http://dx.doi.org/10.4322/2357-9730.73266

Original Article

Alterations in the hypothalamic–pituitary–thyroid axis in animals submitted to early-life trauma

Tania Diniz Machado[1], Roberta Dalle Molle[1], Patrícia Pelufo Silveira[1,2]

ABSTRACT

Introduction: Changes in maternal care can affect offspring's thyroid hormone T3 levels. Pups from highly caring mothers have higher levels of thyroid hormone T3. In humans, physical abuse in childhood is related to lower levels of T3 in adolescence. This study aimed at verifying if early-life trauma in rodents is correlated with T3 levels in adulthood.

Methods: From the second day of life, litters of Wistar rats were subjected to reduced nesting material (Early–Life Stress-ELS) or standard care (Controls). In adult life, the animals were chronically exposed to standard diet or standard diet + palatable diet and plasma T3 levels were measured before and after the exposition to diet.

Results: Thyroid hormone T3 levels in adult life correlated negatively with the licking and grooming (LG) scores in the ELS group. This correlation disappeared when the animals had the opportunity to choose between two diets chronically.

Conclusion: The adverse environment affected maternal behavior and caused marks on the metabolism of the intervention group (T3), which were reverted by chronic palatable food consumption.

Keywords: *Trauma; T3; stress*

Clin Biomed Res. 2017;37(3):169-174

1 Post-graduate Program in Child and Adolescent Health, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS). Porto Alegre, RS, Brazil.

2 Department of Psychiatry, McGill University, Douglas Mental Health University Institute. Montreal, QC, Canada.

Autor correspondente:
Tania Diniz Machado
tanianutricionista@yahoo.com.br
Programa de Pós-graduação em Saúde da Criança e do Adolescente, Faculdade de Medicina (FAMED), Universidade Federal do Rio Grande do Sul (UFRGS)
Ramiro Barcelos, 2350.
90035-903, Porto Alegre, RS, Brazil.

Exposure to early-life trauma leads to behavioral changes in adulthood[1,2] as well as to changes in the functioning of the hypothalamic pituitary adrenal (HPA) axis mediated by alterations in maternal care[1].

By observing the natural variations of maternal care in rats, researchers have classified dams as high- and low-caring mothers. These differences appear only in the first week after delivery, since care is greater in the first few days after the birth of the pups, and declines gradually. The definition of care is performed through the frequency of licking and grooming (LG) and the arched position for nursing[3].

Changes in maternal care (or LG score) produce a peripheral variation of triiodothyronine (T3) levels in the pups. Pups from highly caring mothers show an increase in plasma T3 when the mother is in physical contact with them or in LG[4]; however, it is not known whether these changes are persistent throughout life. There is evidence that physical abuse in childhood is related to a decrease in T3 levels in adolescents[5]. Considering that changes in thyroid function are associated with changes in metabolism and risk for psychiatric diseases[6-8],it is important to understand whether neonatal stress is related to a persistent programming of hypothalamic-pituitary-thyroid axis functioning, and which conditions modify this programming. Therefore, this is the purpose of this work.

METHODS

Nulliparous Wistar rats, from *Universidade Federal de Pelotas* (UFPEL), were mated at the Animal Experimentation Unit of Hospital de Clínicas de Porto Alegre (UEA-HCPA), remained in individual box houses, especially designed for

  ISSN 2357-9730

this animal model, measuring 40 cm × 40 cm × 30 cm, with a metal mesh floor (1 cm × 1 cm) that allowed only the passage of feces and urine to a sink located at the bottom of the box. During gestation, females were kept in cages with a floor covered with wood chips and in a controlled environment: light/dark cycle of 10/14 hours (lights went on at 9a.m. and off at 7p.m.), temperature of 22 ± 2 °C, water and food *ad libitum.* Cage cleaning was carried out once a week. The day of the pups birth was considered Day 0. On day 2, females and their litters were randomized into two groups (see below). Maternal behavior was monitored from day 1 to day 9. A total of 17 litters were used to observe maternal care. In day 10, females and their offspring were removed to a standard cage, measuring 46 cm × 31 cm × 16 cm, with the floor covered in wood chips and were kept in the same controlled environment as reported above.

