

NEONATAL EXPOSURE TO CITALOPRAM, A SEROTONIN SELECTIVE REUPTAKE INHIBITOR, PROGRAMS A DELAY IN THE REFLEX ONTOGENY IN RATS

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Abstract – Serotonin influences the growth and development of the nervous system, as well as its behavioral manifestations. The possibility exists that increased brain serotonin availability in young animals modulates their neuro-behavioral responses. This study investigated the body weight gain and reflex ontogeny of neonatal rats treated during the suckling period with two doses of citalopram (5 mg, or 10 mg/kg, sc, daily). The time of the appearance of reflexes (palm grasp righting, free-fall righting, vibrissa placing, auditory startle response, negative geotaxis and cliff avoidance) as well as the body weight evolution were recorded. In general, a delay in the time of reflex development and a reduced weight gain were observed in drug-treated animals. These findings suggest that serotonergic mechanisms play a role in modulating body weight gain and the maturation of most reflex responses during the perinatal period in rats.

KEY WORDS: serotonin, programming, SSRI, citalopram, reflex development.

Exposição neonatal ao citalopram, um inibidor seletivo da recaptação de serotonina, programa retardo na ontogênese reflexa em ratos

Resumo – A serotonina influencia o crescimento e o desenvolvimento do sistema nervoso e sua expressão comportamental. O aumento da disponibilidade de serotonina no cérebro de ratos jovens parece modular as respostas neurocomportamentais. Neste estudo, foram investigados o ganho de peso corporal e a ontogênese dos reflexos em ratos neonatos, tratados diariamente, durante o período de aleitamento, com duas doses de citalopram (5 ou 10 mg/Kg de peso corporal, via subcutânea). Foram avaliados, o tempo de aparecimento dos reflexos (preensão palmar, endireitamento, colocação pelas vibrissas, resposta ao susto, geotáxico negativo e aversão ao precipício), e a evolução do peso corporal. Foi observado atraso no tempo de desenvolvimento de alguns reflexos e redução no ganho de peso corporal. Os achados em ratos sugerem que as alterações no ganho de peso corporal e na maturação dos reflexos são programadas, durante o período perinatal, com participação de mecanismos serotoninérgicos de modulação.

PALAVRAS-CHAVE: serotonina, programação, ISRS, citalopram, desenvolvimento reflexo.

Environmental impacts during the perinatal period can persistently affect morphofunctional patterns in a variety of physiological systems^{1,2}. During this period, the high speed of cellular events results in a higher vulnerability of the nervous system (NS), so that distress acting upon it could alter the structure and function in age-related, developmental processes, such as maturation of most reflex responses and motor responses³. In the NS, the sequence of cellular events during the early period of life, embryogenesis, and nursing, helps determine both the definitive neurochemical composition and the morphofunctional

structure of the mature organism⁴. These events would involve a mechanism called “programming”. In this phenomenon, an environmental alteration during a critical developmental period would have persistent effects on the structure and function of the body^{5,6}. Experimental evidence indicates that serotonin can also influence embryogenesis and growth^{7,8} by acting, presumably, as a developmental signal⁹ or as a neurotrophic factor¹⁰. Furthermore, serotonin seems to play a role in regulating the development of the mammalian brain through actions on the serotonergic neurons themselves^{8,11,12}. Serotonin has been

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shown to have multiple functions as a neurotransmitter, which it realizes by exerting modulating effects on the neural excitability¹³. There is a large amount of evidence of its participation in pain sensitivity, motor activity, body thermoregulation, sleep, feeding behavior, and mood^{14,15}. Encephalic areas that are known to be involved in psychomotor processes, such as the brainstem, cerebellum, diencephalon, basal ganglia and cerebral cortex, are innervated by serotonergic pathways from raphe nuclei¹⁶. Growth and development of the central nervous system occur with great intensity during the rat's gestation and suckling period¹⁷. In these phases, the brain structures are highly vulnerable to several types of insult. In the rat, the first serotonergic neurons appear between the 12th and the 14th day of gestation¹⁸. The second and third week of postnatal life are distinguished by an abundant dendritic arborization of serotonergic axons in the cerebral and cerebellar cortex, in the hippocampus, and in striatum¹⁸. The final density and location of the serotonergic neuron terminals are achieved only during postnatal maturation^{18,19}.

