

# Na<sup>+</sup>,K<sup>+</sup>-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

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## Abstract

This study was undertaken to verify the effects of chronic stress and lithium treatments on the hippocampal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity of rats, as well as to investigate the effects of stress interruption and post-stress lithium treatment on this enzyme activity and on spatial memory. Two experiments were carried out; in the first experiment, adult male Wistar rats were divided into two groups: control and submitted to a chronic variate stress paradigm, and subdivided into treated or not with LiCl. After 40 days of treatment, rats were killed, and Na<sup>+</sup>,K<sup>+</sup>-ATPase activity was determined. In the second experiment, rats were stressed during 40 days, and their performance was evaluated in the Water Maze task. The stressed group was then subdivided into four groups, with continued or interrupted stress treatment and treated or not with lithium for 30 additional days. After a second evaluation of performance in the Water Maze, rats were killed and Na<sup>+</sup>,K<sup>+</sup>-ATPase activity was also measured. Results showed an impairment in Na<sup>+</sup>,K<sup>+</sup>-ATPase activity and in Water Maze performance of chronically stressed rats, which were prevented by lithium treatment and reversed by lithium treatment and by stress interruption. These results suggest that the modulation of Na<sup>+</sup>,K<sup>+</sup>-ATPase activity may be one of the mechanisms of action of lithium in the treatment of mood disorders.

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## 1. Introduction

Na<sup>+</sup>,K<sup>+</sup>-ATPase is the enzyme responsible for the active transport of sodium and potassium ions in the nervous system, maintaining and re-establishing, after each depolarization, the electrochemical gradient necessary for neuronal excitability and regulation of neuronal cell volume. It is present in high concentrations in brain

cellular membranes, consuming about 40–50% of the ATP generated in this tissue (Erecinska & Silver, 1994).

The pathophysiology of some psychiatric disorders is believed to be associated with some perturbation of ion homeostasis, and earlier studies have shown that Na<sup>+</sup>,K<sup>+</sup>-ATPase activity is decreased in patients with depression and other psychiatric disorders (Hokin-Neaverson & Jefferson, 1989; Mynett-Johnson et al., 1998; Rybakowsky, Potok, Strzizewski, & Nowakowska, 1984; Wood et al., 1991). Nevertheless, little is known about this enzyme activity in experimental models of

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depression in animals, although we have previously observed a decreased  $\text{Na}^+, \text{K}^+$ -ATPase activity in the hippocampus of animals submitted to chronic mild stress (Gamaro, Manoli, Torres, Silveira, & Dalmaz, 2003). Exposure to chronic mild stress has been proposed as a model of depression in animal studies (Katz, 1981; Pucilowski, Overstreet, Rezvani, & Janowsky, 1993; Willner, 1990, 1991), and this effect was accompanied by anhedonic behavior, a characteristic of depressive states.

Additionally, we have shown that animals submitted to chronic variate stress present a cognitive deficit in spatial memory, as evaluated by the Morris Water Maze task (Vasconcellos, Tabajara, Ferrari, Rocha, & Dalmaz, 2003). Some authors believe that the impairing effects of chronic stress could result from a disruption in brain energy metabolism (Hoyer, Lannert, Latteier, & Meisel, 2004; Sadowski et al., 2004). This energy deficit could, consequently, affect  $\text{Na}^+, \text{K}^+$ -ATPase activity. Furthermore, a set of studies have suggested the involvement of  $\text{Na}^+, \text{K}^+$ -ATPase activity in memory consolidation (Sato et al., 2004; Wyse et al., 2004).

Lithium salts are widely used for the treatment of affective disorders, and increasing evidence supports the notion that lithium has neuroprotective effects in a variety of insults (Chen & Chuang, 1999; Jope, 1999; Manji, Moore, & Chen, 1999). We observed previously that the memory impairments in rats caused by exposure to chronic stress was prevented by concomitant lithium treatment (Vasconcellos et al., 2003). Although the mechanism of action of lithium remains unclear, it is already known that this cation is able to normalize erythrocyte  $\text{Na}^+, \text{K}^+$ -ATPase activity in patients with mood disorders (Hokin-Neaverson & Jefferson, 1989). Moreover, lithium prevents or delays neurochemical and behavioral effects elicited by ouabain, an inhibitor of  $\text{Na}^+, \text{K}^+$ -ATPase activity (El-Mallakh, Schurr, Payne, & Li, 2000; Hennion, El-Masri, Huff, & El-Mallakh, 2002; Li, el-Mallakh, Harrison, Changaris, & Levy, 1997), suggesting a possible modulation of  $\text{Na}^+, \text{K}^+$ -ATPase activity by this cation.

