Short communication

Modulation of synaptic plasticity by short-term aerobic exercise in adult mice

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ABSTRACT

Physiological effects of different types of continuous and interval aerobic training, have been largely described and studied in the adult man. It was previously indicated that interval training plays an important role in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement even in healthy sedentary subjects. Since the outcome of different aerobic training trials on cognitive processes had never been evaluated, we compared, on an experimental mouse model, the effects of four training exercise protocols, named respectively C100, I100, C50 and I50 depending on the volume and on the type of training proposed, continuous or interval method. Therefore, to assess quantitative and qualitative functional changes, we analyzed several physical parameters before and after 6 weeks training in all four groups with respect to the control sedentary animals and we studied synaptic plasticity, by extracellular in vitro recordings, in hippocampal mouse slices, a region involved in learning and memory processes. We found that all four protocols of exercise applied in this study exerted positive effects on both physical and training parameters inducing weight augmentation, strength endurance and aerobic endurance increase, and potentiation of motor coordination. However, the improvement observed failed to induce an enhancement in synaptic plasticity in three out of four exercise protocols and only in the slices from mice trained with the interval 50% volume exercise the long term potentiation (LTP) increased with respect to the sedentary group. These findings suggest that motor activity exerts positive effects on cognitive processes provided that certain principles are respected, such as the training load and the elements of which it is composed, in order to plan the right quantitative and qualitative parameters and the appropriate recovery periods.

1. Introduction

Exercise has many benefits for the body, but it also improves and ameliorates brain functions by increasing neurogenesis, synaptic plasticity, and performance on learning and memory tasks. The numerous health benefits of physical activity and exercise have been observed following aerobic exercise programs [1].

Scientific evidence based on neuroimaging approaches over the last decade has demonstrated the efficacy of physical activity improving cognitive health across the human lifespan [2]. Moreover physical exercise has positive effects on general health and reduce the incidence of pathological conditions such as diabetes, osteoporosis and cardiovascular disease [3,4].

The impact of workout on cognition is more strongly supported by results from intervention studies, which generally show that older adults who have completed a physical activity program that produces significant increases in cardiorespiratory fitness (indexed by direct measures or estimation of VO2 max) often show enhanced cognitive performance [5]. Moreover physical exercise improves many aspects of brain functions as well as induces neuroprotection [6].

Neurons can modify the ability to communicate each other through intercellular communication as well as through classical synaptic transmission [7]. Neuronal plasticity is the neurobiological correlate of functional and structural alterations in the brain enabling adaptation to the environment and storing information. Furthermore it plays a pivotal role in compensating the degenerative modifications that take place during aging [8]. This is a period of life in which processes responsible for plasticity must be forced to run at the highest possible rate to maintain the morphofunctional substrate of the existing networks as well as to allow the formation of new memory traces [9].

In the last years, the long-lasting synaptic enhancement, known as long-term potentiation (LTP), has been the object of intense investigation since it is widely believed that LTP, largely dependent upon kinase activation and protein synthesis, provides an important key to understanding the cellular and molecular mechanisms by which memories are formed and stored [10-12].
It is well known that voluntary exercise can facilitate both structural and functional plasticity and enhances cell proliferation and neurogenesis [13], synaptic plasticity [14–17] and spatial learning in both rats and mice.

Physiological effects of different types, of continuous and interval aerobic training, have been largely described and studied in the adult man. In human, exercise can be used in either continuous or interval modalities and several authors have shown cardiovascular effect of aerobic interval training in heart failure patients [18]. Even in healthy sedentary subjects interval training seems optimal in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement. Fluctuations of workload and oxygen uptake during training sessions, rather than exercise duration or global energy expenditure, are key factors in improving muscle oxidative capacities. For similar energy expenditure and training duration, endurance training adaptations are different in function of training modalities [19]. Until now, it had never been evaluated whether the different types of trainings might otherwise affect the activity of Central Nervous System, all over the cognitive processes.

