

Study of sensory-motor and somatic development of the offspring of rats (Wistar) treated with caffeine

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The influence of caffeine, administered to rats, on the somatic and sensory-motor development of the offspring was investigated. Female Wistar rats were divided into a control group and a treated group and received drinking water and a 0.1% solution of caffeine orally, respectively. The offspring, also divided into a control group and a treated group, received daily monitoring until the 20th day of life to verify alterations in somatic neural development. The offspring of the treated group had reduced weight on the day of birth and on the 1st, 5th, 15th and 20th days of life; shorter snout-anus length (evaluation done daily); shorter snout-tail length on the day of birth and on the 1st, 5th and 10th days of life, and signs of retardation of somatic and sensory-motor maturation. These results allowed the conclusion that administration of caffeine to rats affects somatic and sensory-motor development of offspring.

Uniterms: Caffeine/effects. Postnatal development. Central nervous system/stimulants.

Estudou-se a influência da cafeína, administrada a ratos, no desenvolvimento somático e sensorial-motor da prole. Ratos Wistar fêmeas foram divididos em grupo controle e grupo tratado e receberam água e solução de cafeína a 0,1%, respectivamente. A prole, também dividida em grupo controle e grupo tratado, foi monitorada diariamente até o 20^o. dia de vida para se observar as alterações no desenvolvimento somático neural. O grupo tratado apresentou peso reduzido no dia do nascimento e nos 1^o, 5^o, 15^o e 20^o. dias de vida; comprimento focinho-ânus mais curto (avaliação efetuada diariamente); comprimento focinho-cauda mais curto no dia do nascimento e nos 1^o, 5^o, 15^o e 20^o. dias de vida e sinais de retardamento da maturação somática e sensorial-motora. Esses resultados permitem que se conclua que a administração da cafeína a ratos afeta o desenvolvimento somático e sensorial-motor da prole.

Unitermos: Cafeína/efeitos. Desenvolvimento pós-natal. Sistema Nervoso Central/estimulantes.

INTRODUCTION

Physiologic adaptation, also called metabolic programming or metabolic impression, can be activated in an organism when exposed to a particular influence in the intrauterine environment or early stages of life, while still passing through critical phases of tissue and organ development (Barker, 2000).

Any disturbances to the maternal organism before pregnancy can affect the offspring into adult life (Silveira,

2004). If the mother consumes certain substances during pregnancy which cross the placental barrier, the fetus is exposed to these substances and the many effects they can cause.

Caffeine intake in animals is a risk factor due to adverse reproductive effects. Caffeine has a rapid absorption in the gastrointestinal system, and passes into blood and fetal tissues including the central nervous system, when it is administered to the mother (Matijasevich, Santos, Barros, 2005). Highest blood caffeine levels are reached between 3 and 120 minutes, during which time it is rapidly distributed throughout body tissues, achieving equilibrium between blood and tissue levels (Golding, 1995).

Although animal studies indicate that caffeine leads to a decrease in fetal intrauterine growth, low birth weight,

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fetal re-absorption and teratogenesis, these results are still inconclusive in human studies (Souza, Sichieri, 2005). There are reports in the literature of decreased intrauterine growth after mother's consumption of caffeine during pregnancy; however, other articles found no connection between caffeine consumption and low birth weight and prematurity (Bicalho, Barros Filho, 2002), or delayed intrauterine growth (Bicalho, Barros Filho, 2002; Bracken *et al.*, 2003).

In rats, longitudinal growth after birth and after weaning, based on tail length with respect to body length, is used to track body growth (Stewart, Preece, Sheppard, 1975; Barbosa, Santiago, 1994). Other studies have evaluated somatic maturation as a form of corporal growth of these rats (Deiró, 1998; Silveira, 2004; Reis, 2005; Oliveira, 2006).

