cycle, and fed with either standard chow (CON) or a cafeteria diet (CAF) (N= 10/group) from weaning. The standard chow (Cooperación, ACA Nutricion Animal, Buenos Aires, Argentina) provided 12.55 KJ/g, 5% energy as fat, 23% protein and 72% carbohydrate. The CAF diet was composed of standard chow and food that reflects variety, palatability, and energy density (parmesan cheese, cheese flavored snacks, crackers, sweet biscuits, pudding, and chocolate). This diet provided an average of 20.29 KJ/g, 49% of energy as fat, 7% as

protein, and 44% as carbohydrate, in addition to that provided by the standard chow. Dietary composition and treatment procedure are described in Lazzarino et al. (2017).

On the 14th week of treatment,when CAF animals were significantly heavier of that than the CON, females were mated with male rats that were housed under standard laboratory conditions during all the experiment (22 ± 2 °C, 12-h light–dark cycle, fed with standard chow).After mating, each dam was single caged and continued with the respectively diet. Five dams per diet groupwere euthanized on embryonic day 21(**E21)**.The rest of the animals (N=5/group) were maintained and at day 1 after delivery each litter was adjusted to 8 pups per dam. At postnatal day 10 (**PND10)**, pups were euthanized.During all the experimental period, dam body weight and energy intake were recorded weekly and litter weight was measured daily. After euthanization, female fetus and pups brains (named as E21-CAF, E21-CON, PND10-CAF and PND10-CON) were removed, frozen on dry ice and stored at −80 °C until sectioning for RNA and DNA analysis.

Micropunches of VTA and NAc

Following the procedure of microdissection technique described by Palkovits (Palkovits and Brownstein, 1988), embryo and pup brains were cut in a cryostat at −12 °C (serial coronal sections of 150μm).To identify and punch VTA and NAc regions, theatlas of the developing rat nervous system(Paxinos et al. 1994) and atlas of the postnatal rat brain in stereotaxic coordinates(Khazipov et al. 2015) were used. Both areas were removed bilaterally using a 0.5 mm stainless steel micropunch needlesand the reproducibility was checked analyzing the topography of the holes under a stereo microscope (Stemi 305, Zeiss, Oberkochen, Germany). Samples were stored at −80 °C until RNA and DNA isolation.

Reverse transcription and real-time quantitative PCR analysis (qRT-PCR)

VTA and NAc areas(N=8/group) were homogenized in TRIzol (Invitrogen, Carlsbad, CA, USA), and total RNA was isolated. 1 μg of RNA were reverse-transcribed into cDNA with Moloney Murine Leukemia Virus reverse transcriptase (10 units; Promega, Madison, WI, USA) as previously described(Rossetti et al., 2015) and final product was diluted with nuclease-free water to a final volume of 60 μl.

Reverse-transcribed products were combined with HOT FIRE Pol Eva Green qPCR Mix Plus (Solis BioDyne; Biocientífica, Rosario, Argentina) and 10 pmol of each primer (Invitrogen) and further amplified in duplicate using Real-Time DNA Step One Cycler (Applied Biosystems Inc., Foster City, CA, USA).The primer pairs used are detailed in Table 1 and the protocol for real-time quantitative PCR is described byRossetti et al. (2015).

Table 1. Sequences of primer oligonucleotides for PCR amplification.

Target	Primer sense	Primer antisense	Temperature of annealing (°C)
L19 (*housekeeping*)	5´- AGCCTGTGACTGTCCATTCC -3´	5´- TGGCAGTACCCTTCCTCTTC -3´	60
TH	5´-TACCAAGATCAAACCTACCAGCC-3´	5´-GGTCAAACTTCACAGAGAATGGG-3´	58
DRD1	5´-TCCAAGGTGACCAACTTCTT-3´	5´-GTTACAAAAGGACCCAAAGG-3´	55
DRD2	5´-CCCAGCAGAAGGAGAAGAAA-3´	5´-CAGGATGTGCGTGATGAAGA-3´	55
DAT	5´-CATCACCACCTCCATTAACTCC-3´	5´-CATTGTGCTTCTGTGCCATG-3´	56
GHSR	5´-GCTCTGCAAACTCTTCCA-3´	5´-AAGCAGATGGCGAAGTAG-3´	56

Bioinformatics

TH, DRD1, DRD2, DAT and GHSR promoters were analyzed for: a) CpG islands using MethPrimer program (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi; *RRID*:SCR_010269); b)restriction sites for *SmaI (*New England BioLabs, Beverly, MA, USA*), BstUI*(New England BioLabs) or *Mae II* (Roche Applied Science, Indianapolis, IN, USA); and c) potential binding sites for transcription factors with the bioinformatic tool PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3; RRID: SCR_016926) (Messeguer et al., 2002). PCR primers were designed with the online software NCBI Primer-BLAST (National Center for Biotechnology; https://www.ncbi.nlm.nih.gov/tools/primer-blast/; RRID: SCR_003095; Table 2).

Table 2. Sequences of primer oligonucleotides for PCR amplification to evaluate methylation sensitive sites in promoters.

Target	Primer sense	Primer antisense	Temperature of annealing (°C)
TH IC	5´- CCATCAGATTTACCTAGAAGC-3´	5´-TGAGACTATGAAGGGACATTG-3´	51.5

TH-*MaeII*	5´-ACAGCAGGCGTGGAGAGGAT-3´	5´-TGGTGGTCCCGAGTTCTGTC-3´	60
TH-*MaeII* b	5´-CCTTAGGAAATCCAGCATGG-3´	5´-ATTGCATCCACTGTCACAGG-3´	57.7
TH-*MaeII* c	5´-CATGTGGCTGCTCCTATGTA-3´	5´-GAGAGAGATTGGCACACACA-3´	52.6
DRD1 IC	5´-GTGGTGAGAATCCCCTCAGG-3´	5´-AGTTCCACAGGCGGAGAACC-3´	55
DRD1-*MaeII*	5´-CAGGCAAAGAGGTTCACAAG-3´	5´-CCGCCATCTAAACAGTTACC-3´	54.6
DRD1-*BstUI*	5´-AGCAGGAAACCACAGGCACC-3´	5´-GCTTCTGCGGTCAACTCACG-3´	60
DRD2 IC	5´-AATTCTGTGGTGCCTTCTCCT-3´	5´-ATGGGGTCAATCCAGAGTAGA-3´	55
DRD2-*BstUI*	5´-AGTGCAGAGATAGTTCTGGG-3´	5´-AGAAGCCACAGACTGTCGTT-3´	63
DAT IC	5´-TTTGGGGTCTCAACTAGAAA-3´	5´-TAAGACCTTTTCAGAACCCA-3´	55
DAT-*BstUI* (a)/*MaeII*	5´-CTTCTGACAACCTCGCTGGA-3´	5´-GGGGCTTGACAGGAGTCTTT-3´	60
DAT-*BstUI* (b)/*SmaI*	5´-CGTACAACACCGAAGGAAGA-3´	5´-CGAGGTTGTCAGAAGCAGAT-3´	57.7
GHSR IC	5´-TCCAGCATACTCCTTATCCA-3´	5´-TGGCAATCTTAGAACACACC	54.6
GHSR-*BstUI*(a)/*SmaI*	5´-TACGCCACGGTCCTCACCAT-3´	5´-ACGCTGGACACCCACACCAT-3´	61
GHSR-*BstUI*(b)/*MaeII*	5´-TCTCCCTTTCCTCTCCAAGC-3´	5´-TTCGTCAGGCAGTGAGTCGT-3´	61

IC: Internal Control.

