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AEROBIC EXERCISE PREVENTS AGE-DEPENDENT COGNITIVE DECLINE AND REDUCES ANXIETY-RELATED BEHAVIORS IN MIDDLE-AGED AND OLD RATS

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Abstract—Recent research involving human and animals has shown that aerobic exercise of moderate intensity produces the greatest benefit on brain health and behavior. In this study we investigated the effects on cognitive function and anxiety-related behavior in rats at different ages of aerobic exercise, performed regularly throughout life. We designed an aerobic training program with the treadmill running following the basic principles of human training, and assuming that rats have the same physiological adaptations. The intensity was gradually adjusted to the fitness level and age, and maintained at 60–70% of maximum oxygen consumption (max.VO_2). In middle age (8 months) and old age (18 months), we studied the cognitive response with the radial maze (RM), and anxiety-related behaviors with the open field (OF) and the elevated plus maze (EPM). Aerobically trained (AT) rats had a higher cognitive performance measured in the RM, showing that exercise had a cumulative and amplifier effect on memory and learning. The analysis of age and exercise revealed that the effects of aerobic exercise were modulated by age. Middle-aged AT rats were the most successful animals; however, the old AT rats met the criteria more often than the middle-aged sedentary controls (SC), indicating that exercise could reverse the negative effects of sedentary life, partially restore the cognitive function, and protect against the deleterious effects of aging. The results in the OF and EPM showed a significant decrease in key indicators of anxiety, revealing that age affected most of the analyzed variables, and that exercise had a prominent anxiolytic effect, particularly strong in old age. In conclusion, our results indicated that regular and chronic aerobic exercise has time and dose-dependent, neuroprotective and restorative effects on physiological brain aging, and reduces anxiety-related behaviors.

Key words: aging, exercise, memory, learning, stress, anxiety.

There is growing interest in studying factors related to lifestyle and interventions to improve the cognitive capability of older adults, and reduce the risk of neurodegenerative diseases associated with aging. Physical activity is considered one of the most important and accessible factors to prevent and protect brain functions at low cost (Kramer and Erickson, 2007). Extensive evidence from animal and human studies (Cotman and Berchtold, 2002; Markham and Greenough, 2004) suggests that exercise has a positive impact on cognitive and emotional aspects of behavior. In this context, many publications have reported anxiolytic and antidepressant effects of exercise, resulting in better management of stress (Dishman et al., 2006). In rodent models, young and old, voluntary or forced exercise showed improved hippocampus-dependent memory system and learning, and other hippocampus-independent memory systems. In addition, exercise can reverse the negative effects of physical inactivity, and decrease and/or delay the deleterious effects of aging on cognition (Cotman and Engesser-Cesar, 2002). Van Praag et al. (2005) showed that young mice with free access to wheel running as well as old mice that were sedentary until 18 months and then exposed to voluntary exercise, had a significantly higher cognitive performance than age-matched controls. This work demonstrated that the mechanisms underlying these effects operate throughout life when properly stimulated. Several human epidemiological studies (Dik et al., 2003; Lytle et al., 2004; Kramer et al., 2006) addressed this issue with different designs. Although most of these studies highlight the importance of exercise in all its forms, they also report some difficulties at controlling confounders, defining inclusion criteria, or identifying and quantifying the type of exercise performed by study participants.

Recent research found that aerobic physical activity produces the greatest benefits (Colcombe et al., 2004; Boveris and Navarro, 2008; Kamijo et al., 2009; Baker et al., 2010). This kind of exercise is characterized by being performed continuously or at intervals, with moderate intensity (60–70% of maximum oxygen consumption (max.VO_2)). The aerobic energy system is responsible for supplying oxygen demanded in activities involving large muscle mass, lasting 3 min or more, such as walking, jogging, swimming, rowing, and so forth, and made repeatedly with a characteristic frequency.

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Abbreviations: AT, aerobically trained; EPM, elevated plus maze; ex, exercise; HPA axis, hypothalamic-pituitary-adrenal axis; max.VO_2, maximum oxygen consumption; no-ex, no-exercise; OF, open field; RM, eight-arm radial maze; SC, sedentary control.

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Erickson and Kramer (2009) demonstrated in older adults that, chronic aerobic training performed during 6 months, improved markedly critical aspects of the attention system, the executive control, and the activity of prefrontal and parietal regions. In a recent paper, also performed with older adults, the authors showed that the old brain was plastic and responsive to changes induced by aerobic exercise, and that this effect was modulated by the extent of training. Aerobically trained subjects were evaluated with neuroimaging techniques and showed a significant amplification of connectivity in the frontal and temporal regions that are sensitive to aging. Interestingly, this response was detected after 12 months of training, but not at 6 months. These changes correlated with an improvement in executive function, possibly reflecting a restoration or prevention of neural circuit deterioration. So, cardiovascular fitness can promote a significant increase in efficiency, adaptive capacity, and plasticity of aged brain, and therefore can reduce the biological and cognitive senescence (Voss et al., 2010; Szabo et al., 2011). The results obtained by different authors suggest the need to specifically investigate the time and dose-dependent relationship between aerobic fitness and cognitive performance (Etnier et al., 2006; Studeski et al., 2006). In this regard, animal models offer a unique opportunity to apply different training regimes, whether acute or chronic, and to obtain a more homogeneous and reliable response, at least in some issues. However, few studies attempted to standardize these conditions to model human activity.