During pregnancy or the suckling period, pharmacological or nutritional manipulations can induce drastic morphological and functional changes in the growth and development of the nervous system²⁰⁻²². Furthermore, the reflex maturation constitutes an indicator of the development of the nervous system²³. Retardation of the reflex ontogeny in malnourished rats has been observed²⁴. High levels of 5-HT and 5-HIAA were found in the brains of undernourished animals that were up to 300 days old²⁵. In addition, serotonin and serotonergic drugs have anorexic properties^{26,27}. The selective serotonin reuptake inhibitors increase the availability of serotonin in the synaptic cleft²⁸. Neonatal manipulation with sertraline also caused a delay in somatic ontogenesis and in the maturation of some reflexes²⁹.

Thus, the possibility exists that the use of serotonergic agents in the initial phase of life could have some effects on the body growth and sensorimotor functions. To this end, we observed (in a previous work) that rats neonates, treated with citalopram, showed a retardation of somatic maturation and a decrease in body weight gain³⁰. The objective of this study was to test the hypothesis that the administration of citalopram, a highly selective serotonin reuptake inhibitor³¹, in rats during the suckling period – the so-called brain growth spurt – results in adaptive changes in the appearance time of congenital reflexes.

METHOD

Animals

Wistar male rats from the colony of the Nutrition Department – Federal University of Pernambuco – Brazil were coupled to obtain litters. Between gestation and the end of the experiment, the animals were housed in polyethylene cages (30 × 27 × 47).

Male pups from different mothers were randomly distributed in litters of 6 neonates, 24 hours after their births. Each pup was labeled with a mark of methyl violet solution on its skin, which provided identification during the experiment. Each litter was breastfed by one of the dams until the 21st post-natal day (the day of birth was considered to be day zero). The animals were maintained at a room temperature of 23±1°C, on a light-dark cycle of 12/12 hours (light on 6:00 a.m. to 6:00 p.m.) with free access to meals (Labina-Purina of Brazil) and water. The experimental protocol of this paper was approved by the Ethical Committee for Animal Experimentation (CEEA) from the Federal University of Pernambuco, in agreement with the National Institute of Health guide for the Care and Uses of Laboratory Animals.

Pharmacological treatment and experimental groups

A blind experiment was performed to prevent identification of the experimental groups. The animals of the different groups were simultaneously evaluated. According to the experimental treatment, three groups (n=27 each one) of suckling rats were distributed as follows: group Cit5 (5 mg/kg, sc); group Cit10 (10 mg/kg, sc); and one control group receiving an equivalent volume of saline solution (NaCl 0.9%, sc.). During the experiment, one neonate in the Cit5 group died. Therefore, 80 rats were evaluated during the whole experiment. The time for reflex maturation and the body weight were determined. Citalopram (H.Lundbeck A/S, Copenhagen-Valby, Denmark) was dissolved in a saline solution and injected at a concentration of 1 mL/100g b.w. The treatment was applied daily from the 1st to the 21st postnatal day (suckling period).

Body weight

The measurement of body weight was performed on days 1, 3, 7, 14 and 21, between 10:00 p.m. and 1:00 p.m., using a Marte scale, Brazil-São Paulo, SP (100 mg precision).

Reflex testing

The reflex tests²³ were carried out daily from the 1st to the 21st postnatal day (Table 1), and were conducted between 11:00 a.m. and 1:00 p.m. The progress of the individuals was followed throughout the experiment. The time of the appearance of each reflex was defined as the first day of its occurrence during a period of three consecutive days.

Statistical analysis

After preliminary testing to identify the distribution normality and homogeneity among the groups, statistical analyses were performed: a two way repeated measures ANOVA test on the 1st, 3th, 7th, 14th and 21st day for the body weight followed by a post-hoc Holm-Sidak test were used for multiple comparisons. The Kruskal-Wallis analysis of variance, followed by Dunn's test, was used to compare the time appearance of the reflexes between each citalopram dose and saline. The level of significance was p<0.05.

Table 1. Description of the tests reflexes.

Reflex	Eliciting stimuli	Response
Palmar-grasp	The fore paws of the rat are stroked gently with a cylindrical stick of 1mm thickstick (paper-clip)	Digits flex to grasp the stick. The disappearance date is taken.
Righting (recovery of the decubitus)	The rat is placed with the back on a surface	Its rolls and turns over on to ventral surface, in 10 s.
Free-fall righting (acceleration righting)	The rat is dropped, back downwards, from 35 cm on to cotton wool pad	Its turns in mid-air to land on all fours
Negative-Geotaxis	The rat is placed on the surface tilted to 45°, with its head turned back to the inferior area	Its returns in direction to the superior area of the tilted plan, in 10 s.
Cliff-avoidance	The rat is put on the edge of table, with nose and fore feet just over edge	It moves away from "cliff", in 10 s.
Auditory-startle response	Sound stimulus: snap of rat trap closing on wooden base	Sudden, brief extension of hind limbs (which raise hind-quarters)
Vibrissa-placing	The rat is held by the tail, the head facing the edge of the table, vibrissa just touching vertical surface	It lifts its head and extends the fore limbs in direction the table, in 10 s.

