A strength exercise program in rats with epilepsy is protective against seizures

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A R T I C L E   I N F O
Article history:
Received 29 June 2012
Revised 3 August 2012
Accepted 6 August 2012
Available online 24 October 2012

Keywords:
Exercise
Epilepsy
Pilocarpine
Seizure
Strength training
Resistance training

A B S T R A C T
The beneficial effects of physical exercise on epilepsy, such as a decreased seizure frequency, have been observed following aerobic exercise programs in both clinical and experimental studies. However, it is not well clarified whether other types of exercise, including strength exercise, can provide similar benefits for epilepsy. Forty four animals with epilepsy were continuously monitored 24 h a day for 60 days and divided into two periods of 30 days. The first period was used to determine the number of seizures before beginning the physical exercise program, and the second period was utilized to determine the number of seizures during the strength training. The mean frequency of seizures in the control and SHAM groups increased significantly from period 1 to period 2. Although the frequency of seizures did not change significantly between the two periods of 30 days of observation in the strength exercise group, a significant reduction in the seizure frequency was observed compared with the control and SHAM groups in period 2. Our study demonstrated that a strength exercise program exerted a significant influence on the seizure frequency in animals with epilepsy and strengthens the observed beneficial effect of exercise on epilepsy that has been demonstrated in animal studies. The finding of this nonclinical study can open a new window to verify the beneficial contribution of strength exercise in epilepsy. Further experimental and clinical investigations are necessary to explore the extent to which strength exercise interferes with the epileptic condition.

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1. Introduction

Evidence reported in the literature has demonstrated the beneficial effects of physical exercise on epilepsy. These positive effects have been observed after aerobic exercise programs in both animal and human studies. In the human investigations, a long-term aerobic exercise program (15 weeks) decreased the seizure frequency in women with intractable epilepsy [1]. In another study, however, four weeks of aerobic training program did not change the average frequency of seizures but led to improved cardiovascular and psychological health of persons with uncontrolled epilepsy [2]. An investigation conducted by McAuley and coworkers reported no impact of seizure frequency after 12 weeks of a physical exercise program [3]. In addition, studies have explored whether intensive exercise alters the seizure susceptibility in people with epilepsy, and, from the few studies that explored this subject, people with temporal lobe epilepsy did not experience seizures after incremental physical exercise to exhaustion or during the recovery period of an ergometric test [4,5].

From an experimental point of view, aerobic exercise training was able to retard the development of amygdala kindling [6], reduce seizure frequency [7], and promote positive plastic changes in the hippocampal formation, such as decreased CA1 hyperresponsiveness [8] and pronounced changes in the staining of parvalbumin, a calcium-binding protein, in the dentate gyrus [9] (for a review, see Arida et al. [10,11]).

It is important to note that people usually combine both aerobic and strength training (also known as resistance training or weight lifting) into their exercise program routine. Scientific research has shown that strength training improves muscle strength and increases muscle and bone mass [12–14], flexibility [15], dynamic balance [16], mood, self-confidence, and self-esteem [17]. Thus, the symptoms of several chronic diseases, such as arthritis, depression, type-2 diabetes, and osteoporosis, have been reduced after strength training [14,18–21], reduced falls have also been reported in older adults with functional limitations [22]. From the experimental point of view, only one study has investigated the impact of a strength exercise program on brain plasticity. For instance, an elegant work conducted by Cassilhas and collaborators demonstrated that strength...
exercise induced an increased expression of hippocampal IGF-1 and receptor (IGF-1R), AKT, synapsin 1, and synaptophysin in the hippocampus [23].

Although the beneficial effects of aerobic exercise on epilepsy have been highlighted, it is not well clarified whether other types of exercise, such as strength exercise, can provide similar benefits. Indeed, some studies that analyzed the effect of supervised exercise programs in people with epilepsy included sessions of cardiovascular, strength, and flexibility training [3,24]. Nevertheless, the favorable effect of exercise on seizure frequency might be influenced by combinations of the above mentioned stimuli. Clearly, only the aerobic exercise has been investigated as an isolated variable. Accordingly, the aim of this study was to analyze the effect of a strength training program on spontaneous recurrent seizures (SRS) in rats with epilepsy using the pilocarpine model of epilepsy.

2. Materials and methods

2.1. Animal care

Seventy male Wistar rats, (age: 60-day old, weight: 256 ± 3.1 g) provided by the Center for Development of Experimental Models for Medicine and Biology (CEDEME/UNIFESP), were housed under environmentally controlled conditions (7:00–19:00 h of light in a light/dark cycle; 22–24 °C) and permitted free access to food and water throughout the experiment. All experimental procedures were approved by the University Ethics Committee.