After birth, the animal's behavior throughout life will be a fundamental part of its adaptation to the environment (Deag, 1981). Responsiveness to environmental factors is related to the level of maturation of the nervous system (Rodríguez-Perez, Vicente-Perez, Garcia, 1992). In this context, this reflex can be characterized: an elementary coordinated motor action that corresponds to a specific sensorial stimulus (Kandel, Schwartz, Jessell, 1992). In recent decades, studies involving growth and development of the nervous system have been featured in the scientific literature. All areas of the nervous system are affected by exogenous factors, for example: dietary alterations and pharmacologic manipulations of neurotransmitters (Deiró, 1998).

Caffeine, commonly used as a psychoactive substance, has been widely studied for its effects on cognitive discharge, although these findings are inconclusive (Lieberman *et al.*, 2002; Perod, Roberts, McKinney, 2000; Sawynok, Yaksh, 1993; Shapkin, 2002). Small doses of caffeine (60 mg) alter cognitive function related to alertness, strength, mood, efficiency, and perception of energy (Lieberman, 2001). The substance has also been associated with an increase in motor performance (Fredholm *et al.* 1999; Johnson-Kozlow *et al.*, 2002; Lieberman *et al.*, 2002; Magill *et al.*, 2003).

Caffeine has been shown to be effective in a one-time dose of 25 mg/kg to prevent apnea in infants, but it can also result in considerable reduction in the blood flow velocity in cerebral arteries. When the same dose was administered again after 4 hours, velocity of blood flow in the cerebral arteries was decreased after the 2nd dose, while the velocity in intestinal arteries and left ventricle ejection were unaffected (Hoecker *et al.*, 2006).

Caffeine is found in many popular drinks (for example: coffee, tea, soft drinks) (Graham, 1978; Kaminsky,

Fisberg, 1992) which 80% of the population uses daily; coffee being the most abundant source of caffeine and the most consumed (Camargo, Toledo, 1998). Chocolate and some medicines are other important sources; it has been calculated that approximately 200 non-prescription drugs contain caffeine (Santos *et al.*, 1998).

As it is a non-adaptive drug, when consumed regularly the stimulant effect does not decrease. However, individual sensitivity exists in that small doses can cause tremors, throbbing and anxiety for many hours in some people, while others consuming caffeine daily show no toxic effects for years or decades. Clinical tolerance and habit depend on chronic use of the drug. Individuals drinking 5 or more cups of coffee per day, followed by a period of 12 to 16 hours' abstinence, can present withdrawal symptoms such as headaches, lethargy, irritability and inability to work (Kaminsky, Fisberg, 1992).

Other psychoactive substances causing dependency also present abstinence syndromes: cocaine withdrawal induces somnolence, depression, tiredness, bradycardia; abstinence from marijuana alters sleep and provokes agitation and irritability; nicotine abstinence is associated with appetite increase and weight gain (O'Brien, 2005).

The objective of this study was to investigate the influence of caffeine, administered to rats, on the somatic sensory-motor development during the first 20 days of life.

MATERIAL AND METHODS

This experiment was approved by the Ethics Committee Responsible for Experiments in Animals at the Center of Biologic Science of the Federal University of Pernambuco (document n° 65/06, process n° 013280/2006-31).

In the experiments, twelve rats older than 21 days, weighing between 30-40g were divided into a control group (cg) and treated group (tg) which received a solution of 0.1% of caffeine, a dose method quoted in the literature by Ohnishi *et al.* (1986), managed orally by water fountains. The administration of caffeine started on the 21st day of life, when weaning occurs, and continued until weaning of the offspring; the solution of caffeine and the commercial food were offered *ad libitum*.

After 90 days of life, the rats were mated and during this period the vaginal smear was analyzed with an optical microscope (Carl Zeiss-Germany) to observe the presence of sperm and to detect the beginning of pregnancy. Offspring of the control group (ocg) and the offspring of the treated group (otg), were weighed on a digital scale (Andhr-200, A&D Company) with sensitivity of 0.001 g and length of snout-anus (sa) and snout-tail (st) were measured

with a slide caliper (accuracy 0.05mm) until the 20th day, to test if the somatic neural growth was compromised by treatment.

Indicators adopted for somatic maturation were: ear unfolding (eu), auditory conduit opening (aco), eruption of the lower incisors (ei) and eye opening (eo), as described in the literature (Silveira, 2004; Smart, Dobbing, 1971).