Hence aim of this study was to assess: i) quantitative and qualitative functional changes induced by different training exercise and ii) whether synaptic plasticity is modified in the hippocampus, one of the main brain region involved in learning and memory processes, in relation to training effects of different aerobic exercise. For this purpose, synaptic plasticity was investigated in hippocampal mouse slices by extracellular in vitro recordings.

2. Materials and methods

2.1. Animals and experimental protocol

Twenty four mice eleven weeks old, belonging to the strain BALB/c mice, were used according to the EU Directive 2010/63/EU for animal experiments.

The animals were divided into four groups (4 mice per group), each one submitted to different aerobic training program, and a fifth control group (8 mice), which did not perform any type of training. Each mouse was weighed before and after training. A rotarod (for mice, Cat N° 47600, Ugo Basile srl, Italy,) was employed for the administration of the different aerobic protocols training and for evaluating their effects before and after the four training programs. The protocols were approved by the Ethics Committee.

Before starting the training program, a group of five mice, not belonging to the twenty involved in the experimental procedure, was subjected to daily variable training loads of different volume and intensity (rate per minute), to spot a volume of work that could be sustained with a daily maximum number of three falls from the rotarod.

The volume and intensity identified by preliminary tests have been given the name of Volume 100 (V100) consisting in performing 110 laps and a total distance traveled of 9.90 m.

The V100 is the reference volume of each training session for the first week which was subsequently increased: 2 weeks 110 laps per training session, 2 weeks 133 laps per training session and 1 week 144 laps per training session.

The four groups of mice were randomly divided and trained with four different protocols:

- group I with continuous progressive method (C 100);
- group II with the same method as group A, but carried out with a volume equal to 50% of group A, called (C 50);
- group III, with interval method, which consisted in performing the same volume as group I, by alternating high intensity and low intensity phases (I 100);
- group IV with the same interval training protocol as in group III but a 50% volume.

The total amount of exercise (laps per day of training) and intensity (laps per minutes) was the same in continuous progressive method as in interval method. In continuous training speed it was constant while interval training provided alternating low and high speed.

The trainings were conducted for a total of six weeks, on a three-weekly frequency (18 workouts). At the beginning and at the end of the training program some tests were performed to assess the physical fitness of the animals involved in the study. During each training session the number of falls of the mice from the rotarod and the time in which they occurred were evaluated.

2.2. Evaluation of aerobic-endurance and strength-endurance

Aerobic endurance was estimated by a test that included an exercise on the rotarod at constant speed of 10 rotations per minute. The "time limit" was considered as the time between the start of the test and the occurrence of the third fall. If no fall occurred the test was interrupted at the thirtieth minute of exercise.

The test of the Strength-endurance was performed by the use of a force transducer (Ad Instruments MLT050/D) at which the mouse was suspended by its front legs. The back legs were blocked by the use of an adhesive tape. The time passed between the start of the test and the fall down was measured. The proof was repeated three times without observing any recovery periods between individual efforts. The total time of the three tests was recorded.

2.3. Electrophysiological recordings in mouse hippocampal slices

Basal synaptic transmission (BST) and LTP were examined in the Schaffer collateral/commissural CA1 pathways in mouse hippocampal slices. The animals belonging to the four different groups were sacrificed after the twelve weeks of training as well as the sedentary animals. All efforts were made to minimize the number of animals used and their suffering. The hippocampal slices were prepared according to conventional procedures [20]. Slices were then transferred to a tissue chamber and placed at an interface between oxygenated ACSF and humidified gas (95% O2–5% CO2) at 32–34 °C (pH 7.4). and superfused at a constant flow rate of 1.2 ml/min with ACSF. Extracellular recordings of the population spikes (PSs) were made in the stratum pyramidale of the CA1 subfield, with glass microelectrodes filled with 2 M NaCl (resistance 5–10 MΩ). Orthodromic stimuli (10–500 mA, 20–90 ms, 0.1 Hz) were delivered through a platinum electrode placed in the stratum radiatum (Schaffer collaterals). The test stimulus intensity of 50-ms square pulses was adjusted to give a PS of 2–4 mV at 0.03 Hz. After recording stable signals (20–30 min), a tetanic stimulation (100 Hz, 1 s) was delivered to induce LTP at the same stimulus intensity used for the baseline responses. Responses were acquired, digitized, and stored using a personal computer with standard acquisition software (Axon, Foster City, CA, U.S.A.). Signal was fed to a computer interface (Digidata 1440A, Axon Instruments, Foster City, CA) for subsequent analysis with the software PCLAMP10 (Axon Instruments).