On day 21st of life, pups were weaned and separated by sex, in two or three per cage, and kept in a controlled environment: 12:12 h light-dark cycle (lights went on at 7h and off at 19h), temperature of 22 ± 2 °C, water and food *ad libitum*. Males were destined to another project and only the females were used in this study.

From weaning to the 120th day of life, females (n=30) received standard feed (Nuvilab®). Afterwards, the animals were subdivided into four groups, as described below (see Figure 1).

All procedures were approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (GPPG/HCPA, project number 11-0182).

Neonatal Stress Model: Intervention Group (Adverse Neonatal Environment)

The restriction of the material for nest confection was conducted for 8 days (from day 2 today 9 postnatal), which consists in removing the wood chips from the cage and offering a total of only 2000 cm^3 of paper towel for the confection of a rudimentary nest during this period[9]. Urine and animal feces were deposited in the collector placed at the bottom of the cage, and the metal mesh floor was generally kept clean by the dam. In contrast, dams from the control group were provided with enough supply of wood chips for the construction of the nest. During the intervention period (from postnatal day 1 to 9), maternal behavior of each mother was observed daily in 5 periods, of 72 minutes each, by observers who received standardized training in the research group. The observations occurred in three periods during the light phase (10a.m., 1p.m., 5p.m.) and two periods during the dark phase (7a.m. and 8p.m.). At each observation period, maternal behavior was registered every three minutes. The following maternal behaviors were registered: mother in touch or not with her offspring, mother nursing her offspring in arched back nursing position (positions 1, 2, 3 or 4; greater numbers indicate more arched back) or passive position and mother licking (licking and grooming - LG) any pup[3]. The scores of each behavior were calculated by dividing the frequency of the behavior by the total number of observations. The pure contact score was calculated from the total contact score, subtracting from this value the contact at the

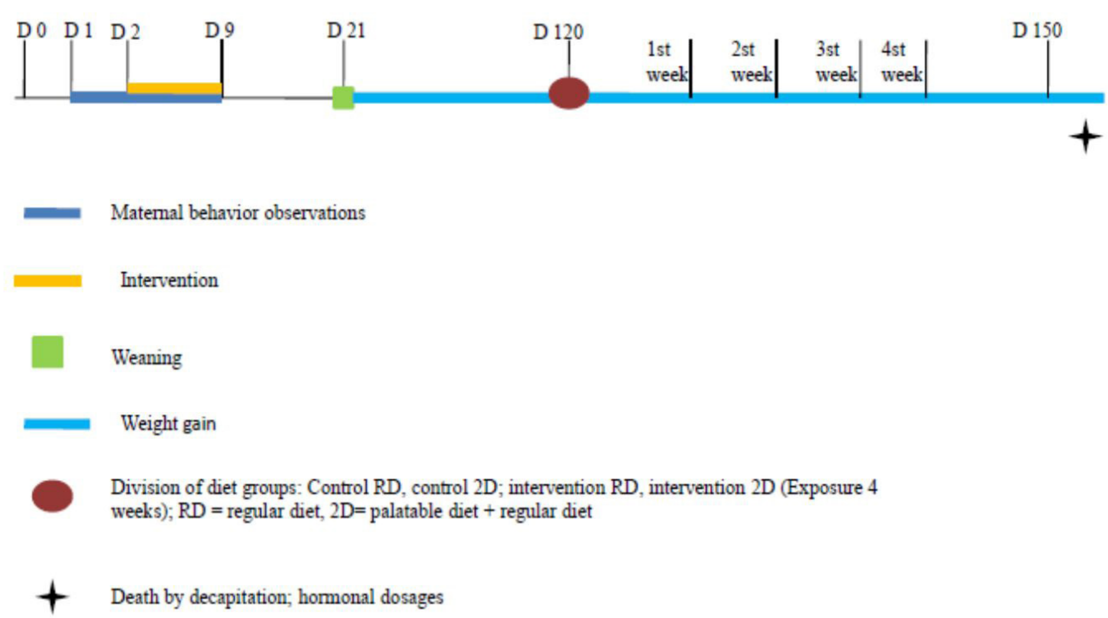

Figure 1: Time line of the experimental procedure.

moment of nursing and the contact at the moment of licking, being considered a low-quality contact (or careless contact).