The aim of the present study was to verify the effect of an animal model of depression (chronic variate stress) on  $\text{Na}^+, \text{K}^+$ -ATPase activity in synaptic plasma membranes from rat hippocampus, and the action of lithium treatment on such effect. In addition, we also evaluated the effects of stress interruption on enzymatic activity and on spatial memory, as well as the effects of administration of lithium after repeated stress.

## 2. Experimental procedures

### 2.1. Animals

One hundred adult male Wistar rats (60 days old; 180–230 g in weight) were used. The experimentally

naive animals were housed in groups of 4 or 5 in home-cages made of Plexiglas material (65 × 25 × 15 cm) with the floor covered with sawdust. Animals were maintained under a standard dark-light cycle (lights on between 7:00 and 19:00 h) in a room temperature of  $22 \pm 2^\circ\text{C}$ . The rats had free access to food and water. All animal treatments were in accordance with the institutional guidelines and according to the recommendations of the International Council for Laboratory Animal Science (ICLAS), and all efforts were made to reduce the number of animals. Animals were further divided in subgroups, control and stressed, and receiving or not lithium treatment; see Table 1 for group divisions and experimental design.

### 2.2. Chronic variate stress model

Chronic variate stress was modified from other models of variate stress (Gamaro et al., 2003; Konarska, Stewart, & McCarty, 1990; Murua & Molina, 1992; Willner, 1990, 1991). The animals were divided into two groups: group 1 (control), that was kept undisturbed in their home cages during the first 40 days of treatment, and group 2 (chronically stressed). A chronic variate-stressor paradigm was used for the animals in the stressed group. The following stressors were used: (a) inclination of the home cages at a  $45^\circ$  angle for 4–6 h, (b) 10–15 min of noise, (c) 1–3 h of restraint, as described below, (d) 1.5–2 h of restraint at  $4^\circ\text{C}$ , (e) forced swimming for 10 or 15 min, as described below, (f) flashing light during 2 to 4 h, and (g) isolation (2–3 days). Animals were exposed to stress starting at a different time everyday, in order to minimize its predictability. We have previously measured plasma corticosterone levels after this chronic stress model, and corticosterone basal levels (i.e., levels at the 40th day of exposure to stress, before being exposed to a stressor) were not different from control animals. After being exposed to a stressor, at the 40th day of treatment, corticosterone levels increased around 90%.

Restraint was carried out by placing the animal in a 25 × 7 cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1 cm hole at the far end for breathing. Forced swimming was carried out by placing the animal in a glass tank measuring 50 × 47 × 40 cm with 30 cm of water at  $23 \pm 2^\circ\text{C}$ . Exposure to flashing light was achieved by placing the animal in a 50 cm-high, 40 × 60 cm open field made of brown plywood with a frontal glass wall. A 100 W lamp, flashing in a frequency of 60 flashes per minute, was used.

### 2.3. Chronic lithium treatment

Lithium was administered through the chow. Lithium chloride ( $\text{LiCl}$ —2.5 mg/g of chow) and sodium

Table 1  
Groups and experimental design for experiments 1 and 2

Groups		Treatment duration			Experiment			
Experiment 1								
20 male Wistar rats	Control (n = 5)	40 days of treatment			Evaluation of Na <sup>+</sup> ,K <sup>+</sup> -ATPase activity			
	Lithium treated (n = 5)							
	Chronically stressed (n = 5)							
	Stressed and treated with lithium (n = 5)							
Group	Treatment duration	Experiment	Subdivision of the groups	Treatment duration	Experiment	Lithium and stress treatment continued	Experiment	
Experiment 2								
85 male Wistar rats	Control n = 25	40 days of treatment	First exposure to the Water Maze task	Control	30 additional days of treatment	Second exposure to the Water Maze task	One week of interval	Evaluation of Na <sup>+</sup> ,K <sup>+</sup> -ATPase activity
	Stress n = 60			Control + lithium				
				Stress continued				
				Stress continued + lithium				
				Stress interrupted + lithium				
				Stress interruption				

Experiment 1: rats were treated for 40 days with the procedures described under Section 2, constituting four experimental groups: control (1), lithium treated (2), chronically stressed (3), and chronically stressed plus lithium treatment (4).

