NEUROSCIENCE

Volume 202, January 27, 2012
ISSN 0306-4522

THE IBRO JOURNAL

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

A. PIETRELLI,[a,b*] J. LOPEZ-COSTA,[b] R. GOÑI,[a] A. BRUSCO[b] AND N. BASSO[a,c]

[a]*School of Health Sciences, Department of Basic Sciences Research, University of Business and Social Sciences (UCES), Buenos Aires, Argentina*

[b]*Institute of Cell Biology and Neuroscience "Prof. E. De Robertis," School of Medicine, University of Buenos Aires (UBA), CONICET, Buenos Aires, Argentina*

[c]*Laboratory of Cardiovascular Physiopathology, School of Medicine, University of Buenos Aires (UBA), Buenos Aires, Argentina*

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. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

*Correspondence to: A. Pietrelli, School of Health Sciences, University of Business and Social Sciences (UCES), Paraguay 1239, floor 12th (C1061ABA), Buenos Aires, Argentina. Tel: +54-11-4815-3290 int. 485; fax: +54-11-4815-3290 int 485.
E-mail address: apietrelli@uces.edu.ar (A. Pietrelli).

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.

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
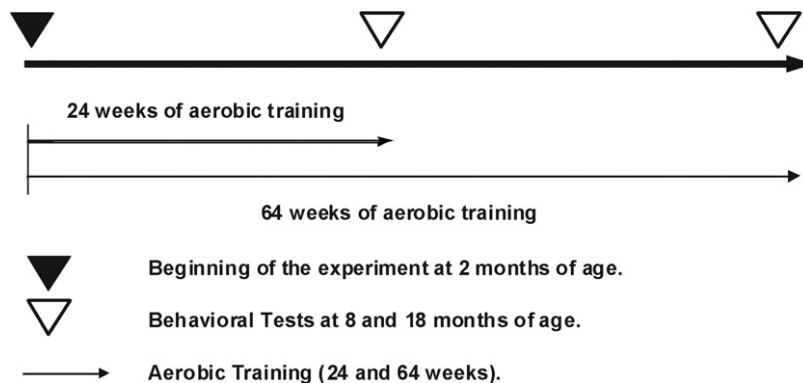
Fig. 1. Overall scheme of the experiment from its beginning at 2 mon old to completion at 18 mon old. Observe the distribution of cut off points (8 and 18 mon, middle age and old age, respectively) for behavioral testing (RM, OF, and EPM), after the period of aerobic training (24 and 64 wk).

speed, and 0 degrees slope. Maximum overload was reached at 6–8 months (60 min, 14 m/min speed, and 10 degrees), which corresponds approximately to a young adult human between 20 and 30 years of age who is in his best physical performance. This routine was maintained between 8 and 12 months with small variations. Afterward, the workload began to decrease following the reverse order: first the slope, then the speed, and at last, the time. At 18 months, the final workload was 30 min, 6 m/min speed, and 0 degrees.

Behavioral tests

At 8 and 18 months, following the period of training (24 and 64 weeks, respectively), three experiments were performed at each age: (1) Radial maze (SC, $n=10$; AT, $n=10$); (2) Open field (SC, $n=12$; AT, $n=12$); and (3) Elevated plus maze (SC, $n=12$; AT, $n=12$). Each rat was randomly assigned to a single test. The animals were not subjected to restrictions on food or drink before the experiments. Fig. 1 shows the overall scheme of aerobic training and cut off points to assess cognitive and behavioral parameters.

Eight-arm radial maze. The radial maze consisted of a round central platform (40 cm in diameter) from which eight arms (12 cm wide×40 cm high×60 cm long) radiated. The entire maze was made of transparent acrylic, standing 30 cm above the floor, and was surrounded by extra maze cues held in constant spatial relations throughout the experiment such as table, chair, balloons, stickers on the walls, including the operator. At 8 and 18 months old, following the period of training, both groups of animals were tested. The protocol consisted of three stages: (1) Handling, (2) Shaping/habituation, and (3) Testing procedure. Formal training began on the 8th day after the first exposure to the maze. Each rat was tested for 10 min. The first 5 min corresponded to the acquisition phase, and the latter to the consolidation phase of memory. The whole procedure was recorded by a video camera mounted on the ceiling of the room. The score was performed manually. The following parameters were determined: (a) Correct entries (%): calculated as the percentage of right (baited) arm entries divided by total arm entries (baited/right and non-baited/wrong), [(number of correct entries/number of arms)×100]; (b) Number of arms (NA, right and wrong): arms visited in the allowed time; (c) Total time (TT, seconds): time needed to visit right arms; (d) Working memory errors (WME): number of reentries into a right arm already visited in the same session; (e) Spatial memory errors (SME): number of entries into a wrong arm; (f) Success: number of trials that met the following criteria: (1) four correct entries; (2) without errors in WME or SME, and (3) in time less than 3 min; (g) Grooming (G): number of times the rat grooms; (h) Fecal pellets (FP): number of fecal pellets left in the maze.

