Loss of memory induced by swimming exercise in mice treated with lithium and anxiolytic-like effects

Perda de memória induzida pelo exercício de natação em camundongos tratados com lítio e efeitos ansiolíticos

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Abstract

Previous studies have indicated that chronic exercise mimics some of the central nervous system effects observed after antidepressant drug treatment. The purpose of this work was to investigate the possible effect of lithium treatment in mice swimmers in the animals models of anxiety and memory. The mice were evaluated at the elevated plus-maze, open-field, Morris water maze and object recognition. Adult male Swiss mice were divided in four groups: control sedentary (CS), lithium sedentary (LS), control swimming exercise (CE), and lithium swimming exercise (LE), treated either with normal chow or with chow containing with 0,25% lithium carbonate for 30 days. The results suggest that lithium treatment, swimming exercise or lithium treatment combined with swimming exercise induces an anxiolytic-like effect in mice in the elevated plus-maze test. These symptoms were observed in LS as an increase in time spent in the open arms and open arms entries (F = 3.238; p<0,05 and F = 4.093; p<0,05; respectively); while CE - LE showed an increase in time spent in the risk assessment (F = 17.644; p<0,001). The open-field tests indicated a reduction of fear and an increase in exploratory activity. The LS - CE reduced the time spent in the freezing (F = 3.274; p<0,05) and CE - LE increased the time spent in the exploratory activity (F = 9.149; p<0,001). Comparison within each group during the five days of Morris water maze test, showed a decrease of latency time of groups LS - CE from the second day of the testing (F = 21.512; p<0,001 and F = 3.688; p<0,001; respectively). In object recognition test, mice of group CE increased in exploration ratio in both short (STM) and long-term object recognition memory (LTM), when compared to CS (F = 9.246; p<0,001). The groups LS - LE did not differ significantly from CS (p>0,05). The present study found that swimming exercise and/or lithium treatment induced an anxiolytic-like effect, indicating that interactions between physical activity and/or lithium may be beneficial. However, the lithium treatment prevents the increase in learning and memory improvement induced by swimming exercise. The results provide evidence that stimulates investigation of the possibility that swimming exercise, in conjunction with lithium treatment, could represent a new approach to improvement of behavioral management in depression.

Key Words: Open-field. Swimming exercise. Morris water maze. Elevated plus-maze. Lithium. Object recognition.

Resumo

Estudos anteriores indicam que o exercício crônico apresenta alguns efeitos sobre o sistema nervoso central, observados após tratamento com antidepressivos. O objetivo deste trabalho foi investigar os possíveis efeitos do tratamento com lítio em camundongos nadadores em modelos animais de ansiedade e memória. Os camundongos foram avaliados no labirinto em cruz elevado, campo aberto, labirinto aquático de Morris e reconhecimento de objetos. Camundongos adultos machos foram divididos em quatro grupos: controle sedentário (CS), lítio sedentário (LS), controle exercício de natação (CE) e lítio exercício de natação (LE), tratados com ração normal ou contendo 0,25% de carbonato de lítio, por 30 dias. Os resultados sugerem que o tratamento com lítio, exercício de natação ou o tratamento com lítio combinado com exercício de natação induz um efeito ansiolítico nos camundongos submetidos ao teste do labirinto em cruz elevado. Estes sintomas foram observados em LS, com aumento no tempo de permanência e entradas nos braços aberto (F = 3.238, p<0,05 e F = 4.093, p<0,05, respectivamente); enquanto CE - LE mostraram um aumento no tempo gasto na avaliação de risco (F = 17.644, p<0,001). Os testes no campo aberto indicaram uma diminuição do medo e um aumento na atividade exploratória. LS - CE diminuíram o tempo gasto no congelamento (F = 3.274, p<0,05) e CE - LE aumentaram o tempo gasto na atividade exploratória (F = 9.149, p<0,001). As comparações realizadas em cada grupo durante os cinco dias de teste no labirinto aquático de Morris mostraram uma diminuição no tempo de latência nos grupos LS - CE a partir do segundo dia de teste (F = 21.512, p<0,001 e F = 3.688, p<0,001; respectivamente). No teste de reconhecimento de objetos, camundongos do grupo CE aumentaram a razão de exploração tanto na memória a curto-prazo (MCP) quanto na de longo-prazo (MLP), quando comparado com o CS (F = 9.246, p<0,001). Os grupos LS - LE não apresentaram diferenças significativas em relação ao CS (p>0,05). Este estudo mostra que o exercício de natação e/ou tratamento com lítio induz efeitos ansiolíticos, indicando que as interações entre a atividade física e/ou tratamento com lítio podem ser benéficas. Entretanto, o tratamento com lítio impede o aumento na aprendizagem e memória, induzidos pelo exercício de natação. Os resultados fornecem evidências que estimulam as investigações sobre a possibilidade do exercício de natação, em conjunto com o tratamento com lítio, representarem uma nova abordagem para a melhoria e a gestão comportamental na depressão.

Palavras-chave: Campo aberto. Exercício de natação. Labirinto aquático de Morris. Labirinto em cruz elevado. Litio. Reconhecimento de objetos.

INTRODUCTION

Lithium or physical exercise has been used as an important in the treatment of manic-depressive[1,2]. It is interesting to note that a combination of behavioral and

pharmacological therapy is considered the most effective clinical intervention for depression[3]. Presently, the neurobiological mechanisms underlying the decreased anxiety and increased learning and memory by lithium treatment and/or physical exercise are still unclear.

