Universidade Estadual de Londrina

HELOÍSA MARIA COTTA PIRES DE CARVALHO

DIFERENÇAS DE GÊNERO EM MODELOS ANIMAIS DE DEPRESSÃO E ANSIEDADE: O EFEITO DA EXPOSIÇÃO AO ESTRESSE CRÔNICO

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Análise do Comportamento da Universidade Estadual de Londrina.

Orientadora: Prof. Dr. Célio Roberto Estanislau

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Dissertação apresentada para cumprimento dos requisitos para a obtenção do título de Mestre em Análise do Comportamento.

COMISSÃO EXAMINADORA

Orientador: Prof. Dr. Célio Roberto Estanislau
Universidade Estadual de Londrina

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RESUMO

Os transtornos de depressão e ansiedade representam alguns dos problemas de saúde mais comuns, no mundo todo. Modelos animais têm sido elaborados e amplamente utilizados para se estudar a depressão e a ansiedade, como o nado forçado e o labirinto em cruz elevado, respectivamente. Modelos animais são particularmente úteis para se estudar depressão e ansiedade e a interação entre esses dois transtornos, principalmente, em situações nas quais o impacto do estresse não pode ser estudado em humanos, devido a questões éticas. Adicionalmente, a maioria das pesquisas utiliza somente ratos machos como sujeitos, havendo, assim, uma escassez de pesquisas que levem em consideração o comportamento de fêmeas e as diferenças existentes entre os gêneros. Diante disso, o presente estudo tem como objetivo avaliar as possíveis diferenças de gênero nos comportamentos exibidos durante o procedimento de nado forçado repetido e durante o teste no labirinto em cruz elevado. Ratos Wistar machos e fêmeas foram distribuídos em três grupos: nado forçado (n=24), manuseio (n=24) e controle (n=24). Após 14 dias de exposição a cada condição, os animais foram testados no labirinto em cruz elevado por 10 min. Não houve diferenças de gênero durante as sessões de nado forçado, havendo somente diferença da primeira sessão para com as demais. No labirinto em cruz elevado, o número de entradas nos braços abertos de fêmeas que nadaram e o tempo gasto nestes braços de machos que nadaram foram menores do que os de seus grupos controle. Houve menos entradas nas extremidades dos braços abertos para machos e fêmeas que nadaram do que para seus grupos controle. O tempo nos braços fechados dos animais que nadaram foi maior do que o do grupo controle, para ambos os gêneros.

Palavras-chave: gênero, nado forçado, labirinto em cruz elevado, ansiedade, depressão, modelo animal.

ABSTRACT

The anxiety and depression disorders are the most common health problem in the entire world. Animal models has been elaborated and widely used for the study of depression and anxiety, like forced swimming and elevated plus-maze, respectively. Animal models are particularly useful for the study of anxiety and depression and the relationship between both disorders, mainly, in situations where the impact of stress cannot be studied in humans for ethics questions. Additionally, the most of researches uses only male rats and few researches utilize females and evaluate the gender differences. Therefore, the aim of the study is evaluate the possible gender differences in the repeated forced swimming and in the elevated plus-maze. Male (n=36) and female (n=36) Wistar rats were divided in three groups: forced swimming (n=24), handled (n=24) and control (n=24). After 14 days of exposition in each condition, the animals were tested in the elevated plus- maze for ten minutes. There was no gender difference in the forced swimming, only session difference. In the elevated plus-maze, the numbers of entries in the open arms from females swam and the time spent in the open arms from males swam were less than their control groups. There were fewer entries in the open arms extremities to males and females swam than their respective control groups. Time spent in the closed arms from males and females swam was longer than their control groups.

Key-words: gender, forced swimming, elevated plus-maze, anxiety, depression, animal model.

1. Introduction

Animal models have been widely used as tools to study the genetic, behavioral and neurobiological basis of anxiety and depression. However, little attention has been given to the potential utility of these experimental models to study the relation between both disorders (Hinojosa, Spricigo Jr, Izídio, Brüske, Lopes & Branches, 2006).

The forced swimming stress is a putative animal model of depression, which emulates the behavioral despair paradigm of depression (Porsolt el al., 1978). The forced swim procedure is commonly used to investigate the potential antidepressant of drugs and the neurobiological and behavioral factors involved in the depression (Andreatini & Bacellar, 2000). Moreover, forced swim can be used as a stressor too (Boyce-Rustay, Cameron & Holmes, 2007). It is adopted because increasing evidence implicates stress as an important factor in the vulnerability to depression and other behavioral disorders (Qi, Lin, Li, Pan & Wang, 2006). Some studies (Dal-Zotto, Martí & Armario, 2000; Boyce-Rustay et al., 2007; Stone, Lehmann, Lin & Quartermain, 2007) demonstrated that repeated exposition to the forced swimming procedure alters the same behaviors that in other studies had shown sensible to the antidepressant action (see Cryan, Markou & Lucki, 2002).

The elevated plus-maze has been used for investigate the behavioral effects induced from chronic exposition to different stressful events, for example: restraint (Vyas, Mitra, Rao & Chattarji, 2002; Vyas & Chattarji, 2004; Gameiro, Gameiro, Andrade, Pereira, Arthuri, Marcondes & Veiga, 2006), elevated open platform (Storey, Robertson, Beattie, Reid, Mitchell & Balfour, 2006), forced swimming (Qi, Lin, Li, Pan & Wang, 2006; Qi, Lin & Li, 2006) and variable stress (Vyas et al., 2002; Vyas & Chattarji, 2004; Matuszewich, Karney, Carter, Janasik, O'Brien & Friedman, 2007; Zurita, Martijena, Cuadra, Brandão & Molina, 2000). In these experiments, except the variable stress, there were less entries or time spent in the open arms from animals submitted to chronic stress, suggesting that these animals were more anxious. Therefore, results from these researches indicate that the elevated plus-maze is an animal model which contributes to understanding the behavioral processes associated to anxiety.

The association between anxiety and depression has been little studied in the animal research (Andreatini & Bacellar, 1999). Few authors studied the relationship between these disorders (Braw, Malkesman, Dagan, Bercovich, Lavi-Avnon, Schroeder, Overstreet & Weller, 2006; Hinojosa et al., 2006; Qi, Lin, Li, Pan & Wang, 2006; Qi, Lin & Li, 2006). Braw et al. (2006) and Hinojosa et al. (2006) analyzed the influence of genetic variables using different littermate of rats in the development of comorbidity between anxiety and depression. The others authors (Qi, Lin, Li, Pan & Wang, 2006; Qi, Lin & Li, 2006) studied the influence of chronic forced swimming in the elevated plus-maze test. Nevertheless, any of these researches compared the effects of forced swimming on the male and female behaviors in the elevated plus-maze.