Experiment 2: rats were treated for 40 days with the procedures described under Section 2, constituting two experimental groups: control and chronically stressed. At the end of this period, these animals were submitted to the first exposure to the Water Maze task. Afterwards, the control group was divided in two other groups: control (1) and lithium treatment (2), and the stressed group was subdivided in four other groups: stress continued (3), stress continued plus lithium (4), stress interrupted plus lithium (5), and stress interrupted (6). The animals were treated during 30 additional days, and then submitted to a second exposure to the Water Maze task. One week after Water Maze procedures, and 24 h after the last stress exposure, the animals were killed by decapitation and brains were removed for enzymatic measurements.

chloride (NaCl—17 mg/g) were added to the food, as described by Rocha and Rodnight (1994). 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 0.6–1.2 mM (Rocha & Rodnight, 1994; Vasconcellos et al., 2003), similar to the levels observed in treated patients.

#### 2.4. Water Maze apparatus and procedures

This task was adapted from the paradigm originally described by Morris (1984). The Water Maze was a black circular pool (180 cm diameter, 60 cm high), filled with water (depth 30 cm; 24 ± 1 °C), placed in a room that was rich in consistently located spatial cues (including a large wood door, two prominent posters on one wall, and the experimenter). An escape platform (10 cm diameter) was placed in the middle of one of the quadrants, 1.5 cm below the water surface, equidistant from the sidewall and middle of the pool. The platform provided the only escape from the water and was located in the same quadrant in every trial. The position of the animal in the pool was recorded during the entire experiment. Four different starting positions were equally spaced around the perimeter of the pool. On each of the training days, all four start positions were used once in a random sequence (i.e. four training trials per day). A trial began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape within

60 s, it was gently conducted to the platform by the experimenter. The rat was allowed to stay there for 10 s. The intertrial interval was 10 min. After each trial the rats were dried and were returned to their cages at the end of the session. Animals were trained for 5 days. Twenty-four hours after the last training session, the rats were submitted to a test session. Before this session, the submerged platform was removed. The retention test consisted of placing the animals in the water for 1 min. The latency in reaching the original position of the platform, the number of crossings in that place, and the time spent in the target quadrant compared to the opposite quadrant were measured. Training and test sessions were always performed between 13 and 17 h.

The protocol for the second exposure to the Water Maze task was identical to the explained above, except by the modification of the platform position during training.

#### 2.5. Preparation of synaptic plasma membrane from hippocampus

After chronic treatments (Experiment 1; see Table 1) and 1 week after exposure to the Water Maze task (Experiment 2; see Table 1), animals were killed by decapitation without anesthesia, the brain was rapidly removed, and the hippocampus was dissected to prepare synaptic plasma membranes according to the method of Jones and Matus (1974), with some modifications (Wyse et al., 2000).

The hippocampus was homogenized in ten volumes of a 0.32M sucrose solution containing 5mM Hepes and 1mM EDTA. The homogenate was centrifuged at 1000g for 20min and the supernatant removed and centrifuged at 12,000g for a further 20min. The pellet was then resuspended in hypotonic buffer (5.0mM Tris–HCl buffer, pH 8.1), incubated at 0 °C for 30min, and applied on a discontinuous sucrose density gradient consisting of successive layers of 0.3, 0.8, and 1.0M. After centrifugation at 69,000g for 2h, the fraction at the 0.8–1.0M sucrose interface was taken as the membrane enzyme preparation.

### 2.6. $\text{Na}^+, \text{K}^+$ -ATPase activity assay

The reaction mixture for the  $\text{Na}^+, \text{K}^+$ -ATPase assay contained 5.0mM  $\text{MgCl}_2$ , 80.0mM NaCl, 20.0mM KCl, and 40.0mM Tris–HCl buffer, pH 7.4, in a final volume of 200  $\mu\text{L}$ . The reaction was started by the addition of ATP (disodium salt, vanadium free) to a final concentration of 3.0mM. Control was assayed under the same conditions with the addition of 1.0mM ouabain.  $\text{Na}^+, \text{K}^+$ -ATPase activity was calculated by the difference between the two assays (Wyse et al., 2000). Released inorganic phosphate (Pi) was measured by the method of Chan, Delfer, and Junger (1986). Enzyme specific activity was expressed as nmol Pi released per min per mg of protein. All assays were performed in duplicate and the mean was used for statistical analysis.

### 2.7. Protein measurement

Protein was measured by the method of Bradford (1976), with bovine serum albumin used as standard.

### 2.8. Statistical analysis

Data were expressed as means  $\pm$  SEM.  $\text{Na}^+, \text{K}^+$ -ATPase activity was analyzed using one- or two-way analysis of variance, followed by the Duncan multiple range test when the *F* test was significant. Water Maze performance was analyzed using Student's *t* test (when comparing two groups) or one-way analysis of variance, followed by the Duncan's multiple range test when the *F* test was significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software.

