UNIVERSIDADE FEDERAL DO PAMPA

CAROLINE BITENCOURT SOARES

MULTICOMPONENT TRAINING PREVENTS MEMORY DEFICIT RELATED TO AMYLOID-β PROTEIN-INDUCED NEUROTOXICITY

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Trabalho de Conclusão de Curso apresentado ao Curso de Enfermagem da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Enfermagem.

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RESUMO

A doença de Alzheimer (DA) é atualmente a forma mais comum de demência. Os principais desafios enfrentados pela DA incluem a falta de biomarcadores confiáveis para o diagnóstico precoce, bem como a falta de estratégias e tratamentos preventivos eficazes. Assim, pesquisas que investigam estratégias para prevenir essa doença têm buscado o uso de ferramentas acessíveis com potencial para intervir nos diversos marcadores patológicos da DA, incluindo a neurotoxicidade amilóide. No presente estudo, utilizando ratos Wistar, mostramos que um treinamento multicomponente (MT) de longo prazo, incluindo exercícios físicos aeróbicos, anaeróbicos e cognitivos, é capaz de impedir déficits de memória de reconhecimento relacionados à neurotoxicidade amilóide. Além disso, o MT é capaz de evitar a peroxidação lipídica do hipocampo, restaurar a capacidade antioxidante do hipocampo e aumentar os níveis de glutationa no hipocampo.

Palavras-chaves: Doença de Alzheimer; Exerecício físico; Exercício cognitivo; Treino multicomponente.

ABSTRACT

Alzheimer's disease (AD) is currently the most common form of dementia. The major challenges facing AD include the lack of reliable biomarkers for early diagnosis, as well as the lack of effective preventive strategies and treatments. So, researches investigating strategies to prevent this disease have been look for the use of accessible tools with the potential to intervene in the various pathological markers of AD, including the amyloid-β neurotoxicity. In the present study, using a Wistar rats, we show that a long-term multicomponent training (MT), including aerobic and anaerobic physical exercise, and cognitive exercise, is capable to prevent recognition memory deficits related to amyloid-β neurotoxicity. Additionally, MT is able to avoid hippocampal lipid peroxidation, restore hippocampal antioxidant capacity and increase glutathione levels in hippocampus.

Key-words: Alzheimer’s disease; physical exercise; cognitive exercise; multicomponent training.

SUMÁRIO

MULTICOMPONENT TRAINING PREVENTS MEMORY DEFICIT RELATED TO AMYLOID-β PROTEIN-INDUCED NEUROTOXICITY

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1 INTRODUCTION

Recent estimates suggest that 46.8 million people worldwide live with dementia, and is expected by 2030 an increase in this number to 74.7 million, as the population ages [1]. Dementia represents a condition of progressive cognitive impairment that directly impacts quality of life, causing dependence, disability and mortality [2].

Currently, Alzheimer's disease (AD) is the most common form of dementia, accounting for the majority of cases [3]. One of the major challenges facing AD is the lack of reliable biomarkers for early diagnosis, as well as the lack of effective preventive strategies and treatments [4, 5]. AD is a progressive neurodegenerative disease that primarily affects areas of the hippocampus and cerebral cortex [6, 7].

The neuropathological and neurochemical features of AD include synaptic loss and selective neuronal death, decrease in specific neurotransmitters levels, and abnormal protein deposits, mainly the amyloid-β protein (Aβ) in extracellular spaces, what result in memory-related cognitive decline [8]. Aβ protein induces neurotoxicity by multiple mechanisms that lead to synaptic dysfunction in the neuron network, stimulating the generation of reactive oxygen species (ROS) [9] and inducing a cascade of cell imbalance.

AD and other dementias are linked to a number of potentially modifiable risk factors, so, it as a consensus that some lifestyle changes are able to prevent or retard the development of this type of disease [10]. Lifestyle interventions involving diet, exercise, cognitive training, and vascular risk reduction have shown benefits for some cognitive measures [1, 11].

Physical exercise (PE) is considered a potent non-pharmacological preventive agent, easily accessible, and capable to protect brain function [12, 13]. The cognitive effects of aerobic exercise are already well established. Aerobic exercise has been shown to increase hippocampal neurogenesis and cell proliferation [14-16], besides acting on memory persistence through neurotransmitter modulation, increased pre and post synaptic protein levels in the hippocampus [17-19], and increased brain-derived neurotrophic factor (BDNF) levels and insulin-like growth factors (IGF-1) that may act on the nervous system [16, 20, 21]. Due to the multifactorial beneficial mechanisms, which lead to reduction of amyloidogenesis and taupatology, suppression of neuroinflammation and oxidative stress, in addition to preserving the energetic metabolic function in the hippocampus region [22, 23], aerobic PE has been reported as an important neuroprotective strategy [22, 24, 25].

Anaerobic exercise, on the other hand, has other important benefits, especially in elderly, such as great impact on the improvement of muscle strength and balance [26]. The anaerobic PE effects in cognition have been recognized more recently, by studies that shown the action of this type of PE preventing the effects of Aβ on oxidative stress and lipid peroxidation increase in the hippocampus [13, 27].

Another potential non-pharmacological preventive strategy is the cognitive exercise (CE), which can maintain neural functions, promoting cognitive flexibility, decreasing oxidative stress and improving patients' quality of life [28-30]. Recently, using AD rats, we have shown that CE can be effective in protecting against memory deficits and hippocampal oxidative stress related to Aβ neurotoxicity, maintaining the tissue organization, when performed prior to neurotoxicity induction [25].

