

## Effect of Aerobic Physical Exercise in Pinealectomized Animals Submitted to the Pilocarpine Model of Epilepsy

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### ABSTRACT

**Objective:** To better clarify the positive effects of physical exercise in the epilepsy, we analyzed the effect of the pinealectomy in animals with temporal lobe epilepsy (TLE) induced by pilocarpine submitted to an aerobic physical program. **Material and methods:** Forty adults Wistar rats were used: 1) PX + CHRONIC – Pinealectomized animals (PX) with TLE (CHRONIC) without exercise (n = 9); 2) PX + CHRONIC + EXERCISE – submitted to an aerobic physical exercise program (n = 5); 3) CHRONIC – without exercise (n = 8); 4) CHRONIC + EXERCISE (n = 8); 5) CTRL – control without exercise (n = 5); 6) CTRL + EXERCISE (n = 5). The physical exercise program consisted of 1 hour of treadmill, 5 days/week, during 30 days, at 60% VO<sub>2max</sub>. The Nissl and neo-Timm methods were used. **Results:** The pinealectomy increased the frequency of seizures in animals with epilepsy. It was observed a reduction of the neuronal death and mossy fiber sprouting in the animals with epilepsy submitted to an aerobic physical exercise program. However, the physical exercise program did not modify the frequency of the seizures in the pinealectomized animals.

**Key words:** epilepsy, melatonin, physical exercise, pilocarpine and pinealectomy.

### RESUMO

*Efeito do exercício físico aeróbio em animais pinealectomizados submetidos ao modelo de epilepsia induzida por pilocarpina*

**Objetivo:** Buscando elucidar os efeitos positivos do exercício físico aeróbio na epilepsia, analisamos a influência da pinealectomia em animais com epilepsia do lobo temporal (ELT) induzida por pilocarpina e submetidos a um programa de exercício físico. **Material e métodos:** Quarenta ratos Wistar adultos foram usados: 1) PX + CRÔNICO – pinealectomizados (PX) com ELT (CRÔNICO) sem exercício (n = 9); 2) PX + CRÔNICO + EXERCÍCIO – submetidos a um programa de exercício físico aeróbio (n = 5); 3) CRÔNICO – sem exercício (n = 8); 4) CRÔNICO + EXERCÍCIO (n = 8); 5) CTRL – controle sem epilepsia, sem exercício (n = 5); 6) CTRL + EXERCÍCIO (n = 5). O programa de exercício físico consistiu de corrida em esteira, 5 dias/semana (30 dias) a 60% VO<sub>2max</sub>. Os métodos de Nissl e neo-Timm foram utilizados. **Resultados:** A pinealectomia aumentou a frequência de crises em animais com epilepsia. Foi observada uma diminuição da morte neuronal e do brotamento de fibras musgosas em animais com epilepsia, submetidos ao programa de exercício físico. Entretanto, este programa não alterou a frequência de crises em animais pinealectomizados.

**Unitermos:** epilepsia, exercício físico, melatonina, pilocarpina e pinealectomia.

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## INTRODUCTION

Physical exercise programs are frequently contraindicated for patients with epilepsy due to the idea that physical exercise provokes seizures<sup>(1)</sup>. In opposition to this idea, in studies using animal models of epilepsy, physical exercise has demonstrated favourable effects in the control of the seizure frequency in the pilocarpine model of epilepsy<sup>(2)</sup> and in the inhibition of amygdala kindling development in rats<sup>(3)</sup>. Thus, the mechanisms involved in the control of the seizures induced by physical exercise still remain unknown.

Several reports have also demonstrated anticonvulsant effects of melatonin<sup>(4,5)</sup>, as well as increased plasmatic melatonin levels induced by physical activity<sup>(6,7)</sup>.

To better clarify the mechanisms involved in this process, we had as objective to analyse the influence of pinealectomy in animals with epilepsy induced by pilocarpine, and thus evaluate the effect of endogenous melatonin as a possible mediator of the positive effects of the physical exercise.