## 3. Results

### 3.1. Experiment 1: Effect of chronic stress and chronic lithium treatment upon hippocampal $\text{Na}^+, \text{K}^+$ -ATPase activity

After the treatments, hippocampal  $\text{Na}^+, \text{K}^+$ -ATPase activity was measured. The effect of 40 days of chronic

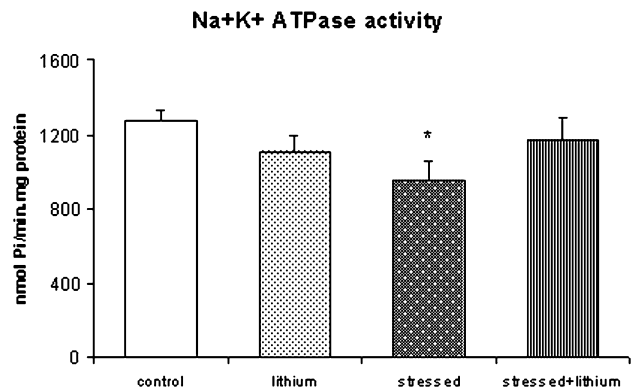


Fig. 1. Effect of chronic variable stress and chronic lithium treatment on  $\text{Na}^+, \text{K}^+$ -ATPase activity in synaptic plasma membranes from rat hippocampus. Control represents the normal group, lithium represents the group treated just with LiCl for 40 days, stressed represents the group submitted to a chronic variable stress paradigm for 40 days, and stressed+lithium represents the group stressed for 40 days and concomitantly submitted to lithium treatment. Data are expressed as means  $\pm$  SEM, for 5–7 animals in each group. There was a significant interaction between stress and lithium treatments (two-way ANOVA,  $P < 0.05$ ). \*Significantly different from the control group (Duncan's multiple range test,  $P < 0.05$ ).

variable stress and concomitant chronic lithium treatment upon the  $\text{Na}^+, \text{K}^+$ -ATPase activity is shown in Fig. 1. A two-way analysis of variance showed a significant interaction between stress and lithium treatment [two-way ANOVA,  $F(1, 20) = 4.08$ ,  $P < 0.05$ ,  $N = 5-7$  animals/group]. A Duncan's multiple range test demonstrated that just the stressed group was different from the control group, indicating that lithium, although not presenting any effect by itself, prevented the stress-induced inhibition in the enzyme activity.

### 3.2. Experiment 2: Effect of chronic stress interruption and post-stress chronic lithium treatment upon spatial memory in the Water Maze task and upon hippocampal $\text{Na}^+, \text{K}^+$ -ATPase activity

To verify whether lithium treatment could reverse the cognitive impairment and the decrease in  $\text{Na}^+, \text{K}^+$ -ATPase activity after these effects of chronic stress were already established, rats were initially divided into two groups: control and chronically stressed. After 40 days of chronic stress exposure, animals were submitted to the Water Maze task (Fig. 2), and the stressed rats showed impaired performance in this task. Regarding the number of times that the animals crossed the platform location, results showed that chronically-stressed rats presented a decreased number of crossings [Student's *t* test;  $t(31) = 2.804$ ;  $P < 0.01$ ], as shown in Fig. 2A. These results are not related to reduced motor activity, since no difference is observed in the number of crossings and rearings between groups when these animals are exposed to an open field (data not shown). When analyzing the