It is generally accepted that there is a sex difference related to stress response and depressive-like behavior in various experimental procedures (Rivier, 1999; Palanza, 2001). There are few experimental studies that have focused simultaneously on the effects of depression-like models in both sexes (Drossoupoulou, Antoniou, Kitraki, Papathanasiou, Papalexi, Dalla & Papadopoulou-Daifoti, 2004). Behavioral, physiological and neurochemical data published until now used mainly males as subjects. Very few behavioral observations were performed with females (Barros & Ferigolo, 1998). In animal research, different effects between males and females have been found in stress conditions like maternal separation, prenatal stress and restraint (Barna, Balint, Baranyi, Bakos, Makara & Haller, 2003; Bowman, MacLusky, Sarmiento, Frankfurt, Gordon & Luine, 2004; Bowman, Ferguson & Luine, 2002). However, few researches has been planned to evaluate gender differences (Dalla, Antoniou, Drossopoulou, Xagoraris, Kokras, Sfikakis & Papadopoulou-Daifoti, 2005; Barros & Ferigolo, 1998; Drossoupoulou et al., 2004; Estanislau e Morato, 2006). Barros and Ferigolo (1998) and Drossoupoulou et al. (2004) evaluated behavioral measures from males and females in the forced swimming test-retest. Dalla et al. (2005) analyzed behavioral measures from males and females in the forced swimming test-retest after chronic mild stress exposition and Estanislau and Morato (2006) investigated the influence of prenatal stress in male and female rats at different ages tested in the elevated plus-maze. The results obtained in these studies, except from Drossoupoulou et al. (2004), were opposite from clinical literature, suggesting necessity of more researches.

This study investigated the possible gender differences in the behaviors exhibited in the forced swimming sessions and in the elevated plus-maze test, after exposure to chronic forced swimming procedure.

2. Materials and methods

2.1. *Subjects*

Thirty six male and thirty six virgin female Wistar naïve rats (weighing 250-300 g), fifthy days old, were housed in polypropylene cages (40 cm x 34 cm x 17 cm) in same sex groups of three. All the rats in a cage were submitted to the same treatment. In this cage there were food and water available *ad libitum*. In the room, where the cages were housed, the temperature was maintained between 21-25°C and it was established a 12:12h light/dark photoperiod (lights on at 7:00 a.m.). Each animal was weighed in the

1^{st}, 5^{th}, 10^{th} and 14^{th} days. No experimental procedure was made before passed 72 h the animals arrived to the *vivarium*. All procedures were approved by the Ethics Committee for Experimental Procedures of the Londrina State University.

2.2. *Forced swimming*

The 36 animals of each gender were divided into three groups. Twelve rats were submitted to forced swimming (FSW) which was performed between 08:00h and 12:00h in a black plastic cylinder (height: 50 cm, diameter: 22 cm) filled with water to a depth of 30 cm (25 ± 2°C). Each rat was forced to swim individually for 15 min each day for 14 consecutive days. Water was always changed and the cylinder was cleaned (ethanol 5 %) between sessions. The animals were dried with a cloth (10-20 s) after the sessions and returned to the cages. Twelve rats were submitted to the same procedure but in a cylinder without water as a handling control (HAN). Twelve rats were kept at the *vivarium* for the same period and were used later as a control group (CTL). The time spent in the following behaviors were measured: *floating* (behavior during which the animal uses just enough limb movement to keep the nose or the head above surface of water); *swimming* (move constantly the forelimbs or hindlimbs); *climbing* (all four limbs in vertical motion against the wall of the cylinder) and *diving* (when the whole body, included the head, stayed underwater). In addition, the *latency to start floating* was recorded. The diving behavior from males and females was more frequent in the first session of forced swimming. In the others sessions, it stayed proximate from zero. All the measures were made for a trained observer with intra-observer concordance of 85%, at least.

2.3. *Elevated plus-maze test*

In the day following the last session of forced swimming, all of the rats were tested individually in a wooden elevated plus-maze. Briefly, the apparatus consisted of two opposite open arms (50 cm x 12 cm) and two opposite closed arms (50 cm x 12 cm x 40 cm). The arms were connected by a central square (12 cm x 12 cm). To avoid falls, open arms were provided with 1-cm Plexiglas edges. The apparatus was 50 cm above the floor. It was cleaned with a 5%-ethanol solution and dried with a paper towel between sessions. The experimental room was lit by a 60-W bulb placed two meters above the elevated plus-maze. All sessions were video recorded by a camera placed 1.8 m above the apparatus. Each rat was gently placed in the center of the maze facing one of the closed arms. Ten minutes later, the rat was returned to its home cage. Plus-maze sessions were performed between 14:00 and 18:00 h. Observations were done from the TV set screen. Time spent in the open and closed arms and the number of entries into both arms was recorded. Whenever a rat entered with all four paws into an arm an entry was recorded. The frequency and time spent in the following behaviors were also measured: *head out* (sticking the head outside the maze border with at least one of the ears outside the border; as performed in the closed arms it correlates positively while in the open arms it correlates negatively with anxiety (Espejo, 1997)); *stretching attend postures* (an exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward; closed arm stretching correlates positively with anxiety while open arm stretching correlates with approach-avoidance conflict (Espejo, 1997)); *flat back approach behavior* (exploratory locomotion where the animal stretches to its full length and cautiously moves forward;); *rearing* (rising on the hind limbs both touching and not touching a wall surface) and *freezing* (the lack of all movements apart from those necessary for respiration). The head out, stretching attend postures and flat back approach behaviors were divided into regions on the plus-maze where they occurred: protected area – closed arms and central square – and unprotected area – open arms (Rodgers & Johnson, 1995).

The grooming behavior was analysed through a protocol modified from Kalueff and Tuohimaa (2004). Grooming was divided in four patterns, i.e.: (1) paw licking and nose/face wash; (2) head wash; (3) body grooming and leg licking; (4) tail/genitals grooming and (0) indicating no grooming. These grooming patterns normally proceed in a cephalocaudal progression (Kalueff & Tuohimaa, 2005) which corresponds to expected transitions in this study. Unexpected transitions did not follow this progression. The total frequency and total duration of grooming behavior and the latency to start grooming were measured. In addition, were recorded the percentage of unexpected transitions between grooming patterns, the total number of transitions (the sum of expected and unexpected transitions), the frequency and time spent in each grooming pattern, frequency and duration of the interruptions (interruptions longer than 5 s determine separate grooming episodes) and percentage of interrupted bouts.

For the three female groups, a vaginal smear was collected immediately after the elevated plus-maze test. The estrous cycle phase for all female rats was determined by vaginal smears cytology always by the same investigator. It was found that there were similar numbers of rats in each cycle phase among the groups. Therefore, no further analyses were performed in respect to estrous cycle phase.

2.4. *Adrenal weight*

Adrenal glands were removed, dissected from fat and weighted.

2.5. *Data analysis*

In the forced swimming data comparisons between the groups were performed using two-way analyses of variance (ANOVA) for repeated measures, with the gender as one factor (two levels: male and female) and session as the repeated measure factor (fourteen sessions). In the elevated plus-maze data comparisons between the groups were performed using three-way ANOVAs for repeated measures, with the factors gender (two levels: male and female), chronic treatment (three levels: FSW, HAN and CTL) and elevated plus-maze session period as the repeated measure (two levels: first five minutes and last five minutes). Whenever appropriate, *post hoc* Fisher LSD test was performed. The significance level was set at $P < 0.05$.

3. Results

3.1. Forced swimming

Comparisons between the sessions of forced swimming showed that latency to start floating ($F_{[13,286]} = 19.086$, $P < 0.001$), climbing ($F_{[13,286]} = 16.081$, $P < 0.001$), swimming ($F_{[13,286]} = 2.468$, $P < 0.01$) and floating ($F_{[13,286]} = 2.727$, $P < 0.01$) were modified by the reexposure to the forced swimming procedure. It is shown at Figure 1. Post hoc comparisons showed that latency to start floating of females decreased since the second forced swimming session while latency of males decreased since the third session, both in comparison with the first forced swimming session. Additionally, climbing behavior of the females decreased since the fourth forced swimming session while climbing of the males decreased since the third session, in comparisons with the first forced swimming session. Further, swimming behavior of the females increased only in the sessions thirteen and fourteen while swimming of the males increased only in the session fourteen. Finally, floating behavior of the females increased in the sessions four, nine and ten in comparisons with the first forced swimming session. Floating behavior of the males increased from session four to session nine and in the sessions twelve and thirteen in comparisons with the first forced swimming session. For all the behavioral measures studied, there was no significant difference between male and female rats in the forced swimming sessions.