Variable (f) was considered as the main index of cognitive performance level achieved because it summarizes the remaining variables, and variables (g) and (h) were considered as indicators of anxiety-related behaviors.

Open field. It consisted of a box of 1×1 m², and 40 cm high, built in white opaque acrylic, which was placed on a platform of the same size, 30 cm above the floor. The space defined by the walls was covered with white non-slip rubber, and divided into 36 numbered squares, approximately 16×16 cm². We defined a central area consisting of four squares, and a peripheral sector comprising the outer perimeter of the arena. The open field was placed in an isolated room, and under the same standardized conditions, with no objects or clues that could represent signals and alter patterns of behavior. The whole procedure was recorded by a video camera mounted on the ceiling of the room. Each animal was tested for 5 min. The score was performed manually. The following parameters were determined: (a) Latency (seconds): time to leave the central platform; (b) Distance (meters): number of squares crossed by the animal's body length (18 cm), measured from the snout to the root of the tail. It was considered a square crossed when the animal entered with its four paws; (c) Time spent in the central square (%): it refers to the total duration of the reentries to the central square. Calculated as the percentage of the time in the central square divided by total test time [(time spent in central platform/5 min)×100]; (d) Time spent in peripheral squares (%): calculated as the percentage of the time in the periphery divided by total test time [(time spent in peripheral squares/5 min)×100]; (e) Rearing and thigmotaxis: number of times the rat stands on its two hind paws. Records were also considered valid when the rat touched the walls; (f) Grooming: number of times the rat grooms its head or genitals; (g) Fecal pellets: number of fecal pellets; (h) Urine: number of times that rats urinate. Variables (a), (c), (d), (f), (g), and (h) were considered major indexes of anxiety-related behaviors. Variable (b) was indicative of locomotive activity, and variable (e) was indicative of exploratory behavior.

Elevated plus maze. It consisted of a platform built of wood, 50 cm high, covered with a white non-slip rubber, cross shaped: two arms, 60 cm long and 12 cm wide, remained free (open), and surrounded by a small edge of 1 cm to prevent falls; the other two arms (closed) were enclosed in black acrylic walls, 12 cm wide, 40 cm high, and 60 cm long, closed at its end. The union of the four arms left an open central area of approximately 12×12 cm². The maze was installed in an isolated room, with no objects or signals. The entire procedure was recorded by a video camera placed on the ceiling. Each animal was tested for 5 min. The score was performed manually. The following parameters were determined: (a) Latency (seconds): time to leave the central platform; (b) Open

arms: number of entries in open arms. Entries were considered as correct when the animal crossed the central platform with its four paws; (c) Closed arms: number of entries in closed arms; (d) Time spent in open arms (%): calculated as the percentage of the time spent in open arms divided by total test time [(time spent in open arms/5 min)×100]; (e) Time spent in closed arms (%): calculated as the percentage of the time spent in closed arms divided by total test time [(time spent in closed arms/5 min)×100]; (f) Rearing and thigmotaxis: number of times the rat stands on its two hind paws. Records were also considered valid when the rat touched the walls in the closed arms; (g) Grooming: number of times the rat grooms its head or genitals; (h) Fecal pellets: number of fecal pellets; (i) Urine: number of times that rats urinate. Variables (a), (b), (d), (e), (g), (h), and (i) were considered as the major indexes of anxiety-related behaviors. Variable (c) was indicative of loco-motor activity, and variable (f) was indicative of exploratory behavior.

Statistical analysis

The radial maze data were analyzed by three-way ANOVA with repeated measures (RM ANOVA) on time factor (factors between groups: age, two levels; group, two levels), followed by Bonferroni's test for multiple comparisons. Each week was considered as a block of 5 days (factor within the groups: time/block, six levels). Fecal pellets parameter was not analyzed or plotted because of the lack of records greater than zero. Statistical analysis was performed with SPSS v. 18.0, 2010, IBM, USA. All the variables were plotted with the GraphPad Prism v. 5.0, CA, USA, 2010. Results were expressed as mean±SEM. Asterisks represent the significant [* $P<0.05$] and highly significant [*** $P<0.001$] differences.

