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IVERMECTIN ADMINISTERED IN JUVENILE AGE DISRUPT SEXUAL DIMORPHIC BEHAVIORS IN RATS EXPOSED OR NOT TO STRESS

(A ivermectina administrada na idade juvenil prejudica comportamentos sexualmente dimórficos em ratos expostos ou não ao estresse)

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ABSTRACT: Objective: The behavioral effects of two therapeutic ivermectin (IVM) doses administered in the juvenile period of male and female rats, submitted or not to stress, were observed. The open field and elevated plus maze tests were employed. Methods: Male and female rats that were 29 and 45 days of age were divided in six groups: two controls groups injected with the control solution and four experimental groups injected with 0.2 or 1.0 mg/kg of IVM. Twenty-four hours after the last treatments, only half of these rats were submitted to stress and all groups observed in the open field and elevated plus maze. The plasmatic corticosterone levels were measured in stressed and non-stressed rats. Results: 1) 0.2 mg/kg IVM did not affected the sexual dimorphism in the open field and elevated plus maze tests but, the 1.0 mg/kg dose disrupt the sexual dimorphism in both tests; 2) only females treated with the high dose of ivermectin showed increased levels of plasmatic corticosterone levels; 3) stress only impaired the sexual dimorphism in the elevated plus maze, 4) no differences were observed in the plasmatic levels of corticosterone between all groups exposed to stress. Conclusions: IVM high dose disrupt the sexual dimorphism in the open field and elevated plus maze behaviors in the juvenile period. Stress disrupts the sexual dimorphism only in the elevated plus maze, mainly in female rats. These data suggest that female rats were more sensible to IVM, particularly related to anxiety behavior.
Keywords: avermectin, restraint stress, open field, elevated plus maze, rats, behavior

RESUMO: Objetivo: foram observados os efeitos comportamentais das doses terapêuticas de ivermectina (IVM) administradas no período juvenil de ratos machos e fêmeas, submetidos ou não ao estresse. Foram utilizados os testes de campo aberto e labirinto elevado. Métodos: ratos machos e fêmeas com idade entre 29 e 45 dias foram divididos em seis grupos: dois grupos controle foram injetados com a solução de controle e quatro grupos experimentais injetados com 0,2 ou 1,0 mg / kg de IVM. Vinte e quatro horas após os últimos tratamentos, metade desses ratos foram submetidos ao estresse e a outra metade não sendo todos os grupos observados no campo aberto e labirinto em cruz elevado. Os níveis plasmáticos de corticosterona foram medidos em ratos estressados e não estressados. Resultados: 1) 0,2 mg / kg IVM não afetou o dimorfismo sexual em campo aberto e teste de labirinto elevado, mas a dose de 1,0 mg / kg prejudicou o dimorfismo sexual em ambos os testes; 2) apenas as fêmeas tratadas com a maior dose de ivermectina apresentaram maiores níveis plasmáticos de corticosterona; 3) o estresse prejudicou apenas o dimorfismo sexual no labirinto elevado, 4) não foram observadas diferenças nos níveis plasmáticos de corticosterona entre todos os grupos expostos ao estresse. Conclusões: A maior dose de IVM prejudicou o dimorfismo sexual no campo aberto e os comportamentos no labirinto em cruz elevado no período juvenil, principalmente nas fêmeas. Esses dados sugerem que as fêmeas foram mais sensíveis à IVM, em comportamento relacionados à ansiedade.
Palavras-chave: avermectinas, estresse por contenção, campo aberto, labirinto em cruz elevado, ratos, comportamento.

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INTRODUCTION

Avermectins are broad-spectrum antiparasitic agents that are widely used in agricultural, and domestic animals (BLOOMQUIST, 2003) (CASIDA; DURKIN, 2013) Campbell, 2016). In human clinical practice, avermectins is used to treat lymphatic filariasis, onchocerciases, rosacea, scabies, head lice and others (KIRCIK et al., 2016). Ivermectin (IVM) belongs to the macrocyclic lactone class of endectocides and consists of a mixture of two homologous compounds, 22, 23-dihydroavermectin B1a (H2B1a; not b80%) and 22,23-dihydroavermectin B1b (H2B1b; not N20%). In vertebrates, IVM, the first macrocyclic lactone synthesized avermectin (ELGART; MEINKING, 2003), can produce γ-aminobutyric acid (GABA)-mimetic effects by acting as agonists at GABA receptors and stimulating GABA release (DAWSON et al., 2000; ESTRADA-MONDRAGON; LYNCH, 2015; SHOOP; MROZIK; FISHER, 1995). Mammals are less susceptible to the toxic effects of macrocyclic lactones because GABA-mediated nerves occur only in the central nervous system (CNS), and macrocyclic lactones do not readily cross the blood-brain barrier (YANG, 2012)