Subdivision of Neonatal Groups in Different Diets

Neonatal groups (control and intervention) were randomized by body weight. This was done by dividing rats into three body weight groups (higher, medium and lower body weight) and assigning them to the different dietary groups, to avoid that most higher-body-weight females were allocated to the same dietary group. The neonatal groups (control and intervention) were subdivided into:

a) Control with regular diet (n=5);
b) Control with regular diet + palatable diet (n=5);
c) Intervention with regular diet (n=10);
d) Intervention with regular diet + palatable diet (n=10).

All groups received new diets for 4 weeks; therefore, even the standard rat chow (regular diet) was changed to a new brand. This was done so that all animals would face the effects of food neophobia and therefore this bias could be diluted among the different experimental groups. The composition of the regular feed was: 22% protein, 4% fat, 45.5% carbohydrate, 3.06Kcal/g (in 1Kg of feed); the palatable diet consisted of 14% protein, 34% fat, 30% carbohydrate (being 20% sucrose); 4.82Kcal/g (in 1Kg of feed). Both diets were produced by the Prag Soluções Biociências® Laboratory (São Paulo, Brazil), aiming to minimize possible biases. Data from food intake can be found in another publication[1].

Biochemical Analysis

The plasma triiodothyronine (T3) and thyroxine (T4) levels were measured at the Laboratory of Biochemistry of Hospital de Clínicas de Porto Alegre, by means of direct chemiluminescent immunoassay.

Statistical Analysis

All data collected were entered and analyzed with the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., IBM Company, Chicago, USA). One-way ANOVA was used to analyze maternal care data, using as factor the neonatal group adjusted for litter size. Pearson's correlation was used to correlate LG with plasma T3 levels. In all analyzes significance was established at 5%.

RESULTS

Mean LG was similar between the control group and the intervention group; however, the standard deviation for LG was 2.7 times lower in the intervention group. The percentage of time the rat mothers in the intervention group nursed their infants was higher [F(1, 14)=6.00, p=0.028], but a longer nursing time was found in positions 1 and 2 [F(1, 14)=16.67, p=0.001], considered less effective postures, in the same group (Table 1).

At weaning, which occurred at 21 days of age, females from the two neonatal groups did not present a statistically significant difference in body weight. However, in adult life, the intervention group had lower body weight. This difference disappears after the offer of new diets (see Tables 2 and 3).

Table 1: Comparison between the neonatal groups in relation to the means and variables related to maternal care.

Variables (observed frequency)	Control (n=8)	Intervention (n=9)	*p* Value
LG	8.06 ± 2.02	8.73 ± 0.73	0.630
Passive nursing	5.05 ± 2 .28	3.80 ± 2.37	0.299
Nursing positions 1 and 2	32.86 ± 6.97	40.76 ± 5.85	0.001*
Nursing positions 3 and 4	11.73 ± 4.70	11.53 ± 4.96	0.635

One-way ANOVA; using the size of the litter as a co-variable; values expressed as mean ± SD. *p<0.05 LG= licking and grooming.

Table 2: Comparison between the weight variables of the control and intervention neonatal groups.

Variables	Control (n=10)	Intervention (n=20)	*p* Value
Weight at weaning (21d)	44.25 ± 5.36	40.59 ± 6.85	0.137
Weight in adult life (60d)	212.34 ± 15.47	194.27 ± 15.47	0.004*
Weight before initiating diets (120d)	259.42 ± 17.89	238.05 ± 18.04	0.005*

Student's *t* test; values expressed as mean ± SD. *p<0.05 d=days of life.

Table 3: Comparison of weight between groups at the end of the experiment.