## MATERIAL AND METHODS

Forty adult male Wistar rats (200-250 g) were housed under environmentally controlled conditions with 12/12 h light/dark cycle and were divided into six groups: 1) PX + CHRONIC – Pinealectomized animals (PX) with TLE (CHRONIC) without exercise (n = 9); 2) PX + CHRONIC + EXERCISE – submitted to an aerobic physical exercise program (n = 5); 3) CHRONIC – without exercise (n = 8); 4) CHRONIC + EXERCISE (n = 8); 5) CTRL – control without exercise (n = 5); 6) CTRL + EXERCISE (n = 5).

Pinealectomy was performed following the method described by Siuciak & Dubocovich<sup>(8)</sup>.

Pilocarpine hydrochloride (350 mg/kg, Sigma, St. Luis, MO)<sup>(9)</sup> was used for inducing *status epilepticus* and the surviving animals were continuously video-monitored 24 h/day until 30 days after characterization of the chronic period. Pinealectomized groups were submitted to the pilocarpine model 15 days after the surgical procedure.

The animals of the exercise groups were submitted to an aerobic exercise program after familiarization with the apparatus (Columbus instruments) and to provide a measure of trainability, we rated each animal treadmill performance on a scale of 1-5<sup>(10)</sup> including just the animals with a mean rating 3 or higher. The physical exercise program consisted of 1 hour of treadmill, 5 days/week, during 30 days, at 60%  $VO_{2max}$ <sup>(2)</sup>.

After the behavioural period, all animals were perfused and their brains processed for histological analysis through the Neo-Timm<sup>(11)</sup> evaluating the supragranular mossy fiber

staining using a score ranging of 0 to 5<sup>(12)</sup> and Nissl method using coronal adjacent sections (40  $\mu$ m thick) to analyse the survival of the cells in the hilus of the dentate gyrus and in the hippocampal subfields.

One-way Analysis of Variance (ANOVA) followed by Bonferroni Multiple Comparisons Test and unpaired t test with Welch correction was used and  $p < 0.05$  was accepted as significant.