2.2. Pilocarpine-induced epilepsy model and behavioral analyses

Sustained seizures were induced by a single i.p. administration of pilocarpine hydrochloride (350 mg/kg; Sigma, St. Louis, MO). Scopolamine methylnitrate (Sigma) was injected (1 mg·kg·s.c.) 30 min before the pilocarpine to reduce peripheral cholinergic effects [25]. Following the status epilepticus period, the surviving animals (44/70) were continuously monitored for 24 h using a video system to detect the first spontaneous recurrent seizure (SRS). Infrared emitting lights were used during the dark periods to allow for the video recording of the animals’ activity during this time. After the first SRS had been detected, the animals were continuously monitored 24 h a day for 60 days. The animals with epilepsy were divided into three groups: the strength group (n = 17), SHAM group (n = 13), and control group (n = 14). The behavioral analysis was divided into two periods of 30 days, period 1 (1–30 days) and period 2 (31–60 days). For the strength group, the first period was used to quantify the number of seizures before the strength exercise program, and the second period was used to analyze the number of seizures during the strength exercise program.

2.3. Strength exercise training

2.3.1. Familiarization with the vertical ladder

The animals were subjected to a familiarization protocol for an 80° inclined vertical ladder apparatus (110 cm high × 18 cm wide, with 2-cm grid steps). A housing chamber (L×W×H = 20×20×20 cm) was located at the top of the ladder and served as a shelter during the resting period (Fig. 1a) [23,26–30]. The familiarization protocol consisted of three trials per day for three days. In the first trial, the rats were kept in the housing chamber for 60 s and then placed on the ladder, 35 cm from the top. In the second trial, the rats were placed in the middle of the ladder. In the third trial, the rats were placed at the bottom of the ladder as previously described [23].

2.3.2. Strength exercise group

The animals of the strength group were subjected to a progressive strength exercise, 5 sessions per week for 4 weeks. Each session consisted of 8 climbing series with a progressively heavier load fixed to the proximal part of the animal’s tail with a Coastlock Snap Swivel and Scotch 23 Rubber Tape (Scotch 3 M). For each series, the animals had to make 8–12 dynamic repetitive movements to reach the housing chamber. In the first two series, the load was 50% of the animal’s total body mass; in subsequent series, the load was progressively increased to a final load of 100% (Fig. 1b). The average body weight was 492 g (range 470–550). The rest interval between the series was 60 s. The training sessions for each animal were between 20 and 30 min. Animals from SHAM group spent 20 min in the training apparatus (at the side top of the vertical ladder, Fig. 1a). This procedure was performed five times per week for 4 weeks.

![Fig. 1. a) The training apparatus. Ladder with 110 cm, 2-cm between grid steps and 80° incline. Housing chamber (L×W×H = 20×20×20 cm) located centrally at the top of the ladder served as a shelter during the resting period for the exercising animals, while the housing chamber located laterally served the SHAM group. b) A rat climbing ladder with weights fastened to the tail.](image)
2.4. Tissue collection and processing

Twenty-four hours after the last training session, the rats were euthanized by decapitation. The striated skeletal muscle *flexor digitorum longus* (FDL — an ankle extensor muscle that participates in the climbing movement), of the right leg was dissected, and each FDL muscle was coated with embedding medium (Tissue-Tek OCT, Miles, Naperville, IL, USA) and immersed in liquid nitrogen-cooled isopentane. All of the tissues and sera were stored at −80 °C until use.

2.4.1. Measurement of fiber cross-sectional areas of the FDL

Transverse sections (8 μm thick) were cut from the mid-bellies in a cryostat at −20 °C, melted onto poly-l-lysine-coated microscopic slides (Superfrost, Fisher Scientific) and stained with Meyer’s hematoxylin-eosin (H&E). Digital images of the H&E-stained sections were captured using an Olympus brightfield microscope BX50, camera DP71 (Melville, NY) with a 40× objective. The blinded analysis of the cross-sectional areas of 100 fibers per muscle sample was performed using the software Axio Vision 4.6 (Carl Zeiss MicroImaging GmbH).