The reported effects of lithium on many tests of fear and anxiety are not consistent: for example, in the conditioned freezing model of fear, in which the duration of the freezing response of electric shock is measured, lithium has been shown to enhance, to inhibit, or to have no impact upon freezing[4,5,6]. In addition, in rodents the anxiolytic effects of exercise has been somewhat more variable. In studies allowing animals voluntary access to a running wheel there are reports of anxiolytic effects[7,8,9], no effects[10] or increases in anxiety-like behavior following exercise.[11]

Reports on the effect of lithium on human cognition are inconsistent. Lithium treatment inhibits learning, memory, and speed of information processing in patients with bipolar disorders and to some extent in control subjects[12,13]. It has also been reported that lithium enhances memory in some tasks, and attenuates memory impairments induced by other factors[14,15]. As in humans, the effect of lithium on cognitive functioning in rodents is ambiguous. One group found an improvement in spatial reference memory, as measured by the Morris water maze; however, other reports of spatial working memory have yielded inconclusive results[15,16,17]. With respect to animal studies on learning and memory, physical exercise has been shown to have a beneficial effect on water maze[18,19,20], passive avoidance[21,22], pole-jumping active avoidance[23], fear conditioning to the context[24] and radial arm maze[25]. However, there are also a few studies using similar tasks that have reported no effects[26,27,28] or even a negative effect.[27,29]

Exercise has demonstrated efficacy as a monotherapy[30] as well as in combination with other treatments for depression[31] when used as a first step treatment. Exercise is associated with increases in hippocampal neurogenesis, monoamine neurotransmission and synaptic growth[32]. These effects are similar to effects seen with medications, suggesting that a common physiological mechanism or pathway is involved. These data indicate that interactions between lithium and physical activity are complex. In this study, we have used the swimming exercise model in order to analyze the performance of mice in elevated plus-maze, open-field, Morris water maze and object recognition tests, after chronic lithium treatment.

MATERIAL AND METHODS
Animals

Male Swiss mice, aging 45 days and weighing 25-30 g were used in all experiments. Mice were reared in air-conditioned rooms (23±1ºC), with a 12 h light-dark cycle; ten mice to a cage, with food and water *ad libitum*. All procedures followed a protocol approved by the local Institutional Animal Care and Use Committee.

Chronic lithium treatment

The mice were randomly assigned to one of two experimental groups: sedentary and swimming exercise. The mice were fed rat chow with 0,25% lithium carbonate added or fed its control diet (VETEC, Brazil) with supplemental saline (0,9%) in their home-cages. This treatment has been previously used, and at the end of a period of 4 weeks or more animals present lithium levels in the range of 0,4 – 1,2 mM[16,17,33], similar to the levels observed in treated patients. These diets were the only source of nutrition for the animals and were administered throughout the swimming exercise. After 4 weeks exposure to the control or lithium diets, mice began behavioral tests.

Adaptation to the water

All the mice were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the animals in shallow water at 32±1°C, 5 days during one week in sessions lasted 10 min, from 08.00 a.m. to 05.30 p.m. The purpose of the adaptation was reducing the stress without, however, promoting physical training adaptations.

Exercise training

The mice of the exercise group were trained to swim 30 min/day, 5 days a week, during 8 weeks in a progressively increasing moderate swimming program with no weight loading in free style. This has been validated by previous reports[34,35]. Daily swimming exercise was performed in a large water tank (100 cm × 40 cm × 90 cm) at 32±1æ%C a depth of 60 cm. Exercise sessions lasted 10 min on the first day of the training period and was increased by 10 min each 7 days. At the end of the 7th day the animals swam continuously for 20 min. Continuous exercise (30 min) was performed from the 14th day until the end of the training period. Sedentary mice placed in shallow water at 32±1°C, 30 min, 5 days/week, were used as controls. At the end of the training period and twenty-four hours after the last exercise bout, all rats were submitted individually to behavioral tests. All experiments were realized between 07:30 and 11:30 a.m. Those experiments were carried out in a sound-attenuated and temperature-controlled (23±1ºC) room, illuminated with one 40-W fluorescent light placed 1.3 m away from the apparatus.

Behavioral tests
Elevated plus-maze test

The elevated plus-maze test involves conflict between the desire to explore and the desire to avoid the anxiogenic stimuli of open and high spaces[36]. The test is considered to be a measure of generalized anxiety

since agents used to alleviate generalized anxiety disorder symptoms modify defensive behaviors evoked by the model[37]. The equipment consisted of a "plus" wooden shaped maze with two opposite open arms (30 x 7,5 x 0,3 cm) and two enclosed arms (30 x 7,5 x 21,5 cm) extended from a central platform (7,5 x 7,5 cm). The floor of the maze was painted with impermeable epoxy resin, to avoid urine impregnation. The maze was elevated to a height of 30 cm above the floor. A 0,3 cm high edge of plexiglas circumscribed the open arms to avoid incidental falls. Each individual animal was tested for 5 min starting from the platform facing the closed arm. The time spent in the open arms and enclosed arms as well as the entries into these compartments were measured. An open-arm entry was defined as all four of the paws being placed in the open arm. We also included ethologically derived measures related to the defensive pattern of risk assessment behavior, which has been proven very sensitive to changes in anxiety[38]. Risk assessment is measures comprised for time spent in the head-dipping (exploratory movement of head/shoulders over the side of the maze), and stretched attend postures (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion). Thus, the closed arms and center platform were designated as "protected" areas (i.e., offering relative security) and the "time protected" for head-dipping and stretched attend postures calculated as the time of these behaviors displayed in or from the protected area.[39,40]

Open-field test

The open-field test relies on a rodent's innate exploratory behavior counteracted by its natural aversion to open space. The time spent in the interior of the box is related to the exploratory behavior of mice and inversely related to the anxiety level[41]. The open-field apparatus consisted of circular wooden box (61 cm in diameter and 24 cm high) with an open top. In order to record locomotor activity, the open-field was subdivided into 17 parts. Animals were placed into the center of the open-field and allowed to explore freely for 5 min. The following parameters were recorded: time the movements of the animal between parts (ambulation); time for which the animal did not move at all (freezing); time rearing (rising on the hind paws) and time the animal performed self-cleaning (grooming). The total time spent ambulation and freezing was determined as a measure of activity. Exploration behavior in the open-field has also been used as a measure of defensive behavior, where increased line rearing responses are suggestive of a decrease in defensive behaviors.[42]

Morris water maze test

A spatial memory was performed by the method of Morris (1984)[43]. The Morris water maze was a circular pool (90 cm diameter and 30 cm height) filled with water

(32 °C) to a depth of 14 cm. The pool was located in a dimly lit, soundproof test room with a various visual cues, including a white-black colored poster on the wall, a halogen lamp and the experimenter. The maze was divided into four quadrants, and three equally spaced points served as starting positions around the edge of the pool. The order of the release positions varied systematically throughout the experiment. A circular escape platform (6 cm diameter and 12 cm high) was located in one quadrant 1 cm below the water surface during the test. One familiarization and five acquisition sessions were performed using the Morris water maze. During the familiarization session and acquisition phase of the experiment, each mouse was given three trials. The delay between the trials was 60 s, and a 1 day interval was used between each session. For each trial, the mouse was taken from the home cage and placed into the water maze at one of three randomly determined locations with its head facing the center of the water maze. After the mouse had found and climbed on to the platform, the trial was stopped, and the escape latency was recorded. If the mouse did not climb onto the platform in 60 s, the trial was stopped, and the experimenter guided the mouse to the platform. The time escape latency was used as measures for the development of spatial memory.