Variable	Standard Feed		Two Diets	
	Control (n=5)	Intervention (n=10)	Control (n=5)	Intervention (n=10)
Weight at the end of the experiment (150d)	261.13 ± 25.38	233.79 ± 17.93	257.07 ± 24.39	244.98 ± 20.50
p Value	0.074		0.373	

Student's *t* test; values expressed as mean ± SD. d=days of life.

There was no statistically significant difference between the groups in relation to T3 and T4 values (see Table 4).

When analyzing the correlation between the LG score (which reflects the quality of maternal care) and the plasma T3 levels in adult life between the groups, we observed that there is no correlation between these variables in the control group, but there is a statistically significant correlation in the intervention group. Therefore, in the rats belonging to the intervention group, there was a negative correlation between the LG score in the neonatal period and the peripheral T3 levels in adult life. However, after chronic exposure to the palatable diet, the intervention group equaled the control group and the correlation was no longer observed (see Tables 5 and 6 below).

DISCUSSION

In this study it was observed that the mothers of the intervention group spent more time in lower-quality nursing posture. There was a correlation between LG and T3 measurements only in the neonatal intervention group, a phenomenon that disappeared after chronic exposure to the palatable diet in adult life.

Regions such as the medial pre-optic area when injured inhibit maternal behavior as well as nesting and retrieval behavior in rats. There was no consensus about the maintenance of nursing in this type of damage, although there is a shorter duration[10]. The implantation of estradiol or prolactin in the medial pre-optic area is able to promote attraction toward the pups, stimulating maternal behavior[10]. Our data suggest that the intervention affects the sensitivity/functionality to hormones in this region of the rat mothers' brains, since they presented a lower-quality nursing pattern.

Another hypothesis for the fact that the mothers of the intervention group stayed longer in a less arched posture (posture 1 and 2) is that they were tired from the effort expended in spending more time in the nest with the pups. It was demonstrated in this same model of neonatal trauma that the mothers present higher levels of corticosterone (stress hormone) when compared to the controls[9].

The trauma experienced in the neonatal period interfered with the metabolism of the females of the intervention group, since the LG correlated negatively with the T3 in adult life. The correlation observed suggests a relation between maternal LG and the functioning of the HPT axis in adult life. The same did not occur with the neonatal control group.

Table 4: Comparison between the levels of T3 among groups.

Variables	Standard Feed		Two Diets		p Value
	Control (n=14)	Intervention (n=15)	Control (n=5)	Intervention (n=10)	Group Diet Interaction
T3	52.21 ± 8.25	55.08 ± 11.92	55.46 ± 8.26	64.49 ± 11.05	0.08 0.07 0.37
T4	2.58 ± 0.76	2.72 ± 0.90	2.88 ± 0.49	2.26 ± 0.77	0.36 0.75 0.16

Two-way ANOVA using as factor the neo group and diet; values expressed as mean± SD.

Table 5: Correlation between LG and T3 in neonatal groups with Nuvilab® standard feed at 120 days of life.

	Standard Feed	
	Control (n=9)	Intervention (n=5)
Correlation between LG and T3	0.638	-0.958
P	0.064	0.010*

Pearson's Correlation. It was not observed correlation between LG, TSH and T4 in any of the groups (data not disclosed). *Statistically significant results (p=0.010). LG= licking and grooming.

Table 6: Correlation between LG and T3 in the groups that received different diets at 150 days of life.

	Regular feed		Two Diets	
	Control (n=5)	Intervention (n=10)	Control (n=5)	Intervention (n=10)
Correlation between LG and T3	**-0.553**	**-0.711**	**0.546**	**0.150**
P	0.334	0.021*	0.341	0.679

Pearson's correlation. There was no correlation between LG, TSH and T4 in any of the groups (data not shown). *Statistically significant results (p=0.021). LG= licking and grooming.