2.4.2. Immunohistochemistry

Immunohistochemical studies were performed in cryostat sections (8 μm) from FDL skeletal muscle. Cryostat sections (8 μm) from FDL were incubated for 10 min in phosphate buffer (PBS: 137 mM NaCl, 2.68 mM KCl, 8.03 mM Na2HPO4, 1.47 mM KH2PO4, pH 7.4). The sections were incubated with blocking solution (3% albumin, w/v, in PBS) for 1 h at room temperature. After a PBS wash, sections were incubated overnight at 4 °C with primary antibody against slow myosin and fast myosin (1:100), respectively diluted in blocking solution. Following six 5-min washes in blocking solution, sections were incubated for 1 h at room temperature with the appropriated secondary antibody conjugated to peroxidase (1:200) diluted in blocking solution. Peroxidase activity was revealed using PBS containing 3,3-diaminobenzidine (DAB, 0.05%, w/v) and hydrogen peroxide (0.01%, v/v) for 3 min at room temperature. The sections were washed in PBS and then air-dried and coveredslipped with Permount (Fisher Scientific, Pittsburgh, PA, USA). Negative control experiments were performed by omission of primary antibody. Sections were visualized using Olympus B50 microscope (Olympus, Japan, JP). Images were digitalized using a CoolSNAP-Pro charge-coupled device digital camera and Image-Pro Express Software (both from Media Cybernetics, Silver Spring, MD, 40× USA).

2.5. Statistical analysis

The Statistica 5.0 software was used for all analyses. The Shapiro-Wilk W test was used to verify data normality. To determine whether the frequency of seizures varied or not between the two periods of 30 days of observation for the three groups, to investigate whether any difference could be found between the same periods of the three groups, and to examine whether sleep duration (measuring “freezing”) varied or not between the two periods of 30 days of observation for the three groups, the ANOVA for repeated measurements followed by Fisher post-hoc test was adopted. To verify the difference of cross-sectional area fibers between groups, the one-way ANOVA followed by Fisher post-hoc test was adopted. The significance level was set at 5%, and the data were presented as the mean± standard error.

3. Results

3.1. Behavior

The behavioral features of pilocarpine-induced seizures during the acute period were similar to those reported previously[25,31]. The behavioral pattern of SRS showed the same characteristics described by Cavalheiro et al.[32]. Briefly, the SRS consisted of facial automatisms, forelimb clonus, rearing, loss of postural control, and generalized clonic seizures.

3.2. Seizure frequency

The first spontaneous seizure was observed between 3 and 71 days (control group, between 27 and 71 days; SHAM group, between 3 and 29 days; and strength group, between 14 and 64 days) after the pilocarpine administration, and the mean seizure silent period lasted for 39.0±3.3 days, 13.0±2.6 days, and 33.0±6.3 days (mean±S.E.) for the control, SHAM, and strength groups, respectively. The mean frequency of seizures in the control group increased significantly from period 1 (mean 11.3±2.5) to period 2 (mean 19.1±2.4) (p<0.05). Similarly, the mean frequency of seizures in the SHAM group increased significantly from period 1 (mean 11.9±1.3) to period 2 (mean 19.4±3.4) (p<0.05). In the group of animals submitted to the strength training program, the frequency of seizures did not change significantly from period 1 (mean 13.0±1.8) to period 2 (mean 12.0±1.9). When the same periods of the three groups were analyzed together, a significant reduction in the seizure frequency was observed when comparing the training group with the control and sham groups during period 2 (Fig. 2).

The analysis of the SRS over the light/dark cycle showed an increase in the seizure frequency during the diurnal period (07:00–18:59 h), compared with the nocturnal period (19:00–06:59 h) for all groups (control group, p<0.001; strength group, p<0.001; and SHAM group, p<0.001). However, when seizure frequency per unit time (6 hour blocks) was analyzed separately for the active and inactive periods, animals from the strength group had a reduction of seizures from 13:00 h to 18:59 during the training program (second period) compared to the

![Fig. 2](image-url) Mean frequency of seizures from control (n=14), SHAM (n=13), and strength (n=17) groups. Each period of behavioral observation consisted of 30 days (period 1; 1–30 days and period 2; 31–60 days). * Different from control and SHAM groups (p≤0.05); ** Different from control and SHAM groups in the second period (p≤0.05). Analysis of variance for repeated measurements followed by Fisher post-hoc test. Data are presented as mean± standard error.
control and SHAM groups. No significant difference was found between the control and SHAM groups (Fig. 3). Thus, we carried out a blind rater score for sleepiness to verify whether exercised rats had the same sleep duration as non-exercised rats. For this purpose, we performed 24 h/day video recording in our dataset (observing rats in their home cage) using video tracking software capable of measuring "freezing" or movement. The mean freezing (control: first period = 818.6 ± 25.5 min and second period = 808.2 ± 24.6 min; SHAM: first period = 894.8 ± 26.4 min and second period = 798.2 ± 24.7 min; and strength group: first period = 825.1 ± 26.0 min and second period = 794.9 ± 25.8 min; mean ± SE) did not differ statistically among groups (p > 0.05).