PTP and LTP were measured by calculating the PS amplitude prior to and after the tetanus. The PS amplitude was calculated every minute as the average of six recordings performed every 10 s. Changes in the amplitude of PS after tetanization were expressed as percentages of the basal PS amplitude (PS/PS basal p100, where PS basal is the mean PS amplitude before tetanization). The overall effects on PTP and LTP were measured by calculating the average, for each slice, of the PS amplitudes recorded during the 5-min period before the tetanus (BST), during the first minute after the tetanus (PTP), and during the 60 min period after the tetanus (LTP).

2.4. Statistical analysis

The results are expressed as mean and standard deviation. The normal distribution was verified using plot normality and Kolmogorov-Smirnov test. Multiple comparisons were performed and Tukey’s test was set for post hoc analysis. The statistical significance was set to 0.05.
The effects of the four types of aerobic training on the expression of short- and long-term synaptic plasticity were analyzed in the CA1 region of mice hippocampal slices. In the slices from mice trained with C100 and I100, a complete blockade of the expression of PTP and the induction of LTP is observed (Fig. 1 n = 5) while no significant differences are detected in the maintenance phase; the values at different times after tetanic stimulation are reported in Table 2. The effect of C50 on synaptic plasticity is characterized by a slight reduction of PTP, while the LTP expression and the maintenance phase overlap the trend of the sedentary group (Fig. 2 n = 5). The analysis of the slices obtained from the mice trained with I50 shows a lack of modification of short term potentiation while the expression of the LTP maintenance phase results extremely enhanced with respect to the sedentary group (Fig. 2 n = 6). The PS values recorded in the trained slices are reported in Table 2, where the values of significance are also indicated.

3. Results

3.1. Effects of different training protocols on physical and performance parameters

We have analyzed several physical parameters before and after 6 weeks training in all four groups with respect to the control animals. The mouse weight augmented of 5 ± 2 gr increasing from 15% to 24% after each kind of training protocol. The aerobic endurance was also enhanced after the training period reaching the time limit of thirty minutes without the insurgence of a fall. The evaluation of the strength enhanced after the training period reaching the time limit of thirty minutes.

<table>
<thead>
<tr>
<th></th>
<th>C 100</th>
<th>I 100</th>
<th>C 50</th>
<th>I 50</th>
</tr>
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<tr>
<td></td>
<td>Pre ± sd</td>
<td>Post ± sd</td>
<td>Pre ± sd</td>
<td>Post ± sd</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>23.36</td>
<td>28.72</td>
<td>23.06</td>
<td>28.31</td>
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<td>Aerobic endurance (s)</td>
<td>181.136</td>
<td>1200.0</td>
<td>176.65</td>
<td>1200.0</td>
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<td>Strength endurance (s)</td>
<td>61.19</td>
<td>117.47</td>
<td>64.67</td>
<td>158.82</td>
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<td>effect size “d” for weight</td>
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<td>4.0</td>
<td>4.2</td>
<td>2.7</td>
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<tr>
<td>effect size “d” for endurance</td>
<td>10</td>
<td>22.0</td>
<td>3.7</td>
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<tr>
<td>effect size “d” for strength</td>
<td>1.5</td>
<td>1.2</td>
<td>0.4</td>
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</tbody>
</table>

We investigated the association between the LTP values recorded in 5 groups measured on the 1st, 5th, 10th, 20th, 30th, 40th, 50th and 60th minute. The Wilcoxon test for paired data was chosen to study associations between time 1 (pre workout) and time 2 (after training). For these purposes “in vivo” and “in vitro” studies were performed.