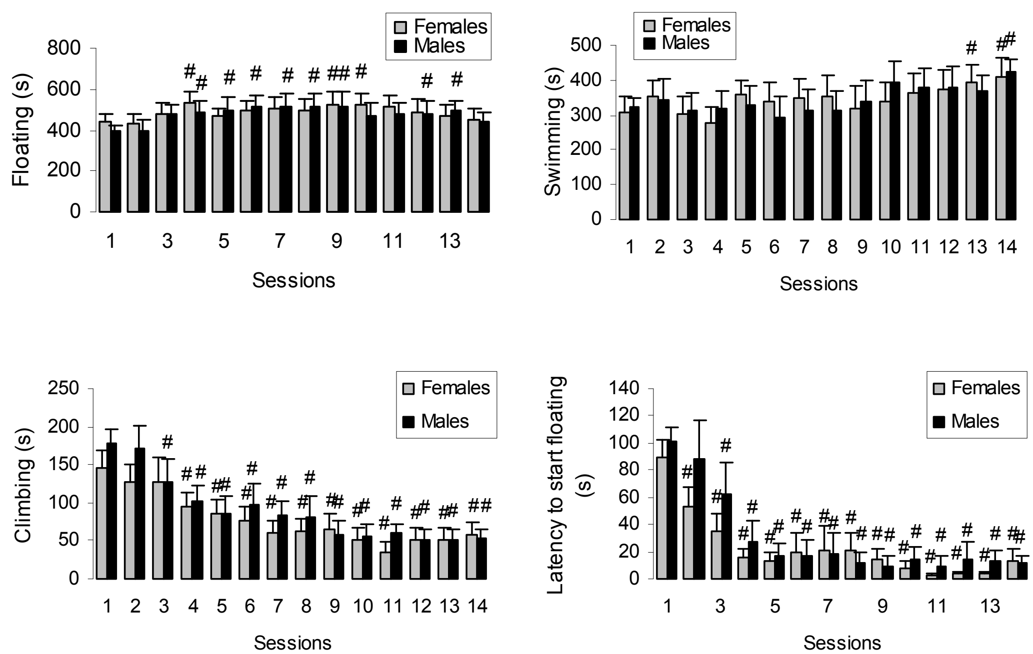

Figure 1. Durations of floating, swimming, climbing behaviors and the latency to start floating from males and females along forced swimming sessions. #difference from the first forced swimming session of respective gender.

3.2. Elevated plus-maze

Number of entries into the open arms is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 6.844$, $P < 0.01$) and session period ($F_{[1,66]} = 104.676$, $P < 0.001$). Additionally, a chronic treatment per session period ($F_{[2,66]} = 4.525$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that within handled groups, female's entries into the open arms were more frequent than that from their correspondent male group in the 6-10 session period. In addition, it was found that male and female handled groups had more entries into the open arms than their respective control groups during the 1-5 session period. Such effect extended to the 6-10 session period only for females. Females submitted to forced swimming entered into the open arms less than their correspondent control group during the 1-5 session period. Furthermore, number of entries into the open arms decreased from 1-5 to 6-10 session period for all the groups, except for males submitted to forced swimming.

Number of entries into the closed arms is shown at Table 1. Main effects were found for the factors gender ($F_{[1,66]} = 5.033$, $P < 0.05$) and session period ($F_{[1,66]} = 70.666$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that control and handled females, during the 1-5 session period, had more entries into the closed arms than their correspondent male groups. Such effect extended to the 6-10 session period only for control group. Also, it was found that males submitted to forced swimming, during the 1-5 session period, had more entries into the closed arms than their respective control group, such effect extended to the 6-10 session period. Handled males had more entries into the closed arms than their respective control group in the 6-10 session period. Moreover, number of entries into the closed arms decreased from 1-5 to 6-10 session period for all the groups.

Percentage of entries into the open arms is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 5.888$, $P < 0.01$) and session period ($F_{[1,66]} = 23.074$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that percentage of entries into the open arms decreased from 1-5 to 6-10 session period only for male and female control groups and for handled males.

Number of entries into the open arm extremities is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 6.791$, $P < 0.01$) and session period ($F_{[1,66]} = 80.218$, $P < 0.001$). In addition, a chronic treatment per session period ($F_{[2,66]} = 12.292$, $P < 0.001$) significant interaction was found. Post hoc comparisons showed that handled females had more entries into the open arm extremities than their correspondent male group in the 6-10 session period. Additionally, it was found that male and female handled groups had more entries into the open arm extremities than their respective control groups during the 1-5 session period. Male and female rats submitted to forced swimming entered into the open arm extremities less than their correspondent control groups in the 1-5 session period. Furthermore, number of entries into the open arm extremities decreased from 1-5 to 6-10 session period for all the groups, except for male and female rats submitted to forced swimming.

Number of entries into the closed arm extremities is shown at Table 1. Main effects were found for the factors gender ($F_{[1,66]} = 6.513$, $P < 0.05$) and session period ($F_{[1,66]} = 96.720$, $P < 0.001$). Also, a chronic treatment per session period ($F_{[2,66]} = 4.974$, $P < 0.01$) significant interaction was found. Post hoc comparisons showed that control and handled females entered into the closed arm extremities more than their correspondent male groups in the 1-5 session period. Such effect extended to the 6-10 session period only for control group. Additionally, males submitted to forced swimming had more entries into the closed arm extremities than their respective control group in both session periods. Handled males had more entries into the closed arm extremities than their control group during the 6-10 session period. Moreover, number of entries into the closed arm extremities decreased from 1-5 to 6-10 session period for all the groups, except for handled males.

Time spent in the open arms is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 8.049$, $P < 0.001$) and session period ($F_{[1,66]} = 26.126$, $P < 0.001$). In addition, chronic treatment per session period ($F_{[2,66]} = 6.176$, $P < 0.01$) and chronic treatment per gender per session period ($F_{[2,66]} = 3.841$, $P < 0.05$) significant interactions were found. Post hoc comparisons showed that females submitted to forced swimming spent shorter time in the open arms than their correspondent male group in the 6-10 session period. Also, it was found that male and female handled groups spent longer time in the open arms than their respective control groups in the 1-5 session period. Such effect extended to the 6-10 session period only for females. Additionally, male rats submitted to forced swimming stayed shorter in these arms in the 1-5 and longer in the 6-10 session period than their control groups. Furthermore, time spent in the open arms decreased from 1-5 to 6-10 session period for all the groups, except for male and female rats submitted to forced swimming.