Object recognition test

The object recognition test measures non-spatial working memory in the mice and takes advantage of the mice unprompted nature to explore its surroundings[44]. This model is advantageous as it does not require punishment or reward and is quick and simple to implement. Mice are first exposed to two identical objects and then, following a specific delay, the rat is presented with one of the familiar objects and also a novel object. When the subject remembers the previous exposure to the familiar object, the rat will explore the novel object to a greater degree than that of the familiar one[45]. All animals were habituated to the experimental arena in the absence of any specific behavioral stimulus for 20 min/day during 4 days. The objects, made of metal or glass, were fixed to the arena's floor with adhesive ribbon. In the first day (training session) the animals were placed in the arena containing two different objects (A and B) and left to explore them freely for 5 minutes. The test was repeated 2 hours later to test short-term memory (STM) or 24 hours later to evaluate long-term memory (LTM) after the pre-test. In the tests, one of the objects was changed for a new object (C, for STM or D, for LTM) and the mice were introduced in the arena for more 5 minutes. The positions of the objects (familiar or novel) were randomly permuted for each experimental animal and the arena was cleaned between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on or turning around the object was not considered

exploratory behavior. The main index of retention was the exploration ratio - the proportion of total object-exploration time during the test phase that was spent exploring the novel object; tnovel/(tnovel+tfamiliar).

Statistical analysis

All data presented are expressed as the means ± S.E.M., and each value reflects the mean of 10 animals per group. The means were compared by analysis of variance (ANOVA), followed by Newman-Keuls multiple comparisons test. A probability level of 0,05 were used to test for statistical significance.

RESULTS

Analysis of variance revealed significant differences between groups on the time spent by mice in the open arms of the plus-maze (F = 3.238; p<0,05). The experimental group lithium sedentary (LS), increased the time spent in the open arms on the day of experiment (Figure 1, A; p<0,05). In case of the number of entries in the open arms, there were significant differences between groups (F = 4.093; p<0,05). The experimental group lithium sedentary (LS), increased the entries onto the open arms (Figure 1, B; p<0,05). There was no significant difference in groups in the time spent in the enclosed arms (F = 2.546; p>0,05; Figure 1, C) and entries onto the enclosed arms (F = 0.1926; p>0,05; Figure 1, D). Analysis of variance revealed significant differences between groups on the risk assessment time (F = 17.644; p<0,001). The experimental groups control swimming exercise (CE; p<0,01) and lithium swimming exercise (LE; p<0,001) increased the risk assessment time (Figure 1, E).

Analysis of variance revealed significant differences between groups on the time spent by mice in the ambulation of the open-field (F = 3.837; p<0,05). The experimental group lithium sedentary (LS), increased the time spent in the ambulation when compared to control sedentary (CS) (p<0,05; Figure 2, A). In case of freezing time, there were significant differences between groups (F = 3.274; p<0,05). As shown in Figure 2B, lithium sedentary (LS) and control swimming exercise (CE) significantly decreased the time spent in the freezing (p<0,05). Analysis of variance revealed significant differences between groups on the time spent by mice in the rearing (F = 9.149; p<0,001). The experimental groups control swimming exercise (CE) and lithium swimming exercise (LE) increased time spent in the rearing (p<0,05; Figure 2, C). There was no significant difference in groups to the open-field, when considering the time spent in the grooming (F = 1.103; p>0,05; Figure 2, D).

Analysis of variance revealed significant enhancement in spatial learning as decreased of latency to the platform in the Morris water maze. The group control sedentary (CS) decreased the latency time after five days (F = 5.835; p<0,01; Figure 3, A). Mice of group lithium sedentary (LS) (F = 21.512; p<0,001; Figure 3, B)
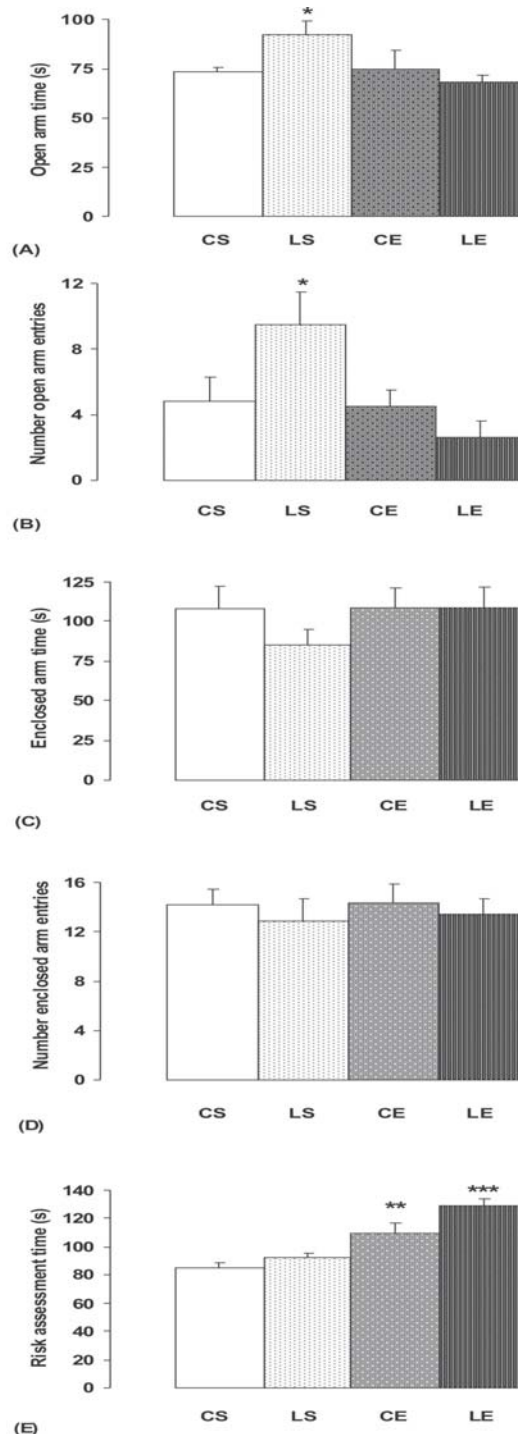

