

NEONATAL ADMINISTRATION OF FLUOXETINE DID NOT ALTER THE ANXIETY INDICATORS, BUT DECREASED THE LOCOMOTOR ACTIVITY IN ADULT RATS IN THE ELEVATED PLUS-MAZE

Valdenilson Ribeiro Ribas³, Helena Karine Rufino Aniceto⁶,
Hugo André de Lima Martins³, Ketlin Helenise dos Santos Ribas⁷,
Renata de Melo Guerra-Ribas⁵, Simone do Nascimento Fraga⁴,
Valéria Ribeiro-Ribas³, Célia Maria Mendes Vasconcelos⁶,
Marcelo Tavares Viana², Raul Manhaes-de-Castro¹

Abstract – The objective of this study was evaluate the anxiety and locomotor activity (LA) in 52 Wistar adult male rats, being 26 treated with fluoxetine (10 mg/Kg – sc) in the neonatal period. These same rats received foot shock (FS) (1.6-mA – 2-s) in the 90th day. The anxiety and LA were appraised by plus-maze. The time spent in the open arms was used as anxiety index and the LA was measured by number of entries in closed arms (NECA) and the total of entries (TE). T-test was used with $p < 0.05$ and expresses data in mean \pm SEM. There were reductions with the fluoxetine group in the NECA (2.35 \pm 0.33) and in the TE (3.96 \pm 0.61) compared to the controls (4.65 \pm 0.52) and (6.96 \pm 0.94). The neonatal administration of fluoxetine did not alter the anxiety, but reduced the LA in the animals that received FS.

KEY WORDS: anxiety, plus-maze, serotonin, fluoxetine, neonatal.

Administração neonatal de fluoxetina não alterou os indicadores de ansiedade, mas diminuiu a atividade locomotora em ratos adultos no labirinto elevado em cruz

Resumo – O objetivo deste estudo foi avaliar a ansiedade e a atividade locomotora (AL) em 52 ratos Wistar adultos machos, sendo 26 tratados no período neonatal com fluoxetina (10 mg/Kg – sc) e no 90^o dia, receberam estímulos elétricos nas patas (1,6-mA-2-s). A ansiedade e a AL foram avaliadas por meio do labirinto elevado em cruz. O tempo de permanência dos animais nos braços abertos (BA) foi utilizado como índice de ansiedade e a AL medida pelo número de entradas nos braços fechados (NEBF) e pelo total de entradas (TE) nos BA e BF. O teste t foi utilizado, com ($p < 0,05$) e os dados apresentados em média \pm erro padrão. Os animais tratados reduziram o NEBF (2,35 \pm 0,33) e o TE (3,96 \pm 0,61) comparados a seus controles (4,65 \pm 0,52) e (6,96 \pm 0,94). A administração neonatal de fluoxetina não alterou a ansiedade, mas diminuiu a AL dos animais que receberam EE.

PALAVRAS-CHAVE: ansiedade, labirinto elevado em cruz, serotonina, fluoxetina, neonatal.

The serotonin (5-hydroxytryptamine, 5-HT) controls several functions in the central nervous system¹. Animals models have contributed to demonstrate the role of serotonin in affective disorders like depression^{2,3}, in the aggressive behavioral regulation^{4,5} and anxiety^{6,7}. Adult rats

treated with fluoxetine during the neonatal stage (days 1 to 21, suckling period) have demonstrated behavioral alterations in the experimental models of anxiety as elevated plus-maze^{8,9}. During the acute administration, there is an increase in extracellular serotonin in several subcorti-

Departamento de Nutrição, Universidade Federal de Pernambuco (UFPE), Recife PE, Brasil; ¹Doutor em Farmacologia Experimental e Clínica da Universidade de Paris VI, Professor Adjunto do Departamento de Nutrição, UFPE; ²Doutorando em Bases Experimentais da Nutrição, Departamento de Nutrição, UFPE; ³Mestre em Neuropsiquiatria, Pós-graduação em Neuropsiquiatria e Ciências do Comportamento, UFPE; ⁴Mestranda em Bases Experimentais da Nutrição, Departamento de Nutrição, UFPE; ⁵Graduada em Nutrição, Pós-graduanda em Gestão da Qualidade e Vigilância Sanitária em Alimentos, Hospital Barão de Lucena, Recife PE (HBL); ⁶Graduada em Nutrição, Chefe do setor de Nutrição do HBL; ⁷Estagiário do Laboratório de Imunopatologia Keizo Asami LIKA, UFPE.

Received 14 May 2008, received in final form 11 September 2008. Accepted 2 October 2008.

Dr. Valdenilson Ribeiro Ribas – Avenida Armindo Moura 581 / Quadra D / Bloco 02 / Apto 201 / Conj WXL - 51130-180 Recife PE - Brasil. E-mail: ribaspsy@ufpe.br

cal brain regions due to reuptake blockade¹⁰. In the chronic administration, there is an increase of extracellular concentrations of 5-HT at cortical and subcortical levels, and the long-term 5-HT reuptake blockade provokes desensitization of somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors, respectively leading to a disinhibitory effect on raphe neurons firing and to reduced feedback inhibition of 5-HT release^{11,12}. Some studies show chronic administration of selective serotonin reuptake inhibitor (SSRI) during the suckling period induces several morphologic^{13,14}, functional¹⁵ and behavioral changes¹⁶. These alterations can become irreversible depending on the magnitude of the aggression^{15,17}.

