

Influence of a 60 Hz, 3 μ T, electromagnetic field on the somatic maturation of wistar rat offspring fed a regional basic diet during pregnancy

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Abstract

The aim of the present study was to observe how the exposition of pregnant rats to an electromagnetic field (EMF), with frequency of 60 Hz, and a magnetic field of 3 μ T for 2 hours per day and/or using the so-called Regional Basic Diet (RBD), influenced the somatic maturation in their offspring. Four groups were formed: Group A (casein), B (casein and EMF), C (RBD) and D (RBD and EMF). The diet manipulation occurred during pregnancy. The somatic maturation indexes - assessed daily between 12:00 AM and 2:00 PM - were: Eye Opening (EO), Auricle Opening (AO), Auditory Canal Opening (ACO), Low Incisor Eruption (LIE), and Upper Incisor Eruption (UIE). The association between EMF and deficient diet caused a delay in all Somatic Maturation Indexes (SMI) and the RBD caused delay only in the AO. Furthermore, the EMF caused delay in AO, ACO, LIE. In relation to the body weight, the EMF associated with the deficient diet caused change in the twenty-first day of life. The RBD, during pregnancy, caused lower body weight in the offspring in the first and third day of life. The body weight of the offspring whose mothers were fed casein and exposed to the EMF during pregnancy was lower in the third and sixth day of life. In conclusion, the EMF associated with under-nutrition caused delay in all SMI. In relation to the body weight, the EMF associated with under-nutrition caused a decrease in the body weight at the sixth day of life.

Keywords: casein, electromagnetic field, neurodevelopment, somatic maturation, regional basic diet.

Influência do campo eletromagnético de 60 Hz, 3 μ T, na maturação somática de filhotes de ratos wistar alimentados pela dieta básica regional durante a prenhez

Resumo

O objetivo deste estudo foi observar a influência do campo eletromagnético (CEM), com frequência de 60Hz, campo magnético de 3 μ T, durante 2 horas por dia, associado ou não à dieta básica regional (DBR) no desenvolvimento somático da prole. Quatro grupos foram formados: Grupo A (caseína), B (caseína e CEM), C (DBR) e D (DBR e CEM). A manipulação dietética ocorreu durante a prenhez. Os índices de maturação somática – Abertura dos Olhos (AO), Abertura do Pavilhão Auditivo (APA), Abertura do Conduto Auditivo (ACA), Erupção do Incisivo Inferior (EII), e Erupção do Incisivo Superior (EIS) - foram avaliados diariamente entre 12 e 14 horas. A associação entre o CEM e a dieta deficiente causou retardo em todos os índices de maturação somática (IMS) e a DBR causou retardo somente na APA. O CEM causou retardo na APA, ACA, EII. Em relação ao peso corporal, o CEM associado à dieta deficiente causou mudanças no 21º dia de vida. A DBR, durante a prenhez, causou diminuição do peso corporal dos filhotes no 1º e no 3º dia de vida. O peso corporal dos filhotes, cujas mães foram alimentadas pela caseína e expostas ao CEM, durante a prenhez, apresentaram uma diminuição no 3º e 6º dia de vida. Conclusão: o CEM, associado com a desnutrição, causou retardo em todos os IMS. Em relação ao peso corporal, o CEM, associado à desnutrição, causou uma diminuição no peso corporal no 6º dia de vida.

Palavras-chave: caseína, campo eletromagnético, desenvolvimento nervoso, maturação somática, dieta básica regional.

1. Introduction

Epidemiological studies have implicated maternal protein-calorie deficiency as an important public health problem in developing countries (Pissaia et al., 1980; Olubodun, 1992). In North-Eastern Brazil the diet that is consumed by the population living in the area of sugarcane cultivation in coastal Pernambuco is known as the Regional Basic Diet or RBD (Teodósio et al., 1990).

The RBD was prepared by Teodósio et al. (1981) according to data from food consumption surveys in the Pernambuco coastal forest strip. The RBD is made from the most common foods and in the same proportion as that consumed by the population, as detected by surveys. Pioneer studies have indicated that this experimental diet, RBD, produces in rats a type of under-nutrition similar to that prevalent among children from this region of Brazil, which is associated with nutritional dwarfism and some clinical signs of marasmus (Silva, 1987). When this diet is compared with the standard one, it is seen to be deficient in proteins (content and quality), calories, fat, vitamins and minerals (Pessoa et al., 2000). From the obtained results, the RBD has been consolidated as a malnourishment experimental model (Guedes et al., 1987; Teodósio et al., 1990; Rocha de Melo and Guedes, 1997; Pessoa et al., 2000).