Sensory-motor maturation was evaluated by observation of ontogenic reflexive actions of righting (r), cliff avoidance (ca), auditory startle (as) and free-fall righting (fr) as described elsewhere (Smart, Dobbing, 1971; Rodriguez-Perez, Vicente-Perez, Garcia, 1992; Saste *et al.*, 1998; Silveira, 2004).

Comparisons between treated and control group were performed for each individual period using Student's t-Test for independent samples with $*p < 0.05$. The results were expressed as mean \pm standard deviation in each treatment group (calculations performed with Microsoft Excel®).

RESULTS

The rats whose mothers were treated with caffeine had lower weight on the day of birth and on the 1st, 5th, 15th and 20th days of life with differences of 10.75%, 18.95%, 19.49%, 15.74%, 35.42%, respectively, compared to the control mothers (Figure 1). During the treatment, there was no significant difference in the treated mothers' body weight compared to the control group. The quantity of diluted caffeine consumed versus the consumption of water was an average of 53.28 mL and 57.85 mL, respectively.

Offspring of rats treated with caffeine (otg) had shorter snout-anus (fa) length of 8.62%, 6.39%, 13.94%,

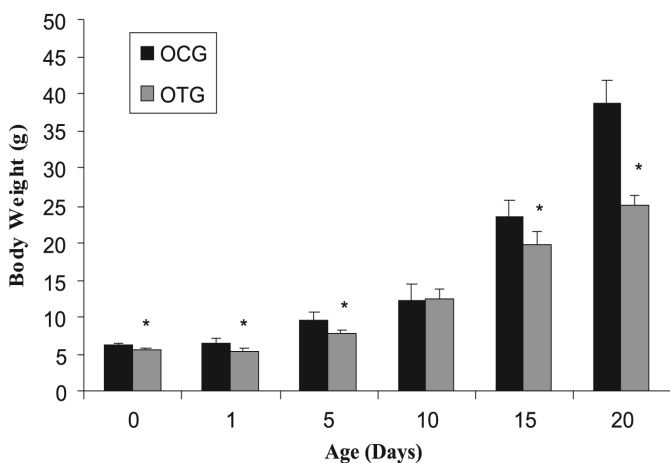


FIGURE 1 - Body weight (grams) as a function of age (days) of the offspring of control rats (ocg) and the offspring of treated rats (otg). Values are expressed as mean \pm standard deviation, $n=15$ and $*p < 0.05$.

11.54%, 10.37% and 34.67% on the day of birth and on the 1st, 5th, 15th and 20th days of life, respectively, compared to the offspring of the control group (ocg). Snout-tail (fc) length, in the treated group (otg) showed lower values by 8.43%, 7.59%, 13.74% and 12.54% on the day of birth and on the 1st, 5th, 15th and 20th days of life, respectively, compared to the ocg group. (Figures 2 and 3)

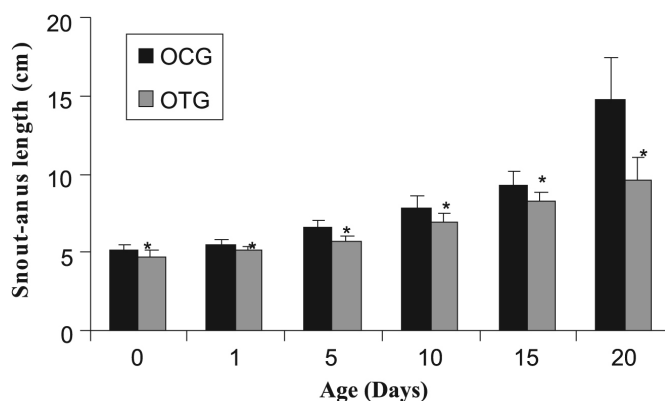


FIGURE 2 - Snout-anus length (centimeters) as a function of age (days) of offspring of control rats (ocg) and of offspring of treated rats (otg). Values were expressed as mean \pm standard deviation, $n=20$ and $*p < 0.05$.