Methylation-sensitive analysis

Genomic DNA from VTA and NAc areas (N= 8/group) was isolated using the phenol/chloroform/isoamyl alcohol extraction and digested with 1 unit of *SmaI*/*Mae II* or 10 units of *BstUI* and 1X enzyme buffer for 1 h at 25°C, 60 °C or 50 °C, respectively. After purification with the Wizard SV gel and PCR Clean-Up System Kit (Promega, Madison, WI), an optimized qRT-PCR protocol was used to analyze the relative methylation levels of various regions of the TH, DRD1, DRD2,DAT and GHSR promoters (Table 2). The procedure for DNA amplification was previously described by our group in several studies (Lazzarino et al., 2017; Rossetti et al., 2018; Rossetti et al., 2016; Rossetti et al., 2015).

Statistical analysis

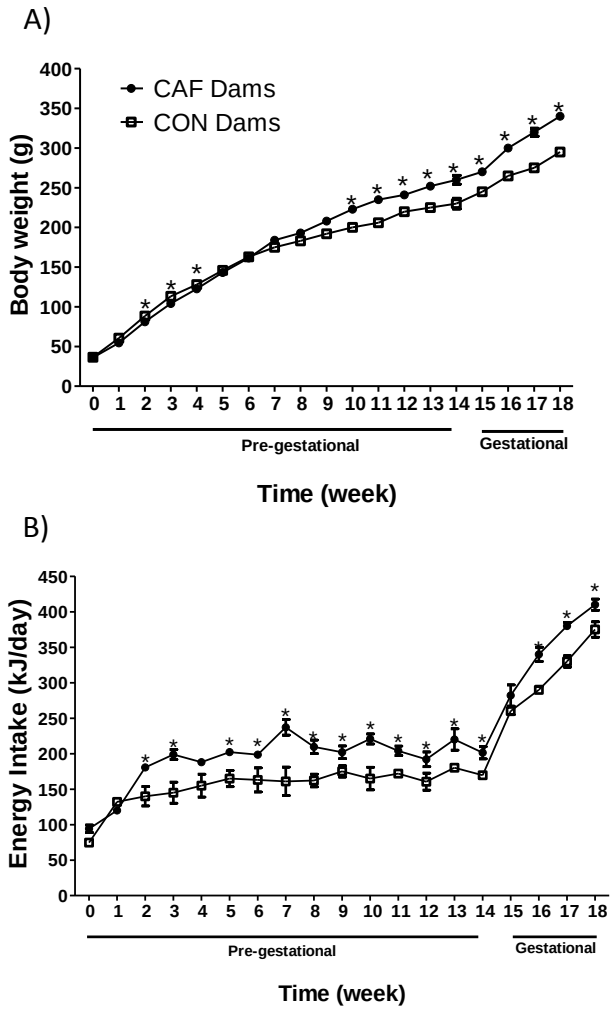
G Power software (http://www.gpower.hhu.de/; RRID:SCR_013726)was used to determine the sample size (Faul et al., 2007). To confirm the normal distribution of the data and variance homogeneity,Shapiro–Wilk test and Levene's test were performed. Weekly body weights,nutrient intake and energy intakewere analyzed using Student's T test; while a two-way ANOVA followed by Bonferroni post-test was implemented to study the ageand diet

effects on mRNA and DNA methylation.All the datais expressed as the means ± SEM and was statistically analyzed using the IBM SPSS Statistics 19 software (IBM Inc.; RRID:SCR_002865), considering significant differences at $p<0.05$.

Results

Effects of CAF diet on dams´body weight, nutrient intake andenergy intake.

The body weights of dams fed with CAF diet increased from week 10 of dietary intervention and on the 14th week of treatment (CAF: 266.5±2.92 g; CON: 240.5±3.1 g; $p<0.05$) females were mated with male rats. During pregnancy, CAF dams significantly increased gestational weight gain in comparison with those fed the control diet (Fig 1a). In addition, energy intakes over the pre-pregnancy (Week 2 to 14) and pregnancy (Week 16 to 18) period remained significantly higher in CAF rats when compared with CON animals (Fig. 1b). Moreover, CAF ratsconsumed a significantly greater percentage of their daily energy intake as fat, and significantly less as protein than CON rats (Fig 1c), during all the experiment.



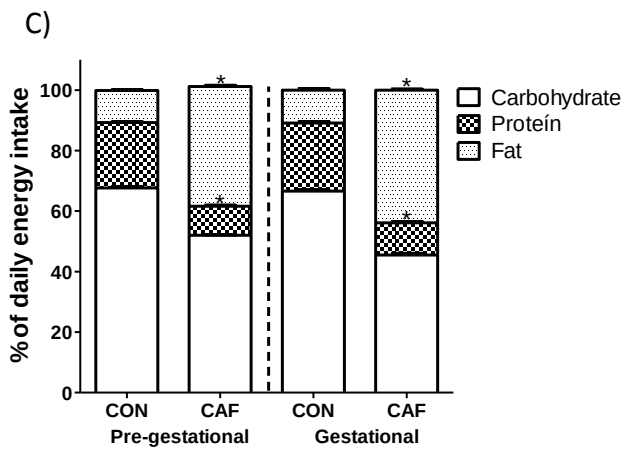


Fig 1. Body weight (A), energy intake (B) and nutrient intake (C) of dams fed with a control (CON) or a cafeteria (CAF) diet during pre-gestation (Week 1 to 14) and gestation (Week 15-18) periods(N=10/Group). * indicates significant differences at $p < 0.05$ vs. CONgroupby Student's T test.

Effects of CAF diet on the body weight of the offspring.

Pups of dams fed with a CAF diet had a significantly lower weight ($p<0.05$) than those from a CON diet from birth to PND10 (Fig. 2). It is interesting to note that during this period no apparent differences were detected in maternal behavior between CON and CAF groups. At E21no significant differences were detected (data not shown).

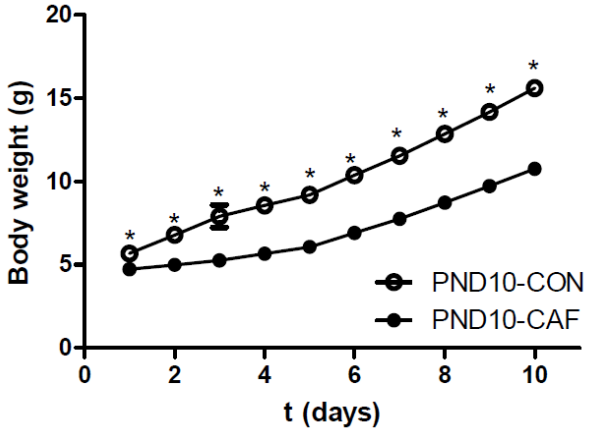


Fig 2. Body weightof pups from dams fed with a control (PND10-CON) or a cafeteria (PND10-CAF) diet from birth up to post-natal day 10 (PND10). Values are means, with standard errors represented by vertical bars(N=16/Group). * indicates significant differences at $p < 0.05$ vs. CON groupby Student's T test.

Maternal CAF diet modifies the mRNA expression of dopamine-related genes in the reward brain system of the offspring at early postnatal development.

To analyze the effect of maternal CAF diet on dopaminergic reward system in the offspring before and during gestation and lactation periods, we analyzed the expression of molecules that are involved in the synthesis, transport and reuptake of DA in two key regions of the reward system, VTA and NAc, at E21 and PND10.

In VTA, the two-way ANOVA revealed interactions between age and maternal diet for the expression of TH ($p < 0.01$, $F = 8{,}804$), DRD2 ($p < 0.05$, $F=4.875$), DAT ($p<0.01$, $F=13.93$) and GHSR ($p<0.001$, $F=8.458$) (Fig 3A). Maternal CAF diet decreased the expression of these genes in the offspring in PND10 (DPN10-CAF vs DPN10-CON, $p < 0.05$), without affecting expression in E21 (E21-CAF vs E21-CON, $p > 0.05$). In addition, the increase in age generated an increase on their transcription (E21 vs DPN10, $p<0.05$). Related to DRD1, mRNA levels were not found to be modified by maternal diet in VTA, but age increased the expression in the offspring (E21 vs PND10, $p < 0.01$, $F = 48.86$, Fig 3A).