So, both aerobic and anaerobic PE, and also CE, are potential stimuli that may influence brain function and health, and can be used as preventive strategies to protect brain from cognitive impairments and dementias. However, they are only a few studies that investigate the effects of different types of exercise combination on cognition [31]. Interventions using exercises' combination are generally recommended for pre-frail and frail elderly to improve muscle strength, gait speed, balance and physical performance, including endurance, aerobics, balance and flexibility tasks [29, 30, 32].

Recently, multicomponent exercise training (MT), a type of protocol that combine different exercises modalities, showed be more effective than isolated aerobic exercise training in the improvement of fall risk factor [31], and we hypothesized that this type of exercise could also have beneficial effects on cognition. Here we demonstrate the beneficial effects of a MT in the prevention of recognition memory deficits related to Aβ neurotoxicity. We also show that this type of training prevents hippocampal oxidative stress and antioxidant capacity decrease.

2 MATERIAL AND METHODS

2.1 Animals and experimental design

Adult male Wistar rats (3 months old; n = 48) purchased from the vivarium of the Federal University of Santa Maria (RS/Brazil) were used. They were housed four per cage and maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a temperature of 23 ± 2°C and humidity of 50 ± 10%) with free access to food and water. All experiments were conducted in accordance with the

"Principles of laboratory animal care" (NIH publication n. 80-23, revised 1996) and experiments were approved by the Animal Use Ethics Committee of the Federal University of Pampa (RS/Brazil) (Protocol 031/2018).

Initially the animals were divided into two groups, according to the intervention: Sedentary (control) and Multicomponent Training (MT). The animals from the MT group underwent to 8 weeks of exercise including three different modalities (aerobic exercise, anaerobic exercise and cognitive exercise). The training was performed 6 times a week, with 1 different modality each day, resulting in 2 exercise sessions of each modality per week (table 1).

Multicomponent training

Days of week	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Training	Aerobic	Anaerobic	Cognitive	Aerobic	Anaerobic	Cognitive	Free

Aerobic exercise	Endurance/running training in a treadmill- 60-70% of max. indirect VO_2, 2 times a week, for 40 min, without inclination on the treadmill.
Anaerobic exercise	Strength training in a ladder with increasing workload - 50%, 75%, 90%, 100% of body weight, 2 series each weight, 60s rest interval between series, 2 times a week.
Cognitive exercise	Cognitive flexibility training using an adaptation of Barnes Maze - 2 times a week, every ten days the escape cage was changed to another location.

Table 1. Multicomponent training. The protocol involved the training of three components (endurance, strength, and cognitive flexibility), with sessions 6 times/week, with 1 different modality each day, resulting in 2 exercise sessions of each modality per week, during 8 weeks.

After the intervention period, the animals underwent stereotactic surgery for amyloid-β or saline infusion and were subdivided into 4 groups (n = 12/group): Control, Amyloid-β (Aβ), Multicomponent training (MT), and, Multicomponent training + Amyloid-β (MT+Aβ). Ten days after, the animals were submitted to behavioral tests to assess memory recognition and monitor control behavioral parameters. At the end, the rats were euthanized and brain tissues were collected for biochemical (n = 6) analyses (Fig. 1).

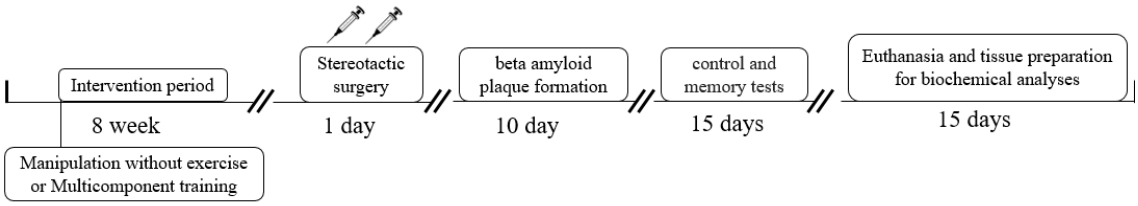

Fig. 1. **Experimental Design.** In the first week the groups were divided according to the intervention into: multicomponent exercise training (MT) or control. The MT was conducted for 8 weeks. After, all rats undergo to a surgery to hippocampal beta-amyloid protein or saline (vehicle) infusion; 10 days after surgery, time required for beta-amyloid protein aggregation, animals were submitted to control and memory tests. After the tests, the rats were euthanized and the hippocampal tissues were removed for further biochemical analysis.

2.2 Amyloid-β Preparation and infusion

Aβ peptide 25–35 (A4559; Sigma Aldrich) was dissolved in saline solution (i.e., vehicle) at a concentration of 100 μM and incubated at 37°C for 4 d to induce Aβ 25–35 aggregation initiation.

To mimic one of the main characteristics of AD the animals were anesthetized interperitoneally with ketamine and xylazine (75 mg/kg and 10 mg kg, respectively) and submitted to a stereotaxic surgery. Based on the Paxinos and Watson Atlas coordinates for hippocampus (anterior-posterior = - 4.2 mm; lateral-lateral, ± 3.0 mm; ventro-medial, - 3.0 mm) [33] and using a Hamilton syringe and an infusion pump, 2.0 μl of Aβ protein or vehicle were infused bilaterally in the hippocampal CA1 region. Afterwards, the rats returned to their cages and were monitored for 10 days to recovery and Aβ aggregation [34, 35].