Figure 1, A-E - Behavioral responses on the elevated plus-maze of control and swimming exercise mice 30 days after treated with lithium: (A) open arm time; (B) number open arm entries, (C) enclosed arm time, (D) number enclosed arm entries, and (e) risk assessment time. The experimental groups are: control sedentary (CS), lithium sedentary (LS), control swimming exercise (CE), and lithium swimming exercise (LE). Bars represent the means for each group and the vertical lines represent the standard errors of the means. Newman-Keuls test showed that groups with asterisks are significantly different (n = 10; *p<0,05, **p<0,01 and ***p<0,001).
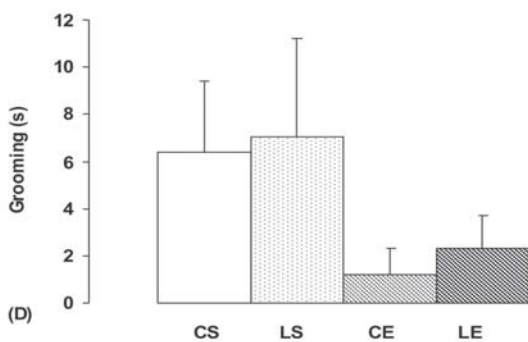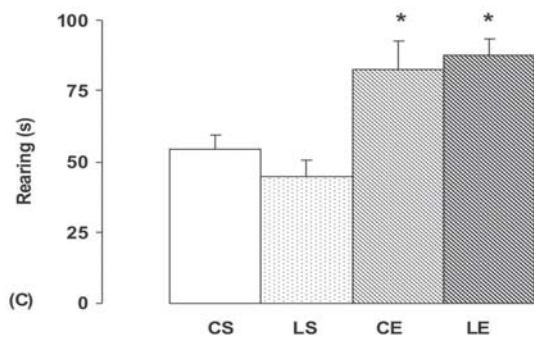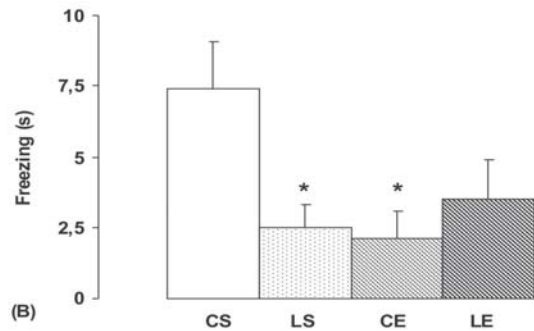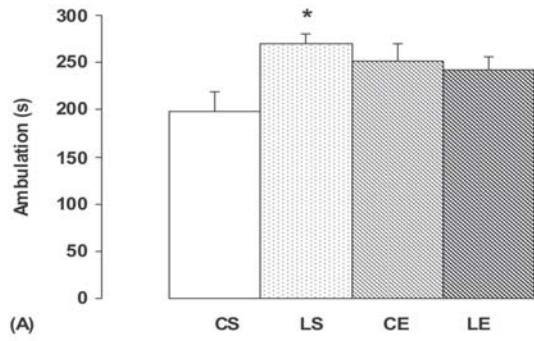
Figure 2, A-D - Behavioral responses on the open-field of control and swimming exercise mice 30 days after treated with lithium: (A) ambulation; (B) freezing; (C) rearing, and (D) grooming. The experimental groups are: control sedentary (CS), lithium sedentary (LS), control swimming exercise (CE), and lithium swimming exercise (LE). Bars represent the means for each group and the vertical lines represent the standard errors of the means. Newman-Keuls test showed that groups with asterisks are significantly different (n = 10; *p<0,05).

Figure 3, A-D - Behavioral responses on Morris water maze of control and swimming exercise mice 30 days after treated with lithium: (A) latency CS; (B) latency LS, (C) latency CE, and (D) latency LE. The experimental groups are: control sedentary (CS), lithium sedentary (LS), control swimming exercise (CE), and lithium swimming exercise (LE). Bars represent the means for each group and the vertical lines represent the standard errors of the means. Newman-Keuls test showed that groups with asterisks are significantly different (n = 10; ***p<0,001).
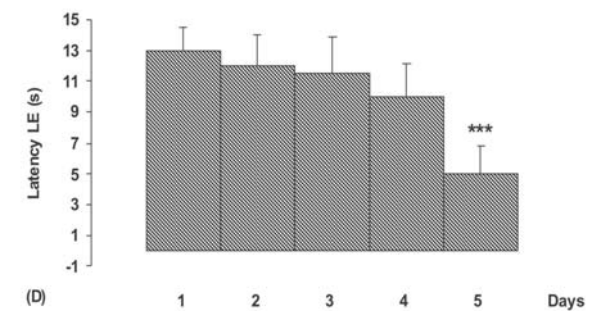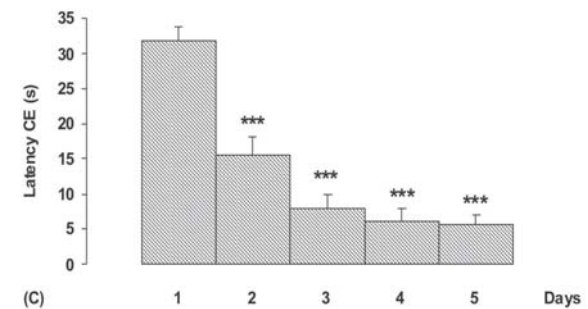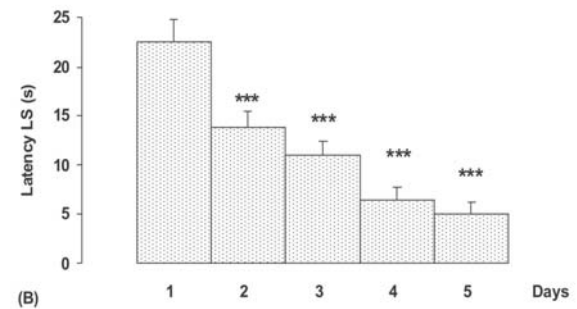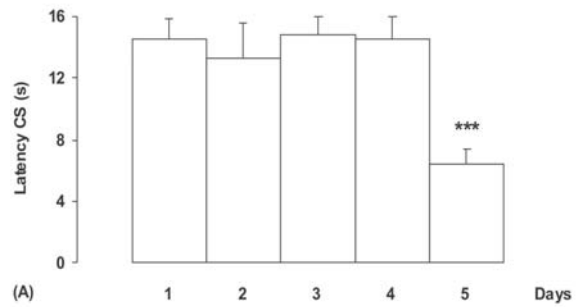
and control swimming exercise (CE) (F = 3.688; p<0,001; Figure 3, C) found the platform in significantly shortest time in the Morris water maze in all testing days. Comparison within each group during the five days of Morris maze testing, the latency time revealed a significant progressive decrease in groups lithium sedentary (LS) and control swimming exercise (CE) from the second day the testing. In this period, also the latency significantly decreased in groups lithium swimming exercise (LE) (F = 3.638; p<0,01; Figure 3, D) whose latencies decreased after five days.