In NAc, the expression of DRD1 ($p<0.05$,$F=7.369$), DRD2 ($p<0.01$,$F=10.01$) and DAT ($p<0.01$, $F=15.29$)was affected by the interactions between age and maternal diet (Fig 3B). Their expression decreased in the offspring of dams fed with CAF diet in PND10 (DPN10-CAF vs DPN10-CON, $p < 0.05$); however, no expression changes were observed in E21. Age generated an increase in the transcription of these genes (E21 vs DPN10, $p<0.05$). TH and GHSR mRNA levels were not found to be modified by maternal diet in NAc, but age improved the expression in the offspring (E21 vs PND10, $p < 0.05$, $F_{TH} = 10.31$, $F_{GHSR}=5.29$, Fig 3B).

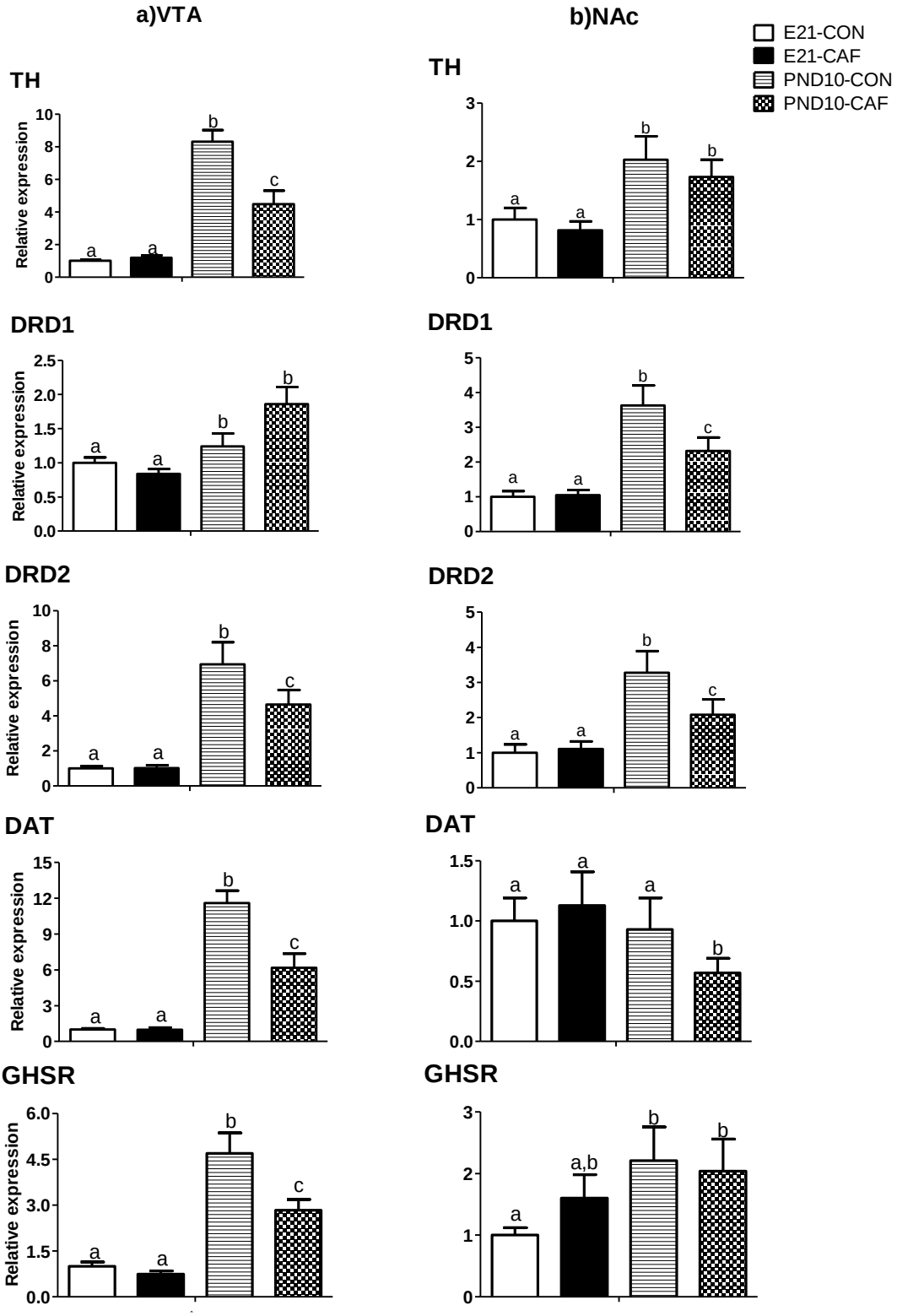

a)VTA
b)NAc
E21-CON
E21-CAF
PND10-CON
PND10-CAF
TH
DRD1
DRD2
DAT
GHSR
Relative expression

Fig 3. Analysis of relative mRNA levels of dopaminergic related-genes and ghrelin receptor in ventral tegmental area (VTA, A) and nucleus accumbens (NAc, B) of the offspring in embryonic day 21 and on post-natal day 10 from dams fed with control (E21-CON and PND10-CON, respectively) or cafeteria (E21-CAF and PND10-CAF, respectively) diet. Relative amounts of mRNA in E21-CAF, PND10-CON and PND10-CAF are showedas fold changes from those of E21-CON. The means ± SEM (N=8/group) are represented by columns and error bars. Significant differences at $p < 0.05$ by Bonferroni's test after two-way ANOVA are denoted by different letters. TH: tyrosine hydroxylase, DRD1: dopamine receptor 1, DRD2: dopamine receptor 2, DAT: dopamine transporter, GHSR: ghrelin receptor.

Transcriptional regulation of dopaminergic-related genes by DNA methylation in the reward brain system during development and in response to the maternal diet.

To determine if the changes observed in the transcript levels of those genes are related to DNA methylation modifications, we analyzed *in silico*the promoter regions of TH, DRD1, DRD2, DAT and GHSR and we determined the methylation state in the E21-CON, E21-CAF, PND10-CON and PND10-CAF groups (Fig. 4 and 5).

In VTA, DNA methylation levels of TH-*MaeII* c ($p <0.05$, $F =5.545$, Fig 4A), DAT-*SmaI* ($p <0.05$, $F =6.943$, Fig 4D) and GHSR-*SmaI* ($p <0.05$, $F =26.91$, Fig 5) sites were affected by the interactions between age and maternal diet. In TH-*MaeII* c and DAT-*SmaI*sites, maternal CAF diet increases methylation in the offspring in PND10 (DPN10-CAF vs DPN10-CON, $p <0.05$); in GHSR-*SmaI* this occurs in E21 (E21-CAF vs E21-CON, $p <0.05$); and in DRD2-*BstUI* this arises at both stages (E21 and PND10, $p<0.05$, $F=21.95$, , Fig 4C). On the other hand, age decrease methylation levels of TH-*MaeII* c site in the offspring of dams fed with CON diet in PND10 (E21-CON vs DPN10-CON, $p<0.05$). In addition, age decrease methylation levels of DRD1-*BstUI* and GHSR-*MaeII* sites in the offspring of dams fed with both CON and CAF diet in PND10 (E21 vs DPN10, $p<0.0001$, $F_{DRD1}= 42.35$ and $F_{GHSR}=68.51$, Fig 4B and 5). No differences were observed in methylation in the others studied sites (data not shown).

In NAc, DNA methylation levels of DAT-*SmaI*($p <0.05$, $F =15.17$), TH-*MaeII* b($p <0.05$, $F =23.66$) and DRD1-*MaeII* ($p <0.05$, $F =29.88$) siteswere affected by the interactions between age and maternal diet. In the first site, maternal CAF diet increased methylation in the offspring in PND10 (DPN10-CAF vs DPN10-CON, $p <0.05$). Age also increased methylation levels in this site in the offspring from dams fed with CAF diet (E21-CAF vs DPN10-CAF). Contrary, age decreased DNA methylation levels of TH-*MaeII* b ($p <0.005$,

F = 13.26) and DRD1-*MaeII* ($p < 0.0001$, F = 33.07) sites in the offspring from dams fed with CON diet (E21-CON vs DPN10-CON). No differences were observed in methylation of DRD2, DAT and GHSR sites (data not shown).