2.3 Multicomponent training (MT)

The MT protocol involves the training of three components: aerobic/endurance, anaerobic/strength, and cognitive. Training sessions were performed 6 times/week, over 8 weeks, and in each day one of the components were trained (Table 1). Each component followed a specific protocol, as described bellow.

2.3.1 Aerobic exercise

To aerobic exercise training the rats were familiarized to a treadmill specific for rodents (Insight Ltda, São Paulo, Brazil), to avoid stress effects. Familiarization was conducted for 5 days (treadmill velocity starts from 0 to 2 a 5 m/min for 10 min/day) [13, 24]. On the last day of familiarization, an indirect oxygen consumption test (VO_2)

was performed to determine the individual training intensity of each animal. To do this, each rat started at a slow speed and gradually increased by 5 m/min every 3 minutes, until the rat cannot continue running. Fatigue time (min) and workload (m/min) were considered as an indirect measure of maximal VO_2 [36].

Aerobic exercise started in the following week and was performed at an intensity of 60-70% of maximal indirect VO_2, 2 times a week, once a day, for 40 min, without inclination on the treadmill, during 8 weeks. After 5 weeks of aerobic training, and the last week of training, the same VO_2 test was repeated to adjust exercise intensity and/or to verify aerobic gain [12].

2.3.2 Anaerobic exercise

The apparatus used for the strength exercise were a vertical ladder with an inclination of 80 degrees (110 cm high, 18 cm wide, 2 cm between steps). At the top of the stairs was a box (25 x 25 x 20 cm) that provides shelter to the animal. Before starting the training, the animals were familiarized to the apparatus, performing 4 trials per day during 3 days. In the first trial, the rat was kept in the housing chamber for 60 s; in the second trial the rat was put 35 cm below to the top, in the third trial in the bottom half of the ladder, and, in the last trial, in the bottom of the ladder.

After familiarization, anaerobic training was initiated, and 8 series were performed, with 12 repetitions/climbing with a fixed progressive load on the proximal part of the animal's tail. In the first week, the training load was 50% of the total body mass of the animal. In the second week, a maximum load test was performed, which consists of climbing with 75% of body weight and an addition of 30 grams in each new climb. The maximum load is determined and recorded when the animal can no longer perform the repetitions. In the following weeks, the series were performed with progressively increased load percentage (50%, 75%, 90%, 100%), determined from the maximum load. A 60s rest interval between sets was defined. The maximal load test was performed every two weeks of training to adapt the training protocol to the animals' gain [12].

2.3.3 Cognitive Exercise (CE)

The CE was performed according an adaptation of the Barnes Maze Spatial Memory Task proposed by Daré et al (2018). The Barnes Maze apparatus is a circular platform with 20 potential escape holes, equally distributed on the periphery of the

platform, and only one of these roles leads to a scape cage. Negative reinforcement (bright lights in the top of the platform) is used to motivate the animal to escape into the escape cage hidden under one of the holes. Geometric clues around the maze are used as visual cues to make spatial learning possible.

To perform the CE, the animals were trained 2 times per week during 8 weeks. With each new training the rats were able to perform the CE more efficiently, meaning they found the escape cage faster using the spatial cues. Thus, every ten days, the escape cage was changed to another location, consequently the animals had to do a new reorganization of spatial memory, which required cognitive flexibility [37].

2.4 Control behavioral tasks

Considering that memory assessment depends on behavioral observation, behavioral control tasks were used to ensure that surgery, MT, or other procedures do not impair exploratory and motor performance by altering memory test results. The Elevated Plus Maze (PM) and the Open field (OF) test were used to evaluate anxiety state and to analyze exploratory and locomotor activities, respectively.

To PM test the rats were placed in the center of maze. The maze consists of two open arms, 50 x 10 cm, and two enclosed arms, 50 x 10 x 40 cm, with an open roof, arranged such that the two open arms were opposite to each other. The maze is elevated to a height of 50 cm. The total number of entries and the time spent into the four arms were recorded over a 5 min session [38]

For the OF test, the rats were placed in the left quadrant of a 50 × 50 × 39 cm box made of white painted wood with a front glass wall. Black lines were drawn on the floor to divide it into 12 equal quadrants. The number of crossings, as measures of locomotor activity, was measured over 5 minutes. [39].

2.5 Memory tests

2.5.1 Object Recognition Memory Task (OR)

Initially, the rats were individually habituated to the OR apparatus and left to freely explore it for 20 min during four consecutive days before the training session. In the fifty day, the OR training was performed. In the training two different objects (named A and B) were placed in the apparatus, and rats were allowed to freely explore them for 5 min. Three hours and 24 h after, the short (STM) and long-term (LTM)

memory were evaluated, respectively [40]. In each testing session, one of the objects was randomly replaced by a novel object (C and D, respectively) and the rats were reintroduced into the apparatus for additional 5 min period of free exploration. The time spent exploring the new and the familiar object were recorded.

2.5.2 Social Recognition Memory Task (SR)

The SR memory task is an adaptation of the social interaction test [24]. The task was completed in 3 days. First, the rats were placed in an arena for habituation with two small cages during 20 min of free exploration. In the following day, a training session was performed with the inclusion of one unfamiliar rat in one of the cages for 60 min of free exploration. After 24 h, a testing session was performed, when the same rat from the training (now a familiar rat) and a new rat were placed for exploration for 60 min. The time spent exploring the new and the familiar rat were recorded. Exploration of the conspecific animal was defined as sniffing or touching the small cages with the nose and/or forepaws.