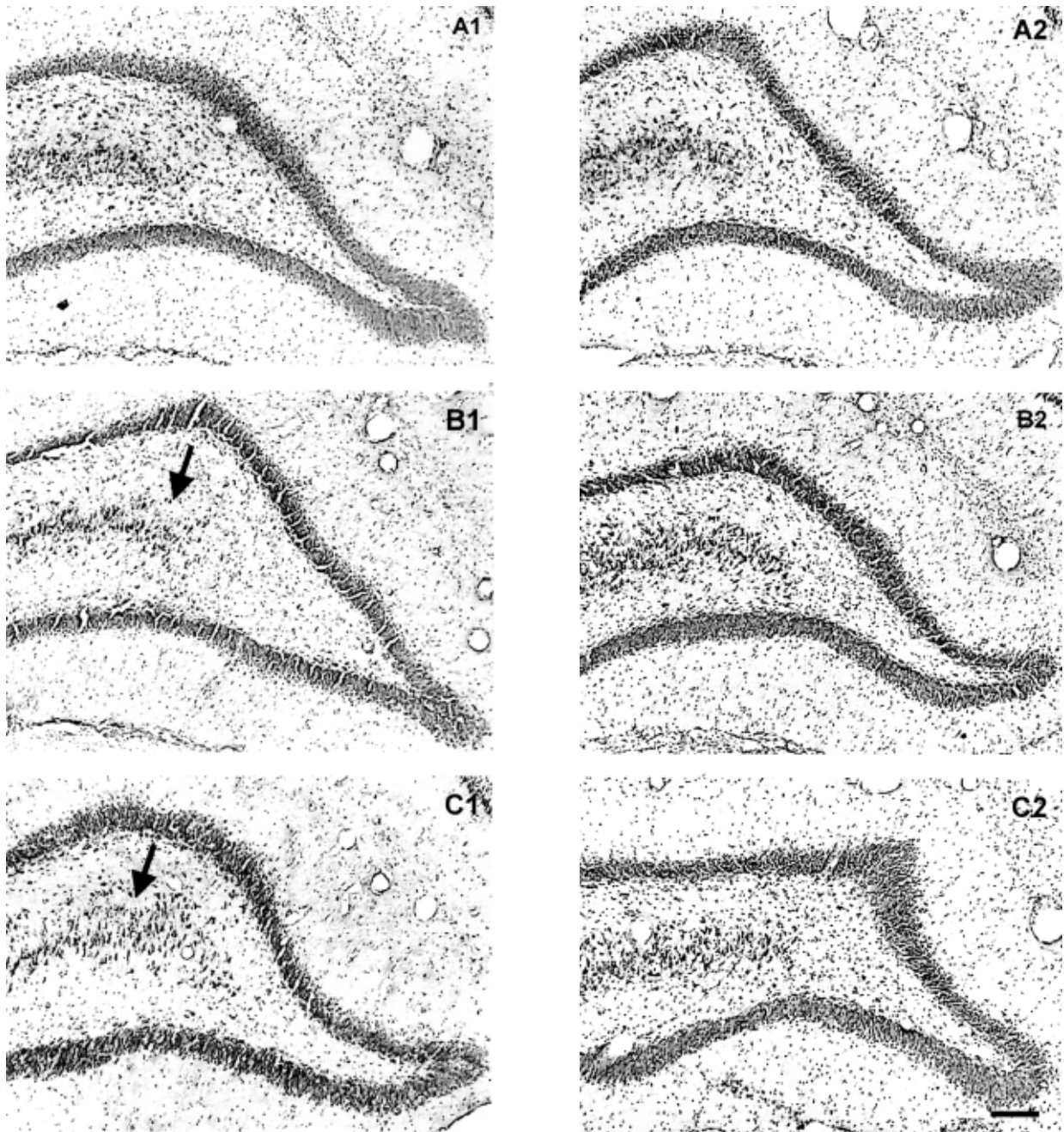
## RESULTS

Pilocarpine treatment in PX animals sequentially induced the similar behavioural changes as previously reported<sup>(9,13)</sup> evolving to *status epilepticus* (SE). After SE, animals returned to normal over a 3 to 5-day period<sup>(9,13)</sup>. The chronic period was characterized by the occurrence of spontaneous recurrent seizures<sup>(13)</sup>.