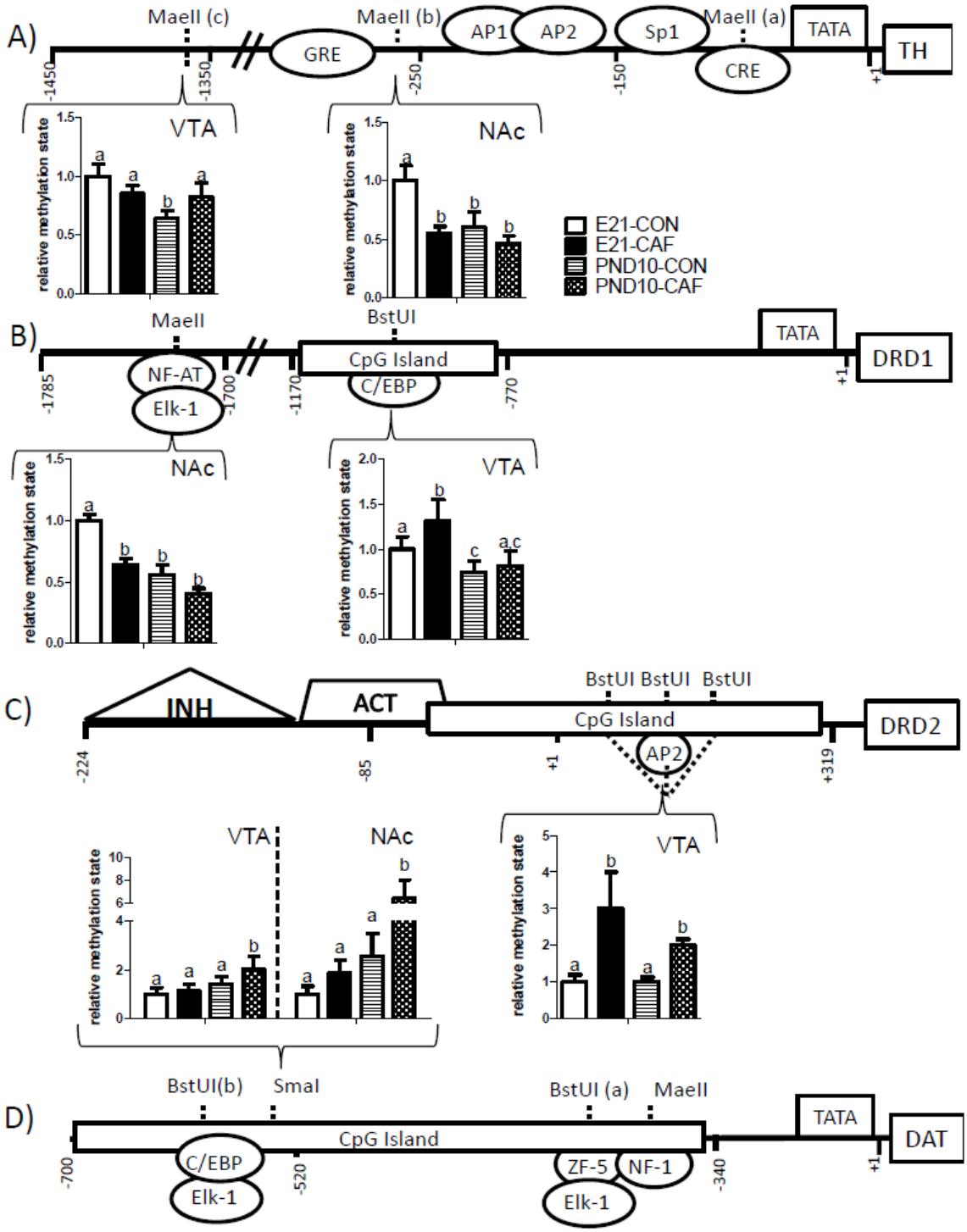


Fig 4. Methylation analysis of dopaminergic related genes. Tyrosine hydroxylase (TH, A), dopamine receptor 1 (DRD1, B),dopamine receptor 2 (DRD2, C) and dopamine transporter (DAT, D) promoters were studied in the ventral tegmental area (VTA) and/or nucleus accumbens (NAc).TATA box, predicted binding sites for transcription factor, CpG islands and CG target sites for digestion by the methylation-sensitive restriction enzymes are indicated.Offspring on embryonic day 21 and on post-natal day 10 from dams fed with control (E21-CON and PND10-CON, respectively) or cafeteria (E21-CAF and PND10, respectively) diet was evaluated. Methylation levels of promoters in E21-CAF, PND10-

CON and PND10-CAF are showedas fold changes from those of E21-CON. The means ± SEM (N=8/group) are represented by columns and error bars. Significant differences at $p < 0.05$ by Bonferroni's test after two-way ANOVA are denoted by different letters.ACT: activator, AP: activator protein, C/EBP: CCAAT/enhancer-binding protein, CRE: cAMP response element-binding protein, CREB: cAMP response element-binding protein, GRE: Glucocorticoid response element, INH: inhibitor, NF-1: nuclear factor 1, NF-AT: nuclear factor of activated T cells (NF-AT), Sp1: selective promoter factor 1.

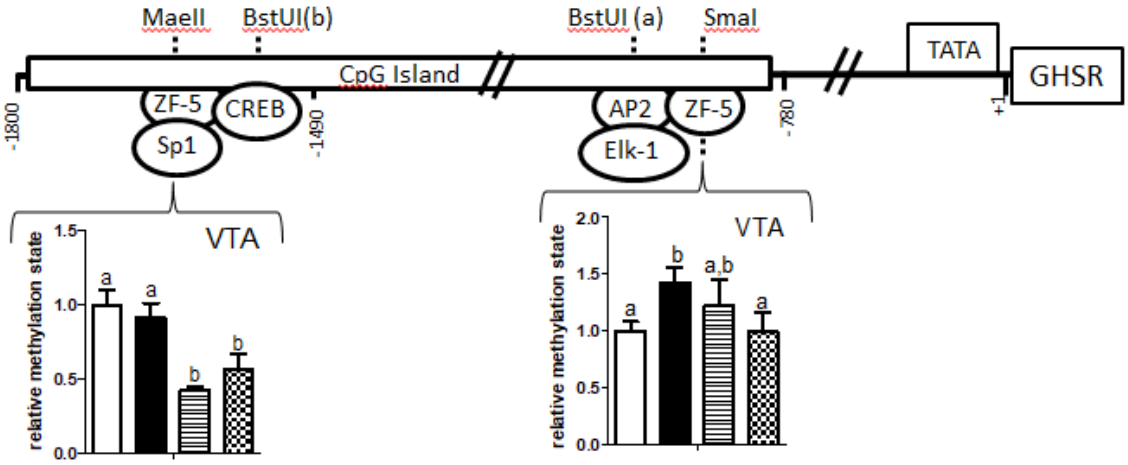


Fig 5. Methylation analysis of ghrelin receptor (GHSR) promoter in the ventral tegmental area (VTA). The offspring brain on embryonic day 21 (E21) and on post-natal day 10 (PND10) from dams fed with control (CON) or cafeteria (CAF) diet wasstudied. TATA box, activator protein (AP), cAMP response element-binding protein (CREB), selective promoter factor 1 (Sp1), CpG islands and CG target sites for*BstUI*, *MaeII* and *SmaI*are described.Methylation levels of promoters in E21-CAF, PND10-CON and PND10-CAF are indicated as fold changes from those of E21-CON. The means ± SEM (N=8/group) are represented by columns and error bars. Significant differences at $p < 0.05$ by Bonferroni's test after two-way ANOVA are denoted by different letters.