2.6 Biochemical Testing

After the euthanasia, the brain tissues of some animals (n = 6) were quickly removed in an iced surface, and then the hippocampal tissues were immediately isolated from the brain and cleaned using ice-cold saline. Tissue samples were frozen in liquid nitrogen and stored at -80°C until biochemical analysis performance.

For biochemical experiments, the tissue samples were homogenized in 50 mM TrisHCl, pH 7.4. The homogenates were centrifuged at 2,400 g for 20 min at 4°C to obtain supernatants that were used for the analysis of the all biochemical variables.

2.6.2 Detection of lipid peroxidation (TBARS)

Hippocampal lipid peroxidation levels were evaluated by TBARS test [41]. Samples were incubated with a 0.8% thiobarbituric acid solution, acetic acid buffer (pH 3.2), and SDS solution (8%) at 95°C for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nanomoles of malondialdehyde per milligrams protein.

2.6.3 Ferric reducing/antioxidant power (FRAP) assay

The total antioxidant capacity was measured by FRAP assay (ferric reducing/antioxidant power). The working FRAP reagent was prepared by mixing 25

mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl3·6H2O solution. The homogenate (10 μL) was added to the 300 μL working FRAP reagent in microplate [42]. Additionally, a standard curve with 10 μL Trolox concentrations (15, 30, 60, 120, and 240 mM) and 300 μL working FRAP reagent was used. The microplate was incubated at 37°C for 15 min before reading in a SpectraMax M5 Microplate Reader at 593 nm.

2.6.4 Glutathione (GSH) Levels

GSH levels were fluorometrically determined [28]. An aliquot of homogenized sample was mixed (1:1) with perchloric acid ($HClO_4$) and centrifuged at 3000 for 10 min. This mixture was centrifuged, the protein pellet was discarded, and free thiols (SH) groups were determined in the clear supernatant. An aliquot of supernatant was incubated with orto-phthaladehyde, and fluorescence was measured at excitation of 350 nm and emission of 420 nm. Results were expressed as nmol g^{-1} of tissue.

2.7 Statistical analysis

First, the data normality was evaluated by Shapiro-Wilk test. Object exploration time in OR task and rats' exploration time in SR task were converted to percentage of total exploration time, and an one-sample t-test was used to compare the percentage of the total time of exploration spent on each object/rat with a theoretical mean of 50%. The OF and PM data were analyzed by an one-way ANOVA. Biochemical results were compared using a two-way ANOVA test followed by Sidak's post hoc. Results are expressed as mean and standard deviation. The significance level was set at 0.05 for all variables.

3 RESULTS

3.1 Control Behavior Tasks

The intrahippocampal infusion of Aβ or saline, as the other procedures involved in the experimental design, did not affect the locomotor behavioral during the 5 min long free exploration session at the open field ($P = 0.58$; number of crossings; table 2). The exploratory behavioral was no affected either ($P > 0.05$; total exploration time on

OR training and tests; table 2). Additionally, no effects in the anxiety were detected on PM test (P = 0.39; time spent at open arms; table 2).

	Control	Aβ	MT	MT+Aβ	P value
Open field					
Crossings (n)	86.13 ± 29.43	76.33 ± 21.90	78.33 ± 18.30	87.08 ± 16.84	0.58
OR total exploration time (s)					
Training	82.75 ± 26.85	81.78 ± 31.86	69.42 ± 23.94	74.92 ± 26.86	0.66
Test (3h)	70.38 ± 26.35	77.78 ± 33.70	75.50 ± 30.89	67.75 ± 29.27	0.87
Test (24h)	81.63 ± 30.84	76.22 ± 25.99	69.83 ± 27.29	72.75 ± 23.32	0.79
Plus maze					
Time in open arms (s)	71.25 ± 63.97	109.8 ± 22.86	99.83 ± 57.26	104.5 ± 43.78	0.39

Table 2. The infusion of Aβ or saline, as the other procedures involved in the study design, have no effect on locomotor activity evaluated in an open field, on exploratory behavior, evaluated in OR training and tests, and on anxiety, evaluated in plus maze test (P > 0.05; one-way ANOVA). Data are expressed as mean ± SD (n = 8-12 per group).

3.2 Strength and oxygen uptake (VO_2) gains

The aerobic gains were estimated by indirect VO_2 measures performed 3 times along the MT protocol (Fig. 2A). Strength gains were estimated by the maximum carrying load (MCL) measures performed every two weeks (Fig. 3B, data showed represents only 3 measures). Trained animals presented a continuous increase on VO_2 and MCL towards the end of the training (Fig. 2).