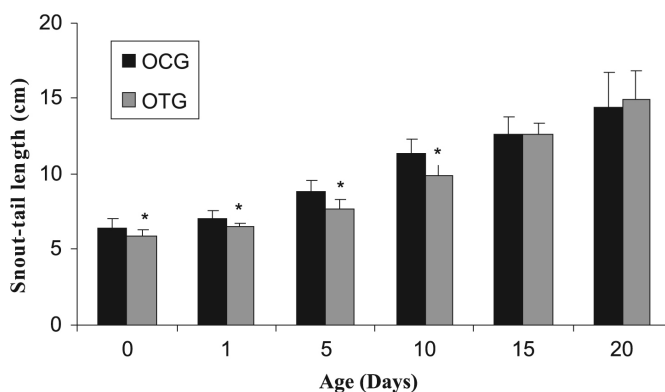


FIGURE 3 - Snout-tail length (centimeters) as a function of age (days) of offspring of control rats (ocg) and offspring of treated rats (otg). The values were expressed as mean \pm standard deviation, $n=20$ and $*p < 0.05$.

The offspring of rats that consumed caffeine presented retardation in somatic development. The differences in percentage found for the indicators of eu, aco, ei and eo, after comparison of maturation signals between otg and ocg, were 28%, 22.3%, 11.1% e 4.1%, respectively (Table I).

The offspring of rats which were administered caffeine presented retardation on the day of appearance of the sensory-motor maturations investigated. According to the day of appearance of the reflexes of r, ca, as and fr, the differences in percentage found, comparing otg

TABLE I - Somatic development of the offspring of control rats (ocg) and the offspring of rats treated with caffeine (otg). Values expressed as mean \pm standard deviation of age (days), n=20 *p<0.05

| Somatic development | Ocg | Otg | Difference |
|---------------------|-----------------|-------------------|------------|
| Eu | 2.50 \pm 0.76 | *3.2 \pm 0.41 | -28% |
| Aco | 9.85 \pm 0.81 | *12.05 \pm 0.68 | -22.3% |
| Ei | 12.1 \pm 0.96 | *13.45 \pm 0.51 | -11.1% |
| Eo | 14.6 \pm 0.99 | *15.2 \pm 0.61 | -4.1% |

Legend: Eu – ear unfolding; Aco – auditory conduit opening; Ei – eruption of the lower incisors; Eo – eye opening

TABLE II - Sensory-motor development of the offspring of control rats (ocg) and of the offspring of rats treated with caffeine (otg). Values expressed as mean \pm standard deviation of age (days), n=20 *p<0.05

| Sensory-motor development | Ocg | Otg | Difference |
|---------------------------|-----------------|-------------------|------------|
| R | 1.83 \pm 0.71 | *5.58 \pm 0.79 | -204.9% |
| Ca | 3.16 \pm 0.85 | *6.11 \pm 0.83 | -93.35% |
| As | 11.4 \pm 0.88 | *13.25 \pm 0.71 | -16.22% |
| Fr | 14.3 \pm 0.94 | *16.4 \pm 0.96 | -14.68% |

Legend: R – righting; As – auditory startle; Ca – cliff avoidance; Fr – free-fall righting

and ocg, were of 204.9%, 93.35%, 16.22% and 14.68%, respectively (Table II).

DISCUSSION

Rats submitted to treatment with caffeine during pregnancy and lactation yielded a higher volume of milk, although the values of the daily total production of proteins, lactose and triglycerides was similar to values observed in females without treatment (Hart, Grimble, 1990).

This could affect the body weight of both offspring groups of this study. However, although caffeine caused weight modifications, only more detailed studies can conclude if the alterations were of metabolic origin (Barker, 2000; Silveira, 2004), or due to congenital metabolic alterations exclusively in the offspring. The findings of weight difference between the groups, corroborate other studies available in the literature (Soyka, 1979; Aeschbacher *et al.*, 1980; Evereklioglu *et al.*, 2003).

During the period of fetal and neonatal development, body tissues are growing (histogenesis) and the so animals grow (Bernardi, 1996). However, appropriate conditions are necessary for a species to reach the correct body gro-

wth for age and exposure to some substances can interfere significantly with the growth and maturation process.