DISCUSSION

The principal aim of the present study was to determine whether exposure to maternal CAF feeding had an impact on dopaminergic reward pathways during perinatal period, selecting a representative point of the embryonic stage (E21) and the lactation period (PND10). Additionally, we analyzed the developmental profile of dopaminergic-related genes between both stages (E21 vs. PND10). We hypothesized that epigenetic modifications may be involved in the transcriptional control of these genes. To our knowledge, this is the first study reporting that: 1)- increase mRNA expression of TH, DRD1 and GHSR genes from E21 to PND10 in females is regulated by methylation mechanisms in VTA and/or NAc;

and 2) the offspring from dams fed with CAF diet showed alterations in the transcriptional regulation of TH, DRD2 and DAT genes in VTA and NAcat PND10.

Changes in dopamine-relatedgene expression in VTA and NAc during early development are regulated by methylation mechanisms.

The development of the dopaminergic system of the striatum in the rat begins during embryonic life and continues up to the 3rd postnatal week(Antonopoulos et al., 2002). The first mesolimbic dopamine neurons can be identified in the rat brain in E12, although dopamine axon innervations are not complete until the 3rd week of postnatal life(Ong et al., 2012). TH mRNA was found in brain tissues on early embryonic developmentE10-E12 and its specific activity increased from gestation to adulthood(Berger et al., 1985; Burgunder and Young, 1990; Marin et al., 2005). DRD1 and DRD2 receptors were detected in neural tissues on E14 and on E18 their localization was already similar to that observed in the adult brain. At birth, expression of mRNA for both dopamine receptor subtypes in the striatum approximated that seen in mature rats(Ong et al., 2012). In addition, DAT mRNA was first detected in neurons of the ventrocaudal mesencephalon on E14. By E18, intensely expressing neurons in the VTA and substantia nigra resembled the pattern found in adult midbrain(Fujita et al., 1993; Galineau et al., 2004).GHSR mRNA was found in the brain and spinal cord as early as E12 and continued to be expressed in these tissues during postnatal life(Steculorum and Bouret, 2011).Along the same line, we detected the mRNA presence of TH, DRD1, DRD2, DAT and GHSR in VTA and NAc areas at E21. Moreover, we showed that the transcriptional levels of these genes increased from E21 to PND10, suggesting that the development of the dopaminergic system in the rat continues from embryonic stage to the first weeks of life.

We found that early changes in gene expression of TH, DRD1 and GHSR in VTA and NAc in female rats are accompanied by alterations in promoter DNA methylation. In PND10-CON, we observed hypomethylation at the TH and DRD1 promoters (in VTA and NAc) and GHSR gene (in VTA), which may explain the increased mRNA expression of these genes, compared to E21-CON. We found that the DRD1 promoter was mostly methylated in two sites, one of it is located in a CpG Island (in VTA), while the other is a potential

binding site for the nuclear factor of activated T-cells (NF-AT) and for Elk-1 (in NAc). On the other hand, a potential binding site for ZF5 and forselective promoter factor 1 (Sp1) was predicted in the mostly methylated site within the GHSR promoter. Interestingly, these binding sites have been suggested to have a role in the regulation of dopaminergic related-genes transcription; particularly, DRD1 and DAT (Groth et al., 2008; Lee et al., 2004; J. Wang and Bannon, 2005). Moreover, changes in the methylation patterns of these sites could be related to the brain area involved (as occurs in DRD1 promoter). Although the increase expression of DRD1, GHSR and TH mRNA in the rat brain was previously related to changes in methylation patterns (Gozen et al., 2013; Inoue et al., 2011; Vucetic et al., 2012); we showed for the first time a relation between changes in the expression of these genes and age-associated methylations mechanisms.

Maternal CAF diet decreased body weight and affects the transcriptional regulation of dopaminergic related-genesin the offspring during perinatal period.

Maternal CAF diet significantly decreased the body weight of the offspring from birth to PND10.

Dams fed with a palatable diet administered from weaning to adulthood significantly increased energy intakeand body weight gain compared to animals fed with the standard chowduring pre-pregnancy and pregnancy periods, as was previously reported by several authors(Akyol et al., 2009; Goularte et al., 2012; Lalanza et al., 2014; Lazzarino et al., 2017). However, maternal CAF exposure significantly reduced the body weight of the pups, generating a decrease from birth (17%) to PND10 (30%). Bayol et al. (2007)found similar results in the offspring of dams fed with CAF diet at PND1 and PND21; while Ong and Muhlhausler (2011) observed a significant decrease in body weight at PND3. Contrary to our results, the offspring from dams fed with a high fat diet (HFD) showed no body weight differences at birth, while at PND 16 and PND 19 the body weight of the HFD-offspring was 30% higher compared to control animals (Chen et al., 2008; Purcell et al., 2011). These results suggest that the effects of CAF diet on the body weight of the offspring is opposite tothat observed in the offspring from dams fed with other obesogenic

diets, such as HFD. However, both low and high birth weights have been associated with the risk of diseases on adult ages, such as glucose intolerance, type II diabetes mellitus, syndrome X, dyslipidemia and obesity(Gluckman et al., 2008; Reyes and Manalich, 2005).

The decrease in the body weight of pups from dams fed with a CAF dietcould be due to the excess of maternal body weight that acts as a programming agent per se or due to other aspects of the CAF diet that drive the fetal responsesas was previously suggested(Akyol et al., 2009).In fact, the increased energy intakes of the CAF-fed dams were accompanied by a significant change in the composition of their intakes: they consumed a much greater proportion of their daily energy consumption from fat and less from protein, as was previously reported (Akyol et al., 2009; Bayol et al., 2007; Esteve et al., 1994; Llado et al., 1995; Shafat et al., 2009). In this sense, Bayol et al. (2007)reported that the reduction in protein intake during gestation and lactation in CAF-fed dams would be a key factor in explaining the reduced birth and weaning weights observed and that maternal protein intake rather than overall energy intake would play a major role in regulating the offspring's body mass at birth and at weaning. Importantly, the effect observed on the body weight of CAF-PND10 pups is similarly to those reported in the offspring of dams fed with alow protein diet model(Bieswal et al., 2006; Langley-Evans and Nwagwu, 1998). On the other hand, it would be possible that the limited protein intake of the CAF diet also affects the production and composition of breast milk. Although this factor was not analyzed here, Rolls et al. (1986)showed that the milk of CAF-fed rats contained more energy, with more fat and long-chain fatty acid content but less protein and medium-chain fatty acid content than that of control rats. Contrary, other authors reported that there were no differences in the protein content of either the early or mid-lactation milk between CON and CAF dams, despite the lower protein intake of the CAF dams during both pregnancy and lactation(Grigor et al., 1987; Pine et al., 1994; Vithayathil et al., 2016). It is important to note that we found no differences in breeding success between the control and CAF dams, as was previously suggested by Akyol et al. (2009).

Maternal CAF diet affects the transcriptional regulation of dopaminergic related-genes in VTA and NAc regions at PND10.

We found a decrease in the expression of TH and GHSR in VTA in the offspring of CAF fed-dams at PND10.The diminished expression of TH in VTA has been previously related to a reduced DA production(Naef et al., 2008). In addition, it has been reported that ghrelin impacts in VTA and induces DA release (Fulton, 2010), proposing that GHSR-TH coexistence coordinates regulation of DA levels. Ghrelin is thought to incentivize food intake by increasing acetyl choline levels in the VTA, increasing DA levels in the NAc, activation of dopaminergic projections from the VTA to the NAc, and activation of DRD1 and DRD2 in the NAc(Murray et al., 2014). Contrary, in mice, absence of the GHSR gene was associated with lower insulin-like growth factor 1 concentrations and lower body mass, independently of food intake(Chanoine et al., 2009).Although these studies have been performed in adult rats, some works suggestthat ghrelin and GHSR have a role in linear growth and development in early life (Chanoine et al., 2009; Steculorum and Bouret, 2011). Interestingly, the decrease in mRNA expression of TH-GHSR in VTA was correlated with a decreased in mRNA levels of DAT and DRD2 in VTA and reduce levels of DRD1, DRD2 and DAT transcripts in NAc.The decrease in the synthesis of DA accompanied by lower levels of it transporter and receptors and therefore, in the actions of the DA, suggests a reduced dopamine signaling in the reward system of these animals. Importantly, during this period, permanent alterations in the function of this pathway could be established and could have a long-lasting effect later in life and in adulthood.