Time spent in the closed arms is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 4.034$, $P < 0.05$) and session period ($F_{[1,66]} = 22.840$, $P < 0.001$). In addition, chronic treatment per session period ($F_{[2,66]} = 10.847$, $P < 0.001$) and chronic treatment per gender per session period ($F_{[2,66]} = 4.863$, $P < 0.05$) significant interactions were found. Post hoc comparisons showed that handled females spent shorter time in the closed arms than their correspondent male group in the 6-10 session period. Female rats submitted to forced swimming spent longer time in the closed arms than their correspondent male group during the 6-10 session period. Also, it was found that handled males spent shorter time in the closed arms than their respective control group in the 1-5 session period. Male and female rats submitted to forced swimming spent longer time in the closed arms than their respective control groups in 1-5 session period. During the 6-10 session period, handled females and males submitted to forced swimming spent shorter time in the closed arms than their correspondent control groups. Moreover, time spent in the closed arms increased from 1-5 to 6-10 session period for all the groups, except for those females submitted to handling or to forced swimming. Time spent in the closed arms decreased from 1-5 to 6-10 session period only for male rats submitted to forced swimming.

Time spent in the open arm extremities is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 5.506$, $P < 0.01$) and session period ($F_{[1,66]} = 19.101$, $P < 0.001$). Additionally, a chronic treatment per session period ($F_{[2,66]} = 9.065$, $P < 0.001$) significant interaction was found. Post hoc comparisons showed that handled females spent longer time in the open arm extremities than their correspondent male group in the 6-10 session period. Also, it was found that male and female handled groups spent longer time in the open arm extremities than their respective control groups in the 1-5 session period. Such effect extended to the 6-10 session period only for females. Male rats submitted to forced swimming spent shorter time in the open arm extremities than their respective control group in the 1-5 session period. Furthermore, time spent in the open arm extremities decreased from 1-5 to 6-10 session period only for control and handled males.

Time spent in the closed arm extremities is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 4.177$, $P < 0.05$) and session period ($F_{[1,66]} = 8.227$, $P < 0.01$). In addition, a chronic treatment per session period ($F_{[2,66]} = 4.030$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that females submitted to forced swimming spent longer time in the closed arm extremities than their correspondent male group in the 6-10 session period. Also, it was found that handled females spent shorter time in the closed arm extremities than their respective control group in both session periods. Males submitted to forced swimming spent shorter time in the closed arm extremities than their respective control group in the 6-10 session period. Moreover, time spent in the closed arm extremities increased from 1-5 to 6-10 session period only for control and handled males.

Time spent in the central square is shown at Table 1. No main effect was found. A significant interaction between chronic treatment and session period ($F_{[2,66]}$ = 5.500, $P < 0.01$) was found. Post hoc comparisons showed that those females submitted to handling or to forced swimming spent longer time in the central square than their respective control groups in the 6-10 session period. Furthermore, time spent in the central square decreased from 1-5 to 6-10 session period only for control females.

Distance traveled into the open arms is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]}$ = 8.443, $P < 0.001$) and session period ($F_{[1,66]}$ = 94.209, $P < 0.001$). In addition, a chronic treatment per session period ($F_{[2,66]}$ = 9.528, $P < 0.001$) significant interaction was found. Post hoc comparisons showed that handled females traveled more into the open arms than their correspondent male group in the 6-10 session period. Additionally, it was found that male and female handled groups traveled more into the open arms than their respective control groups in the 1-5 session period. Male and female rats submitted to forced swimming traveled less into the open arms than their respective control groups during the 1-5 session period. Furthermore, distance traveled into the open arms decreased from 1-5 to 6-10 session period for all the groups, except for males submitted to forced swimming.

Distance traveled into the closed arms is shown at Table 1. Main effects were found for the factors gender ($F_{[1,66]}$ = 8.227, $P < 0.01$) and session period ($F_{[1,66]}$ = 102.276, $P < 0.001$). In addition, a chronic treatment per session period ($F_{[2,66]}$ = 5.324, $P < 0.01$) significant interaction was found. Post hoc comparisons showed that control and handled females traveled more into the closed arms than their correspondent male groups in the 1-5 session period. Such effect extended to the 6-10 session period only for control group. Also, it was found that males submitted to forced swimming traveled more into the closed arms than their respective control group, in both session periods. Handled males traveled more into the closed arms than their respective control group during the 6-10 session period. Moreover, distance traveled into the closed arms decreased from 1-5 to 6-10 session period for all the groups, except for handled males.

The rearing frequency is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]} = 6.424$, $P < 0.05$). Additionally, an significant interaction between chronic treatment and gender ($F_{[2,66]} = 8.215$, $P < 0.001$) was found. Post hoc comparisons showed that rearing behavior of handled females was more frequent than that from their correspondent male group in the 1-5 session period. Females submitted to forced swimming had less frequent rearing behavior than their correspondent male group in both session periods. In addition, in both session periods, it was found that while for females forced swimming decreased the rearing frequency, for males it increased such measure. For handled males rearing frequency was larger than that from their control group in the 6-10 session period.

The rearing duration is shown at Table 1. A main effect was found for the factor gender ($F_{[1,66]} = 4.376$, $P < 0.05$). Also, chronic treatment per gender ($F_{[2,66]} = 4.007$, $P < 0.05$) and chronic treatment per session period ($F_{[2,66]} = 5.153$, $P < 0.01$) significant interactions were found. Post hoc comparisons showed that rearing duration from females submitted to forced swimming was shorter than that from their correspondent male group in the 1-5 and 6-10 session periods. Additionally, it was found that rearing duration from males submitted to forced swimming was longer than that from their respective control group in the 1-5 and 6-10 session periods. During the 6-10 session period, rearing duration from handled males was longer than that from their respective control group. Complementarily, rearing duration increased from 1-5 to 6-10 session period only for handled males.

The freezing frequency is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]} = 14.215$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that freezing frequency from females submitted to forced swimming was larger than that from their correspondent male group in the 6-10 session period. In addition, it was found that freezing frequencies from male and female handled groups were smaller than that from their respective control groups in the 6-10 session period. Males submitted to forced swimming showed a similar effect in the same session period. Furthermore, freezing frequency increased from 1-5 to 6-10 session period only for males and female control groups.

The freezing duration is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]} = 5.776$, $P < 0.05$). No interaction was found. Post hoc comparisons showed that freezing duration from females submitted to forced swimming was longer than that from their correspondent male group in the 1-5 and 6-10 session period. Freezing duration from control females was shorter than that from their correspondent male group in the 6-10 session period. Also, it was found that freezing duration from females submitted to forced swimming was longer than that from their respective control group in both session period. Freezing durations from males submitted to handling or to forced swimming were shorter than that from their respective control group in the 6-10 session period. Moreover, freezing duration increased from 1-5 to 6-10 session period only for control males and for females submitted to forced swimming.

The unprotected head out frequency is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 6.548$, $P < 0.01$) and session period

($F_{[1,66]}$ = 93.529, $P < 0.001$). In addition, a chronic treatment per session period ($F_{[2,66]}$ = 6.944, $P < 0.01$) significant interaction was found. Post hoc comparisons showed that unprotected head out frequency from females submitted to forced swimming was smaller than that from their correspondent male group in the 6-10 session period. Additionally, it was found that unprotected head out frequency from handled males was larger than that from their respective control group in the 1-5 session period. The unprotected head out frequencies from male and female rats submitted to forced swimming was smaller than that from their respective control groups in the 1-5 session period. Furthermore, unprotected head out frequency decreased from 1-5 to 6-10 session period for all the groups, except for males submitted to forced swimming.