However, the data obtained in animal studies using SSRI antidepressants are contradictory¹⁸, specifically, in the effect of fluoxetine on animal models of anxiety, above all in chronic effects, using elevated plus-maze¹⁹. This is a widely used animal model of anxiety involving unconditioned responses based on exploration. Although an anxiogenic effect or lack of effect has been found in some of the studies⁸, anxiety evaluation in adult rodents treated with fluoxetine in neonatal period is still very scarce.

The objective of this study was test the hypothesis that the administration of a SSRI, fluoxetine, to suckling rats, promotes changes in anxiety behavior in the elevated plus-maze in adult rats.

METHOD

Animals

The animals were Wistar male rats maintained at a room temperature of 23±2°C, on a light-dark cycle of 12:12 hours (light on at 7:00 a.m.), with free access to water and food. The animals were assigned randomly to two groups (6 pups per litter) 24 h after birth. One group (fluoxetine group) received fluoxetine (10 mg/kg, sc, dissolved in saline solution, 1 ml/kg), and the other (control group) received an equivalent volume of saline (NaCl, 0.9%). The treatments were applied every day from the 1st to the 21st postnatal day (suckling period). Body weights were determined at 1st to the 21st (weaning) and 90th day. In the end, there were 52 rats in each group.

Apparatus

A standard wooden elevated plus-maze apparatus consisting of 50×10×40 cm opposite closed arms and 50×10 cm open arms that radiated from a central 10×10 cm space was used. The apparatus was elevated to a height of 50 cm above floor level by a single support.

Behavioral evaluation

The animals aged 90 days, weighing 310-330g, were evaluated with regard to anxiety behavior and locomotor activity, using elevated plus-maze. This model is based on the innate fear rodents have for open and elevated spaces^{19,20}. Rats on the elevated plus-

maze tend to avoid the open arms and prefer to stay in the enclosed arms. When confined to the open arms, rats show behavioral and physiological manifestations of fear, such as freezing, defecation, and increases in plasma corticosteroids²⁰. The avoidance of the open arms occurs primarily because they prevent the rat from engaging in thigmotaxic behavior^{21,22}. Thigmotaxis is a natural defensive response that keeps the rat in contact with a vertical surface, thereby avoiding predators²³.

Each animal was placed in the central area of the maze facing one of the closed arms. The animals were observed for 5 min by a trained observer who sat quietly 1.5 m from the center of the maze and recorded the time spent in and the number of entries into each arm. An entry was recorded when the animal's four limbs had entered an arm. The observer was "blind" to the animal's condition.

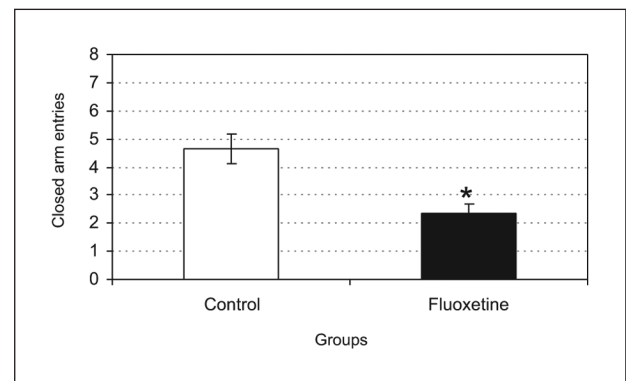


Fig 1. The number of entries in the closed arms. Evaluation in the elevated plus-maze. Fluoxetine chronic effect (10 mg/kg, sc, dissolved in saline solution, 1 ml/kg, n=26), during neonatal period compared to control group treated with saline (NaCl 0.9%, 1 ml/Kg, sc, daily, n=26). The behavioral parameters (expresses mean±SEM) was analyzed by "t" test. The significance level adopted for statistical tests was $p < 0.05$.

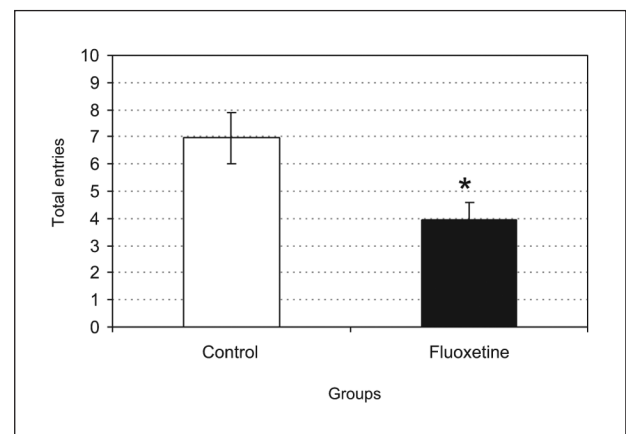


Fig 2. The total of entries. Evaluation in the elevated plus-maze. Fluoxetine chronic effect (10 mg/kg, sc, dissolved in saline solution, 1 ml/kg, n=26), during neonatal period compared to control group treated with saline (NaCl 0.9%, 1 ml/Kg, sc, daily, n=26). The behavioral parameters (expresses mean±SEM) was analyzed by "t" test. The significance level adopted for statistical tests was $p < 0.05$.