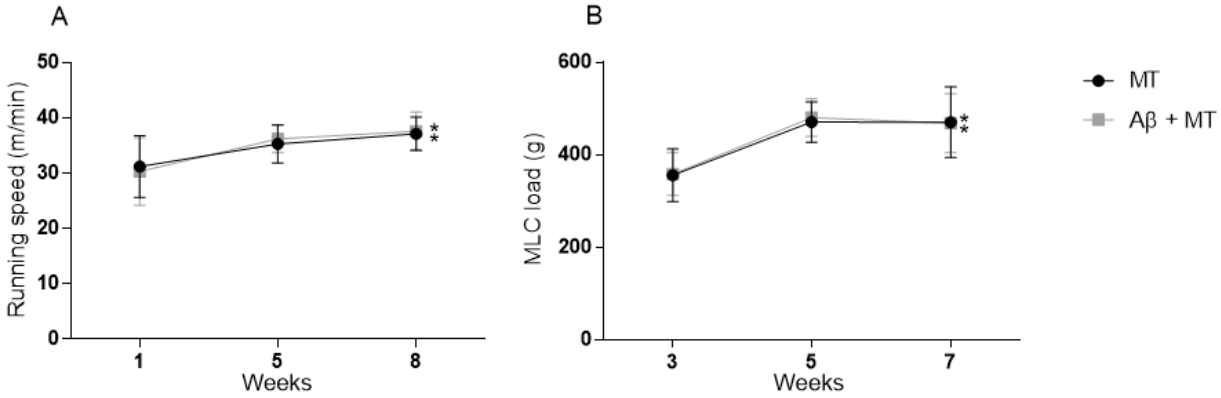

Fig. 2. The VO_2 max and the MLC of trained animals increased along the MT. A: progression of the maximum running speed (m/min), an indirect measurement of VO_2, considering the first, the fifth and the last week of training; *P < 0.0001; Repeated measures ANOVA. **B:** progression of the load (grams), measured by MLC test in the first, the fifth and the last week of training; *P < 0.0001; Repeated measures ANOVA. Data were expressed as mean ± SD (n = 12/group).

3.3 Memory Tasks

3.3.1 Object recognition memory (OR)

In the OR training session the rats explored each object (A and B) for a similar percentage of total exploration time (in the graphs the data is present as the mean of all groups; P > 0.05 for all groups; Control: $t_{(4)} = 1.086$, P = 0.3384; Aβ : $t_{(5)} = 0.3077$, P = 0.7707; MT: $t_{(7)} = 1.452$, P = 0.1897; MT+Aβ: $t_{(10)} = 2.206$, P = 0.0632; Fig. 3A and 3B, tr).

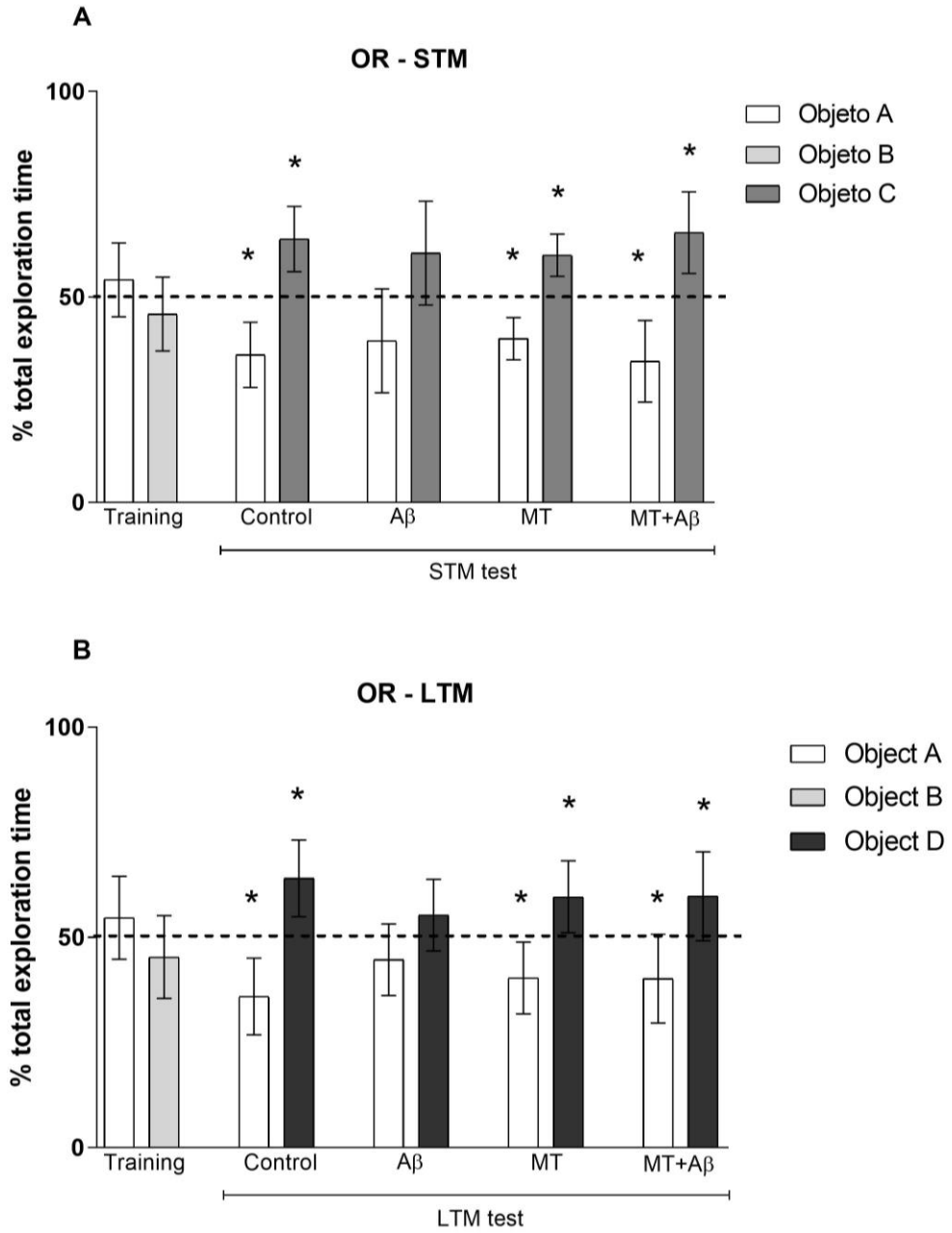

Fig 3. The hippocampal Aβ infusion promotes OR memory deficit. Multicomponent training is able to prevent the OR memory deficit caused by hippocampal Aβ infusion. (A) OR short-term memory (STM); (B) OR long-term memory (LTM). * P < 0.05; one-sample Student *t*-test (theoretical mean 50%). Data are presented as mean ± SD (n = 5-12/group).