The effect of maternal diet on the dopaminergic reward system was not studied in embryos and in early postnatal life; but it was in young and adult rats. Ong and Muhlhausler (2011) reported that the offspring from dams fed with CAF diet decreased DAT expression in NAc in PND42, whereas in adults the expression of DAT increased, compared to control rats. No changes were found in TH, DRD1 and DRD2 expression between groups. Gugusheff et al. (2013)also reported an increase preference for fat, an overall energy intake and bigger fat mass in adult offspring from CAF-fed dams.In the other hand, Vucetic et al. (2010)showed that the offspring from HFD-fed dams have a reduce DA signaling by decreasing the expression of DRD1 and DRD2 receptors and increasing the expression of DAT in adulthood.Contrary, adult offspring from HFD-fed dams displayed increased TH expression in the VTA and NAc and significant increases in DA content in the NAc,

suggesting an elevated DA tone in this target field(Naef et al., 2011).Our results together with the previously mentioned works suggest that maternal diets are critical in the development of the dopaminergic pathways and the effect observed during perinatal period could have a long-lasting impact in the offspring and predispose them to certain behaviors, such as those related to food preferences.In this sense, it will be interesting to evaluate in further studies the period (before pregnancy, during pregnancy or during breastfeeding) in which changes associated with the maternal diet are more relevant.

Some studies showed that the maternal nutritional factors could change the offsprings´ epigenetic marks in association with alterations in gene expression (Glendining et al., 2018; Sinclair et al., 2007; Vanhees et al., 2014). Moreover, it was reported that methylation mechanisms are implicated in the transcriptional control of dopamine-related genes. For example,Vucetic et al. (2010) observed global and gene-specific (DAT and Mu opioid receptor) promoter DNA hypomethylation in the brains of offspring from dams that consumed the HFD.In addition, epigenetic dysregulation of TH and DAT genes in a mouse model of HFD-induced obesity was reported (Vucetic et al., 2012). Sanchez-Hernandez et al. (2016) also reported that the male offspring from dams fed with diet with high levels of vitamin A had increased levels of DNA methylation in the DRD2 promoter region compared to control group. Here, we reported for the first time that maternal CAF diet affects the transcriptional regulation of genes TH, DRD2 and DAT involved in dopaminergic reward system by DNA methylation mechanisms in an early stage of development (PND10).The fact thatDRD2 and DAT promoters were mostly methylated at two sites located in a CpG Island supports the idea that these methylation-sensitive sites could be potential regulatory sites.To reinforce this hypothesis, it would be interesting to perform further experiments using DNMT inhibitors that block the epigenetic effects of maternal CAF diet in offspring.

The reduce dopamine signaling found in the offspring from CAF-fed dams is consistent with changes in the reward pathway observed in adult obese animals and in animals exposed to drugs, such as cocaine or alcohol. Particularly, several studies showed that genetic and functional alterations of the DRD2 have already been linked to the pathophysiology.Areduction in striatal density of DRD2 in overweight individuals (Stice et al., 2008; G. J. Wang et al., 2001) and rodents (Huang et al., 2006; Johnson and Kenny,

2010; Thanos et al., 2008)has been reported. Moreover, loss of DRD2 autorreceptors was linked to drug addiction, such as cocaine intake (Bello et al., 2011; Holroyd et al., 2015). In this sense, it has been shown that DRD2 plays an important role in the reward deficiency syndrome, which is related to compulsive and addictive behaviors(Blum et al., 2011). In VTA, a decrease in DRD2 has been linked to a greater motivation for food and the development of obesity(Bello et al., 2011; Koyama et al., 2014). Here, we showed for the first time a downregulation in DRD2 that is correlated with alteration in the methylation levels of it promoter in the offspring of dams fed with a CAF diet at PND10. Considering that these changes could have a long-lasting effect later in life, these results suggest that the epigenetic dysregulation of DRD2 could be an early marker of health diseases related with excessive consumption of food or drugs in adulthood.However, further studies are needed to clarify the cause-effect relationship between early DRD2 dysregulation in response to maternal diet and addictive behaviors in the adult offspring.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

Grants from the National Scientific and TechnicalResearch Council (CONICET) (PIP 11220150100338CO 2016-2019) and Universidad Nacional del Litoral (CAI+D 2016 No 0420150100085LI) supported this work.

Acknowledgments

We gratefully acknowledge the assistance of Juan Grant, Juan C. Villarreal and Stella Vaira from the Facultad de Bioquímica y Ciencias Biologicas (Universidad Nacional del Litoral, Santa Fe, Argentina).

References

Akyol, A., Langley-Evans, S. C., McMullen, S., 2009. Obesity induced by cafeteria feeding and pregnancy outcome in the rat. Br J Nutr. 102, 1601-1610. 10.1017/S0007114509990961

Antonopoulos, J., Dori, I., Dinopoulos, A., Chiotelli, M., Parnavelas, J. G., 2002. Postnatal development of the dopaminergic system of the striatum in the rat. Neuroscience. 110, 245-256.

Baik, J. H., 2013a. Dopamine signaling in food addiction: role of dopamine D2 receptors. BMB Rep. 46, 519-526.

Baik, J. H., 2013b. Dopamine signaling in reward-related behaviors. Front Neural Circuits. 7, 152. 10.3389/fncir.2013.00152

Bayol, S. A., Farrington, S. J., Stickland, N. C., 2007. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. Br J Nutr. 98, 843-851. 10.1017/S0007114507812037

Bayol, S. A., Simbi, B. H., Bertrand, J. A., Stickland, N. C., 2008. Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. J Physiol. 586, 3219-3230. 10.1113/jphysiol.2008.153817

Bello, E. P., Mateo, Y., Gelman, D. M., Noain, D., Shin, J. H., Low, M. J., Alvarez, V. A., Lovinger, D. M., et al., 2011. Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. Nat Neurosci. 14, 1033-1038. 10.1038/nn.2862

Berger, B., Verney, C., Gaspar, P., Febvret, A., 1985. Transient expression of tyrosine hydroxylase immunoreactivity in some neurons of the rat neocortex during postnatal development. Brain Res. 355, 141-144.

Berthoud, H. R., 2006. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. Obesity (Silver Spring). 14 Suppl 5, 197S-200S. 10.1038/oby.2006.308

Bieswal, F., Ahn, M. T., Reusens, B., Holvoet, P., Raes, M., Rees, W. D., Remacle, C., 2006. The importance of catch-up growth after early malnutrition for the programming of obesity in male rat. Obesity (Silver Spring). 14, 1330-1343. 10.1038/oby.2006.151

Blum, K., Chen, A. L., Oscar-Berman, M., Chen, T. J., Lubar, J., White, N., Bowirrat, A., Braverman, E., et al., 2011. Generational association studies of dopaminergic genes in reward deficiency syndrome (RDS) subjects: selecting appropriate phenotypes for reward dependence behaviors. Int J Environ Res Public Health. 8, 4425-4459. 10.3390/ijerph8124425

Burgunder, J. M., Young, W. S., 3rd, 1990. Ontogeny of tyrosine hydroxylase and cholecystokinin gene expression in the rat mesencephalon. Brain Res Dev Brain Res. 52, 85-93.