Modified from Smart and Dobbing (1971).

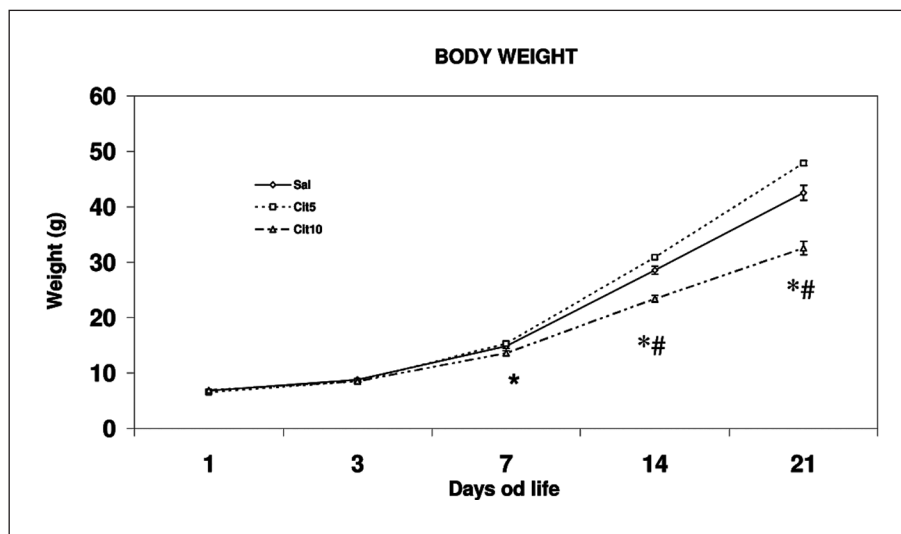


Fig 1. Body weight of suckling rats from the 1st, 3th, 7th, 14th to 21st day of life, treated with citalopram from the 1st to the 21st day of life. Two way repeated measures ANOVA; Holm-Sidak test ($F_{2,399}=13.134$): *Comparisons of Cit 10 mg with saline ($p<0.05$); #Comparisons of Cit 5 mg with saline ($p<0.05$).

Table 2. Maturation of the reflexes of suckling rats treated with citalopram from the 1st to the 21st day of life.

Reflexes (days)	Groups						p-value
	Saline		Cit 5		Cit 10		
	Median	(range)	Median	(range)	Median	(range)	
Palmar grasp	2.5	(1.0–9.0)	6.5	(3.0–5.0) ^a	6.0	(5.0–9.0) ^a	<0.001 [#]
Righting	5.0	(3.0–9.0)	8.0	(4.0–9.0)	9.0	(5.0–10.0) ^a	=0.03 [#]
Vibrissa placing	11.0	(8.0–15.0)	13.0	(11.0–16.0) ^a	13.0	(11.0–17.0) ^a	<0.001 [#]
Cliff avoidance	8.0	(7.0–14.0)	10.0	(8.0–13.0)	11.0	(7.0–18.0)	
Negative geotaxis	12.0	(8.0–16.0)	12.0	(9.0–16.0) ^a	13.0	(12.0–18.0) ^b	<0.001 [#]
Auditory startle	11.0	(11.0–14.0)	12.0	(12.0–18.0)	13.0	(12.0–18.0) ^a	=0.012 [#]
Free-fall righting	14.0	(12.0–18.0)	17.0	(16.0–19.0) ^a	16.0	(14.0–21.0) ^a	<0.001 [#]

^aKruskal-Wallis test; Dunn's test: ^amultiple comparisons with the saline group; ^bmultiple comparisons with the Cit 5 group.

RESULTS

Compared with the saline group, ANOVA revealed a lower body weight in the Cit10 group from the 7th to 21st day ($p < 0.05$), and in the Cit5 group from the 14th to 21st day ($p < 0.05$) (Fig 1).