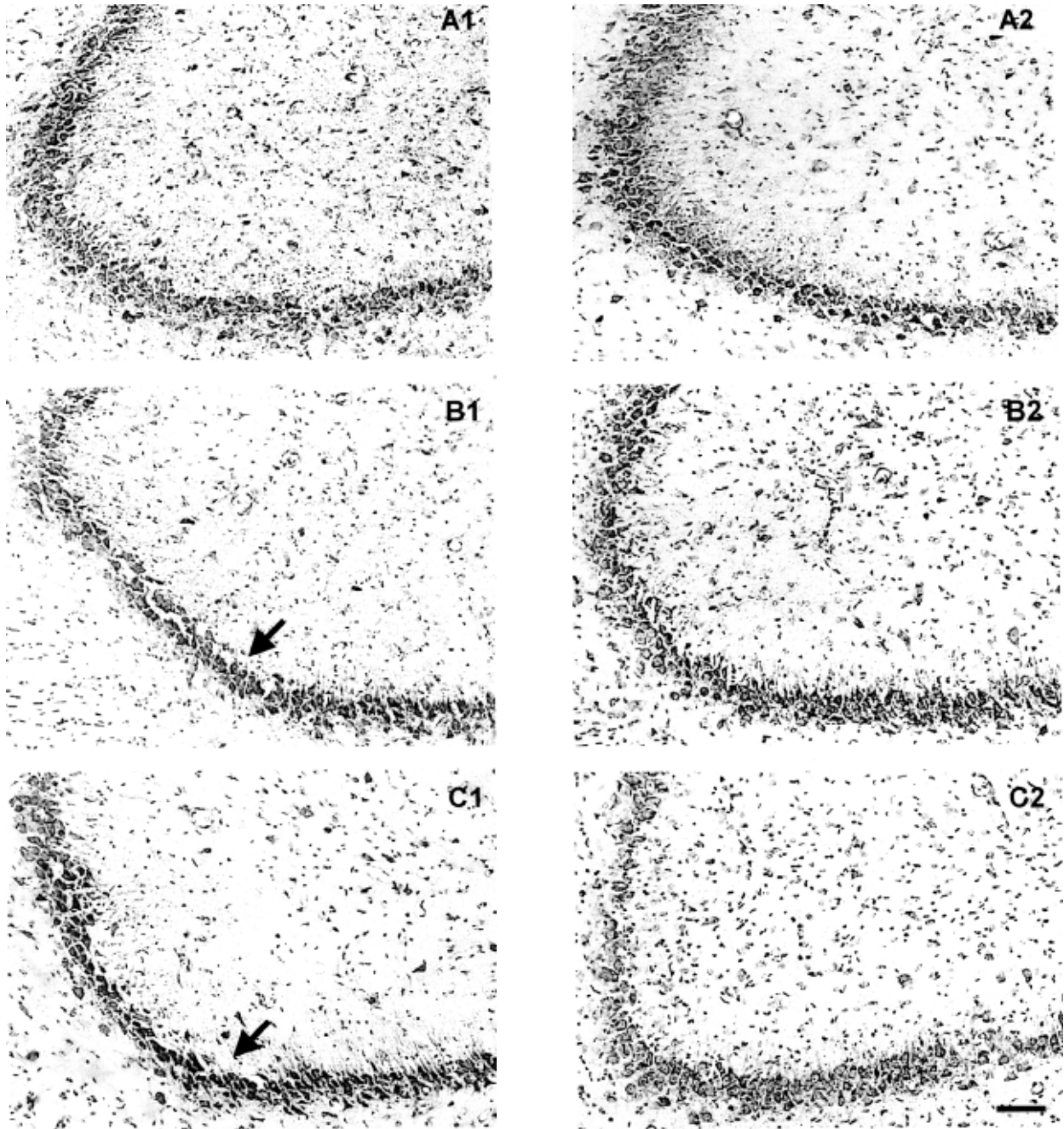
The comparison of seizure frequency among groups showed a significant increase in PX + CHRONIC group ( $2,7 \pm 2,3$  week) when compared with the CHRONIC group ( $1,8 \pm 1,5$ ;  $p < 0,05$ ). No statistical differences were observed between the PX + CHRONIC + EXERCISE group ( $2,2 \pm 2,1$ ) when compared with the PX + CHRONIC group and between the CHRONIC + EXERCISE ( $1,8 \pm 1,5$ ) compared with the CHRONIC group. The analysis done in four periods of one week during the first 30 days of the chronic phase showed an increase in the seizure number in the PX + CHRONIC group during the first week of this period when compared with the second week (1<sup>st</sup> week  $4,5 \pm 2,3$ , 2<sup>nd</sup> week  $1,5 \pm 1,5$ , 3<sup>rd</sup> week  $2,8 \pm 2$ , 4<sup>th</sup> week  $2,1 \pm 2,3$ ). This difference was not statistically significantly in all other groups (CHRONIC 1<sup>st</sup> week  $2,2 \pm 0,9$ , 2<sup>nd</sup> week  $1,5 \pm 1,3$ , 3<sup>rd</sup> week  $1,8 \pm 2,2$ , 4<sup>th</sup> week  $1,6 \pm 1,3$ , CHRONIC + EXERCISE 1<sup>st</sup> week  $2,1 \pm 1,1$ , 2<sup>nd</sup> week  $1,7 \pm 2,1$ , 3<sup>rd</sup> week  $1,6 \pm 1,4$ , 4<sup>th</sup> week  $1,8 \pm 1,7$ , PX + CHRONIC + EXERCISE 1<sup>st</sup> week  $3,4 \pm 3,3$ , 2<sup>nd</sup> week  $1,6 \pm 2$ , 3<sup>rd</sup> week  $1,4 \pm 1,1$ , 4<sup>th</sup> week  $2,6 \pm 1,1$ ).