Previously, we found that IVM at therapeutic dose (0.2 mg/kg) did not alter male rat sexual behavior. However, at a higher dose (0.6 mg/kg) the appetitive phase of sexual behavior in inexperienced male rats was impaired (BERNARDI et al., 2011). The effects of therapeutic and high (1.0 mg/kg) IVM doses were also studied in female rat sexual behavior in physiological and pharmacological conditions. We observed that in both hormonal conditions, 0.2 mg/kg IVM treatment reduced female sexual behavior and the execution of the lordosis reflex(MOREIRA; BERNARDI; SPINOSA, 2014). In addition, other studies performed in our laboratory demonstrated that IVM act as GABAergic agonists and interferes with GABAergic-related behavior, reducing anxiety-like behavior and seizures (DE SOUZA SPINOSA; STILCK; BERNARDI, 2002).

However, no studies were performed in male and female rats treated with IVM in the juvenile period on exploratory/motor and anxiety behaviors. Because male and female rats has different susceptibility to stress (BANGASSER; WICKS, 2017), also the behavioral IVM effects and the plasmatic corticosterone levels were observed after restraint stress .

The period of exposure to IVM was chosen because this drug is used for therapeutic purposes in children who are in a period of hormonal and brain maturation. In addition, in several cases, the effects upon rats were studied only in males or only in females and these variables may be of importance since several modalities of behavior differ qualitatively and quantitatively in male and female rats (FONSECA; SELL; CARLINI, 1976;ROMEO et al., 2016).

MATERIAL AND METHODS

Animals

Male and female Wistar rats 29 days old (male- 88.11± 3.29 g, female- 76.67 ±3.27 g) at the beginning of experiments (Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil) were used. The animals were housed in polypropylene cages (40 × 50 × 20 cm) at a controlled temperature of (20 ± 21 °C) and humidity of (60 ± 5%) under a controlled light/dark schedule (12 h light/12 h dark), with lights on at 10:00 AM for at least 7 days before the experiments. Food (Nuvilab CR1, species-specific ration; Sogorb Ind & Com Ltd, São Paulo, São Paulo, Brazil) and water (filtered in porcelain) were

freely available throughout the study. All of the procedures were reviewed and approved by the Animal Care Committee FMVZ-USP (protocol no. 2881/2013) and conformed with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA(COMMITTEE, 2011).

Drugs

Ivermectin 1% Ivomec injectable, Merial, Paulinia, São Paulo, Brazil) was dissolved in Tween 80 (1 drop/1 ml of 1% Ivomec) and administered subcutaneous (s.c.) at a dose of 0.2 or 1.0 mg/kg. Tween 80 was also administered in the same form as a control solution (1 drop/1 ml of 0.9% NaCl). All of the solutions were prepared immediately before use and administered in a volume of 1 ml/kg body weight.

Procedures

General activity in the open field

An open field (OF) was used to assess the effects of the extract on emotionality and motility and constructed according to Broadhurst (1957). The OF apparatus was a white circular wooden arena based on (FAGGIN; PALERMO-NETO, 1985). The floor of the arena was divided into three concentric circles that were divided into 19 straight segments with equal areas. The circular wooden arena was enclosed inside a wooden case, 48 cm above the floor. The apparatus was placed in a sound-attenuated room with dim light (55 lx at the OF arena). In the OF test, each animal was placed in the center of the arena and observed for 5 min. The animals in the control and experimental groups were alternately observed during the light phase of the light/dark cycle between 9:00 AM and 11:00 AM. The OF was cleaned with a 5% alcohol solution between sessions to remove any odors. We evaluated the total frequency of locomotion, the peripheral locomotion and duration of immobility. One unit of locomotion was defined as the animal entering one area of the arena floor with all four paws. Immobility was defined as the length of time (in seconds) during which the animal did not engage in any motor activity (i.e., the head, trunk, and limbs were still).