Our results indicate that all four protocols of exercise applied in this study exert a positive effects on both physical and training parameters including: i) a weight augmentation; ii) strength endurance and aerobic endurance increase; iii) a potentiation of motor coordination, pointed out by a decrease in the number of falls from the Rotarod.

On the other hand the improvement observed failed to induce an enhancement in synaptic plasticity in three out of four exercise protocols; in fact only in the slices from mice trained with the interval 50% volume exercise, the long term potentiation recorded in CA1 area increased with respect to the sedentary group. In the other experimental conditions we reported i) an inhibition in the induction of LTP in the slices from mice trained both with continuous and interval 100% volume exercise; ii) no changes both in the induction and in the maintenance phase of LTP in the slices of the animals trained with continuous 50% volume with respect to the slices of the sedentary group.

Regarding the cognitive aspect, the lack of positive modulation on synaptic plasticity in the two groups trained with C100 and I100, could be probably due to the presence of a high content of metabolites or corticosteroid hormones, particularly cortisol, released into the circulation during the phases of training. This trend could indicate how the training may have been overly stressful and invasive. There are several studies indicating as CA1 hippocampal area is affected by glucocorticoid secretion. Biagini et al., reported that glucocorticoid receptors immune reactivity in the CA1 hippocampal area of rats exposed to repeated stress was higher than in unstressed control rats and furthermore that the intensity of specific immunoreactivity of hippocampal pyramidal cells can be taken as an index of hypothalamic pituitary adrenocortical (HPA) axis activation and therefore of corticosterone secretion [21]. Moreover, repeated maternal separation periods of 5 h/day during the first week of life produced long lasting effects on the hippocampal regulation of the HPA axis which appear to be associated with increased responsiveness to stress stimuli in adulthood [22].

4. Discussion

In this paper, we have assessed the action of short-term aerobic exercise training, continuous and interval, at two different volumes, both on performance parameters and modulation of synaptic plasticity. For these purposes “in vivo” and “in vitro” studies were performed.

We also observed that the intensity of specific immunoreactivity of hippocampal pyramidal cells can be taken as an index of hypothalamic pituitary adrenocortical (HPA) axis activation and therefore of corticosterone secretion [21]. Moreover, repeated maternal separation periods of 5 h/day during the first week of life produced long lasting effects on the hippocampal regulation of the HPA axis which appear to be associated with increased responsiveness to stress stimuli in adulthood [22].

Scientific studies also claim that the high concentration of corticosteroid hormones, above all cortisol, inhibits the induction of LTP, acting on the postsynaptic NMDA receptors at the hippocampal level, fundamental for LTP induction in the CA1 region [23]. Excessive physical activity leads to overtraining in the body that triggers a defensive
reaction with an overproduction of corticosteroid hormones. Despite the tests carried out at the end of the training period will not detect visible signs of overtraining syndrome, such as a decline in performance, the experiments on synaptic plasticity suggest that animals trained with both continuous and interval 100% volume may however be in a condition of over-reaching. This condition is a sign that these training volumes, with too short recovery times between sessions, have been proved to be particularly stressful.

The administration of training protocols with 100% volume could induce not only the release of cortisol in the circulation but also an excessive production of inflammatory cytokines such as Tumor Necrosis Factor alpha (TNFα), interleukin 6 (IL-6) and interleukin 1 beta (IL-1β). Previous studies have shown that stress due by training causes an increase in the levels of inflammatory cytokines. In particular, TNFα and IL-6 inhibit the induction of LTP in the CA1 region of the hippocampus [24,25].