The development of the nervous system (NS) involves epigenetic processes that activate the genes in a sequential manner at different times (Jessel, 1995). These processes have many environmental and non cellular factors that can change, modulate and direct the next stage of development (Nishi, 1994). Hence, only the genetic information in a living being may be insufficient to specify the whole neuronal interconnections (Jessel, 1995). Morgane et al. (1993) showed that the NS development in mammals happens according to 'time planning' in which the different phases follow a predetermined chronology. This fast growth period of the NS is named the 'critical or vulnerable' period, being considered as the unique opportunity for its development. Therefore, its beginning and duration are different for different species. In humans this 'critical period' begins in the womb (the last three months of pregnancy) and lasts until the beginning of the postnatal phase (two to four years old). In rats this happens after birth (breast-feeding period). The series of events presented by the NS during the prenatal and postnatal development determines the neurochemical composition and the definite morphofunctional structure, present in adults (Morgane et al., 1993).

Much experimental and clinical evidence has shown that aggression during those critical periods can change the ontogenesis sequence of events with diverse and persistent effects on the NS (Morgane et al., 1993; Dobbing, 1970; Lynch et al., 1975; Manhães de Castro et al., 2001). Experimental studies have shown that pre- or postnatal nutritional manipulation may programme adult size, metabolism, blood lipids, diabetes, obesity, behaviour, and learning (Lucas, 1998). In trying to understand

these effects and living beings' capability to adapt to different forms of aggression, many researchers have been investigating the effects of different kinds of nutritional injury to the nervous system in the early phases of its development. These studies have shown morphologic, neurochemical, endocrine and functional alterations (Morgane et al. 1993; Del-Prado et al., 1997).

Over recent decades a remarkable diffusion of electrical equipment and an increased level in electromagnetic fields (EMF) in the environment characterize our society. Recent epidemiological studies of occupational and residential exposure to EMF are concerned with the biological effects of the 50-60 Hz fields (extremely low frequencies or ELF), particularly in relation to determining an increase in cancer incidence in individuals exposed to these types of radiation (Galloni and Marino, 2000). In accordance with Marino (2005), in order to understand the relationship between environmental factors and disease in terms of an internal state variable called stress (for instance, death of a loved one, loss of job, an unhappy marital situation, poor diet, etc.), it is helpful to understand the influence of environmental EMFs.

Experiments investigating the possible effects of EMF associated with under-nutrition during pregnancy on development and somatic maturation of the offspring are scarce. The aim of the present study was to observe how the exposition of pregnant rats to a 60 Hz, 3 μ T, EMF for two hours per day and/or using a RBD influenced the somatic maturation indexes in their offspring.

2. Materials and Methods

2.1. Animals

In this experiment 66 male, newly born rats from the Wistar strain, whose mothers were submitted to four different conditions, were used. The female rats were 90 days old when they mated. Fertilization was detected by the presence of sperm in the vaginal washing of the mated females. Fertilized females were then immediately transferred to cages, two per cage, which were 60 cm long, 50 cm wide and 22 cm high, and put on supports made from polystyrene 35 cm wide, 50 cm long and 35 cm high (Lucena et al., 2002). The pregnant rats were divided in four Groups: Group A, composed of rats that consumed casein without being exposed to EMF; Group B, composed of rats that consumed casein and were exposed to EMF; Group C, composed of rats that consumed the RBD and were not exposed to EMF; and Group D, composed of rats that consumed RBD and were exposed to EMF. They were kept in conditions of constant temperature (23 ± 2 °C) and a light/dark cycle (12/12 hours) with a background magnetic field of less than 0.3 μ T.

When the rats gave birth, the newly born individuals were put together with the other ones which had been born on the same day from mothers in the same conditions, and after that they were selected at random to

form new groups. In this way, the genetic factor did not interfere with the experimental results. Every group from each female was reduced to six individuals on the second day after birth. Only male rats were used. Animal use was approved by the Federal University of Pernambuco Committee on Animal Research.