Elevated-plus maze

Elevated-plus maze (EPM) is an apparatus first conceived by the British psychologist Sheila Handley's group as a model to evaluate anxiety and it is one of the most used for that purpose (PELLOW et al., 1985). The EPM device used was made of wood and had two open arms (23.5 cm × 8 cm) and two enclosed arms of the same size with 20 cm high walls; the apparatus was elevated 80 cm above the ground, it was placed in a sound-proof room with room lamp of 100W (at the floor of apparatus 400 lx). Basically, two strategies can be easily noticed: avoidance of the open arms while staying in the closed arm and escape from the open arm directly to the closed arm (PELLOW et al., 1985). In the present study, the apparatus was used to assess anxiety, and the animals were assayed after being tested in the OF. The animals were allocated in the center of the maze, which was previously cleaned with 5% alcohol and observed during 3 min. Exploratory behavior was determined by the number of crosses in the center of the EPM. The time and entries in open arms and the risk assessment were employed to evaluate the anxiety-like behavior.

Stress Induction (New York Subway System).

The stress model of the New York subway system was described by Dhabhar&. McEwen (DHABHAR; MCEWEN, 1997) , whose laboratory is

located in New York. It was named because it resembles the situation experienced by an individual boarding the subway during a time of great movement of users: restricted capacity to move the body and continuous shaking.

The apparatus to restrict movement consists of a wood laminated board (23.5 cm length) to which six polyvinyl chloride (PVC) pipes (3 cm diameter x 10 cm length) are attached to restrict the movement of individuals. The pipes have closed ends to prevent escape, but with holes at the front for rear ventilation and allowing passage of the tail. To induce the New York subway stress, the apparatus was placed on a mechanical shaker (Shaker Kline – Nova Ética, Model 108, Vargem Grande Paulista), set to 1 vibration/second. We exposed the rats to 1 hour of stress, during which the animals had no access to acid solution or feed. Animals were subjected to 1h of stress at 45 days old.

Corticosterone plasmatic levels

The blood was collected in conical tubes that contained 10% Ethylenediaminetetraacetic acid. The samples were centrifuged, and plasma was obtained. Plasma samples from each animal were aliquoted in several conical tubes for analyses (in duplicate) of corticosterone using commercial enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. Corticosterone levels were determined using an Arbor Assays kit (catalog no. K014-H5, Ann Arbor, MI, USA). The results are expressed as ng/ml.

Experimental design

Forty-two male and forty-two female rats 29 days old were divided into six groups. The following groups were formed: 1) control male and control female groups injected with the control solution (n=14/group); 2) experimental male and female groups injected with 0.2 mg/kg of IVM (n=14/group); 3) experimental male and female groups injected with 1.0 mg/kg of IVM (n=14/group). At 44 days old these treatments were repeated and 24 h after, 1) half of these rats were observed in the OF and EPM; 2)the other half of rats were submitted to 1 h of stress and also observed in the OF and EPM. Immediately after the EPM, the rat trunk blood was collected to evaluate the corticosterone levels.

Statistical analysis

Homogeneity was verified using Bartlett's test. Normality was verified using the Kolmogorov-Smirnov test. Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple-comparison test was used to compare the data. The results are expressed as the mean ± SEM. In all cases, the results were considered significant at $p < 0.05$.

RESULTS

Effects of IVM treatment in male and female rats not exposed to stress.

Fig.1 shows the general activity of male and female rats treated with 0.2 or 1.0 of IVM in the juvenile age observed in the OF at 45 days age.

Concerning the total locomotion (fig.1A) the two way ANOVA did not show interaction between sex and treatments ($F_{2, 36} = 0.82$, $p = 0.45$), the sex influenced the results ($F_{1,36} = 23.3$, $p < 0.0001$) but not the treatments ($F_{2,36} = 2.15$, $p = 0.13$). The Bonferroni test indicates an increased total locomotion in female of control and 0.2 mg/kg IVM relative to control and 0.2 mg/kg IVM groups of males, respectively. No differences were observed between male and female treated with 1.0 mg/kg of IVM.