Analysis of variance revealed significant enhancement in exploration ratio in the object recognition test (F = 9.246; p<0,001). Mice of group control swimming exercise (CE) increased in exploration ratio in both short (STM) and long-term object recognition memory (LTM), when compared to control sedentary (CS) (p<0,001 and p<0,01; respectively). The groups lithium sedentary (LS) and lithium swimming exercise (LE) did not differ significantly from control sedentary (CS) (p>0,05; Figure 4).
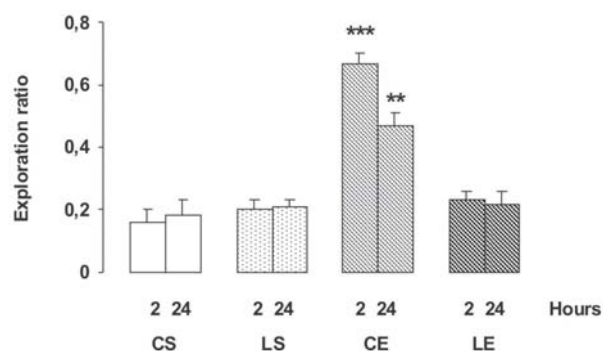

Figure 4 - Exploration ratio on object recognition of control and swimming exercise mice 30 days after treated with lithium. The test was repeated 2 hours later to test short-term memory (STM) or 24 hours later to evaluate long-term memory (LTM) after the pre-test. The experimental groups are: control sedentary (CS), lithium sedentary (LS), control swimming exercise (CE), and lithium swimming exercise (LE). Bars represent the means for each group and the vertical lines represent the standard errors of the means. Newman-Keuls test showed that groups with asterisks are significantly different (n = 10; **p<0,01 and ***p<0,001).

DISCUSSION

The results suggest that lithium treatment, swimming exercise and lithium treatment combined with swimming exercise induced an anxiolytic-like effect in mice in the elevated plus-maze test. These symptoms were observed in LS as an increase in time spent in the open arms and open arms entries (Figure 1, A and 1, B); while CE and LE as an increase in time spent in the risk assessment (Figure 1, E). The behaviors that are typically recorded when rodents are in the elevated plus-maze

are the time spent and entries made on the open and closed arms. Behavior in this task (i.e., activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments[36]. Thus anxious animals will spend most time in the closed arms while less anxious animals will explore open areas longer[37]. Other ethological measure that can be observed in rodents in the maze is time spent in risk assessment. These ethological elements, which include stretched attend postures (SAP) and head-dipping, have been linked through factor analysis to risk assessment, directed exploration, and displacement activity, respectively[38]. Furthermore, pharmacological studies have shown that the incorporation of such measures in plus-maze scoring not only reduces the likelihood of false positives and negatives[39,46], but also enhances the sensitivity of the model to novel anxiolytic[47]. These results strongly indicate that lithium treatment and/or swimming exercise results in an improved coping with aversive situations, thus, leading to a reduced anxiety level.

The differences in anxiotypic behavior expressed by these animals are not limited to their performance on the elevated plus-maze. The novel environment is an established measure of general anxiotypic behavior, and levels of locomotion, rearing and grooming in this paradigm can be used as indices of an anxiety-like state in mice[41]. The LS and CE reduced freezing in the open-field (Figure 2, B), indicated by a reduction of fear and an increase in exploratory activity. In addition, CE and LE increased the time spent in the rearing in open-field (Figure 2, C). Decline in the time spent in the freezing and increase in ambulation in open-field, consistent with reduced anxiety. However, it has also been proposed that changes observed in the time spent in the rearing rather reflect changes in the level of locomotor activity[36]. It is probable that both of them are indices of rearing behavior and both of them depend on the level of anxiety and the level of locomotor activity. These results indicate that lithium treatment and swimming reduces anxiety-like behaviors in two animal tests of anxiety, without a significant change in total activity levels.

Several physiological mechanisms have been suggested to explain the beneficial effects of lithium and physical exercise on anxiety[7,48]. Altho-ugh, it has been shown that lithium and physical exercise can regulate signal transduction pathways in several regions of the brain, and alter the function of several neurotransmitter systems[49,50], and in spite of recent neurochemical and behavioral data concerning a neuroprotective role for lithium and physical exercise[51,52], its primary effects on anxiety and memory in general are debatable. Animal studies also suggest that lithium may improve memory deficits induced by environmental stressors or neuropathological insult[16,17,48,53,54], but whether lithium is effective in altering learning combined with physical exercise is unknown.

Comparison within each group during the five days of Morris water maze test, the latency time revealed a significant progressive decrease in groups LS and CE from the second day the testing (Figure 3, B and 3, C). Furthermore, we may conclude from these behavioral data that the effects of lithium and physical exercise may be specific to particular cognitive processes, especially those involving the hippocampus, as has been previously suggested[48,55]. In support of our findings of lithium enhancements in learning in animals, a clinical report indicated that lithium may be effective in diminishing learning deficits associated with attention-deficit hyperactivity disorder[56]. Lithium also improves memory deficits in animals which were induced by environmental stressors or neuropathological insult[16,17,48]. These results strongly indicate that swimming exercise or treatment with lithium seems to be beneficial to cognition. However, as compared to CS, animals LE showed clear signs of blocking effects in learning. The latency significantly decreased in groups CS (Figure 3, A) and LE (Figure 3, D) after five days.