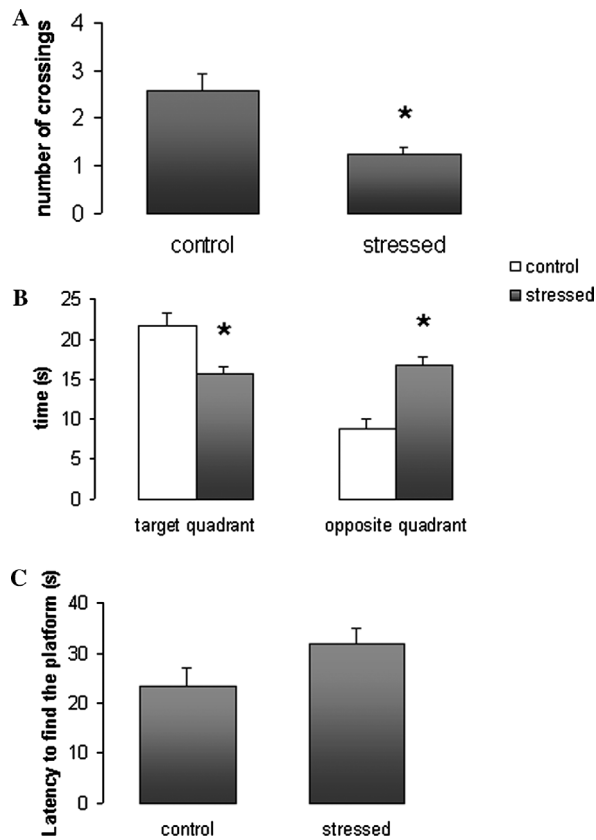


Fig. 2. Effect of treatment with chronic variable stress for 40 days on the performance of rats in the Morris Water Maze task. (A) Number of crossings performed by the animals at the exact location where the platform was; (B) time spent by the animals in the target quadrant and in the opposite quadrant; and (C) latency to find the original location where the platform was. Data are expressed as means  $\pm$  SEM,  $n = 28$  animals in the control group and 60 animals in the stressed group. \*Significant effect of stress treatment (Student's  $t$  test for independent samples,  $P < 0.01$ ).

time spent in the target and in the opposite quadrants, it was observed that chronic stress decreased time spent in the target quadrant [Student's  $t$  test;  $t(44) = 2.932$ ;  $P < 0.05$ ], while increasing time spent in the opposite quadrant [Student's  $t$  test;  $t(81) = 4.610$ ;  $P < 0.01$ ; Fig. 2B]. As may be observed in Fig. 2C, there was no significant difference in the latency to reach the original position of the platform [ $t(50) = 1.219$ ;  $P > 0.05$ ].

Afterwards, the stressed animals were divided into four additional groups, as displayed in Table 1, and the stress treatment was continued (groups 3 and 4) or discontinued (groups 5 and 6), with (groups 4 and 5) or without (groups 3 and 6) lithium treatment. The control group was divided into two groups, treated or not with lithium. Thirty days later, animals were again submitted to the Water Maze task (Fig. 3). The continuously stressed group presented an impaired performance in this task when compared to all the other groups, as demonstrated by an increased latency to find the original