LG relates to the gene expression of the glucocorticoid GR receptor in the hippocampus by modulating epigenetic mechanisms facilitating the transcription of NGFI-A with the transcription promoter site of the gene for this receptor in the DNA. Serotonin as well as the thyroid hormones mediate these effects. Variations in maternal care (measured by the frequency of LG) produce variations in T3 levels, increase of serotonin in the raphe, initiating reactions that culminate in GR transcription. The pups of high caring mothers convert more T4 into T3, regardless of whether the mother is near or far from the pups at the time of collection. Therefore, maternal care influences the ability to transform T4 into T3 in brown tissue[4] during the neonatal period. This result goes in the opposite direction to the results demonstrated in our research. The fact that the evaluation of T3 levels has been performed in another phase of life, adult life, when the animals are no longer in contact with the mother, can help explain such a difference. This study demonstrates that some changes in the HPT axis are still noticeable in this group in adulthood, several weeks after the discontinuation of stress.

Interestingly, after the chronic consumption of a palatable diet by the intervention group, the correlation between LG and T3 disappears. Possibly, the correlation between LG and T3 is mediated by the HPA axis. The HPA axis is one of the main pathways activated in a stress situation. When there is chronic stress and there is a palatable food supply, the latter is used as a "medication," possibly activating mechanisms that decrease the activity of the HPA axis leading to lower hormone production (cortisol in humans/corticosterone in rodents)[11,12]. Consequently, the brain would learn to relieve stress using this mechanism. Although the mechanisms are not yet known, the consumption of a palatable diet would lead to neuroendocrine and behavioral stress inhibition. As the palatable diet is able to inhibit the HPA axis[11], this would help to explain why the correlation is no longer seen in the intervention group that received this diet in a chronic way.

In conclusion, we observed that the adverse environment is associated with changes in maternal behavior. These data are in line with an extensive literature demonstrating that the quality of the neonatal environment leaves persistent marks on the animals' metabolism, as we saw in the intervention group (T3). The possible reversal of these associations by the use of a chronic palatable diet raises questions about the relation of neonatal stress and conditions such as obesity and metabolic syndrome. Future studies should be performed to understand the mechanisms involved.

Acknowledgements

This study was funded by the Brazilian National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES) and the Hospital de Clínicas de Porto Alegre Research and Event Incentive Fund (FIPE/HCPA).

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Machado TD, Dalle Molle R, Laureano DP, Portella AK, Werlang IC, Benetti Cda S, et al. Early life stress is associated with anxiety, increased stress responsivity and preference for "comfort foods" in adult female rats. *Stress*. 2013;16:549-56.

2. Dalle Molle R, Portella AK, Goldani MZ, Kapczinski FP, Leistner-Segal S, Salum GA, et al. Associations between parenting behavior and anxiety in a rodent model and a clinical sample: relationship to peripheral BDNF levels. *Transl Psychiatry*. 2012;2:e195.

3. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav*. 2003;79:359-71.

4. Hellstrom IC, Dhir SK, Diorio JC, Meaney MJ. Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. *Philos Trans R Soc Lond B Biol Sci*. 2012;367:2495-510.

5. Machado TD, Salum GA, Bosa VL, Goldani MZ, Meaney MJ, Agranonik M, et al. Early life trauma is associated with decreased peripheral levels of thyroid-hormone T3 in adolescents. *Int J Dev Neurosci*. 2015;47:304-8.

6. Pompili M, Gibiino S, Innamorati M, Serafini G, Del Casale A, De Risio L, et al. Prolactin and thyroid hormone levels are associated with suicide attempts in psychiatric patients. *Psychiatry Res*. 2012;200:389-94.

7. Hage MP, Azar ST. The link between thyroid function and depression. *J Thyroid Res*. 2012;2012:590648.

8. Premachandra BN, Kabir MA, Williams IK. Low T3 syndrome in psychiatric depression. *J Endocrinol Invest*. 2006;29:568-72.

9. Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience*. 2008;154:1132-42.

10. Numan M, Insel TR. *The neurobiology of parental behavior*. New York: Springer; 2003.

11. Dallman MF, Pecoraro N, Akana SF, la Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: a new view of “comfort food”. *Proc Natl Acad Sci USA*. 2003;100:11696-701.

12. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav*. 2007;91:449-58.

Received: May 10, 2017
Accepted: Aug 11, 2017