2.2. Diets

The ingredients of the multid deficient diet used in this experiment were beans (*Phaseolus vulgaris*), manioc flour (*Manihot esculenta*), dried and salted meat and sweet potato (*Ipomoea batatas*) (Teodósio et al., 1981). The diet was prepared in our laboratory as follows: all ingredients (except manioc flour) were cooked, dehydrated for 24-60 hours (according the type of ingredient) at 60 °C and pulverized. Each component was mixed with manioc flour by humidifying. Meat fat was then added, and the mixture was shaped into balls which were dehydrated for 24 hours at 60 °C. The centesimal composition of the RBD, which was determined by the Department of Nutrition, the Federal University of Pernambuco, is given in Table 1. The caloric adequacy of the RBD was calculated to be about 316 Kcal per 100 g. The control diet provided 18% of protein (commercial casein) and was balanced according to recommendations for pregnant rats (AIN – 93), as shown in Table 2. The diet during the mating period was maintenance pellet food (Purina® do Brasil Ltd., São Paulo, SP, Brazil).

2.3. Feeding protocol

During the mating period all female and male rats were fed a standard balanced diet provided by Purina. In this investigation the diets (RDB and casein) were only used when the rats presented a positive test for pregnancy. The animals had food and water ad libitum. Two Groups were fed RBD (Groups C and D) and two Groups were fed casein (Groups A and B).

2.4. Exposure to EMF

The groups of pregnant rats fed casein and RBD, Groups B and D respectively, were exposed to a 60 Hz senoidal, of 3 µT EMF, measured by a gauss meter, on the scale of 0-100 mG (0-10 µT) for two hours per day for twenty-one consecutive days, one hour in the morning from 8:00 to 9:00 AM hours and one hour in the afternoon from 2:00 to 3:00 PM hours.

They were exposed when pregnancy was detected and removed from exposure when they gave birth. As the radiation source, transformers of 220/110 V of 500 W working with opened secondary and controlled by an electronic timer were used and placed under the polystyrene supports. Control groups of animals, Groups A and C, fed with casein and RBD respectively, were obtained by simply not placing the transformers under the polystyrene supports. The transformers were previously examined and tested to confirm their working

Table 1. Centesimal composition of the Regional Basic Diet (RBD).

Ingredients	g (%)	Proteins (%)	Carbohydrates (%)	Fats (%)	Fibers (%)	Kcal (%)
Beans	18.34	3.99	10.66	0.24	1.09	60.76
Manioc flour	64.81	0.84	48.59	0.12	5.64	198.80
Poor fat dried and salted meat fat	3.74	2.74	-	0.21	-	12.85
Dried and salted meat fat	0.35	-	-	0.35	-	3.15
Sweet potato	12.76	0.30	9.99	0.03	0.48	41.43
Total	100.00	7.87	69.24	0.95	7.21	316.99

Table 2. Centesimal composition of the Control Diet (Casein).

Ingredients	g (%)	Proteins	Carbohydrates	Fats	Calories
Comercial casein*	19.60	18.00	-	-	72.00
Vegetable fat	7.00	-	-	7.00	63.00
Biscomil	63.35	0.18	53.37	-	214.20
Salt Mixture	3.50	-	-	-	-
Vitamin mixture	1.00	-	-	-	-
Fibers	5.00	-	-	-	-
L-Cystine	0.30	-	-	-	-
Choline bitartrate	0.25	-	-	-	-
Total	100.00	18.18	53.37	7.00	349.20

*92% of protein.

parameters at the Biomedical Engineering Department of the Biophysics and Radiobiology Department of the Federal University of Pernambuco. The EMF was measured inside the cage and it remained uniform irrespective of its position. It should be noted that, except for the weekly cage cleaning and the weekly measuring of their weight, the rats were not moved or handled during this experiment. Exposed animals should be compared with control groups that have been derived from the same source and simultaneously handled and assayed in the same way, except for their exposure to the fields.

2.5. Somatic maturation indexes

The following somatic maturation indexes were investigated on a daily basis, between 12:00 AM and 2:00 PM hours from the second day after birth until when somatic maturation had occurred, namely Eye Opening (EO), Auricle Opening (AO), Auditory Canal Opening (ACO), Low Incisor Eruption (LIE) and Upper Incisor Eruption (UIE) (Smart and Dobbing, 1971). They were considered positive when the two eyes, the two auricles and the two auditory canals were opened and the two lower and upper incisors had appeared.