The unprotected head out duration is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]}$ = 4.361, $P < 0.05$) and session period ($F_{[1,66]}$ = 37.149, $P < 0.001$). Also, a chronic treatment per session period ($F_{[2,66]}$ = 3.881, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that unprotected head out duration from females submitted to forced swimming was shorter than that from their correspondent male group in the 6-10 session period. In addition, it was found that unprotected head out duration from male and female rats submitted to forced swimming was shorter than that from their respective control groups in the 1-5 session period. Moreover, unprotected head out duration decreased from 1-5 to 6-10 session period for all the groups, except for male rats submitted to forced swimming.

The protected head out frequency is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]}$ = 30.325, $P < 0.001$). No interaction was found. Post hoc comparisons showed that protected head out frequency decreased from 1-5 to 6-10 session period only for male and female control groups and for handled males.

The protected head out duration is shown at Table 1. No main effect was found. A significant interaction between chronic treatment and session period ($F_{[2,66]}$ = 3.738, $P < 0.05$) was found. Post hoc comparisons showed that protected head out duration from males submitted to forced swimming was longer than that from their respective control group in the 6-10 session period. Furthermore, protected head out duration decreased from 1-5 to 6-10 session period for control males and increased for male rats submitted to forced swimming.

The protected stretching attend posture frequency is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]}$ = 12.680, $P < 0.001$). Additionally, a chronic treatment per gender ($F_{[2,66]}$ = 3.416, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that protected stretching attend posture frequency from handled females was smaller than that from their respective control group in the 1-5 session period. Besides, protected stretching attend posture frequency decreased from 1-5 to 6-10 session period only for females submitted to forced swimming.

The protected flat back approach behavior is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]}$ = 20.580, $P < 0.001$). No interaction was found. Post hoc comparisons showed that protected flat back approach behavior decreased from 1-5 to 6-10 session period only for control male group, handled females and male rats submitted to forced swimming.

The unprotected stretching attend posture is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]}$ = 6.881, $P < 0.05$). No interaction was found. Post hoc comparisons showed that exhibitions of unprotected stretching attend posture from control and handled females were more frequent than that from their respective male groups in the 1-5 session period. Further, unprotected stretching attend posture decreased from 1-5 to 6-10 session period only for control and handled females.

The unprotected flat back approach behavior is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]} = 13.013$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that unprotected flat back approach behavior decreased from 1-5 to 6-10 session period only for female control group.

The grooming duration is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 7.561$, $P < 0.01$) and session period ($F_{[1,66]} = 18.393$, $P < 0.001$). Additionally, a chronic treatment per session period ($F_{[2,66]} = 4.333$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that grooming durations from control and handled females were longer than that from their respective male groups in the 6-10 session period. In addition, grooming duration from females submitted to forced swimming was shorter than that from their correspondent control group during 6-10 session period. Moreover, grooming duration increased from 1-5 to 6-10 session period only for control and handled females.

The grooming pattern transitions are shown at Table 1. A main effect was found for the factor chronic treatment ($F_{[2,66]} = 3.613$, $P < 0.05$). In addition, a chronic treatment per gender per session period ($F_{[2,66]} = 3.420$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that grooming pattern transitions from control females were larger than that from their correspondent male group in the 1-5 session period. Also, it was found that grooming pattern transitions from females submitted to handling or forced swimming were smaller than that from their control group during the 1-5 session period. Furthermore, grooming pattern transitions increased from 1-5 to 6-10 session period only for handled female group.

The fore paws/nose grooming duration is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 5.149$, $P < 0.01$) and session period

($F_{[1,66]} = 7.200$, $P < 0.01$). No interaction was found. Post hoc comparisons showed that fore paws/nose grooming duration from control females was longer than that from their male group during the 6-10 session period. Additionally, it was found that fore paws/nose grooming duration from females submitted to forced swimming was shorter than that from their control group in the 6-10 session period. Supplementary, fore paws/nose grooming duration increased from 1-5 to 6-10 session period only for control and handled females.

The body/rear paws grooming frequency is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 5.340$, $P < 0.01$) and session period ($F_{[1,66]} = 9.797$, $P < 0.01$). No interaction was found. Post hoc comparisons showed that body/rear paws grooming frequency from handled females was larger than that from their male group in the 6-10 session period. In addition, it was found that body/rear paws grooming frequency from females submitted to forced swimming was smaller than that from their control group in the 6-10 session period. Further, body/rear paws grooming frequency increased from 1-5 to 6-10 session period only for handled females.

The body/rear paws grooming duration is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 5.838$, $P < 0.01$) and session period ($F_{[1,66]} = 11.132$, $P < 0.01$). Also, a chronic treatment per session period ($F_{[2,66]} = 4.120$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that body/rear paws grooming durations from control and handled females were longer than that from their respective male groups in the 6-10 session period. Additionally, it was found that body/rear paws grooming duration from females submitted to forced swimming was shorter than that from their control group in the 6-10 session period.

Moreover, body/rear paws grooming duration increased from 1-5 to 6-10 session period only for control and handled females.

The tail/genital grooming frequency is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 3.275$, $P < 0.05$) and session period ($F_{[1,66]} = 15.184$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that tail/genital grooming frequency increased from 1-5 to 6-10 session period only for male and female handled groups.

The tail/genital grooming duration is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 3.727$, $P < 0.05$) and session period ($F_{[1,66]} = 14.148$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that tail/genital grooming duration increased from 1-5 to 6-10 session period only for handled female group.

The interruptions frequency is shown at Table 1. Main effects were found for the factors chronic treatment ($F_{[2,66]} = 3.395$, $P < 0.05$) and session period ($F_{[1,66]} = 5.960$, $P < 0.05$). In addition, a chronic treatment per gender ($F_{[2,66]} = 3.513$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that interruptions frequency from handled females were larger than that from their respective control group in the 6-10 session period. Further, interruptions frequency increased from 1-5 to 6-10 session period only for handled female group.

The interruptions duration is shown at Table 1. A main effect was found for the factor session period ($F_{[1,66]} = 8.444$, $P < 0.01$). Additionally, chronic treatment per gender ($F_{[2,66]} = 6.825$, $P < 0.01$) and chronic treatment per gender per session period ($F_{[2,66]} = 4.306$, $P < 0.05$) significant interactions were found. Post hoc comparisons showed that interruptions duration from male and female handled groups were longer than that from their respective control groups in the 6-10 session period. Moreover, interruptions duration increased from 1-5 to 6-10 session period only for male and female handled groups.

Percentage of unexpected transitions between grooming patterns is shown at Table 2. A main effect was found for the factor chronic treatment ($F_{[2,66]} = 4.216$, $P < 0.05$). No interaction was found. Post hoc comparisons showed that percentages of unexpected transitions from females submitted to handling or to forced swimming was smaller than that from their control group.

Percentage of interrupted bouts of grooming is shown at Table 2. A main effect was found for the factor gender ($F_{[1,66]} = 5.853$, $P < 0.05$). Also, a chronic treatment per gender ($F_{[2,66]} = 4.280$, $P < 0.05$) significant interaction was found. Post hoc comparisons showed that percentage of interrupted bouts from control females was smaller than that from their male group. Further, percentage of interrupted bouts from males submitted to handling or to forced swimming was larger than that from their control group.

For the measures latency to start grooming, mean grooming latency, grooming frequency, fore paws/nose grooming frequency and head grooming (frequency and duration) no main effects neither interaction were found.

3.3. *Adrenal weight*

The adrenal weight is shown at Table 2. A main effect was found for the factor gender ($F_{[1,30]} = 26.262$, $P < 0.001$). No interaction was found. Post hoc comparisons showed that adrenal weights from all the female groups were larger than that from their respective male groups.