Since we were interested in determining the effects of lithium treatment and/or swimming exercise on spatial learning, testing this aspect of exploratory behavior allowed furthering characterizing the possible effects of lithium and/or swimming exercise in eliciting a response to novelty. Object recognition memory, is agreed to be dependent on the perirhinal cortex[57]. Whether it is a hippocampal or non-hippocampal dependent task is still in debate. While some studies reported that the task does not require the hippocampus[58], several studies support the fact that the hippocampus contributes to learned object familiarity.[59,60,61]

Mice of group CE increased in exploration ratio in both short (STM) and long-term object recognition memory (LTM), when compared to CS. The groups LS and LE did not differ significantly from CS (Figure 4). Object recognition memory is assessed by the preference that normal animals display for exploring novel, rather than familiar, complex objects[44]. In the present study, although the groups LS and LE discriminated between the two objects during acquisition training, CE exhibited much of explorative behavior for the objects when compared to CS. This data agrees with previous findings showing that swimming exercise increases the approaches to novel objects[62]. It should be noted, however, that there was no significant difference in object recognition between the group receiving lithium alone and the group receiving both lithium and swimming exercise prior to the test. This suggests that the inclusion of swimming exercise did not add significantly to the behavioral improvement brought about by lithium treatment. In addition, Vasconcellos et al.[17] demonstrated that rats chronically treated with lithium showed effects in reference memory errors but not in working memory errors in the Morris water maze.[17]

Consequently, we may conclude from these behavioral data that the effects of lithium may be specific to particular cognitive processes, especially those involving the hippocampus, as has been previously suggested[13,63]. The observed differences cannot be attributed to changes in anxiety when faced by a new object, since no differences between groups in the specific anxiety tests carried out in plus-maze and open-field (Figure 1 and 2). Thus, it is likely that the differences observed in performance during the two retention sessions are attributable to a modulation of learning and memory processes. However, due to the presence of the treatment before the acquisition sessions, it is not possible to establish whether the changes in the performance during the retention sessions are attributable to a modulation of the information processing during the sessions, a modulation of memory consolidation after the sessions or a combination of both possibilities.

The present study addressed several questions, including assessing if the effects of lithium combined with swimming exercise were similar and if distinct changes are seen in different animal models of learning and memory in Morris water maze and object recognition. Interestingly, we demonstrated that improve memory formation occurs with each intervention alone than with the combination of swimming exercise and lithium treatment. This suggests that lithium treatment blocks the behavioral improvement induced by swimming exercise. It is interesting to note that a combination of behavioral and pharmacological therapy is considered the most effective clinical intervention for depression. The possible mechanism is the effects antagonistic established interaction of lithium and physical exercise with the serotonergic system. Lithium administration has been shown to increased 5-HT release[64] and 5-HT synthesis[65] in the rat dorsal hippocampus. On the other hand, chronic swimming exercise reduced 5-HT and 5-HIAA levels in the hippocampus[66]. It is thus plausible to assume that swimming exercise might influence the 5-HT system to modulate other neural transmitter systems which collectively improve spatial learning, with different mechanisms for lithium. Furthermore, it has been pointed that the effects of a specific neural transmitter on learning and memory can often be demonstrated by a variety of tasks[67], indicating that several neural systems may be affected by a neurotransmitter. As multiple memory systems interact extensively in the mice brain, swimming exercise and/or lithium treatment is expected to have broad effects on learning and memory in general. The discussion suggested possible pharmacological bases for this shift; however, the precise mechanism underlying this change is currently unknown. Whatever these changes are, they underscore the need for more studies to be performed on the functional interaction between the various individual

hippocampal subfields. Furthermore, we may conclude from these behavioral data that the effects of swimming exercise and/or lithium may be specific to particular cognitive processes, especially those involving the hippocampus, as has been previously suggested.[13,68]

In conclusion, a great deal of scientific evidence supports exercise and lithium treatment as being beneficial to mental health. These benefits include a reduction in anxiety, depression and negative mood. The present study found that swimming exercise and/or lithium treatment induced an anxiolytic-like effect, indicating that interactions between physical activity and/or lithium may be beneficial. It is interesting to note that a combination of behavioral and pharmacological therapy is considered the most effective clinical intervention for depression. However, the lithium treatment blocks the learning and memory improvement induced by swimming exercise in mice. Our results provide evidence that stimulates investigation of the possibility that swimming exercise, in conjunction with lithium treatment, could represent a new approach to improvement of behavioral management in depression.

REFERENCES

1. SYLVIA, L. G.; AMETRANO, R. M.; NIERENBERG, A. A. Exercise t3stress markers in rat brain. **Neurochem. Int.**, Oxford, v. 49, n. 4, p. 387-392, 2006.

2. TUNDO, A. et al. Treating bipolar depression - antidepressants and alternatives: a critical review of the literature. **Acta Neuropsychiatr.**, Leiden, v. 23, n. 3, p. 94-105, 2011.

3. IVY, A. S. et al. Noradrenergic and serotonergic blockade inhibits BDNF mRNAactivation following exercise and antidepressant. **Pharmacol. Biochem. Behav.**, Phoenix, v. 75, n. 1, p. 81-88, 2003.

4. KITAICHI, Y. et al. Effect of co-administration of subchronic lithium pretreatment and acute MAO inhibitors on extracellular monoamine levels and the expression of contextual conditioned fear in rats. **Eur. J. Pharmacol.**, Amsterdam, v. 532, n. 3, p. 236-245, 2006.

5. MURAKI, I. et al. Effect of subchronic lithium carbonate treatment on anxiolytic-like effect of citalopram and MKC-242 in conditioned fear stress in the rat. **Eur. J. Pharmacol.**, Amsterdam, v. 383, n. 3, p. 223-229, 1999.

6. YOUNGS, R. M. et al. Lithium administration to preadolescent rats causes long-lasting increases in anxiety-like behavior and has molecular consequences. **J. Neurosci.**, Baltimore, v. 26, n. 22, p. 6031-6039, 2006.

7. BINDER, E. et al. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. **Behav. Brain Res.**, Amsterdam, v. 155, n. 2, p.197-206, 2004.

8. DISHMAN, R. K. Brain monoamines, exercise, and behavioral stress: animal models. **Med. Sci. Sports. Exerc.**, Madison, v. 29, n. 1, p. 63-74, 1997.

9. DISHMAN, R. K. et al. Increased open field locomotion and decreased striatal GABAA binding after activity wheel running. **Physiol. Behav.**, Elmsford, v. 60, n. 6, p. 699-705, 1996.

10. PIETROPAOLO, S. et al. The role of voluntary exercise in enriched rearing: A behavioral analysis. **Behav. Neurosci.**, Washington, v.120, n. 4, p. 787-803, 2006.