2.6. Somatic growth

2.6.1. Pup body weight

The animals were weighed on the 1st, 3rd, 6th, 9th, 12th, 15th and 21st day of life. The scale used was a digital model, Quimis, with 0.1 g sensitivity.

2.7. Statistical analysis

The results of the somatic maturation development were evaluated by the Kruskal-Wallis analysis of variance, followed by Dunn's test for multiple comparisons among groups, with the significant level considered to be $p < 0.05$.

The Variance Analysis for Repeated Measures followed by the Tukey test was employed for comparisons of the body weight. When the normality test had been passed, the One Way Analysis of Variance (ANOVA)

was used followed by the Tukey Test for multiple comparisons among groups. When the normality test had been failed, the Kruskal-Wallis analysis of variance was used followed by Dunn's test for multiple comparisons among groups, considering the significant level considered to be $p < 0.05$.

3. Results

3.1. Somatic maturation indexes

Comparing the suckling group from mothers exposed to EMF and malnourished, during pregnancy (Group D) to the suckling group from mothers fed casein (Group A), a delay was observed in the day (in median) that the following somatic maturation indexes appeared: AO, ACO, EO, UIE and LIE, ($p < 0.05$) (Table 3).

When the different diets were considered, the offspring whose mothers were fed with a deficient diet and exposed to the EMF during pregnancy (Group D) showed a delay in the AO and EO indexes when compared to Group B whose mothers were fed casein and exposed to the EMF during pregnancy ($p < 0.05$) (Table 3).

When comparison was made between the offspring whose mothers were fed RBD, one group of which was exposed and the other group not exposed to (Groups D and C), during pregnancy, only a delay in the AO somatic index was noticed ($p < 0.05$) (Table 3). When comparing the groups whose mothers were fed casein diet, one group of which was exposed to EMF and the other group not during pregnancy (Groups B and A), Group B presented a delay in the following somatic maturation indexes: AO, ACO and LIE ($p < 0.05$) (Table 3).

3.2. Body weight

After performing the statistical analysis of the body weight of the suckling over the first twenty-one days of live, there was no statistical difference observed between the weights measured from the first to the third day. However, from the fourth day a statistical weight gain was observed in all the groups.

Table 3. Somatic maturation times (age in days) of offspring from rats subjected to four different treatments during pregnancy.

Groups*/SMI**	A (n = 12)	B (n = 24)			C (n = 18)	D (n = 12)
		Md (Q1 - Q3)				
EO	12.5 (10-13) ^a	13 (11-14) ^b	13 (13-14)	14 (14-14) ^{ab}		
AO	1.5 (1-2) ^{cde}	3 (3-3) ^{cf}	3 (3-4) ^{dg}	4 (4-4) ^{efg}		
ACO	10.5 (10-11) ^{hi}	13 (12-13) ^h	12 (12-13)	13 (13-13.5) ⁱ		
LIE	10 (9-10) ^{jk}	12 (12-13) ^j	10.5 (10-14)	12 (12-12) ^k		
UIE	8 (8-8.5) ^l	9 (8-13)	9 (7-11)	11 (10-11) ^l		

*A = casein; B = casein + electromagnetic field; C = regional basic diet; D = regional diet + electromagnetic field; **EO = Eye Opening, AO = Auricle Opening, ACO = Auditory Canal Opening, LIE = Low Incisor Eruption, UIE = Upper Incisor Eruption. Equal superscripts indicate significant differences between the groups. Md = median, Q1 = quartile 1, Q3 = quartile 3. $p < 0.05$.

Table 4 shows the suckling average weight \pm standard error for the Groups A, B, C and D during the 1st, 3rd, 6th, 9th, 12th, 15th and 21st days after birth. At the twenty first day of life it was observed that the diet associated with the EMF, taken together, changed the body weight of offspring, where as Group C (formed from offspring of rats fed RBD but not subjected to EMF) showed lower body weight than Group B (formed from offspring of rats fed casein and subjected to EMF during pregnancy).