The time it takes for the maturation of physical features (Table 2) showed statistically significant alterations when compared to the saline group in the palmar grasp ($H=14.842$; $p < 0.001$); the development of this reflex was delayed in the Cit5 ($p < 0.05$) and Cit10 groups ($p < 0.05$). The righting reflex also differed between groups ($H=11.827$; $p=0.003$), where a delay in this physical characteristic occurred in the Cit10 group ($p < 0.05$). In the placing of the vibrissa, presented statistical differences between groups ($H=15.531$; $p < 0.001$); this physical feature was delayed in Cit5 ($p < 0.05$) and Cit10 groups ($p < 0.05$). The acoustic startle response ($H=8.835$; $p=0.012$) was delayed in the Cit10 group ($p < 0.05$). The free-fall righting response showed statistical differences between groups ($H=23.443$; $p < 0.001$), with a delay occurring in the Cit5 ($p < 0.05$) and Cit10 groups ($p < 0.05$). The negative geotaxis reflex ($H=21.132$; $p < 0.001$) showed delays in the Cit5 group ($p < 0.05$) when compared to the saline group, and in the Cit10 group ($p < 0.05$) when compared to the Cit5 group. The Cliff avoidance was not statistically significant different among the groups.

DISCUSSION

The present study showed that the chronic administration of citalopram during the critical period of brain development in rats delays the body weight gain and the appearance time of reflexes. The action of the drug on serotonergic signaling pathways is likely responsible for these effects since SSRIs increase the serotonin release in the synaptic cleft²⁸.

Some studies in humans have observed the use of SSRIs in young children, suggesting repercussions such as affective alterations³². The neonatal manipulation of the 5-HT system with SSRI causes repercussions in adult rats such as the occurrence of depression-like effects³³ and a reduction of aggressiveness²². In the present study, we show an anorexic effect of citalopram in neonatal rats. Recently, we showed a decrease of body weight gain and a reduction of aggressiveness in the adult rats treated with citalopram early in life as well^{22,33}. These findings were hypothesized to be associated with serotonergic pathways because of serotonin's inhibitory action on food intake^{27,34,35}.

Alterations in the appearance of reflexes indicate a correlation between the biochemical and structural development and ontogenesis of the nervous system³⁶. For example, the repercussions of other neonatal manipula-

tions – such as early malnutrition – on the development of the structural, neurochemical and functional integrity of the nervous cells are well known⁹. The amount of brain monoamines present during development increases more quickly after birth³⁷. Rats submitted early in life to low protein diets reveal altered brain levels of noradrenaline, dopamine, and serotonin³⁸⁻⁴⁰. High levels of 5-HT and 5-HIAA were found in the brains of undernourished animals that were up to 300 days old²⁵. It is well established that undernourished rats exhibit a delay in the development of reflexes, such as palm grasping, the startle response, and free-fall righting²⁴.

Corroborating these hypotheses, it is possible that increased serotonin availability, when provoked by the SSRI treatment, could cause the delay in the reflexes. In this sense, an increase of the latency of the startle response in adult rats that were treated with fluoxetine during the neonatal period was shown⁴¹. This fluoxetine effect was reduced by *m*-chlorophenylpiperazine, a 5-HT_{1B/2C} agent, thus suggesting serotonergic modulation in the reflex elicitation⁴¹. In addition, treatment with fluoxetine diminished the locomotor activity in young rats⁴². Furthermore, there is evidence of the involvement of 5-hydroxytryptamine_{1B} autoreceptors in the enhancement of serotonin turnover in the mouse brain following repeated treatments with fluoxetine⁴³.

Collectively, the observations in the present study indicate that serotonergic mechanisms of neurotransmission after chronic treatment with citalopram, a SSRI, are involved in the body weight gain and the emergence of the reflexes. Several lines of evidence indicate that the immediate post-natal period is of particular importance for the long-term programming of nervous functions in the rodent. Notably, the development of the hypothalamic appetite regulatory network in rats and mice occurs predominantly after birth⁴⁴⁻⁴⁷. It therefore remains to be determined to what extent the altered regulatory action of 5-HT on the ontogenesis of reflexes and on body weight control is dependent on the type and subtype of serotonergic receptors, as well as on the developmental period during which serotonin modulation takes place.

In conclusion, the results showed that treatment with citalopram, during the suckling period, delayed the body weight gain and the development of early behavioral expressions. This evidence supports the hypothesis that persistent morphological or functional alterations were produced during the period of fast brain development, indicating the participation of the serotonergic mechanisms in these events. These data are consistent with the idea that changes in reflex ontogeny are programmed with important participation of serotonergic modulation during the perinatal period in rats.

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