Chanoine, J. P., De Waele, K., Walia, P., 2009. Ghrelin and the growth hormone secretagogue receptor in growth and development. Int J Obes (Lond). 33 Suppl 1, S48-52. 10.1038/ijo.2009.17

Chen, H., Simar, D., Lambert, K., Mercier, J., Morris, M. J., 2008. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. Endocrinology. 149, 5348-5356. 10.1210/en.2008-0582

Cragg, S. J., Rice, M. E., 2004. DAncing past the DAT at a DA synapse. Trends Neurosci. 27, 270-277. 10.1016/j.tins.2004.03.011

Deaton, A. M., Bird, A., 2011. CpG islands and the regulation of transcription. Genes Dev. 25, 1010-1022. 10.1101/gad.2037511

Esteve, M., Rafecas, I., Fernandez-Lopez, J. A., Remesar, X., Alemany, M., 1994. Effect of a cafeteria diet on energy intake and balance in Wistar rats. Physiol Behav. 56, 65-71.

693 Faul, F., Erdfelder, E., Lang, A. G., Buchner, A., 2007. G*Power 3: a
694 flexible statistical power analysis program for the social,
695 behavioral, and biomedical sciences. Behav Res Methods. 39, 175-
696 191.
697 Fujita, M., Shimada, S., Nishimura, T., Uhl, G. R., Tohyama, M., 1993.
698 Ontogeny of dopamine transporter mRNA expression in the rat brain.
699 Brain Res Mol Brain Res. 19, 222-226.
700 Fulton, S., 2010. Appetite and reward. Front Neuroendocrinol. 31, 85-103.
701 10.1016/j.yfrne.2009.10.003
702 Galineau, L., Kodas, E., Guilloteau, D., Vilar, M. P., Chalon, S., 2004.
703 Ontogeny of the dopamine and serotonin transporters in the rat
704 brain: an autoradiographic study. Neurosci Lett. 363, 266-271.
705 10.1016/j.neulet.2004.04.007
706 Glendining, K. A., Fisher, L. C., Jasoni, C. L., 2018. Maternal high fat
707 diet alters offspring epigenetic regulators, amygdala glutamatergic
708 profile and anxiety. Psychoneuroendocrinology. 96, 132-141.
709 10.1016/j.psyneuen.2018.06.015
710 Gluckman, P. D., Hanson, M. A., Cooper, C., Thornburg, K. L., 2008.
711 Effect of in utero and early-life conditions on adult health and
712 disease. N Engl J Med. 359, 61-73. 10.1056/NEJMra0708473
713 Goularte, J. F., Ferreira, M. B., Sanvitto, G. L., 2012. Effects of food
714 pattern change and physical exercise on cafeteria diet-induced
715 obesity in female rats. Br J Nutr. 108, 1511-1518.
716 10.1017/S0007114511006933
717 Gozen, O., Balkan, B., Yildirim, E., Koylu, E. O., Pogun, S., 2013. The
718 epigenetic effect of nicotine on dopamine D1 receptor expression in
719 rat prefrontal cortex. Synapse. 67, 545-552. 10.1002/syn.21659
720 Grigor, M. R., Allan, J. E., Carrington, J. M., Carne, A., Geursen, A.,
721 Young, D., Thompson, M. P., Haynes, E. B., et al., 1987. Effect of
722 dietary protein and food restriction on milk production and
723 composition, maternal tissues and enzymes in lactating rats. J
724 Nutr. 117, 1247-1258. 10.1093/jn/117.7.1247
725 Groth, R. D., Weick, J. P., Bradley, K. C., Luoma, J. I., Aravamudan, B.,
726 Klug, J. R., Thomas, M. J., Mermelstein, P. G., 2008. D1 dopamine
727 receptor activation of NFAT-mediated striatal gene expression. Eur
728 J Neurosci. 27, 31-42. 10.1111/j.1460-9568.2007.05980.x
729 Gugusheff, J. R., Vithayathil, M., Ong, Z. Y., Muhlhausler, B. S., 2013.
730 The effects of prenatal exposure to a 'junk food' diet on offspring
731 food preferences and fat deposition can be mitigated by improved
732 nutrition during lactation. J Dev Orig Health Dis. 4, 348-357.
733 10.1017/S2040174413000330
734 Holroyd, K. B., Adrover, M. F., Fuino, R. L., Bock, R., Kaplan, A. R.,
735 Gremel, C. M., Rubinstein, M., Alvarez, V. A., 2015. Loss of
736 feedback inhibition via D2 autoreceptors enhances acquisition of
737 cocaine taking and reactivity to drug-paired cues.
738 Neuropsychopharmacology. 40, 1495-1509. 10.1038/npp.2014.336
739 Huang, X. F., Zavitsanou, K., Huang, X., Yu, Y., Wang, H., Chen, F.,
740 Lawrence, A. J., Deng, C., 2006. Dopamine transporter and D2
741 receptor binding densities in mice prone or resistant to chronic
742 high fat diet-induced obesity. Behav Brain Res. 175, 415-419.
743 10.1016/j.bbr.2006.08.034
744 Inoue, H., Sakamoto, Y., Kangawa, N., Kimura, C., Ogata, T., Fujieda, K.,
745 Qian, Z. R., Sano, T., et al., 2011. Analysis of expression and
746 structure of the rat GH-secretagogue/ghrelin receptor (Ghsr) gene:
747 roles of epigenetic modifications in transcriptional regulation.
748 Mol Cell Endocrinol. 345, 1-15. 10.1016/j.mce.2011.06.034

Johnson, P. M., Kenny, P. J., 2010. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci. 13, 635-641. 10.1038/nn.2519

Koyama, S., Mori, M., Kanamaru, S., Sazawa, T., Miyazaki, A., Terai, H., Hirose, S., 2014. Obesity attenuates D2 autoreceptor-mediated inhibition of putative ventral tegmental area dopaminergic neurons. Physiol Rep. 2, e12004. 10.14814/phy2.12004

Lalanza, J. F., Caimari, A., del Bas, J. M., Torregrosa, D., Cigarroa, I., Pallas, M., Capdevila, L., Arola, L., et al., 2014. Effects of a post-weaning cafeteria diet in young rats: metabolic syndrome, reduced activity and low anxiety-like behaviour. PLoS One. 9, e85049. 10.1371/journal.pone.0085049

Langley-Evans, S. C., Nwagwu, M., 1998. Impaired growth and increased glucocorticoid-sensitive enzyme activities in tissues of rat fetuses exposed to maternal low protein diets. Life Sci. 63, 605-615.

Lazzarino, G. P., Andreoli, M. F., Rossetti, M. F., Stoker, C., Tschopp, M. V., Luque, E. H., Ramos, J. G., 2017. Cafeteria diet differentially alters the expression of feeding-related genes through DNA methylation mechanisms in individual hypothalamic nuclei. Mol Cell Endocrinol. 450, 113-125. 10.1016/j.mce.2017.05.005

Lee, K. H., Kwak, Y. D., Kim, D. H., Chang, M. Y., Lee, Y. S., 2004. Human zinc finger protein 161, a novel transcriptional activator of the dopamine transporter. Biochem Biophys Res Commun. 313, 969-976.

Llado, I., Pico, C., Palou, A., Pons, A., 1995. Protein and amino acid intake in cafeteria fed obese rats. Physiol Behav. 58, 513-519.

Marin, F., Herrero, M. T., Vyas, S., Puelles, L., 2005. Ontogeny of tyrosine hydroxylase mRNA expression in mid- and forebrain: neuromeric pattern and novel positive regions. Dev Dyn. 234, 709-717. 10.1002/dvdy.20467

Messeguer, X., Escudero, R., Farre, D., Nunez, O., Martinez, J., Alba, M. M., 2002. PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics. 18, 333-334.