Effects of caffeine on body growth of rats are debatable. Caffeine is thought to diminish bone mineral density, which may occur through a change in osteoblast viability, leading to an increase in apoptosis in these cells (Tsuang *et al.*, 2006). Caffeine, associated with a protein diet in rats, is capable of altering the bone mineral content of the mandibula and femur of the offspring. However, treatment with zinc supplements promotes greater than normal bone development in the offspring (Sasahara, Yamano, Nakamoto, 1990).

Effects on growth depend on the concentration of the dose selected for treatment. Daily doses of caffeine, starting on the rats' 5th day of life, were associated with an increase in the blood concentration of growth hormone (GH); however this applies only to the acute effect of the dose. The chronic effect reflected a discreet increase in this concentration, which led some authors to conclude that caffeine was responsible for the depletion of GH in the pituitary gland (Clozel *et al.*, 1983). The way growth hormone (GH) is secreted is an important factor in its metabolic and somatic performance in rodents (Jaffe *et al.*, 2002).

It is possible that the interferences caused by caffeine in the somatic development of the offspring, could be due to toxicological alterations: a substance which can cause retardation in growth or development of specific organs and/or systems can create anatomic and biochemical alterations that generally occur in organisms in the late period of development (Bernardi, 1996).

Another way of accompanying the development of rats is through analysis of reflexive actions. The tests of neurologic analysis support confirmation of retardation or precocity of these actions (Silveira, 2004) and provide information about the capacity of an organism to be responsive to an environmental stimulus without having had previous experience. Neurologic analysis can thus measure effects of environmental factors in maturation of the nervous system and also detect changes in cerebral functions in the presence or absence of answers (Rodriguez-Perez, Vicente-Perez, Garcia, 1992).

Caffeine is one of the substances capable of stimulating the central nervous system (Clozel *et al.*, 1983; Martin *et al.*, 2005). However, it is also a substance that interferes in the formation of the nervous system by acting as an antagonist of adenosine receptors, (the neuromodulator that controls the release of acetylcholine) an important neurotransmitter for cerebral development. It is also described as capable of promoting an increase in the activity of acetylcholinesterase, an enzyme that inactivates the action of

acetylcholine (Silva *et al.*, 2008). Another reported injury to the central nervous system due to caffeine is retardation in time of the decubitus reflex in the offspring of rats that consumed caffeine, under the same conditions used in this study (Peruzzi, 1985).

Associating the results on somatic neural development with this information, the values found in the offspring show that, when administered to females, caffeine is responsible for late development in the nervous system of the offspring.

The critical period of cerebral development of the rat, in terms of maturation and sexual differentiation of the brain, occurs between the 6th and 13th day after mating, around the 13th-14th day of pregnancy until the 21st postnatal day of the offspring (weaning day) (Bernardi, 1996). However according to some studies, manipulations using a psychoactive substance, after the critical period of development, are also capable of altering cerebral development. A chronic dosage of cocaine, when administered subsequent to weaning of the male offspring, exacerbates sexual behavior and diminishes the development of hippocampal cells (Andersen *et al.*, 2007).

According to the data described, an explanation for the fact that the differences between the groups analyzed were more discrepant in the first two cerebral maturation stages was that animals were only mid-way into their development time, where retardation could be the acute cerebral effect of the exposition to the external agent. Smaller differences in the day of appearance of the two last signs of reflex maturation, could be related to the fact that these manifest themselves in a more advanced period of cerebral maturation, suggesting that even when exposed this substance, the cerebral modifications appeared to a lesser degree probably because the nervous system had matured sufficiently to counter act the effects caused by caffeine.

CONCLUSION

The current study allows us to conclude that the administration of caffeine, during the period between the 21st and 120th days of life, in Wistar rats affects somatic and sensory-motor development of the offspring in the neonatal period, causing: lower weight and corporal size, retardation of somatic maturation signs of auditory external conduct opening, eruption of inferior incisive teeth, auditory pavilion and eye opening, and lateness of sensory-motor maturation signs of decubitus recuperation, of aversion to precipice, of startle and acceleration reactions.

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