In peripheral locomotion (fig.1B) the two way ANOVA did not show interaction between sex and treatments ($F_{2,36} = 0.68$, $p = 0.52$), the sex influenced

the results (F 1,36=6.91, p<0.01) but not the treatments (F2,36=2.67,p=0.08). The Bonferroni test indicates an increased peripheral locomotion in female of control group relative to control males. No differences were observed between male and female treated with both IVM doses.

Relative to immobility (fig.1C) no interaction between sex and treatments (F2,36=2.03,p=0.15) was observed; the sex influenced the results (F1,36=8.42, p<0.006) but not the treatments (F2,36=1.45,p=0.25). The Bonferroni test indicates a decreased immobility in female of control group relative to control males. No differences were observed between male and female treated with both IVM doses.

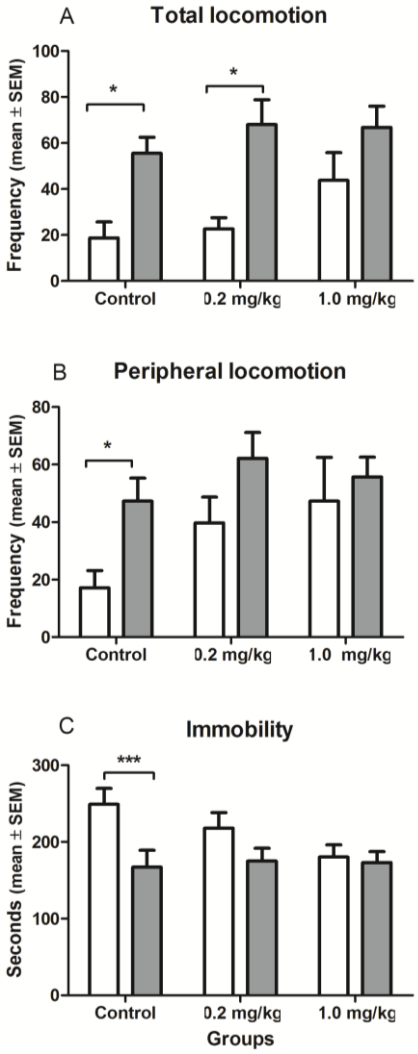

Figure 1- General Activity of male and female rats treated with 0.2 or 1.0 of IVM in the juvenile age observed in the open field at 45 days age. A- Total locomotion frequency; B-peripheral locomotion frequency; C- immobility duration (sec). Data are presented as means ± SEM. N=7/group. Two way ANOVA followed by the Bonferroni test. *p<0.05, ***p<0.0001, relative to the male rat of the same treatment.

Fig.2 illustrates the effects of IVM treatment in male and female rats observed in the EPM at 45 days of age.

Relative to time in open arms (fig.2A), no interaction between sex and treatments (F2,36=1.08,p=0.35) and between treatments (F2,36=0.17,p=0.84) were observed but sex influenced the results (F1,36=4.51,p=0.04). Female rats of control and 0.2 mg/kg of IVM remained more time in the open arms than males of the same treatments. No differences were detected between male and female rats treated with 1.0 mg/kg of IVM.

Concerning the number of entries in the open arms (fig.2C) and the number of crosses (fig.2D), no differences were observed between sex and treatments without interactions between factors (entries in open arms- interaction –F2,36=2.85,p=0.07; treatments- F2,36=1.10,p=0.32; sex- F1,36=0.01,p=0.93; number of crosses- . interaction –F 2,36=1.15, p=0.33; treatments- F2,36 =0.91, p=0.42; sex- F1,36=3.3, p=0.07).

Relative to risk assessment (fig.2D) differences were observed between treatments (F2,36= 4.76,p=0.03) but not relative to sex (F1,36=0.03,p=0.97) without interaction between factors (F2,36=2.33,p=0.11). Female rats of control group showed increased risk assessment than male of control group. No differences were observed between male and female treated with both IVM doses.