The anxiety evaluation was performed in two stages. In the first, the rats received a stimulus. This stimulus (an electric foot shock) consisted of a 1.6-mA – 2-s current pulse. In the second, the animals were naïve. The spent time in the open arms was anxiety index and the closed arms entries number and the total entries number were used as locomotor activity.

Data analysis

For each animal, the spent time in the open arms, the number of entries into the closed arms and the total number of entries (open+closed arms) were computed. The behavioral parameters (expresses mean±SEM) were analyzed by Student's "t" test. The significance level adopted for statistical tests was $p < 0.05$.

RESULTS

Compared to the control group, there was not statistical significance, in both stages, with time spent in the open arms of fluoxetine group. There were reductions within the fluoxetine group in the number of entries in the closed arms (2.35 ± 0.33 , $p = 0.001$) (Fig 1) and in the total of entries (3.96 ± 0.61 , $p = 0.010$) (Fig 2) only in the second stage of the anxiety experiment, after the electric shock stimulus on the foot, when compared to the control group (4.65 ± 0.52 ; 6.96 ± 0.94).

DISCUSSION

This study used fluoxetine in the neonatal period as a tool for manipulation of the serotonin neurotransmission. Besides the drug administration has been in the suckling period, there was also stress stimulus with incentive foot shock due the hypothesis that the behavioral effects of drugs that alter serotonin neurotransmission may be more apparent when the animal is more stressed²⁴. The chronic administration of fluoxetine in rats, during the critical period of development of the nervous system, did not provoke alterations in the experimental anxiety profiles. However, the drug decrease locomotor activity in rats submitted to the foot shock stimulus before the elevated plus maze experiment.

There are studies demonstrating after acute administration of fluoxetine there is increase in extracellular serotonin in several subcortical brain regions due to reuptake blockade²⁵. This extracellular 5-HT seems to inhibit the firing of raphe neurons and thus to reduce 5-HT release from nerve terminals⁸. It is often reported that the initial effect of fluoxetine administration in humans is an exacerbation of anxiety²⁵. During chronic administration there is an increase of extracellular concentrations of 5-HT at cortical and subcortical levels. The long-term 5-HT reuptake blockade provokes desensitization of somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors, leading to a disinhibitory effect on raphe neurons firing and to reduced feedback inhibition of 5-HT release²⁵⁻²⁸.

Acute or chronic treatment with fluoxetine in adult animal seems to increase the levels of 5-HT, facilitating anxiogenic effect in the experimental models of anxiety⁸. However, the manipulation of the serotonergic neurotransmission still producing contradictory results in the anxiety studies using antidepressants²⁹ and especially, when it involves fluoxetine and experimental models of anxiety¹⁸.

The animals treated with fluoxetine, in the neonatal period, submitted to foot electrical shock stimulus showed reductions with the fluoxetine group in the number of entries in the closed arms ($p < 0.05$) and in the total of entries ($p < 0.05$), when compared to the control groups. These results seem to indicate reduction of the locomotor activity, suggesting a sedative effect. This suppression could be due to predominant effects in the dorsal raphe reducing serotonergic transmission in the forebrain²⁵. This study did not find alterations in the anxiogenic or anxiolytic profiles of experimental anxiety.

Differently from our work, Ansorge et al.⁹ found anxiogenic effect in mice tested in the elevated plus-maze. However, this work corroborate Silva and Brandão¹⁹ that used fluoxetine (10 mg/Kg, PO) in chronic administration and did not find effect in none of the measures space-time (entrance and exit of the arms or spent time in some of the elevated plus-maze's arms). Although both works have been accomplished with SSRI, using the elevated plus-maze, it is make necessary to present some methodological differences. The first of them refers the lineage of the animal and the second to the period from pharmacologic treatment. In the first work⁹, the animals were mice, while in our work the animals were rats and in the second¹⁹, fluoxetine administration did not happen in the neonatal period. Similar results, also using the elevated plus-maze, were presented before²⁹.

Another question to be considered is the heterogeneity of symptoms presented by the anxiety disorder. The fluoxetine, for example, does not respond appropriately at treatment from widespread anxiety. However, it is used with effectiveness in compulsive obsessive disorder, social phobia, panic disturbance and nervous bulimya⁸.

The pharmacological treatment happened in the suckling period and the behavior tests were made in the adult age in this work, thus the present study showed the increase of serotonin due to the selective serotonin reuptake inhibition in different areas of the brain seem facilitate permanent behavior alterations.

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