Murray, S., Tulloch, A., Gold, M. S., Avena, N. M., 2014. Hormonal and neural mechanisms of food reward, eating behaviour and obesity. Nat Rev Endocrinol. 10, 540-552. 10.1038/nrendo.2014.91

Naef, L., Moquin, L., Dal Bo, G., Giros, B., Gratton, A., Walker, C. D., 2011. Maternal high-fat intake alters presynaptic regulation of dopamine in the nucleus accumbens and increases motivation for fat rewards in the offspring. Neuroscience. 176, 225-236. 10.1016/j.neuroscience.2010.12.037

Naef, L., Srivastava, L., Gratton, A., Hendrickson, H., Owens, S. M., Walker, C. D., 2008. Maternal high fat diet during the perinatal period alters mesocorticolimbic dopamine in the adult rat offspring: reduction in the behavioral responses to repeated amphetamine administration. Psychopharmacology (Berl). 197, 83-94. 10.1007/s00213-007-1008-4

Nestler, E. J., Carlezon, W. A., Jr., 2006. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry. 59, 1151-1159. 10.1016/j.biopsych.2005.09.018

Ong, Z. Y., Gugusheff, J. R., Muhlhausler, B. S., 2012. Perinatal overnutrition and the programming of food preferences: pathways and mechanisms. J Dev Orig Health Dis. 3, 299-308. 10.1017/S204017441200030X

Ong, Z. Y., Muhlhausler, B. S., 2011. Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. FASEB J. 25, 2167-2179. 10.1096/fj.10-178392

Pine, A. P., Jessop, N. S., Oldham, J. D., 1994. Maternal protein reserves and their influence on lactational performance in rats. 3. The effects of dietary protein restriction and stage of lactation on milk composition. Br J Nutr. 72, 815-830.

Purcell, R. H., Sun, B., Pass, L. L., Power, M. L., Moran, T. H., Tamashiro, K. L., 2011. Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. Physiol Behav. 104, 474-479. 10.1016/j.physbeh.2011.05.012

Reyes, L., Manalich, R., 2005. Long-term consequences of low birth weight. Kidney Int Suppl. S107-111. 10.1111/j.1523-1755.2005.09718.x

Rolls, B. A., Gurr, M. I., van Duijvenvoorde, P. M., Rolls, B. J., Rowe, E. A., 1986. Lactation in lean and obese rats: effect of cafeteria feeding and of dietary obesity on milk composition. Physiol Behav. 38, 185-190.

Rossetti, M. F., Varayoud, J., Andreoli, M. F., Stoker, C., Luque, E. H., Ramos, J. G., 2018. Sex- and age-associated differences in episodic-like memory and transcriptional regulation of hippocampal steroidogenic enzymes in rats. Mol Cell Endocrinol. 470, 208-218. 10.1016/j.mce.2017.11.001

Rossetti, M. F., Varayoud, J., Lazzarino, G. P., Luque, E. H., Ramos, J. G., 2016. Pregnancy and lactation differentially modify the transcriptional regulation of steroidogenic enzymes through DNA methylation mechanisms in the hippocampus of aged rats. Mol Cell Endocrinol. 429, 73-83. 10.1016/j.mce.2016.03.037

Rossetti, M. F., Varayoud, J., Moreno-Piovano, G. S., Luque, E. H., Ramos, J. G., 2015. Environmental enrichment attenuates the age-related decline in the mRNA expression of steroidogenic enzymes and reduces the methylation state of the steroid 5alpha-reductase type 1 gene in the rat hippocampus. Mol Cell Endocrinol. 10.1016/j.mce.2015.05.024

Sampey, B. P., Vanhoose, A. M., Winfield, H. M., Freemerman, A. J., Muehlbauer, M. J., Fueger, P. T., Newgard, C. B., Makowski, L., 2011. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. Obesity (Silver Spring). 19, 1109-1117. 10.1038/oby.2011.18

Sanchez-Hernandez, D., Poon, A. N., Kubant, R., Kim, H., Huot, P. S., Cho, C. E., Pannia, E., Reza-Lopez, S. A., et al., 2016. High vitamin A intake during pregnancy modifies dopaminergic reward system and decreases preference for sucrose in Wistar rat offspring. J Nutr Biochem. 27, 104-111. 10.1016/j.jnutbio.2015.08.020

Sarker, G., Berrens, R., von Arx, J., Pelczar, P., Reik, W., Wolfrum, C., Peleg-Raibstein, D., 2018. Transgenerational transmission of hedonic behaviors and metabolic phenotypes induced by maternal overnutrition. Transl Psychiatry. 8, 195. 10.1038/s41398-018-0243-2

Shafat, A., Murray, B., Rumsey, D., 2009. Energy density in cafeteria diet induced hyperphagia in the rat. Appetite. 52, 34-38. 10.1016/j.appet.2008.07.004

Sinclair, K. D., Allegrucci, C., Singh, R., Gardner, D. S., Sebastian, S., Bispham, J., Thurston, A., Huntley, J. F., et al., 2007. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine

status. Proc Natl Acad Sci U S A. 104, 19351-19356. 10.1073/pnas.0707258104
Steculorum, S. M., Bouret, S. G., 2011. Developmental effects of ghrelin. Peptides. 32, 2362-2366. 10.1016/j.peptides.2011.06.021
Stice, E., Spoor, S., Bohon, C., Small, D. M., 2008. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science. 322, 449-452. 10.1126/science.1161550
Thanos, P. K., Michaelides, M., Piyis, Y. K., Wang, G. J., Volkow, N. D., 2008. Food restriction markedly increases dopamine D2 receptor (D2R) in a rat model of obesity as assessed with in-vivo muPET imaging ([11C] raclopride) and in-vitro ([3H] spiperone) autoradiography. Synapse. 62, 50-61. 10.1002/syn.20468
Valdivia, S., Patrone, A., Reynaldo, M., Perello, M., 2014. Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model. PLoS One. 9, e87478. 10.1371/journal.pone.0087478
Vanhees, K., Vonhogen, I. G., van Schooten, F. J., Godschalk, R. W., 2014. You are what you eat, and so are your children: the impact of micronutrients on the epigenetic programming of offspring. Cell Mol Life Sci. 71, 271-285. 10.1007/s00018-013-1427-9
Vithayathil, M. A., Gugusheff, J. R., Gibson, R. A., Ong, Z. Y., Muhlhausler, B. S., 2016. Effect of a maternal cafeteria diet on the fatty acid composition of milk and offspring red blood cells. Prostaglandins Leukot Essent Fatty Acids. 109, 58-65. 10.1016/j.plefa.2016.03.016
Vucetic, Z., Carlin, J. L., Totoki, K., Reyes, T. M., 2012. Epigenetic dysregulation of the dopamine system in diet-induced obesity. J Neurochem. 120, 891-898. 10.1111/j.1471-4159.2012.07649.x
Vucetic, Z., Kimmel, J., Totoki, K., Hollenbeck, E., Reyes, T. M., 2010. Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology. 151, 4756-4764. 10.1210/en.2010-0505
Wang, G. J., Volkow, N. D., Logan, J., Pappas, N. R., Wong, C. T., Zhu, W., Netusil, N., Fowler, J. S., 2001. Brain dopamine and obesity. Lancet. 357, 354-357.
Wang, J., Bannon, M. J., 2005. Sp1 and Sp3 activate transcription of the human dopamine transporter gene. J Neurochem. 93, 474-482. 10.1111/j.1471-4159.2005.03051.x
Zigman, J. M., Bouret, S. G., Andrews, Z. B., 2016a. Correction to 'Obesity Impairs the Action of the Neuroendocrine Ghrelin System': [Trends in Endocrinology and Metabolism, 27 (2016) 54-63]. Trends Endocrinol Metab. 27, 348. 10.1016/j.tem.2016.02.007
Zigman, J. M., Bouret, S. G., Andrews, Z. B., 2016b. Obesity Impairs the Action of the Neuroendocrine Ghrelin System. Trends Endocrinol Metab. 27, 54-63. 10.1016/j.tem.2015.09.010