On STM test, the control animals spent more than 50% of the total exploration time exploring the new object (Control: $_{t(4)}$ = 2.788 , P = 0.0494; fig. 3A), showed that they remember the familiar object, i.e., formed STM. The same was observed on MT group, which animals spent more time exploring the new object (MT: $t_{(7)}$ = 5.597, P = 0.0008; fig.3A). The Aβ infusion impaired STM, since the rats spent about 50% of the total exploration time exploring each object (Aβ: $t_{(5)}$ = 2.073, P = 0.0929, figure 3A). The MT was able to prevent the deficits caused by Aβ neurotoxicity, since the animals submitted to Aβ infusion but previously to the multicomponent training were able to form OR STM, i.e., they explored more than 50% of total exploration time the new object (MT+Aβ: $t_{(7)}$ = 4.460, P= 0.0342; fig. 3A)

Twenty-four hours after the training the LTM was tested; the results are quite similar to STM. Rats from Control and MT groups explored the novel object for more than 50% of the total exploration time (Control: $t_{(4)}$ = 3.444 , P = 0.0262; MT: $t_{(7)}$ = 3.190, P = 0.0153; LTM, fig. 3B). On the other hand, animals from Aβ group presented impaired LTM, since they spent about 50% of total exploration time in each object (LTM; Aβ: $t_{(5)}$ = 0.1525, P = 0.1878, fig. 3B). Multicomponent training was able to prevent Aβ protein-induced damage, as the Aβ+MT group spent more than 50% of the total exploration time exploring the new object (MT+Aβ: $t_{(7)}$ = 2.623, P = 0.0342; Fig. 3B).

3.3.2 Social recognition memory (SR)

In the SR test session, Control and MT rats explored the new rat for more than 50% of the total exploration time (Control: $t_{(5)}$ = 4.458, P = 0.0066; MT: $t_{(11)}$ = 4.893, P = 0,0005; Fig. 4). Animals from Aβ group, however, explored for ~50% of the total exploration time each rat (Aβ: $t_{(11)}$ = 0.6406, P = 0.5394; Fig 4), demonstrating memory deficit. Multicomponent training prevented the deleterious effect of Aβ protein on SR memory, since the Aβ trained animals spent more than 50% of the total exploration time exploring the new rat (MT+Aβ: $t_{(11)}$ = 4.239, P = 0.0014; Fig. 4).

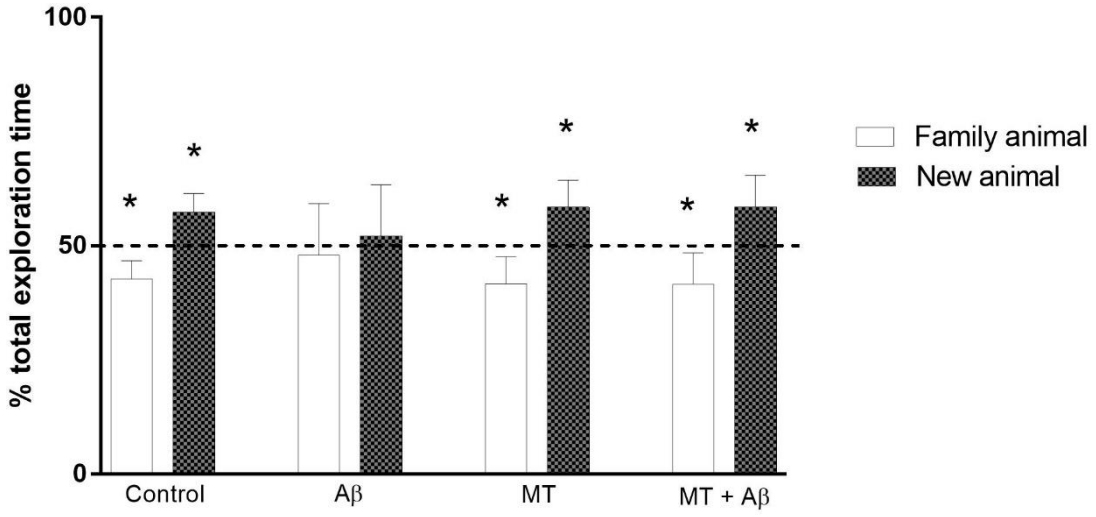

Fig 4. The hippocampal infusion of Aβ promotes SR memory deficit. Multicomponent training is able to prevent SR memory deficit caused by Aβ infusion into the hippocampus. * $P < 0.05$; one-sample Student *t*-test (theoretical mean 50%). Data are presented as mean ± SD (n = 5-11/group).