To ensure the effectiveness of the exercise protocol, we performed a cross-sectional area analysis of the FDL. As shown in Fig. 4, the muscle fiber area of the strength group was increased compared with the control and SHAM groups (F(2,61) = 11.266; p < 0.005), indicating that the protocol applied during the four weeks of the experiment was sufficient to cause muscle hypertrophy. Post hoc Fisher analysis revealed differences between the two control group vs strength group (p = 0.01) and SHAM group vs strength group (p = 0.004). However, the hypertrophy was evident in fast twitch fibers which were marked by the immunohistochemical method against type II myosin. Flexor digitorum longus muscle is compounded by fast twitch fibers predominantly, which are more responsive to strength training (Fig. 5).

4. Discussion

The present work demonstrated that a strength exercise program exerted significant influence on the seizure frequency of animals with epilepsy. The mean number of seizures in the animals subjected to the strength training did not differ significantly from period 1 (before physical training) to period 2 (the physical training period). Although a reduction in the number of seizures was not observed in these animals after the strength training, our data suggest that strength training can exert a positive effect on the seizure frequency, based on the fact that the control and SHAM groups demonstrated an increased frequency of seizures during the two periods of behavior observation. Indeed, it has already been reported that there is a maturation process during the early stages of recurrent seizures in the pilocarpine model of epilepsy [7]. The analysis of the same period of the three groups demonstrated a significant difference between the training group and the control and SHAM groups in period 2. Our findings are in accordance with previous studies that analyzed the seizure frequency of animals with epilepsy after an aerobic exercise program [7–9,33].

Stress is among the most frequently self-reported precipitants of seizures in people with epilepsy [34–36]. Considering that strength exercise involves intense muscle contractions, we could presume that it is an exhaustive effort. Although animal and human studies have reported that intensive exercise does not alter seizure susceptibility [4,5,7,37], it remains unclear whether strength exercise is helpful, harmful, or essentially has no impact on the frequency of seizures. Strength training or weight training is used by many people and for many different reasons. The obvious benefit of strength training is an increase in strength, which, in turn, can prevent injuries and improve posture and performance in sports and day-to-day activities, in addition to reducing the symptoms of the several chronic diseases cited above.

While the effects of strength exercise on brain plasticity or cognitive performance are not well explored, some studies have reported positive findings. One investigation demonstrated that the significant increase in muscular strength in trained elderly subjects was associated with enhance psychological well-being and cognitive functioning [38]. A meta-analysis reported that older adults with cognitive impairment who participate in exercise rehabilitation programs had similar strength and endurance training outcomes, suggesting the positive impact of strength exercise in rehabilitation programs [39]. Furthermore, recent investigations have revealed positive findings of strength exercise on cognition. Cassilhas et al. [40] demonstrated that 6 months of moderate- or high-intensity resistance training improved memory among senior men. This finding has been extended by other research groups [41]. Because it is important to indicate the effectiveness of the strength exercise protocol and to demonstrate the positive impact of strength exercise in the present investigation, we confirmed by histological analysis of the FDL muscle that the strength exercise protocol was efficient in causing muscle hypertrophy [23,28,29] as confirmed by H&E analysis.

Although information about the light/dark cycle is well documented [32,42–45], one study has demonstrated that the seizure frequency in pilocarpine-treated rats was not related to the circadian rhythm [46]. Accordingly, a literature search has demonstrated that the seizure frequency per unit time over the light/dark cycle for each group. Each period (30 days) of behavioral observation consisted of 6 hour blocks. * Different from control and SHAM groups (p ≤ 0.05). Analysis of variance for repeated measurements followed by Fisher post-hoc test. Data are presented as mean ± standard error.
threshold is reduced during sleep (for a review, see Matos et al. [47]). In our investigation, the animals subjected to the strength exercise exhibited the same pattern of seizure frequency as the control and SHAM groups over the light/dark cycle, which is in accordance with previous experimental studies reporting an increase in seizure frequency during the diurnal period compared with the nocturnal period [7,48,49]. On the other hand, the strength exercise protocol significantly affected the seizure frequency during the light period, suggesting that exercise per se may interfere in seizure susceptibility.

In conclusion, the novel finding presented here reinforces the beneficial effect of exercise on epilepsy that has been demonstrated in animal studies [6–9,33]. The mechanisms by which physical training is able to induce such changes are not completely understood and deserve further experimental investigation.

Acknowledgments

This research was supported by CAPES, FAPESP, CNPq, INNT, and CInAPCe (Brazil).

References


Fig. 5. Flexor digitorum longus muscle immunohistochemistry for slow myosin (a, c, and e, one arrow) and fast myosin (b, d, and f, two arrows). a and b, control group; c and d, SHAM group; and e and f, strength group.