The morphological analysis showed that animals from the CHRONIC + EXERCISE and PX + CHRONIC + EXERCISE groups presented reduced cell dispersion in the hilus of the dentate gyrus when compared with their respective controls (CHRONIC and PX + CHRONIC) (Figure 1). Cell loss in the CA3 hippocampal region was noted in all chronic groups but was observed a preservation of cells in the animals of the CHRONIC + EXERCISE and PX + CHRONIC + EXERCISE groups (Figure 2).

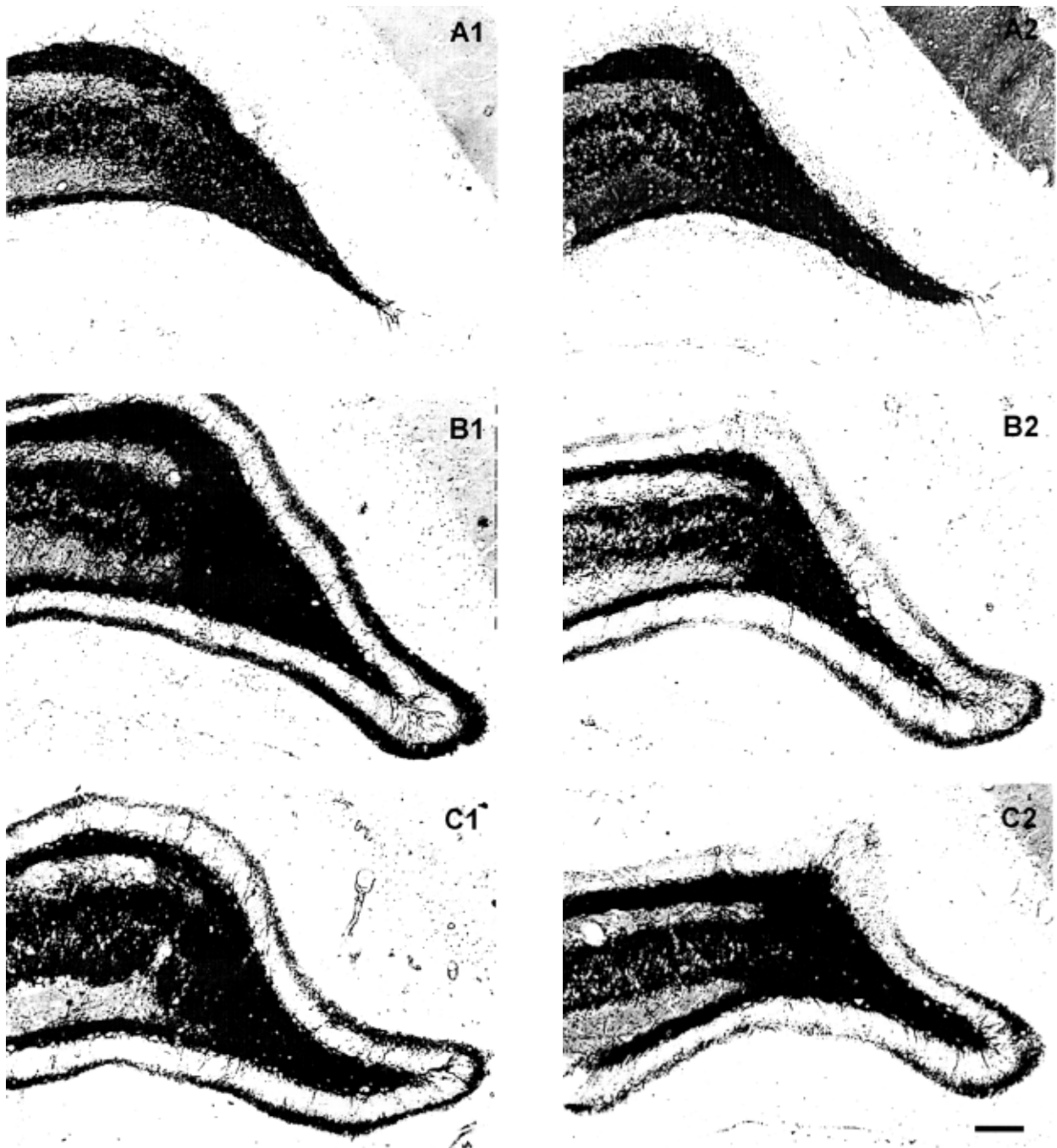
The neo-Timm staining showed supragranular mossy fiber sprouting in a lesser degree in the CHRONIC + EXERCISE and PX + CHRONIC + EXERCISE groups (grade 3) when compared to their respective controls (CHRONIC, grade 5 and PX + CHRONIC, grade 4) (Figure 3).