3.4 Biochemical Results

In the lipid peroxidation determined by TBARS an effect for the beta-amyloid infusion ($F_{(1,19)} = 8.007$; $P = 0.0107$; Fig. 5A) and multicomponente training ($F_{(1,19)} = 6.643$; $P = 0.0185$; Fig. 5A). Additionally, the interaction between the factors was significant ($F_{(1,19)} = 14.63$; $P = 0.0011$; Fig. 5). Aβ group presented increased lipid peroxidation in comparison to control ($P = 0.0012$; Fig. 5A) and MT ($P = 0.0056$; Fig. 5A). Aβ rats that underwent to MT did not increase the lipid peroxidation ($P = 0.0015$ Aβ group vs. MT+Aβ group; Fig. 5A).

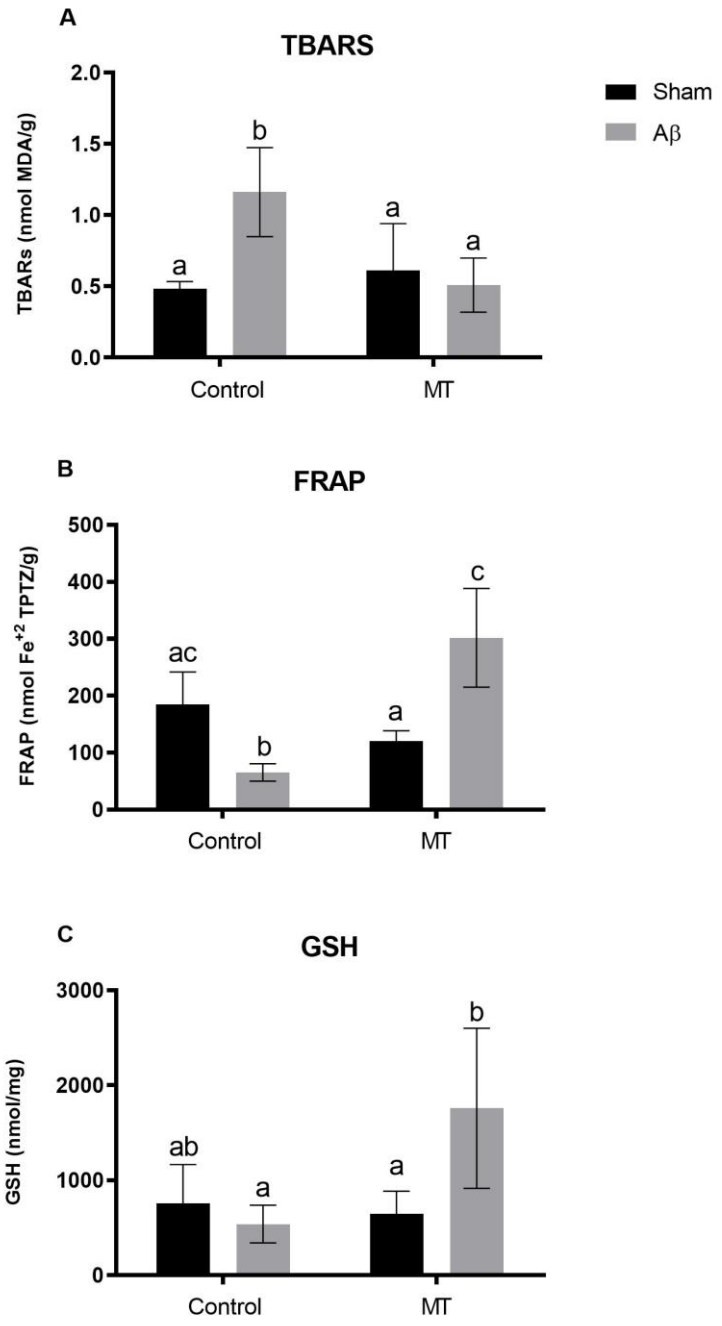

Fig. 5. The hippocampal infusion Aβ promotes increased in lipid peroxidation (TBARS, A), decreased in the total antioxidant capacity (B) and decreased on GSH levels (C). Multicomponent training performed for 8 weeks prevents changes in lipid peroxidation (A), antioxidant capacity (B) and GSH levels (C). Data are presented as mean ± SD and were analyzed by two-way ANOVA, followed by Sidak's post hoc. Bars with different letters are statistically different ($P < 0.05$).

In the total antioxidant capacity (i.e., ferric reducing/antioxidant power – FRAP) an effect of intervention (MT) was observed ($F_{(1,18)} = 13.00$; $P = 0.0020$; Fig. 5B). Additionally, the interaction between the factors was significant ($F_{(1,18)} = 39.74$; $P = <0.0001$; Fig. 5B). Aβ infusion decreases antioxidant capacity compared to the control

group (P = 0.0075; Fig. 5B). Aβ rats that underwent MT increased their antioxidant capacity compared to group Aβ (P = <0.0001; Fig.5.B) and MT (P = 0.0002; Fig. 5B) groups.

Considering the GSH levels, an effect of the MT was observed ($F_{(1,16)}$ = 5.290; P = 0.0352; Fig. 5C). Additionally, the interaction between the factors was significant ($F_{(1,16)}$ = 7.607; P = 0.0140; Fig. 5C). Aβ-infused animals that previously were submitted to MT increased GHS levels in comparison to Aβ (P = 0.0099; Fig. 5C) and MT (P = 0.0258; Fig. 5C) animals.