To analyze and plot the data from the open field and the elevated plus maze, two-way ANOVA (age, two levels×group, two levels) followed by Bonferroni's test was performed with the GraphPad Prism software. Results were expressed as mean±SEM. Asterisks represent the significant [* $P<0.05$] and highly significant [*** $P<0.001$] differences by the exercise effect, whereas the age effect was featured as numeral symbols [# $P<0.05$] and [### $P<0.001$], respectively.

RESULTS

Radial maze

Fig. 2a: The percentage of correct entries was significantly affected by the time effect [$F(5,985)=44.18$, $P=0.0001$], age [$F(5,985)=15.84$, $P=0.0001$], and exercise [$F(1,197)=9.17$, $P=0.002$]. Further analysis indicated that, although all animals showed a clear trend to increase the number of correct entries, AT rats showed a better performance than age-matched controls. When we analyzed the effects of age, we noted that middle-aged rats had a larger number of correct entries than old ones. The exercise×age inter-action was very significant, indicating that the effect of training was different in middle age than in older animals.

Fig. 2b: The number of arms visited (right and wrong) was very significantly affected by the time effect [$F(5,985)=44.26$, $P=0.0001$] and by exercise [$F(1,197)=19.84$, $P=0.0001$]; however, age did not affect this response. The time×exercise interaction was significant, indicating that exercise enhanced the effects of learning on AT rats. Consistent with data reported in (a), this interesting result indicated that AT rats learned the task faster than SC rats.

Fig. 2c: The total time spent in finding the right arms showed highly significant differences [$F(5,985)=74.64$,

$P=0.0001$] by the time, age [$F(1,197)=33.90$, $P=0.0001$], and exercise effect [$F(1,197)=13.73$, $P=0.0001$]. Although both, runners and controls, showed a strong trend to re-duce the time to find the correct arms, AT rats, regardless of age, were significantly ($P=0.0001$) faster in the first week than SC.

Fig. 2d: In the case of working memory, we detected highly significant differences by the time effect [$F(5,985)=27.42$, $P=0.0001$]. All animals made fewer mistakes throughout the sessions. Age markedly affected the work-ing memory [$F(1;197)=12.53$, $P=0.0001$], showing that middle-aged rats made fewer errors than old ones. Exer-cise had no impact on this variable.

Fig. 2e: The spatial memory errors showed highly sig-nificant differences [$F(5,985)=37.21$, $P=0.0001$] by time and exercise [$F(1,197)=18.32$, $P=0.0001$]. We detected a gradual trend in all animals to make fewer errors by time effect. Time×exercise interaction again indicated that training amplified the effects of learning in AT rats. Age did not affect this response. We observed that AT rats made fewer errors than SC rats. Similar to that reported in (b) and (c) data, the old runners appear initially overcoming middle age sedentary controls.

Fig. 2f: The success variable refers specifically to the number of trials that met the criterion (see in section Eight-arm radial maze). Three-way RM ANOVA showed highly significant effects of time [$F(5,85)=19.08$, $P=0.0001$] and exercise [$F(1,17)=24.73$, $P=0.0001$], and significant ef-fects by age [$F(1,17)=15.73$, $P=0.001$]. Although all ani-mals showed an increase in the number of successful trials, block×exercise interaction showed that exercise did not have the same effect along the sessions. The age×exercise interaction showed that AT middle-aged rats were the most efficient, and although outperformed the older runners, both reached the criterion more times than SC rats.

Fig. 2g: The number of grooming revealed significant differences [$F(5,985)=19.04$, $P=0.0001$] by the time ef-fect. All animals showed some degree of anxiety only in the first week of the trial. This behavior was not affected either by exercise or by age. No interaction was detected be-tween sessions, exercise, or age.

Variable (h), fecal pellets: was not analyzed or plotted because of the scarcity of values greater than zero in both groups over the 30 days of testing.

The summary of the three-way RM ANOVA results is widely reported in Table 1.