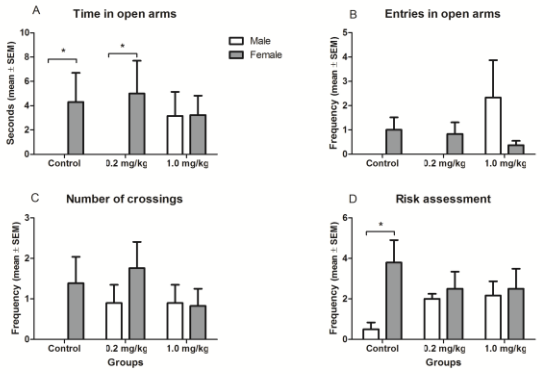

Figure 2- Elevated plus maze behavior of male and female rats treated with 0.2 or 1.0 of IVM in the juvenile age observed at 45 days age. A- Time in open arms; B-number of entries in the open arms; C- number of crossings the center of the elevated plus maze; D- number of risk assessment. Data are presented as means ± SEM. N= 7/group. Two way ANOVA followed by the Bonferroni test. *p<0.05 relative to the male rat of the same treatment.

Fig.3 illustrates the effects of IVM treatment on plasmatic corticosterone levels of male and female rats at 45 days of age. No interaction was observed between sex and treatments (F2/30=0.55, p=0.5); the sex (F1,30=7.35, p=0.01); the treatments (F1/30=3.87, p= 0.03) influenced the results. The post hoc test indicates an increased corticosterone plasmatic level in female rats treated with 1.0 of IVM relative to male treated with the same dose. No differences were observed between male and female of control and treated with 0.2 mg/kg of IVM dose groups.

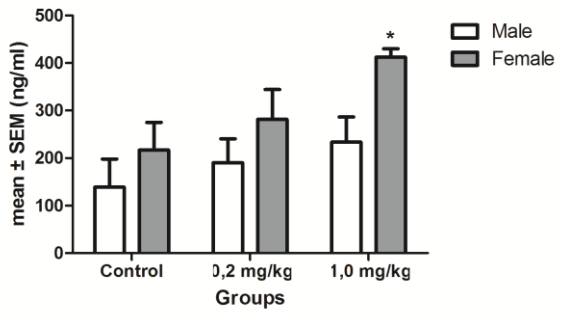

Figure 3- Corticosterone plasmatic levels of male and female rats treated with 0.2 or 1.0 of IVM in the juvenile age evaluated at 45 days age. Data are presented as means ± SEM. N= 7/group. Two way ANOVA followed by the Bonferroni test. *p<0.05 relative to the male rat of the same treatment.

Effects of IVM treatment in male and female rats exposed to stress.

Fig.4 shows the general activity of male and female rats exposed to stress treated with 0.2 or 1.0 of IVM in the juvenile age observed in the OF at 45 days age.

Concerning the total locomotion (fig.4A) the two way ANOVA did not show interaction between sex and treatments (F2,36=0.60, p=0.56), the sex influenced the results (F1,36=5.22, p=0.03) but not the treatments (F2,36=0.76,p=0.48). The Bonferroni test indicates an increased total locomotion in female of control and 0.2 mg/kg IVM relative to control and 0.2 mg/kg IVM groups of males, respectively. No differences were observed between male and female treated with 1.0 mg/kg of IVM.

In peripheral locomotion (fig.4B) the two way ANOVA did not show interaction between sex and treatments (F 2,36=1.01, p=0.38); the sex influenced the results (F1,36= 7.11, p= 0.01) but not the treatments (F2,36=1.92, p=0.16). The Bonferroni test indicates an increased peripheral locomotion in female of control and 0.2 mg/kg IVM relative to control and 0.2 mg/kg IVM groups of males, respectively. No differences were observed between male and female treated with 1.0 mg/kg of IVM.

Relative to immobility (fig.4C) no interaction between sex and treatments (F2,36 =0.52, p=0.60) was observed; the sex (F1,36=0.78, p=0.89) and treatments (F2,36=0.11, p=0.38) did not influenced the results.