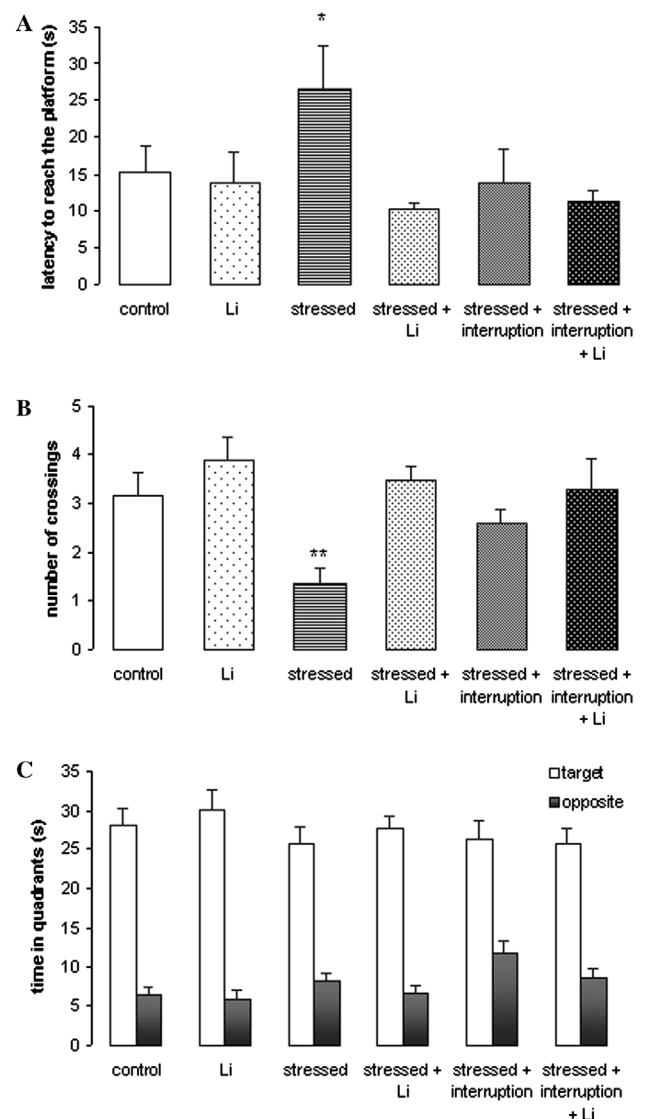


Fig. 3. Effect of stress interruption and of post-stress lithium treatment on the performance of rats in the Morris Water Maze task. (A) Latency to find the original location where the platform was; (B) number of crossings performed by the animals at the exact location where the platform was; and (C) time spent by the animals in the target quadrant and in the opposite quadrant. Control represents the normal group; Li represents the control group that received lithium for 30 days; stressed represents the group that continued to be stressed during all the experimental protocol; stressed + Li represents the stressed group that, after 40 days of stress, started to receive lithium for 30 days; stressed + interruption represents the group that had the stress treatment interrupted, and stressed + interruption + Li represents the group that had the stress treatment interrupted and started to receive lithium treatment. Data are expressed as means  $\pm$  SEM, for 10–15 animals in each group. \*Significantly different from the control and from all the other groups (one-way ANOVA, followed by Duncan's multiple range test,  $P < 0.05$ ); \*\*Significantly different from the control and from all the other groups (one-way ANOVA, followed by Duncan's multiple range test,  $P < 0.001$ ).

place where the platform was localized [ $F(5, 71) = 2.60$ ;  $P < 0.05$ , followed by Duncan's multiple range test,  $N = 10$ –15 animals/group], and a decreased number of

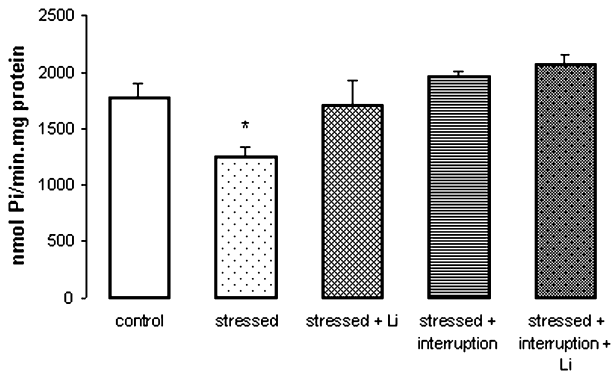


Fig. 4. Effect of stress interruption and of post-stress lithium treatment on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity in hippocampal synaptic plasma membranes. Control represents the normal group; stressed represents the group that continued to be stressed during all the experimental protocol; stressed + Li represents the stressed group that, after 40 days of stress, started to receive lithium for 30 days; stressed + interruption represents the group that had the stress treatment interrupted, and stressed + interruption + Li represents the group that had the stress treatment interrupted and started to receive lithium treatment. Data are expressed as means  $\pm$  SEM,  $n = 5$  animals/group. \*Significantly different from the control and from all the other groups (one-way ANOVA, followed by Duncan's multiple range test,  $P < 0.005$ ).

crossings over the platform [ $F(5, 71) = 4.44$ ;  $P = 0.001$ , followed by Duncan's multiple range test]. Both these effects of stress were reversed by lithium treatment, as well as by stress interruption. There was no difference in the time spent in the target quadrant [ $F(5, 71) = 0.60$ ;  $P > 0.05$ ].

One week after this behavioral task, animals were killed and hippocampal Na<sup>+</sup>,K<sup>+</sup>-ATPase activity was measured. As displayed in Fig. 4, the animals stressed during 70 days presented reduced enzymatic activity, when compared to other groups [ $F(4, 15) = 5.851$ ,  $P < 0.005$ , followed by Duncan's multiple range test,  $N = 4$  animals/group].

#### 4. Discussion

In the present study, we used a chronic stress model adapted from studies concerning models of depression in animals (Echandia, Gonzalves, Cabrera, & Fracchia, 1988; Konarska et al., 1990; Murua & Molina, 1992; Papp, Willner, & Muscat, 1991; Willner, 1990), which consists of exposing rats to different weak stressors for several days. We evaluated the effects of this chronic variate stress model on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity in the hippocampus, since this region is particularly sensitive to stress effects (McEwen, 2000; Sapolsky, 2000). A decreased activity of Na<sup>+</sup>,K<sup>+</sup>-ATPase was observed in stressed animals, in agreement with previous observations both in animals (Gamaro et al., 2003) and in depressed patients (el-Mallakh & Wyatt, 1995). Since decreased activity of this enzyme seems to be an impor-

tant characteristic of depressive disorders (el-Mallakh & Li, 1993; el-Mallakh & Wyatt, 1995), this finding further support this model as an animal model of depression.