Depending on the type and procedure used, different studies have shown that stress differentially affects synaptic plasticity. In fact Tabassum and Frey [26] demonstrate that acute swim stress led to a long-term depression (LTD).

The C50 protocol training may be probably included in a borderline condition [27]. The interval model has been recommended to promote aerobic adaptations due to recovery period that enables the execution of elevated intensity and as consequence, higher workload in relation to aerobic adaptations [19]. Even in healthy sedentary subjects interval training seems optimal in maximizing both peripheral muscle and central cardiorespiratory adaptations, permitting significant functional improvement. Fluctuations of workload and oxygen uptake during training sessions, rather than exercise duration or global energy expenditure, are key factors in improving muscle oxidative capacities. For similar energy expenditure and training duration, endurance training adaptations are different in function of training modalities [19]. The interval model has been recommended to promote aerobic adaptations due to recovery period that enables the execution of elevated intensity and as consequence, higher workload in relation to continuous training.

In conclusion motor activity exerts positive effects on cognitive

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Table 2

<table>
<thead>
<tr>
<th>MIN</th>
<th>CONTROL</th>
<th>C 100</th>
<th>I 100</th>
<th>C 50</th>
<th>I 50</th>
<th>SIGN.</th>
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<tr>
<td>1</td>
<td>227.3 ± 53.7</td>
<td>112.9 ± 27.4</td>
<td>132.0 ± 23.0</td>
<td>172.6 ± 59.0</td>
<td>134.4 ± 30.0</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>224.3 ± 52.0</td>
<td>108.0 ± 37.9</td>
<td>98.8 ± 23.2</td>
<td>162.7 ± 54.0</td>
<td>147.5 ± 48.3</td>
<td>0.04</td>
</tr>
<tr>
<td>10</td>
<td>205.0 ± 46.0</td>
<td>107.8 ± 24.8</td>
<td>107.6 ± 20.2</td>
<td>160.4 ± 53.0</td>
<td>173.1 ± 46.0</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>175.2 ± 47.0</td>
<td>110.0 ± 29.7</td>
<td>112.5 ± 37.0</td>
<td>158.6 ± 47.6</td>
<td>173.8 ± 43.0</td>
<td>0.16</td>
</tr>
<tr>
<td>20</td>
<td>166.7 ± 53.0</td>
<td>113.4 ± 33.0</td>
<td>116.3 ± 27.6</td>
<td>156.2 ± 45.6</td>
<td>187.9 ± 40.4</td>
<td>0.37</td>
</tr>
<tr>
<td>30</td>
<td>152.2 ± 50.8</td>
<td>103.6 ± 29.2</td>
<td>120.8 ± 22.5</td>
<td>153.6 ± 44.2</td>
<td>206.4 ± 52.4</td>
<td>0.22</td>
</tr>
<tr>
<td>40</td>
<td>146.7 ± 48.1</td>
<td>111.5 ± 23.0</td>
<td>117.1 ± 21.6</td>
<td>147.7 ± 40.7</td>
<td>216.3 ± 57.8</td>
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<td>50</td>
<td>148.1 ± 54.1</td>
<td>99.7 ± 36.2</td>
<td>117.8 ± 24.8</td>
<td>150.7 ± 38.9</td>
<td>206.5 ± 51.0</td>
<td>0.04</td>
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<tr>
<td>60</td>
<td>138.9 ± 55.9</td>
<td>98.2 ± 30.2</td>
<td>107.6 ± 24.2</td>
<td>146.4 ± 36.1</td>
<td>233.0 ± 50.7</td>
<td>0.03</td>
</tr>
</tbody>
</table>
processes provided that certain principles are respected, such as the training load and the elements of which it is composed in order to plan the right quantitative and qualitative parameters and the appropriate recovery periods. Further studies will aim to define whether longer time training may affect synaptic and muscular plasticity. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References