**Figure 1.** Photomicrographs of Nissl-stained coronal sections of hilus of dentate gyrus. (A1) Control rat – CTRL; (A2) Control rat submitted to an aerobic exercise program – CTRL + EXERCISE; (B1) Rat with spontaneous recurrent seizures – CHRONIC; (B2) Rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – CHRONIC + EXERCISE; (C1) Pinealectomized rat with spontaneous recurrent seizures – PX + CHRONIC; (C2) Pinealectomized rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – PX + CHRONIC + EXERCISE. The arrows indicate the cell dispersion in the CHRONIC and PX + CHRONIC groups. Magnification –100 $\times$ .



**Figure 2.** Photomicrographs of Nissl-stained coronal sections of CA3 region. (A1) Control rat – CTRL; (A2) Control rat submitted to an aerobic exercise program – CTRL + EXERCISE; (B1) Rat with spontaneous recurrent seizures – CHRONIC; (B2) Rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – CHRONIC + EXERCISE; (C1) Pinealectomized rat with spontaneous recurrent seizures – PX + CHRONIC; (C2) Pinealectomized rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – PX + CHRONIC + EXERCISE. The arrows indicate the death cell in the CHRONIC and PX + CHRONIC groups. Magnification 200 $\times$ .



**Figure 3.** Photomicrographs of neo-Timm stained mossy fiber of the dorsal hippocampus. (A1) Control rat – CTRL; (A2) Control rat submitted to an aerobic exercise program – CTRL + EXERCISE; (B1) Rat with spontaneous recurrent seizures – CHRONIC; (B2) Rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – CHRONIC + EXERCISE; (C1) Pinealectomized rat with spontaneous recurrent seizures – PX+CHRONIC; (C2) Pinealectomized rat with spontaneous recurrent seizures and submitted to an aerobic exercise program – PX + CHRONIC + EXERCISE. Magnification –100 $\times$ .

## DISCUSSION

Our results are in accordance with other studies<sup>(4,5)</sup> showing that a drastic reduction of melatonin, provoked by pinealectomy, causes an increase in the neuronal excitability and in the seizure frequency as observed in the PX + CHRONIC group when compared with the CHRONIC group confirming the study of Lima et al.<sup>(14)</sup>. The mechanisms suggested to be involved in the anticonvulsant action of melatonin are the increase of GABA affinity for its receptors<sup>(15)</sup> and a decrease of glutamatergic activity<sup>(16)</sup>.

The frequency of seizures in PX + CHRONIC group without exercise in the first week of chronic period of model was significantly higher than in the second week. Although not statistically significant, a tendency to present more seizures in the beginning of chronic period was observed in all groups. This data was observed by Arida et al.<sup>(17)</sup> in the pilocarpine model suggesting a maturation process followed by stabilization in the seizure frequency.

The histological analysis of hippocampal formation showed a minimized cell loss and a reduction of mossy fiber sprouting in the animals submitted to the program of physical exercise, suggesting a neuronal protection. The neurogenesis process<sup>(18)</sup> and/or increase of neurotrophic factors<sup>(19,20)</sup> in the hippocampus induced by physical exercise reported by previous studies could explain the preservation of cells (Nissl staining) in trained animals with epilepsy and a lesser regenerative sprouting would be necessary.

Our results show that the physical exercise program used did not influence the frequency of seizures in chronic animals contrasting with previous studies<sup>(2)</sup> but this fact could be justified due to some differences in the exercise protocols used. Even though our study did not show an interference of the exercise in seizure frequency in these animals, morphological beneficial effects were observed but more studies must be done in order to prove the relationship between pinealectomy (absence of endogenous melatonin), exercise and epilepsy.

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