In order to study the potential effects of aging and aerobic exercise practiced regularly throughout life on cognition and emotionality, we designed a longitudinal study in rats with two cut points, representing middle age and old age. Physical training was modeled through a program of moderate and chronic exercise that simulated the regular practice of aerobic activity from puberty to old age, assuming the same cardiovascular, respiratory, and metabolic adaptations that humans have when they practice this type of activity, not for competitive purposes or high performance. The training routine was adjusted according to performance and age, in the same way that humans modify the quality and quantity of physical activity throughout their lives.

The present work aims to study (1) the possible effects of regular and moderate exercise on cognitive, motor, and anxiety-related behaviors of middle-aged adults (age: 8 months) and older adults (age: 18 months); (2) if such changes are age-dependent; and (3) whether the chronic aerobic exercise has neuroprotective and restorative effects of cognitive function, in the long term.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats, WKAH/Hok strain (n=136), weaned from the Animal Facilities of the Faculty of Veterinary Science, University of La Plata (UNLP), weighing 150–200 g, were held in groups of three in standard laboratory cages, and randomly assigned to each of the following groups: (1) Aerobically trained (AT, n=68) or (2) Sedentary control (SC, n=68). All the animals were kept under the same environmental conditions throughout the experiment: 12 h:12 h light–dark cycle (lights off at 6:00 AM), temperature 22±2 °C, humidity 45–55%. In addition, animals were subjected to microbiological monitoring every 6 months and clinically evaluated each week. Food and water were supplied *ad libitum*. Weekly records of body weight, food, and drink were obtained. The housing room remained under the same standardized conditions until the completion of the study. Because physical training and behavioral tests were conducted during the dark period, the testing rooms were illuminated with a 25W red light. All experiments were approved by the Institutional Committee for the Care and Use of Laboratory Animals of the School of Health Sciences (UCES), and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 2011). We also followed the recommendations of specific guidelines: Neuroscience and Behavioral Studies (Van Sluyters et al., 2003), Methods of Behavior Analysis in Neuroscience (Buccafusco, 2009), Animal Exercise Protocols (Kregel et al., 2006), Physical Activity Practice with adults (Haskell et al., 2007) and older adults (Nelson et al., 2007). All efforts were made to minimize the suffering and the number of animals used.

Aerobic training protocol

Rats were trained from 2 to 18 months of age in a motorized treadmill specially built for this experiment: a transparent acrylic box 60 cm width×80 cm length×12 cm height, divided into six lanes of 70 cm length×10 cm width. The treadmill running was connected to a computer and the following values were displayed on the screen: airflow (liters per minute, L/min); O_2 concentration (parts per million, ppm); speed (meters per minute, m/min); slope (degrees, deg); time (min); distance (meters, m); temperature (degrees, °C). The protocol was designed in accordance with the basic principles of training in humans: specificity, progressive overload, and variable intensity (Wilmore and Costill, 2007). As this is a model of forced exercise, we tried to minimize the impact of potentially negative factors (Moraska et al., 2000), and to maintain a strict control of health and animal welfare before, during, and after each training session. In this sense, animals were not daily trained, but three times a week to avoid chronic stress, inflammation, or muscle damage, and to allow the recovery of liver glycogen and muscle glycogen. They received no stimulation (aversive or appetitive) to motivate them to run. Before starting the training, both groups performed the habituation for 2 weeks. The SC was subjected to the same experimental conditions but within the motionless treadmill. Apparatus was thoroughly cleaned between sessions.

Therefore, the objective of our training plan was to progressively and systematically increase the training stimulus to induce and maximize homeostatic adaptation (i.e. cross the threshold of adaptation), and, as a result, improve performance.

Animals were trained with an aerobic routine of moderate intensity (60–70% max.VO_2) with a gradual increase in the workload (volume and intensity). Depending on the performance and age, the duration, speed, and slope were adjusted each 15 days.

In order to determine the proper intensity of training, monthly individual tests of max.VO_2 were performed, considering that max.VO_2 is the best parameter to study the aerobic power (Wisløff et al., 2001). Three rats of the AT group were randomly selected and evaluated as follows: 15 min warm up at 6 m/min (50% intensity) followed by a progressive increase in the speed of 1 m/min every 3 min. The highest record was obtained from the leveling off of oxygen uptake despite the increased workload. Then, from these data, 60–70% max.VO_2 was calculated, thus determining the new running speed. The test was repeated each time the slope was adjusted, starting with 0 degrees, then 5, and finally 10 degrees. Slopes greater than 10 degrees were discarded because they increased the anaerobic component and the risk of injury. At 2 months, the initial workload was 15 min, 6 m/min