11. BURGHARDT, P. R. et al. The effects of chronic treadmill and wheel running on behavior in rats. **Brain Res.**, Amsterdam, v. 1019, n.1-2, p. 84-96, 2004.

12. PACHET, A. K.; WISNIEWSKI, A. M. The effects of lithium on cognition: an update review. **Psychopharmacology.**, Berlin, v. 170, n. 3, p. 225-234, 2003.

13. STIP, E. et al. A double-blind, placebo-controlled study of the effects of lithium on cognition in healthy subjects: mild and selective effects on learning. **J. Affect Disord.**, Amsterdam, v. 60, n. 3, p. 147-157, 2000.

14. LIN, D. Y. et al. Encoding social signals in the mouse main olfactory bulb. **Nature (Lond.).**, London, v. 434, n. 7032, p. 470-477, 2005.

15. TSALTAS, E. et al. Enhancing effects of lithium on memory are not by-products of learning or attentional deficits. **Behav. Brain Res.**, Amsterdam, v. 180, n. 2, p. 241-245, 2007.

16. VASCONCELLOS, A. P. et al. Na+, K(+)-ATPase activity is reduced in hippocampus of rats submitted to an experimental model of depression: effect of chronic lithium treatment and possible involvement in learning deficits. **Neurobiol. Learn. Mem.**, Orlando, v. 84, n. 2, p. 102-110, 2005.

17. VASCONCELLOS, A. P. et al. Effect of chronic stress on special memory in rats is attenuated by lithium treatment. **Physiol. Behav.**, Elmsford, v. 79, n. 2, p. 143-149, 2003.

18. DING, Q. et al. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. **Neuroscience.**, Oxford, v. 140, n. 3, p. 823-833, 2006.

19. UYSAL, N. et al. The effects of regular aerobic exercise in adolescent period on hippocampal neuron density, apoptosis and spatial memory. **Neurosci. Lett.**, Amsterdam, v. 383, n. 3, p. 241-245, 2005.

20. VAYNMAN, S.; YING, Z.; GOMEZ-PINILLA, F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. **Eur. J. Neurosci.**, Oxford, v. 20, n.10, p. 2580-2590, 2004.

21. ALAEI, H. et al. Treadmill running reverses retention deficit induced by morphine. **Eur. J. Pharmacol.**, Amsterdam, v. 536, n. 1-2, p. 138-141, 2006.

22. RADAK, Z. et al. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. **Neurochem. Int.**, Oxford, v. 49, n. 4, p. 387-392, 2006.

23. RADAK, Z. et al. Regular exercise improves cognitive function and decreases oxidative damage in rat brain. **Neurochem. Int.**, Oxford, v. 38, n. 1, p. 17-23, 2001.

24. BARUCH, D. E.; SWAIN, R. A.; HELMSTETTER, F. J. Effects of exercise on pavlovian fear conditioning. **Behav. Neurosci.**, Washington, v. 118, n. 2, p. 1123-1127, 2004.

25. SCHWEITZER, N. B. et al. Exercise-induced changes in cardiac gene expression and its relation to spatial maze performance. **Neurochem. Int.**, Oxford, v. 48, n. 1, p. 9-16, 2006.

26. BARNES, C. A. et al. Exercise does not modify spatial memory, brain autoimmunity, or antibody response in aged F-344 rats. **Neurobiol. Aging.**, New York, v. 12, n. 1, p. 47-53, 1991.

27. BLUSTEIN, J. E.; MCLAUGHLIN, M.; HOFFMAN, J. R. Exercise effects stress-induced analgesia and spatial learning in rats. **Physiol. Behav.**, Elmsford, v. 89, n. 4, p. 582-586, 2006.

28. SAMORAJSKI, T. et al. Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. **Neurobiol. Aging.**, New York, v. 6, n. 1, p. 17-24, 1985.

29. BRASZKO, J. J. et al. Diverse effects of prolonged physical training on learning of the delayed non-matching to sample by rats. **Neurosci. Res.**, Limerick, v. 39, n. 1, p. 79-84, 2001.

30. BLUMENTHAL, J. A. et al. Exercise and pharmacotherapy in the treatment of major depressive disorder. **Psychosom. Med.**, New York, v. 69, n. 7, p. 587-596, 2007.

31. TRIVEDI, M. H. et al. Exercise as an augmentation treatment for nonremitted major depressive disorder: a randomized, parallel dose comparison. **J. Clin. Psychiatr.**, Memphis, v. 72, n. 5, p. 677-684, 2011.

32. DISHMAN, R. K. et al. Neurobiology of exercise. **Obesity (Silver Spring).**, Silver Spring, v. 14, n. 3, p. 345-356, 2006.

33. YOUDIM, M. B. H.; ARRAF, Z. Prevention of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) dopaminergic neurotoxicity in mice by chronic lithium: involvements of Bcl-2 and Bax. **Neuropharmacology.**, Oxford, v. 46, n. 8, p. 1130-1140, 2004.

34. DAWSON, C. A.; HORVATH, S. M. Swimming in small laboratory animals. **Med. Sci. Sports.**, Madison, v. 2, n. 2, p. 51-78, 1970.

35. LIU, X. et al. Swimming exercise effects on the expression of HSP70 and iNOS in hippocampus and prefrontal cortex in combined stress. **Neurosci. Lett.**, Amsterdam, v. 476, n. 2, p. 99-103, 2010.

36. LISTER, R. G. Ethologically-based animal models of anxiety disorders. **Pharmacol. Ther.**, Oxford, v. 46, n. 3, p. 321-340, 1990.

37. PELLOW, S.; FILE, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. **Pharmacol. Biochem. Behav.**, Phoenix, v. 24, n. 3, p. 525-529, 1986.

38. CRUZ, A. P.; FREI, F.; GRAEFF, F. G. Ethopharmacological analysis of rat behavior on the elevated plus-maze. **Pharmacol. Biochem. Behav.**, Phoenix, v. 49, n. 1, p. 171-176, 1994.

39. HOLMES, A.; RODGERS, R. J. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. **Neurosci. Biobehav. Rev.**, New York, v. 23, n. 7, p. 971- 980, 1999.

40. TREIT, D.; MENARD, J.; ROYAN, C. Anxiogenic stimuli in the elevated plus-maze. **Pharmacol. Biochem. Behav.**, Phoenix, v. 44, n. 2, p. 463- 469, 1993.