A common feature of chronic stress exposure and major depression is the activation of the hypothalamus–pituitary–adrenal (HPA) axis, which culminates in the synthesis and release of glucocorticoids (Barden, 2004). Glucocorticoids present a negative feed-back upon corticosteroid receptors (GR), located at the level of pituitary, hypothalamus, and limbic brain areas (Barden, 2004). Sapolsky, Krey, and McEwen (1984) first demonstrated that elevated glucocorticoid levels lead to loss of hippocampal GR-containing cells that mediate the glucocorticoid-induced suppression of HPA axis, what could lead to its persistent hyperactivation. An extensive literature has shown that prolonged stress or prolonged exposure to glucocorticoids can have adverse effects on the rodent hippocampus, which may include neuronal atrophy and explicit memory deficits, and more recent findings suggest a similar phenomenon in the human hippocampus of patients with neuropsychiatric disorders, such as major depression (Sapolsky, 2000). What is not well established is whether the hippocampal atrophy arises from the neuropsychiatric disorder or precedes and predisposes toward it (Barden, 2004; Campbell & MacQueen, 2004; Sapolsky, 2000). The fact is that this atrophy, with a reduction in the number of branch points and synapses, may be involved in several chronic stress effects, including reduced Na<sup>+</sup>,K<sup>+</sup>-ATPase activity.

Several studies have demonstrated that major alterations of the HPA axis can be successfully reversed or prevented by treatments with antidepressants (Holsboer & Barden, 1996; Peiffer, Veillux, & Barden, 1991), which can act by increasing glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) expression and function, and this, in turn, is associated with enhanced negative feedback by endogenous glucocorticoids.

Considering that lithium treatment is able to increase GR in rat brain (Semba, Watanabe, Suhura, & Akanuma, 2000), we also verified the effect of lithium treatment on the reduction of Na<sup>+</sup>,K<sup>+</sup>-ATPase activity caused by stress. Although lithium did not present any effect by itself, it was able to prevent chronic stress effects on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity, in such a way that animals that were chronically stressed and treated with lithium showed the same levels of enzyme activity as controls. This cation is able to normalize erythrocyte Na<sup>+</sup>,K<sup>+</sup>-ATPase activity in bipolar patients, a disturbance that is characterized by surges of mania intercalated with periods of depression (Hokin-Neaverson & Jefferson, 1989; Wood, Elphick, & Grahame-Smith, 1989), and these results of our work suggest that lithium treatment may be useful also in the treatment of major depression. Since Na<sup>+</sup>,K<sup>+</sup>-ATPase is essential to brain normal function, modulation of this enzyme might contribute to the therapeutic efficacy and neuroprotective effects of lithium.

To mimic clinical conditions, when drug therapy is applied after a mood disorder has been diagnosed, we evaluated the effects of chronic lithium treatment after the establishment of chronic stress effects. Lithium treatment was initiated after 40 days of chronic variate stress, when both behavioral (spatial memory) and neurochemical ( $\text{Na}^+, \text{K}^+$ -ATPase activity) deficits had already been installed. It was observed that, in this condition, lithium treatment was able to reverse the biochemical and behavioral alterations studied. This is an important property of this salt, since in most cases a treatment is required after the damage has been installed.

Different morphological and neurochemical effects have been reported in hippocampus and other brain structures after depressive states or prolonged exposure to stress situations. On the other hand, lithium treatment has been shown to reverse illness-related atrophy and to increase the brain gray matter volume in humans (Gray, Zhou, Du, Moore, & Manji, 2003; Moore, Bebachuk, Wilds, Chen, & Manji, 2000). Some mechanisms have been proposed to explain these effects, such as the robust increase the expression of the cytoprotective protein bcl-2 in the CNS (Chen et al., 1999), activation of signaling cascades utilized by endogenous growth factors, like the extracellular signal-regulated kinase (ERK), mitogen-activated protein (MAP) kinase pathway (Manji & Chen, 2002), and altered levels of brain-derived neurotrophic factor (BDNF). Beside the ability to normalize  $\text{Na}^+, \text{K}^+$ -ATPase activity, verified in this and other studies from the literature (El-Mallakh et al., 2000; Hennion et al., 2002), these data argues in favor of the possible trophic and neuroprotective effects of chronic lithium treatment.

We observed that interruption of chronic stress by itself was able to reverse stress effects upon memory and  $\text{Na}^+, \text{K}^+$ -ATPase activity, since the stress-interrupted group presented similar values of enzymatic activity and a similar performance in the Water Maze task when compared to the control group. Several studies have shown hippocampal neuronal loss and/or atrophy after chronic-stress situations, particularly after severe stress paradigms (Kuipers, Trentani, Den Boer, & Ter Horst, 2003; McEwen, 2002). Factors underlying this cellular remodeling include elevated glucocorticoids levels, as mentioned above, which are implicated in decreased neurogenesis and increased activity of excitatory amino acid neurotransmitter, what, by this way, could result in both potentially reversible remodeling and irreversible cell death (Campbell & MacQueen, 2004). Considering that the interruption of exposure to stress was able to reverse the effects observed, we believe that they were not due to neuronal loss, but to neurochemical changes induced by this chronic mild stress model in the rat hippocampus, such as the decreased  $\text{Na}^+, \text{K}^+$ -ATPase activity.