4. Discussion

4.1. Forced swimming

Forced swim stress is currently one of the most frequently used animal models of depression, which includes the components of anxiety and desperation induced by psychological stress when animals are exposed to novel situations and confronted with life threats (Qi, Lin, Li, Pan & Wang, 2006). Our study demonstrated that there was a decrease in floating latency for both genders, since second session for females and since third for males. There was a decrease, too, in climbing duration to both genders, since third session to males and since fourth to females. On the other hand, there was an increase in floating duration since fourth session to both genders. This increase was kept in most of the remaining sessions to males, otherwise, to females this increase was only reestablished in the ninth and tenth sessions. Swimming duration varied between the sessions, showing an increase in session thirteen to females and in session fourteen to males. Swimming and floating results were opposed to some other studies (Dal-Zotto, Martí & Armario, 2000; Boyce-Rustay et al., 2007; Stone, Lehmann, Lin & Quartermain, 2007) that had employed chronic forced swim. In these studies, there was an increase of floating duration (Dal-Zotto, Martí & Armario, 2000; Stone, Lehmann, Lin & Quartermain, 2007) and in the floating duration percentage (Boyce-Rustay et al., 2007). It was observed too a decreasing in swimming duration (Dal-Zotto, Martí & Armario, 2000) and in the distance of swimming (Stone, Lehmann, Lin & Quartermain, 2007). Any of these studies neither verified the climbing and diving behaviors nor verified the behavioral changes along forced swim sessions. Moreover, in the study of Drossopoulou et al. (2004) and Barros and Ferigolo (1998), which was used the test-retest forced swim procedure, were found gender differences in respect floating, swimming and climbing. Using chronic forced swim, in the present study, it was not observed this gender difference in the duration of none of that behaviors. In addition, in the studies that employed forced swimming like a chronic stress model (Qi, Lin, Li, Pan & Wang, 2006), rats behavior along swimming sessions were not evaluated, what does not avoid a comparison between the obtained results. In conclusion, it may be asserted that there was an influence of chronic exposition to forced swimming along the swimming sessions, there was not gender differences.

4.2. Elevated plus-maze

The elevated plus-maze paradigm is currently one of the most widely used animal models where the behavioral repertoire of rodents is used to detect effects of anxiety (Espejo, 1997). Our study demonstrated that the spatiotemporal measures in the elevated plus-maze, in the first session period were: the number of entries in the opened arms was smaller in females submitted to forced swim than the control females; there was no difference in male results between forced swim and control groups. With regard to entries percentage in opened arms, there was not difference between forced swim and control groups to both genders. About the entries in the opened arms extremities, males and females submitted to forced swim entered less than their control groups. Opened arms time, males submitted to forced swim spent less time in these arms than its controls; and in females results this difference was not observed. Time spent in the closed arms increased for males and females submitted to forced swim in comparison to its control groups. All this measures are highly related with anxiety, by the way, the smallest number, time and percentage of entries in the opened arms and the smallest number of entries in the opened arms extremities, greater is the anxiety. In addition, the largest time spent in the closed arms, greater is the anxiety. Results found to time in the opened arms are agreeing to studies that used chronic forced swim like a stressor (Qi, Lin, Li, Pan & Wang, 2006; Qi, Lin & Li, 2006). Differently, in these two studies, the number of entries in the opened and closed arms were not modified. In none of these anxiety measures, were not found gender differences.

The number of entries in the closed arms, that reflect motion activity, was larger to males submitted to forced swim in comparison with its control groups. This difference was not found in females results. Nevertheless, female control and handling groups showed a greater motion activity in comparison to males in these groups. This difference was not found comparing males and females submitted to forced swim. Rearing behavior, whit regard to vertical motion activity, was less frequent in females submitted to forced swim, in comparison with its control group and too with males submitted to forced swim. These group males reared too with more frequency than its control group. This difference of males submitted to forced swim in relation to females and its control group was found to rearing latency too. There was any gender difference in control groups related to rearing. This result is opposed to that found by Estanislau and Morato (2006), which was found a larger rearing frequency in females of control group than in comparison with males of the same group.

The head-out behavior, differently of others studies (Rodgers & Cole, 1993; Rodgers & Cole, 1993; Cole & Rodgers, 1994), was observed with larger frequency in the unprotected region of the maze. Males and females submitted to forced swim had less frequency and duration of this behavior in comparison with its control groups. This behavior, according to Cruz et al. (1994) and Espejo (1997), indicates anxiety, so that the larger time spent in head-out the smaller is the anxiety. According to Rodgers and Johnson (1995), this behavior reflects exploration and gathering of information. Hence, the results found can be viewed in two ways: the animals submitted to forced swim showed more anxiety in the plus-maze test, in comparison to its controls and, too, reduced the new places exploration. This last assertion agreed with the result observed by Qi, Lin, Li, Pan & Wang (2006). There were not gender differences in relation to head-out, protected and unprotected.

In regard with time in the central square, that shows the capacity to wait and make decisions in conflict situations, there was not any difference for gender and group. The stretching attend posture (risk-assessment) is related to anxiety and decisions making. In the present study, it was not found differences between control group and forced swim group or gender differences, in regard to protected and unprotected stretching. Similar results were observed by Estanislau and Morato (2006), using the prenatal stress procedure. The flat back approach, related to the motion activity, has not showed differences between the control and forced swim group and nor for gender differences.

Grooming duration, frequency and latency to start grooming do not modified in relation to gender and groups. Latency to start grooming in the study of Kalueff and Tuohimaa (2005), was larger in rats restricted to lighted environments. The unexpected transitions percentage between the grooming patterns decreased in females submitted to forced swim in comparison with its control group. Differently results were found by Kalueff and Tuohimaa (2004), the unexpected transitions percentage was larger in stressed male mices. In the present study, this measure was not modified in the males submitted to forced swimming.

The trasitions between grooming patterns were less frequent in females submitted to handling or forced swimming than their control groups. In regard with males, this difference was not observed. This result was discrepant in relation to Kalueff and Tuohimaa (2005) results, which had an increase in transitions between grooming patterns to male rats restricted in lighted environments. In the present studty, there was, too, a gender difference in control group, with the female transitions between grooming patterns more frequent than males of the same group. The percentage of interrupted bouts increase in males submitted to handling or forced swimming, besides it was less frequent in control females in comparison to males of the same group. This percentage increase of interrupted bouts from males was similar to result of Kalueff and Tuohimaa (2004) to mice. The interruptions duration and frequency were not modified in comparison between gender and groups. In the study of Kalueff and Tuohimaa (2005), the grooming patterns interruptions were more frequent to the group restricted to lighted environment. Duration and frequency of the four grooming patterns were not modified neither by groups nor by genders. These differences found in results may be related to procedural differences (for example, grooming observation in actimeter and not in the elevated plus-maze).

4.3. Adrenal weight

There were not group differences, only gender differences between the three groups.