41. CRAWLEY, J. N. Exploratory behavior models of anxiety in mice. **Neurosci. Biobehav. Rev.**, New York, v. 9, n. 1, p. 37- 44, 1985.

42. ROYCE, J. R. On the construct validity of open-field measures. **Psychol. Bull.**, Washington, v. 84, n. 6, p. 1098-1106, 1977.

43. MORRIS, R. Developments of a water-maze procedure for studying spatial learning in the rat. **J. Neurosci. Methods.**, Amsterdam, v. 11, n. 1, p. 47-60, 1984.

44. ENNACEUR, A. One-trial object recognition in rats and mice: methodological and theoretical issues. **Behav. Brain Res.**, Amsterdam, v. 215, n. 2, p. 244-254, 2010.

45. BLANCHARD, R. J.; SHELTON, V. F.; BLANCHARD, D. C. Historical effects of stimulus exposure: Readiness to eat and object exploration. **Learn. Motiv.**, New York, v.1, n. 4, p. 432-444, 1970.

46. RODGERS, R. J.; COLE, J. C. An ethological analysis of chlordiazepoxide and bretazenil (Ro16-6028) in the murine elevated plus-maze. **Behav. Pharmacol.**, Oxford, v. 4, n. 6, p. 573-580, 1993.

47. SETEM, J. et al. Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. **Pharmacol. Biochem. Behav.**, Phoenix, v. 62, n. 3, p. 515-521, 1999.

48. YAN, X. B. et al. Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia. **Behav. Brain Res.**, Amsterdam, v. 177, n. 2, p. 282-289, 2007.

49. MARMOL, F. Lithium: bipolar disorder and neurodegenerative diseases Possible cellular mechanisms of the therapeutic effects of lithium. **Prog. Neuropsychopharmacol. Biol. Psychiatr.**, Oxford, v. 32, n. 8, p. 1761-1771, 2008.

50. MAZZARDO-MARTINS, L. et al. High-intensity extended swimming exercise reduces pain-related behavior in mice: involvement of endogenous opioids and the serotonergic system. **J. Pain.**, Philadelphia, v. 11, n. 12, p. 1384-1393, 2010.

51. ZHANG, F.; WU, Y.; JIA, J. Exercise preconditioning and brain ischemic tolerance. **Neuroscience.**, Oxford, v. 177, n. 1, p. 170-176, 2011.

52. ZHU, Z. F. et al. Neuroprotective effect and cognitive outcome of chronic lithium on traumatic brain injury in mice. **Brain. Res. Bull.**, Phoenix, v. 83, n. 5, p. 272-277, 2010.

53. DE FERRARI, G. V. et al. Activation of Wnt signaling rescues neurodegeneration and behavioral impairments induced by beta-amyloid fibrils. **Mol. Psychiatry.**, Houndmills, v. 8, n. 2, p. 195-208, 2003.

54. ROCKENSTEIN, E. et al. Neuroprotective effects of regulators of the glycogen synthase kinase-3 signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. **J. Neurosci.**, Baltimore, v. 27, n. 8, p. 1981-1991, 2007.

55. LIU, H. L. et al. Treadmill exercise prevents decline in spatial learning and memory in APP/PS1 transgenic mice through improvement of hippocampal long-term potentiation. **Behav. Brain Res.**, Amsterdam, v. 218, n. 2, p. 308-314, 2011.

56. DORREGO, M. F. et al. A randomized, double-blind, crossover study of methylphenidate and lithium in adults with attention-deficit hyperactivity disorder: preliminary findings. **J. Neuropsychiatry Clin. Neurosci.**, Washington, v. 14, n. 3, p. 289-295, 2002.

57. MURRAY, E. A.; RICHMOND, B. J. Role of perirhinal cortex in object perception, memory, and associations. **Curr. Opin. Neurobiol.**, London, v. 11, n. 2, p. 188-193, 2001.

58. MUMBY, D. G. et al. Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage. **Behav. Brain Res.**, Amsterdam, v. 132, n. 2, p. 215-226, 2002.

59. GASKIN, S.; TREMBLAY, A.; MUMBY, D. G. Retrograde and anterograde object recognition in rats with hippocampal lesions. **Hippocampus.**, New York, v. 13, n. 8, p. 962-969, 2003.

60. HAMMOND, R. S.; TULL, L. E.; STACKMAN, R.W. On the delay-dependent involvement of the hippocampus in object recognition memory. **Neurobiol. Learn. Mem.**, Orlando, v. 82, n. 1, p. 26-34, 2004.

61. PITTENGER, C. et al. Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. **Neuron.**, Cambridge, v. 34, n. 3, p. 447-462, 2002.

62. CRUZ, J. G. P. et al. Effects of Ginkgo biloba extract (EGb 761) and repeated swimming on memory, anxiety and motor activity of rats. **R. Ciênc. Farm Básica Apl.**, Araraquara, v. 31, n. 2, p. 149-155, 2010.

63. ENGELSMANN, F.; GHADIRIAN, A. M.; GROF, P. Lithium treatment and memory: A long-term follow-up study. **J. Clin. Psychopharmacol.**, Baltimore, v. 8, n. 3, p. 207-211, 1992.

64. TREISER, S. L. et al. Lithium increases serotonin release and decreases serotonin receptors in the hippocampus. **Science (Wash.)**, Washington, v. 213, n. 4515, p. 1529-1531, 1981.

65. BRODERICK, P.; LYNCH, V. Behavioral and biochemical changes induced by lithium and L-tryptophan in muridical rats. **Neuropharmacology.**, Oxford , v. 21, n. 1, p. 671-679, 1982.

66. DEY, S.; SINGH, R. H.; DEY, P. K. Exercise training: Significance of regional alterations in serotonin metabolism of rat brain in relation to antidepressant effect of exercise. **Physiol. Behav.**, Elmsford, v. 52, n. 6, p. 1095-1099, 1992.

67. MYHRER, T. Neurotransmitter systems involved in learning and memory in the rat: A meta-analysis based on studies of four behavioral tasks. **Brain Res. Rev.**, Amsterdam, v. 41, n.2-3, p. 268-287, 2003.

68. ADLARDA, P. A.; ENGESSER-CESARA, C.; COTMANA, C. W. Mild stress facilitates learning and exercise improves retention in aged mice. **Exp. Gerontol.**, Oxford, v. 46, n. 1, p. 53-59, 2011.