When the diet was considered, the offspring whose mothers were fed RBD during pregnancy (Groups C and D) had lower weight on the first and third day of life than the offspring whose mothers were fed casein (Groups A and B).

The EMF caused a decrease in the mean weight of offspring from rats fed casein and exposed to the EMF, during pregnancy (Group B), at the third and sixth day of life when compared with offspring of rats fed casein during pregnancy (Group A).

4. Discussion

The choice of intensity of 3 μ T was due to the necessity of using a higher intensity of EMF than in residences and most work places. In those places the average 50/60 Hz magnetic fields, as determined by the National Academy of Science (National Academy of Science, 1996), are between 0.1 and 0.3 μ T. In this study, a value ten times higher than the maximum expected value was used. Depending on the distance between the conductors and the ground, the magnetic average flux densities can reach 22 μ T resulting from the current load in the line (Simon, 1992). This value may also depend on the geographic location and the nature of the magnetic material near the subject area (Repacholi and Greenebaum, 1999).

The RBD was chosen as the experimental diet in this study because it is not only deficient in protein, but also in lipids, vitamins, sodium and other minerals (Teodósio et al., 1990; Pessoa et al., 2000; Monteiro et al., 2001). The pregnant rats were fed casein or RBD when they presented a positive test for pregnancy. According to Frazer and Huggett (1970), the fetus competes with the mother for the nutrients, but only for those that are ingested and stored throughout the pregnancy. They do

not compete for those already absorbed by the mother's tissues at the moment of conception.

In this study, the somatic maturation indexes were investigated from the second day after birth until when somatic maturation had occurred, because this period corresponds to the breast-feeding period and is considered by Dobbing (1970) as the critical period for development. The observation of the normal sequence of the stages of development in the ontogenesis of the reflexes and acquisition of the mature standard of the locomotive activities can be valuable indexes of the maturation and development of the nervous system (Smart and Dobbing, 1971; Walton et al., 1992; Morgane et al., 1993; Deiró et al., 2005). In rats, these patterns are established in the first three weeks after birth (Gramsbergen, 1998).

The offspring from rats exposed to EMF and RBD, taken together, presented a delay in all somatic maturation indexes. In accordance with Anselmo (2005), pregnant rats fed RBD and exposed to a 60Hz, 3 μ T, EMF for two hours per day showed a low dosage of serum T₃, which is in agreement with the results found in this study. According to Porterfield and Hendrich (1993), Reyns et al. (2005), thyroid hormones are very important for the embryonic development in all vertebrates. In humans, this is more evident in the central nervous system where the thyroid hormone deficit during the fetal and the neo-natal periods results in the syndrome of cretinism, which is characterized by hearing impairment, ataxia, mental disability, and abnormal growth. According to Vara et al. (2002), the hypothyroidism which manifests during the development of rats, results in learning difficulties, delay in locomotion and cerebral skills and shortened interneuron connectivity.

In this study offspring from rats fed casein showed delay in the auricle opening, auditory canal opening and lower incisor eruption when exposed to EMF. These results do not match with the results of Zusman et al. (1990), in which they observed a delay in the eye opening of offspring whose mothers were exposed to EMF of 20, 50, and 100 Hz, during pregnancy. On the other hand, the results obtained in this study agree with the results of Sokolova (1998) and Sienkiewicz et al. (1994). Sokolova (1998) reported that exposure to 2 or 20 mT fields did not exert any effect on eye opening in mice, and identical results were presented by Sienkiewicz et al.

Table 4. Offspring weight (in grams) born from rats subjected to four different treatments during pregnancy, on the 1st, 3rd, 6th, 9th, 12th, 15th, 21st days of life.