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Table 1. Alterations in elevated plus-maze measures along the experimental conditions from males and females. Data are expressed as mean ± S.E.M. P < 0.05. *gender difference ^{o}group control difference ^{a}session period difference

	Control				Handled				Forced swimming			
	1-5		6-10		1-5		6-10		1-5		6-10	
Measures	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Entries in the open arms	8.00 ± 0.82	6.50 ± 0.62	4.00 ± 0.72^{a}	3.17 ± 0.58^{a}	9.58 ± 0.76^{o}	8.42 ± 0.47^{o}	5.67 ± 0.50^{oa}	4.08 ± 0.34^{*a}	6.25 ± 0.99^{o}	5.75 ± 0.72	3.67 ± 0.69^{a}	4.5 ± 0.56
Entries in the closed arms	11.1 ± 0.80	8.42 ± 0.40*	7.58 ± 0.76^{a}	5.17 ± 0.76^{*a}	10.8 ± 0.65	9.00 ± 0.66*	8.75 ± 0.75^{a}	7.42 ± 0.65^{oa}	9.75 ± 1.03	10.3 ± 0.77^{o}	7.58 ± 0.97^{a}	8.00 ± 0.71^{oa}
Entries in the open arms (%)	41.3 ± 2.56	42.9 ± 2.94	32.3 ± 3.91^{a}	32.6 ± 5.30^{a}	46.6 ± 2.53	48.8 ± 2.02	39.7 ± 2.54	35.9 ± 2.19^{a}	35.2 ± 4.05	35.2 ± 3.64	28.3 ± 3.65	35.4 ± 3.11
Entries in the open arms extremities	5.75 ± 0.87	4.92 ± 0.91	3.00 ± 0.63^{a}	2.33 ± 0.58^{a}	7.75 ± 0.86^{o}	6.92 ± 0.53^{o}	4.08 ± 0.40^{a}	2.00 ± 0.37^{*a}	3.67 ± 0.72^{o}	3.33 ± 0.83^{o}	2.50 ± 0.48	2.92 ± 0.53
Entries in the closed arms extremities	10.7 ± 0.67	8.17 ± 0.46*	7.58 ± 0.83^{a}	5.00 ± 0.62^{*a}	10.5 ± 0.54	8.33 ± 0.85*	8.17 ± 0.53^{a}	7.75 ± 0.46^{o}	10.9 ± 0.70	11.0 ± 0.69^{o}	7.58 ± 0.97^{a}	7.58 ± 0.76^{oa}
Time in the open arms (s)	83.6 ± 9.46	94.9 ± 11.5	59.1 ± 14.7^{a}	62.8 ± 11.8^{a}	113 ± 8.34^{o}	123 ± 6.34^{o}	92.4 ± 7.24^{oa}	76.8 ± 6.68^{a}	67.0 ± 10.3	70.1 ± 10.4^{o}	52.1 ± 7.82	83.5 ± 11.9^{*o}
Time in the closed arms (s)	160 ± 14.1	156 ± 15.5	206 ± 19.8^{a}	194 ± 20.3^{a}	141 ± 9.51	124 ± 8.44^{o}	158 ± 8.75^{o}	184 ± 9.71^{*a}	190 ± 13.9^{o}	186 ± 12.0^{oa}	197 ± 12.1	161 ± 11.8^{*o}
Time in the open arms extremities (s)	34.9 ± 6.11	39.8 ± 8.17	24.3 ± 5.64	23.0 ± 5.61^{a}	49.8 ± 5.18^{o}	55.3 ± 5.61^{o}	38.7 ± 5.51^{o}	23.3 ± 5.28^{*a}	24.0 ± 4.51	23.5 ± 5.60^{o}	26.0 ± 5.76	28.1 ± 6.68
Time in the closed arms extremities (s)	83.3 ± 9.02	71.9 ± 7.84	104 ± 14.1	104 ± 19.9^{a}	55.4 ± 3.98^{o}	49.6 ± 6.32	62.3 ± 4.76^{o}	85.7 ± 10.22^{a}	86.0 ± 9.37	79.3 ± 6.78	91.8 ± 20.7	62.5 ± 8.09^{*o}
Time in the central square (s)	56.3 ± 5.29	49.6 ± 7.92	34.8 ± 6.22^{a}	43.6 ± 10.9	46.2 ± 4.27	53.4 ± 4.72	49.7 ± 5.47^{o}	39.1 ± 5.15	42.8 ± 6.47	44.1 ± 5.89	51.1 ± 9.65^{o}	55.2 ± 5.23
Distance traveled in the open arms (m)	4.73 ± 0.53	4.08 ± 0.54	2.52 ± 0.46^{a}	2.00 ± 0.38^{a}	5.90 ± 0.54^{o}	5.37 ± 0.36^{o}	3.46 ± 0.30^{a}	2.24 ± 0.20^{*a}	3.22 ± 0.55^{o}	3.05 ± 0.50^{o}	2.17 ± 0.37^{a}	2.61 ± 0.32
Distance traveled in the closed arms (m)	8.71 ± 0.61	6.20 ± 0.35*	6.11 ± 0.66^{a}	3.97 ± 0.49^{*a}	8.41 ± 0.54	6.48 ± 0.53*	6.61 ± 0.52^{a}	6.01 ± 0.43^{o}	8.45 ± 0.60	8.66 ± 0.53^{o}	5.93 ± 0.75^{a}	5.88 ± 0.60^{oa}
Rearing	14.9 ± 1.35	12.5 ± 2.31	12.3 ± 1.65	9.8 ± 1.38	15.7 ± 1.75	11.8 ± 1.84*	13.2 ± 1.24	14.2 ± 1.33^{o}	11.4 ± 1.20^{o}	17.6 ± 0.71^{*o}	8.25 ± 1.22^{o}	15.0 ± 1.06^{*o}
Rearing (s)	18.4 ± 1.91	15.0 ± 3.38	14.7 ± 2.15	15.7 ± 3.75	15.7 ± 2.12	14.5 ± 2.22	17.7 ± 1.96	22.4 ± 3.70^{oa}	13.8 ± 1.49	25.7 ± 2.58^{*o}	11.9 ± 2.03	22.3 ± 2.80^{*o}
Freezing	0.25 ± 0.18	0.17 ± 0.11	1.00 ± 0.43^{a}	1.33 ± 0.73^{a}	0.00 ± 0.00	0.08 ± 0.08	0.17 ± 0.11^{o}	0.50 ± 0.34^{o}	0.33 ± 0.26	0.08 ± 0.08	1.00 ± 0.46	0.17 ± 0.11^{*o}
Freezing (s)	0.27 ± 0.19	0.33 ± 0.26	1.19 ± 0.48	6.34 ± 3.44^{*a}	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.06	0.51 ± 0.37^{o}	9.23 ± 9.08^{o}	0.05 ± 0.05*	13.4 ± 12.7^{oa}	0.08 ± 0.06^{*o}
Unprotected Head-out	16.2 ± 2.96	14.3 ± 1.77	6.25 ± 1.96^{a}	6.08 ± 1.38^{a}	17.8 ± 2.07	19.1 ± 1.94^{o}	8.92 ± 1.42^{a}	7.00 ± 0.71^{a}	9.92 ± 1.78^{o}	9.25 ± 1.89^{o}	3.50 ± 0.54^{a}	8.50 ± 1.72*
Unprotected Head-out (s)	21.3 ± 4.79	26.2 ± 5.23	10.1 ± 3.83^{a}	12.5 ± 3.31^{a}	25.1 ± 3.32	28.0 ± 2.89	15.8 ± 2.94^{a}	18.1 ± 3.31^{a}	13.3 ± 3.26^{o}	14.8 ± 3.23^{o}	5.10 ± 1.48^{a}	16.4 ± 4.17*
Protected Head-out	5.75 ± 0.62	5.17 ± 1.25	2.08 ± 0.50^{a}	2.33 ± 0.58^{a}	4.17 ± 0.68	6.17 ± 0.81	2.25 ± 0.49	2.67 ± 0.74^{a}	4.33 ± 0.72	4.92 ± 0.79	3.00 ± 0.70	4.17 ± 0.64
Protected Head-out (s)	8.00 ± 1.00	8.97 ± 2.76	4.27 ± 1.20	4.22 ± 1.30^{a}	6.06 ± 1.23	9.17 ± 1.31	4.54 ± 1.13	6.82 ± 1.94	7.82 ± 1.80	6.05 ± 1.30	6.46 ± 1.66	10.6 ± 1.91^{oa}
Protected Stretched attend posture	1.33 ± 0.36	1.00 ± 0.41	0.75 ± 0.35	0.25 ± 0.18	0.42 ± 0.23^{o}	0.58 ± 0.36	0.50 ± 0.23	0.08 ± 0.08	1.75 ± 0.52	1.33 ± 0.53	0.58 ± 0.23^{a}	0.92 ± 0.47
Protected Flat back approach	1.25 ± 0.43	2.17 ± 0.81	0.58 ± 0.23	0.75 ± 0.45^{a}	1.42 ± 0.47	1.33 ± 0.54	0.25 ± 0.13^{a}	0.25 ± 0.18	1.83 ± 0.81	2.42 ± 0.86	1.00 ± 0.44	1.17 ± 0.52^{a}