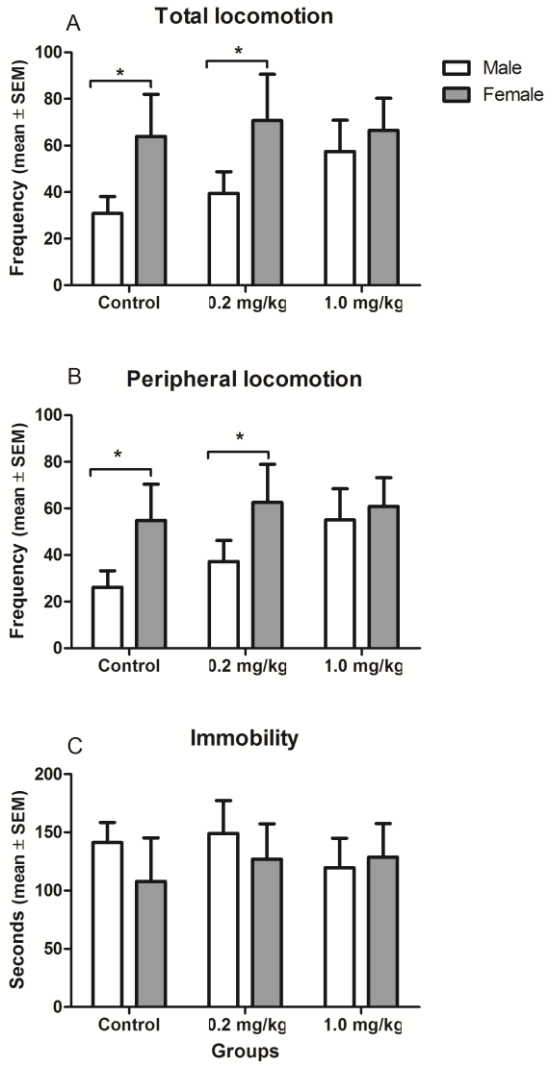

Figure 4- General Activity of male and female rats submitted to stress, treated with 0.2 or 1.0 of IVM in the juvenile age observed in the open field at 45 days age. A- Total locomotion frequency; B-peripheral locomotion frequency; C- immobility duration (sec). Data are presented as means ± SEM. N= 7/group. Two way ANOVA followed by the Bonferroni test. *p<0.05 relative to the male rat of the same treatment.

Fig.5 illustrates the effects of IVM treatment in male and female rats exposed to stress and observed in the EPM at 45 days of age.

Relative to time in open arms (fig.5A), an interaction was observed between sex and treatments (F2,36=4.22, p=0.02), but not between treatments (F2,36=0.64, p=0.43) and sex (F 1,36=0.08, p=0.92)

Concerning the number of entries in the open arms (fig.5B) no differences were observed between sex and treatments without interactions between factors (interaction – F2,36=2.32, p=0.14; treatments- F2,36=0.29, p=0.75; sex-F1,36=0.29, p=0.75).

Relative to the number of crossing (fig.5C), an interaction between factors were observed (F2,36=4.21, p=0.02) but not between treatments (F2,36=2.25, p=0.14) and sex-(F 1,36=0.96, p=0.39). The Bonferroni test indicates that female of control groups showed increased crossings relative to control males.

Relative to risk assessment (fig.5D) no interaction between sex and treatments (F 2,36=0.50, p=0.61, treatments (F2,36=1.22, p=0.28) and sex (F1,36=0.31, p=0.73) were observed.

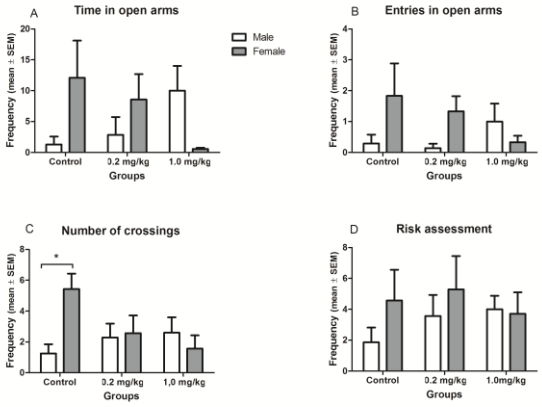

Figure.5 - Elevated plus maze behavior of male and female rats exposed to stress and treated with 0.2 or 1.0 of IVM in the juvenile age observed at 45 days age. A- Time in open arms; B-number of entries in the open arms; C-number of crossings the center of the elevated plus maze; D- number of risk assessment. Data are presented as means ± SEM. N= 7/group. Two way ANOVA followed by the Bonferroni test. *p< 0.05 relative to the male rat of the same treatment.