Groups*	1 st day	3 rd day	6 th day	9 th day	12 th day	15 th day	21 st day
A (n = 12)	7.6 \pm 0.19 ^{ab}	11.6 \pm 0.28 ^{efg}	17.1 \pm 0.57 ^{kl}	20.6 \pm 0.86	25.7 \pm 1.5	32.5 \pm 1.76	49.1 \pm 1.01
B (n = 18)	7.0 \pm 0.13 ^{cd}	8.6 \pm 0.22 ^{chi}	14.6 \pm 0.53 ^j	21 \pm 0.51	27.6 \pm 0.71	34.7 \pm 0.62	52.6 \pm 0.76 ^m
C (n = 15)	5.8 \pm 0.24 ^{ac}	7.6 \pm 0.4 th	12.3 \pm 0.98 ^k	18 \pm 1.6	24.6 \pm 2.38	30.7 \pm 3.07	41.4 \pm 4.18 ^m
D (n = 12)	5.6 \pm 0.06 ^{bd}	7.4 \pm 0.15 ^{gi}	12.5 \pm 0.29 ^l	19.1 \pm 0.38	26.4 \pm 0.46	34.6 \pm 0.72	50 \pm 1.24

*A = casein; B = casein + electromagnetic field; C = regional basic diet; D = regional diet + electromagnetic field; The data show the offspring weight, in grams, mean \pm standard error, on the 1st, 3rd, 6th, 9th, 12th, 15th, 21st days of life. Equal superscripts indicate significant differences between the groups. $p < 0.05$.

(1994) for CD1 mice exposed to 20 mT fields. However, Sienkiewicz et al. (1994) suggest that prenatal exposure to a 50 Hz, 20 mT magnetic field does not engender any gross impairment in postnatal development.

In our experiments we observed that the EMF caused a decrease in the mean weight in Group B (formed from offspring of rats fed casein and subjected to EMF) when compared to Group A (formed from offspring of rats fed by casein) at the third and sixth days of life. This result agrees in part with Nishikawa et al. (1986). They observed that suckling mice exposed to pulsed fields of 1.6 mT during pregnancy suffered a decrease in their body weight between the second and the fifth days of life and an increase between the eighth and the twenty-first days of life. Zusman et al. (1990), showed that the body weight of rats, exposed to the EMF, 20 or 100 Hz during pregnancy was lower on the first day of life, while animals exposed to EMF of 50 Hz suffered a decrease in their body weight between the twenty-first and twenty-sixth days of life.

Our findings did not match the results obtained by Sokolova (1998), Matos et al. (2001) and Sienkiewicz et al. (1994). Matos et al. (2001) observed that rats exposed to an EMF of 60 Hz during the breast-feeding period did not present changes in weight evolution when compared to the control group, but our rats were exposed during pregnancy. This difference makes the comparison difficult. Sokolova (1998) and Sienkiewicz et al. (1994) observed that after exposition to the EMF (50 Hz; 2 and 20 mT) during pregnancy and the postnatal (6 to 90 days) period, there were no changes in the body weight in mice. Rivas et al. (1985) exposed Swiss mice to a 50 Hz pulsed field at either 2.3 mT or 83 μ T from conception until 120 days of age. Male and female mice exposed at the higher field strength were significantly lower in weight at 120 days, but only males were reduced after exposure to the weaker field. The changes in weight were reported to increase throughout the duration of the experiment.

Groups that were fed RBD had a mean body weight lower than the groups fed casein at the first and third days of life. This can be explained by the fact that the suckling depends on its mother for a broad range of physiological functions, i.e. body temperature maintenance, urine excretion and feeding (Friedman, 1975). In approximate terms sucklings have their mother as the only source of food until the fourteenth day of life. The milk availability varies according to the presence of the mother in the nest and her emotional and nutritional states (Friedman, 1975). According to Teodósio et al. (1990), the RBD presents low vegetal protein content. These proteins are considered to be low in quality when compared with animal proteins, because they do not provide essential amino acid levels necessary for normal body development (Teodósio et al., 1990). Another important factor is that when the eye opening occurs (around the fourteenth day of life), the offspring are stimulated to eat solid food when the mother brings it to the nest (Galef and Clark, 1971).

What was done in this experiment can be called programming, because according to Lucas (1994), the nutrition and metabolic changes that act in the early stages of survival of individuals will permanently affect changes in the animal's physiology. Experimental studies have shown that nutritional manipulation pre- and post-birth can program the size of the adult individuals, their metabolism, blood lipid levels, diabetes, obesity, blood pressure, behavior and learning skills (Lucas, 1998; Waterland and Garza, 1999).

5. Conclusion

The total Somatic Maturation Indexes studied in this research were delayed by the association of the EMF and malnutrition during pregnancy. In relation to the body weight, the association between the EMF and malnutrition during pregnancy caused a decrease in the body weight at the sixth day of life.

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