Chronic stress has been shown to induce spatial memory deficits (Bodnoff et al., 1995; Conrad, Galea, Kuroda, & McEwen, 1996; McLay, Freeman, & Zadina, 1998; Nishimura, Endo, & Kimura, 1999; Vasconcellos et al., 2003), and we showed in a previous work that chronic lithium administration is able to attenuate the effect of stress on memory (Vasconcellos et al., 2003). Performance in tasks that measure spatial memory, such as the Water Maze task, is strongly linked to hippocampal function (see Nichols, Zieba, & Bye, 2001, for a review). We found that hippocampal  $\text{Na}^+, \text{K}^+$ -ATPase is diminished in rats chronically stressed. Thereby, this decreased enzymatic activity could be interfering in the energy-dependent memory storage stages (Gibbs & Ng, 1977).

Although the fact that a parallelism of effects was verified between  $\text{Na}^+, \text{K}^+$ -ATPase activity and spatial memory, it does not necessarily mean that the reduced activity of this enzyme would be the only cause of the memory impairment observed. However, there is evidence of a role of  $\text{Na}^+, \text{K}^+$ -ATPase in long-term potentiation (Glushchenko & Izvarina, 1997), and it has been showed that the inhibition of this enzyme activity induces long-term depression (Reich, Mason, & Alger, 2004) as well as spreading depression—a transient breakdown of neuronal function concomitant with a massive failure in ion homeostasis (Kohling et al., 2003). Additionally,  $\text{Na}^+, \text{K}^+$ -ATPase inhibition can lead to memory impairment in the inhibitory avoidance and in the Water Maze tasks (dos Reis, de Oliveira, Lamers, Netto, & Wyse, 2002; Sato et al., 2004; Wyse et al., 2004; Zhan, Tada, Nakazato, Tanaka, & Hongo, 2004), and cognitive deficits have been reported in situations where  $\text{Na}^+, \text{K}^+$ -ATPase was reduced, such as Alzheimer disease and under oxidative stress (Hattori et al., 1998; Lehotsky et al., 1999). Since the  $\text{Na}^+, \text{K}^+$ -ATPase is crucial for maintaining ionic gradients in neurons and is reported to be critically involved in potassium buffering after periods of hyperstimulation (Xiong & Stringer, 2000), it is well acceptable that a reduction in this enzyme activity may impair neuronal activity and memory storage.

The regulation of  $\text{Na}^+, \text{K}^+$ -ATPase activity is a complex matter. This activity seems to be regulated by several factors, including hormones and neurotransmitters, such as catecholamines (Mallick & Adya, 1999; Nishi et al., 1999) and serotonin (Steff & Novakoski, 1997). It should be observed that central catecholaminergic and serotonergic activity may be modulated by exposure to stress situations (Carrasco & Van de Kar, 2003). In addition, regulation of  $\text{Na}^+, \text{K}^+$ -ATPase activity can be divided into a “short-term” and a “long-term” control (Bertorello & Katz, 1993). Although the exact mechanisms underlying these results are not known, it is possible that the “long-term” control is involved, since both lithium and stress treatments were administered during several weeks. This long-term control could be due to an

altered number of pumps, brought about by an increase or decrease in protein synthesis or degradation. In this context, studies demonstrate that chronic lithium treatment may increase Na<sup>+</sup>,K<sup>+</sup>-ATPase number as measured by [<sup>3</sup>H]ouabain binding in lymphocytes (Jenkins, Aronson, & Brearley, 1991; Wood et al., 1991). Additionally, lithium can exert direct effects on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity, or indirect effects on intracellular sodium and calcium concentrations, besides interactions with second messenger transduction and formation, and it is possible that these proposed mechanisms are not mutually exclusive and may even be synergistic (el-Mallakh & Li, 1993; El-Mallakh et al., 2000). These possibilities should be tested in future studies.

In summary, the present study demonstrates that lithium treatment is able to prevent and reverse chronic variate stress effects both on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity and on cognition. Considering the effects observed in these biochemical and behavioral parameters, it is possible that there is a correlation between Na<sup>+</sup>,K<sup>+</sup>-ATPase activity and memory deficits in chronically stressed animals.

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