Fig.6 illustrates the effects of IVM treatment on plasmatic corticosterone

levels of male and female rats exposed to stress at 45 days of age. No interaction between sex and treatments (F2/38 = 0.55, p= 0.5), sex (F1, 38 = 0.15, p=0.70) and treatments (F2/38 = 2.23, p = 0.12) were observed.

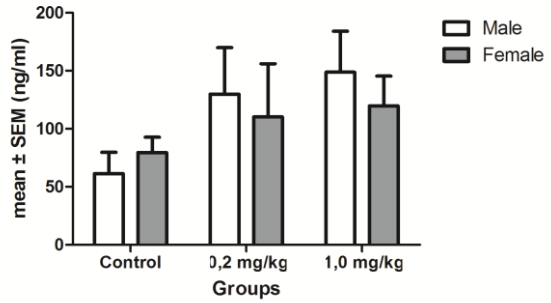

Figure 6- Corticosterone plasmatic levels of male and female rats submitted to stress and treated with 0.2 or 1.0 of IVM in the juvenile age evaluated at 45 days age. Data are presented as means ± SEM. N= 7/group. Two way ANOVA.

DISCUSSION

Sex differences in OF activity appear at defined developmental stages (11) and are maintained in adult life. Females are more active than males (ANDERSON, 1940; BROADHURST, 1957) and it has been suggested that these differences in behavior reflect sex differences in emotionality or susceptibility to the arousal of fear, males being held to be more fearful than females (GRAY, 1971). In developmental studies, these differences were shown to emerge in the peripuberal period at 50-60 days of age (BLIZARD; LIPPMAN; CHEN, 1975).

Our results corroborated with these data because a sexual dimorphic behavior of male and female of control groups was observed at 45 days of age where female rats explore more the OF than males. In fact, the total and peripheral locomotion is higher in female than in males of control groups with reduced immobility in females. However, these differences were attenuated in rats treated with 0.2 mg/kg of IVM (no differences in immobility) and disappears after 1.0 mg/kg.

Decreased locomotion frequency and increased immobility time and vice-versa are interpreted in the OF as interference with motor/exploratory behavior. These data suggest that female rats of control groups showed increased motor/exploratory behavior. Studies in the OF take the ambulation and defecation as a double that express emotionality mainly when studies were performed of sexual dimorphism. Because of the criticisms raised against the defecation score as a measure of the central effects of drugs(CUNHA; MASUR, 1978), in the present experiment we did not measured defecation but the peripheral locomotion. Typically, rats are more active and remain longer in the periphery in the OF in comparison to the central areas, mainly in the first session, a preference that has been interpreted as being determined by thigmotaxis (VALLE, 1970). Thigmotaxis (the tendency to remain close to vertical surfaces) ay related to the grade of aversion to open areas (LAMPREA et al., 2008). Our control group showed increased peripheral locomotion in the OF also suggesting increased aversion to open areas of the OF.

Treatment at 30 and 45 days of age with IVM reduced the sexual dimorphism, mainly in the high dose, by reduced the locomotor of female rats in the OF. Previously we observed that IVM reduces sexual behavior in female rats in natural estrus (physiological condition) in normal cycling rat or induced in intact female rats with estradiol valerate (MOREIRA; BERNARDI; SPINOSA, 2014). In cows, administration of IVM at therapeutic dose induces a sharp decrease in serum FSH, LH and estradiol and increased serum progesterone, prolactin and cortisol that return to normal levels on the 90th day following injection. Serum

sex hormones binding globulin and testosterone were not affected throughout the experiment (SADEK; SHAHEEN, 2015). However, a therapeutic IVM dose had no detrimental effect on the reproductive performance of ewes during the breeding season. In the present study IVM was administered in prepubertal and pubertal ages, critical periods of the brain sexual activation (WILSON; DAVIES, 2007). Thus it is possible that IVM exposure during these periods, by affecting hormonal milieu of male and female rats, affected the sexual dimorphism on behavioral responses observed in the OF.

Another hypothesis to explain the reduced sexual dimorphism here observed is the involvement of the GABAergic system on the regulation of the hypothalamic –pituitary-adrenal axis (HPA) or −gonadal (HPG) axis (CULLINAN; ZIEGLER; HERMAN, 2008). Activation or blockade of the GABA-A receptors during early life induces brain and behavioral abnormalities in adulthood, and may alter physiological phenotypes in a sex-dependent manner in mice (SALARI; AMANI, 2017). Thus, IVM acting as agonists at GABA-A receptors and stimulating GABA release (DAWSON et al., 2000) could affect the hormone regulating sexual activation during puberty.