4 DISCUSSION

Our results show that MT avoids recognition memory deficits, preventing the increase of hippocampal lipid peroxidation and the decrease of hippocampal antioxidant capacity and glutathione levels induced by Aβ neurotoxicity.

AD is the most common dementia, and, despite there is no single animal model of AD that reproduces all the disease's characteristics, the animal models available are important to study its pathophysiology, as well as preventive and therapeutic strategies. Considering that the extracellular Aβ deposition is one of the main features of AD [43], brain Aβ protein infusion is an important model that can contribute to the understanding of the main biological aspects of AD. The use of Aβ25-35 peptide in animal models contribute to the understanding of its effects on mechanisms related to Aβ toxicity [44, 45], since the administration of this fragment of Aβ in the temporal cortex, dorsal hippocampus, or intracerebroventricularlly increases the oxidative stress [45, 46], the membrane lipid peroxidation [35], and the neuroinflammation [47] processes that may contribute to synaptic dysfunction, neuronal death and consequently, cognitive decline [48].

In this study, the intra-hippocampal infusion of Aβ25-35 promoted hippocampal oxidative damage (increased lipid peroxidation levels) and decreased of the total antioxidant capacity, leading to STM and LTM recognition memory deficits observed in OR and SR tests. An important fact to consider is that a high density of Aβ deposits in the brain of AD patients is mainly observed in the hippocampus and cortex, which are two important areas associated with learning and memory [49]. Studies have reported elevated markers of lipid peroxidation and protein oxidation in the hippocampus and

cerebral cortex of individuals with AD [7]. Similarly, in this study, infusion of Aβ into the hippocampus altered memory processes and other biochemical markers.

The relationship between oxidative stress and AD suggests that oxidative stress is an essential part of the pathological process of this dementia, and antioxidants may be a potential target in AD prevention and treatment [50]. Under normal conditions, antioxidant enzymes that act as free radical scavengers. These enzymes include superoxide dismutases (SOD), glutathione peroxidase (GPX), glutaredoxins, thioredoxins and catalase [51]. Glutathione (GSH) is an important endogenous antioxidant found in millimolar concentrations in the brain. GSH levels have been shown to decrease with aging and in various age-related and degenerative diseases, as AD, in particular [52-54]. Our Aβ rats did not show significant hippocampal GSH alterations, but the MT increases the levels of this protein in Aβ rats, suggesting an adaptive brain response as a result of exercise [55].

The effects of physical exercise, especially the aerobic, are well established in the literature [12, 13, 16, 17, 19] . This type of exercise improves brain function, influencing oxidative stress, neurogenesis, and synaptic plasticity, producing a strong neuroprotective effect that can lead to improvements on learning and memory [56, 57]. On the other hand, the anaerobic exercise is less studied and its effects are related to the motor system, including significant improvement of muscle function and balance, preservation of muscle tissue and slow down of sarcopenia process [26, 58]. But effects on cognition have been reported too. Evidences suggested that anaerobic exercise can prevent the neurotoxic effects of Aβ and oxidative stress on the hippocampus [13, 27]. In addition, CE is a potential stimuli that can influence brain function and health and can be used as a preventive strategy to protect the brain from dementia by modulating neuronal excitability, inducing changes in synaptic plasticity and ability to learning [59, 60].

In this study, we observed that the use of a combination of different types of exercise, a multicomponent training, is able to prevent the short and long-term memory deficit caused by amyloid-β. These effects on memory agree with the biochemical observations on MT rats' hippocampus, that did not presented the same oxidative disbalance that non-trained animals. Given this, the execution of a multicomponent training is advantageous since this type of activities can be more attractive for older populations, because they are usually performed in groups and/or outdoors, and the possibility of performing different exercises on alternate days does not make it

monotone and tiring \, resulting in multiple benefits beyond cognition, as improve in muscular, metabolic and functional parameters [26, 58, 61, 62].

Previously work suggested that in the use of jointly performed combination exercise therapies, combinating strength and aerobic exercises, the strength training weakens the cognitive improvements and the hippocampal neurogenesis induced by aerobic exercise in rats [63]. On the other hand, combined physical and cognitive training seems to be effective in improve memory tasks performance [64]. Our results demonstrate that the use of a combination and alternate therapy promotes memory improvement by biochemical mechanisms related to oxidative balance. It is important highlighted that many factors can influence on exercise (combined or not) effects, as intensity, duration, frequency, and training organization [65, 66]. In the cited exercise, for example [63], the strength and aerobic exercises were performed in the same day.

Other important topic to highlighted is that, although there are many treatment-focused studies, here we described the protective effects of a multicomponent exercise-related lifestyle. In summary, the model of intra-hippocampal infusion of Aβ used in this study causes hippocampal oxidative damage and antioxidant capacity decrease, as well as promotes short- and long-term recognition memory deficits that mimic those described or suggested to occur in the hippocampus of AD patients. Fortunately, the multicomponent training, combining aerobic, anaerobic and cognitive exercises, prevents memory deficits by avoiding the increase of lipid peroxidation, and antioxidant system dysfunction. Probably, other mechanisms are involved in the MT effects, as increase of pre and post synaptic proteins and influences on the release of neurotransmitters important for learning consolidation and memory persistence [17-19], neuroprotection and neuroplasticity [12, 13, 24]. Anyway, considering our results we can confirm that the MT has the potential to be used as a preventive therapy against aging-related dementias, as it represents an easily accessible alternative that can be applied to humans.

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