Unprotected Stretched attend posture	1.92 ± 0.56	1.00 ± 0.35*	1.00 ± 0.25[a]	0.67 ± 0.22	2.42 ± 0.48	0.58 ± 0.19*	1.42 ± 0.36[a]	0.67 ± 0.41	1.33 ± 0.38	1.83 ± 0.39	1.17 ± 0.37	1.42 ± 0.38
Unprotected Flat back approach	1.42 ± 0.51	1.25 ± 0.49	0.50 ± 0.23[a]	0.67 ± 0.31	1.50 ± 0.34	1.25 ± 0.39	1.00 ± 0.39	0.50 ± 0.23	1.67 ± 0.26	1.17 ± 0.37	1.08 ± 0.38	0.92 ± 0.34
Grooming duration (s)	19.7 ± 6.64	10.4 ± 2.27	39.1 ± 11.9[a]	16.2 ± 3.15*	16.4 ± 3.95	14.6 ± 2.02	44.6 ± 9.20[a]	27.0 ± 5.21*	12.7 ± 2.59	14.3 ± 2.13	15.4 ± 3.36[o]	19.0 ± 3.13
Grooming frequency	3.33 ± 0.89	2.00 ± 0.41	2.58 ± 0.50	2.08 ± 0.42	2.00 ± 0.39	2.58 ± 0.40	1.83 ± 0.21	2.33 ± 0.31	2.08 ± 0.31	2.50 ± 0.40	2.25 ± 0.48	2.17 ± 0.44
Grooming pattern transitions	8.50 ± 2.20	4.50 ± 0.98*	7.33 ± 1.60	5.00 ± 0.98	5.50 ± 1.32[o]	6.00 ± 0.90	9.83 ± 1.93[a]	7.75 ± 1.43	4.50 ± 0.66[o]	5.25 ± 0.81	5.17 ± 1.11	5.67 ± 0.94
Fore paws/nose grooming	3.92 ± 1.01	2.17 ± 0.46	2.92 ± 0.60	2.08 ± 0.50	2.67 ± 0.62	2.92 ± 0.45	3.67 ± 0.63	3.33 ± 0.63	2.17 ± 0.37	2.58 ± 0.40	2.33 ± 0.45	2.50 ± 0.44
Fore paws/nose grooming (s)	16.3 ± 4.91	9.67 ± 1.88	25.8 ± 5.97[a]	13.1 ± 2.59*	14.8 ± 3.51	13.9 ± 2.00	24.0 ± 3.10[a]	19.1 ± 3.39	12.4 ± 2.68	14.0 ± 1.98	13.9 ± 3.22[o]	14.6 ± 2.48
Head grooming	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.18	0.00 ± 0.00	0.50 ± 0.34	0.17 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Head grooming (s)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.09	0.00 ± 0.00	0.36 ± 0.24	0.12 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Body/rear paws grooming	0.67 ± 0.51	0.25 ± 0.18	1.00 ± 0.39	0.42 ± 0.19	0.25 ± 0.18	0.08 ± 0.08	1.67 ± 0.59[a]	0.58 ± 0.36*	0.00 ± 0.00	0.08 ± 0.08	0.17 ± 0.11[o]	0.33 ± 0.19
Body/rear paws grooming (s)	2.29 ± 1.84	0.44 ± 0.36	9.88 ± 5.91[a]	1.93 ± 0.95*	0.60 ± 0.41	0.10 ± 0.10	13.4 ± 5.30[a]	3.28 ± 2.34*	0.00 ± 0.00	0.12 ± 0.12	0.17 ± 0.11[o]	2.86 ± 1.88
Genital/tail grooming	0.25 ± 0.18	0.00 ± 0.00	0.67 ± 0.36	0.25 ± 0.13	0.00 ± 0.00	0.08 ± 0.08	0.92 ± 0.29[a]	0.67 ± 0.28[a]	0.08 ± 0.08	0.00 ± 0.00	0.25 ± 0.18	0.17 ± 0.11
Genital/tail grooming (s)	0.87 ± 0.64	0.00 ± 0.00	3.27 ± 1.94	0.76 ± 0.42	0.00 ± 0.00	0.27 ± 0.27	4.29 ± 1.41[a]	2.80 ± 1.38	0.12 ± 0.12	0.00 ± 0.00	1.02 ± 0.82	1.04 ± 0.70
Grooming interruptions	0.33 ± 0.19	0.08 ± 0.08	0.17 ± 0.11	0.17 ± 0.11	0.33 ± 0.19	0.33 ± 0.19	1.17 ± 0.34[oa]	0.67 ± 0.26	0.17 ± 0.11	0.08 ± 0.08	0.17 ± 0.11	0.50 ± 0.26
Grooming interruptions (s)	0.23 ± 0.12	0.30 ± 0.30	0.15 ± 0.10	0.41 ± 0.29	0.83 ± 0.53	0.27 ± 0.17	2.49 ± 0.77[oa]	1.68 ± 0.70[oa]	0.16 ± 0.12	0.21 ± 0.21	0.40 ± 0.27	0.45 ± 0.23

Table 2. Grooming measures in the elevated plus-maze test and adrenal weight.

Measures	Control		Handled		Forced swimming	
	Females	Males	Females	Males	Females	Males
Grooming latency (s)	145±34.9	203 ± 26.3	182 ± 41.8	123 ± 27.3	178 ± 13.8	118 ± 16.4
Unexpected transitions (%)	56.9±2.74	53.6 ± 2.34	54.4 ± 1.87º	50.1 ± 0.82	51.7 ± 1.20º	51.0 ± 0.59
Interrupted bouts (%)	5.58±2.63	9.38 ± 5.57*	31.9 ± 7.85	13.1 ± 4.16º	5.56 ± 2.37	12.3 ± 4.96º
Mean grooming latency (s)	139±38.7	162 ± 27.5	152 ± 23.8	121 ± 25.7	137 ± 18.9	122 ± 20.0
Adrenal weight (mg/100g)	24.4±2.73	15.5 ± 2.18*	28.3 ± 1.85	17.1 ± 2.51*	26.9 ± 2.91	17.6 ± 1.55*