Also in the EPM, these behavioral differences between male and female rats were observed. Female rats showed a reduced aversion for the open arms in EPM compared to male rats and tended to make more number of risk assessment indicating a higher overall level of activity in this test. These findings are in agreement with previous report which suggested that female Wistar rats were less anxious than male Wistar rats based on their performance in an EPM test(IMHOF et al., 1993). Treatment with 0.2 mg/kg of IVM did not modify the time in open arms of female but reduced the risk assessment. In male and female rats treated with 1.0 mg/kg this sexual dimorphism disappears.

No sexual dimorphism was observed between control and rats treated with 0.2 mg/kg of IVM on corticosterone plasmatic levels. These sexual differences were observed in rats treated with 1.0 mg/kg where female had increased levels in this hormone relative to male rats. This increased in corticosterone levels could explain the reduced time in the open arms of female treated with 1.0 mg/kg of IVM relative to males of the same treatment, and the lack of sexual dimorphism observed in the EPM at this dose.

Stress exposure had few effects on sexual dimorphism of male and female rats observed in the OF. In fact, control and 0.2 mg/kg of IVM showed a clear dimorphic behavior in total and peripheral locomotion while no differences were observed between male and female rats treated with 1.0 mg/kg of IVM. Only the sexual dimorphism in immobility behavior of control male and female disappear. Thus, our model of stress did not affected the sexual dimorphism effects of IVM in the OF behavior.

The present data indicate sex differences, on behavioral effects, of IVM administered in early and middle adolescence. In both sexes of stressed and non-stressed rats, observed in the OF, female rats are more susceptible than male rats, to the effect of the drug in the OF.

In the EPM, a great variability was observed in female responses relative to males but no differences were observed in all parameters between sexes, except in the number of crosses in the control group. Female control rats crossed more the center of the EPM than male rats.

Concerning the corticosterone levels of stressed rats, no differences were observed between sexes of all groups. Thus, in the EPM and corticosterone levels, stress reduced the sexual dimorphism while no interferences in the OF behavior was observed.

It was found that the adult-like ACTH stress response, from the pituitary, develops during the later stages of adolescence (PND46 to PND59), while the corticosterone response from the adrenal gland changes earlier between the PND30 to PND40 (FOILB; LUI; ROMEO, 2011). These results indicate that shifts in hormonal stress responses occur throughout adolescence and that each gland along this neuroendocrine axis displays a unique developmental trajectory(ROMEO et al., 2016). Thus, in our study, the restraint stress was applied in middle adolescence age of rats, when the ACTH stress response from the pituitary was developed, to verify the effects of IVM in this phase. In addition, gender is one major variable related to differential vulnerability to stress. Most of the data about the effects of stress, during early period of life and adolescence, were performed in male rats. Few studies relative to females and chronic stress during adolescence reported a greater susceptibility to outcomes of female rats than males (DALLA et al., 2005;ROMEO et al., 2016; MCCORMICK; SMITH; MATHEWS, 2008). Moreover, Traslaviña *et al.*, (2014) reported that both, adult behavior and the glucocorticoid stress response , are affected differently in males versus females by adolescent stress. The duration of stressors had a greater effect on corticosterone and progesterone response in males, whereas the nature of the stressor had a greater effect on exploratory behavior in females. Thus, we suggest that our stress model applied during the juvenile period affected mainly female's behavioral responses after IVM high dose. Because IVM is used in children and adolescents, the present data could have clinical implications relative to gender effects.

CONCLUSIONS

A lack of sexual dimorphism was observed in the OF and EPM tests after the IVM high dose administered during the juvenile period. We attribute this effect by a disruption in female rats in the EPM to the high levels of corticosterone. The 0.2 mg/kg/ did not affected the sexual dimorphism in both behavioral tests. Restraint stress was unable to alter the effects of both IVM doses in the OF behavior but, in the EPM, the sexual behavioral dimorphism was impaired in controls and experimental groups as well as no differences were observed in the corticosterone plasmatic levels of male and female of all groups.

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