Open field

Fig. 3a: The latency was very significantly affected by age [$F(1,33)=20$, $P=0.0001$], and significantly by exercise [$F(1,33)=6.70$, $P=0.014$]. The exercise×age interaction indicated that the effect of exercise on the latency de-pended on the age. Post hoc analysis revealed that old AT rats showeda significantly ($P<0.001$) lower latency than the age-matched controls, whereas no differences were detected among middle-aged runners and their respective controls.
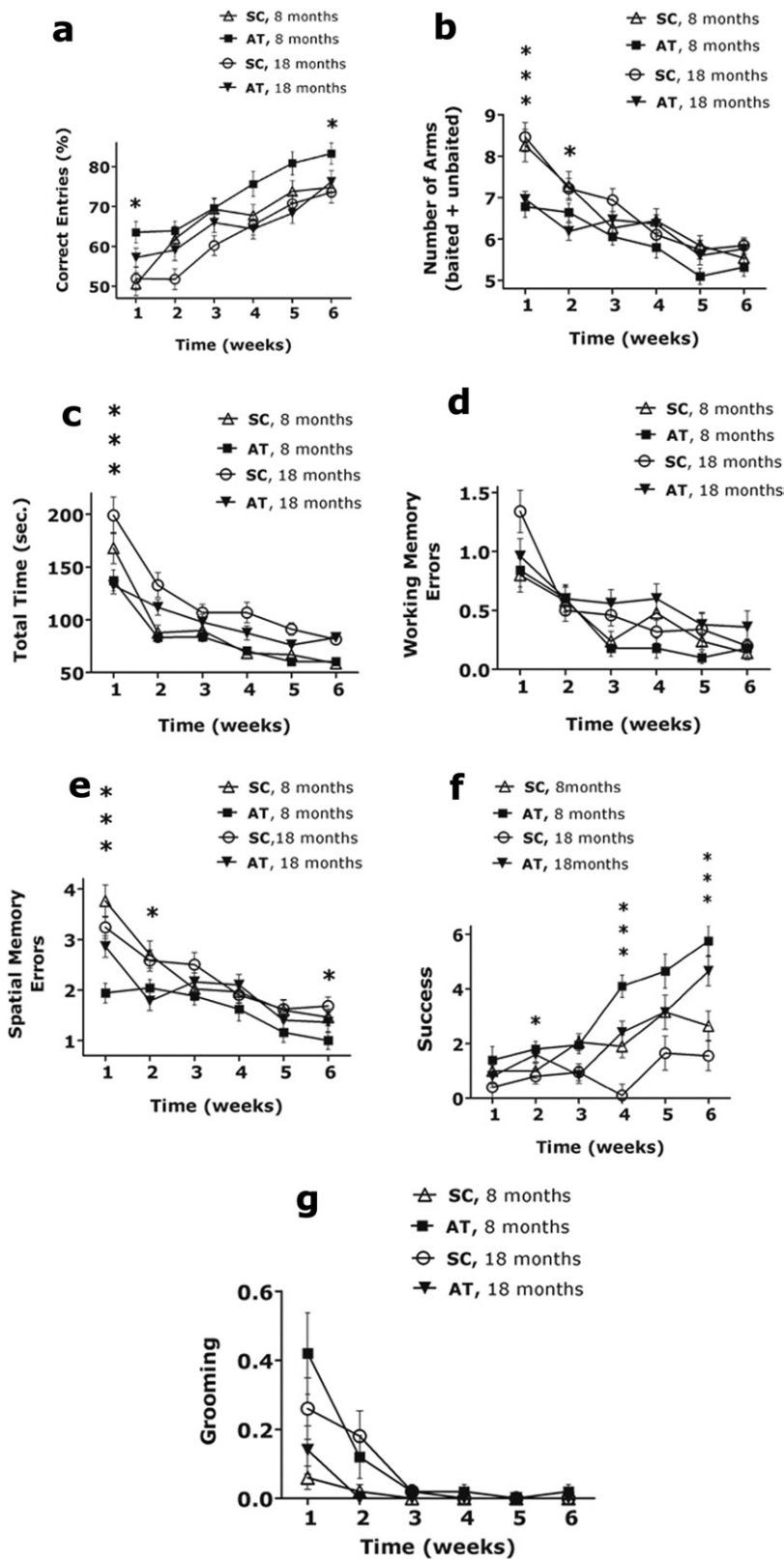
Fig. 2. Effects of aerobic exercise and age on cognitive performance and anxiety-related behaviors in the eight-arm radial maze. Rats subjected to aerobic routine on the treadmill running were trained for 24 and 64 wk. After the training, SC and AT rats were assessed over 30 d in the radial maze. Graphs (a–f) show acquisition and consolidation curves of memory and learning in middle-aged (8 mon) and old rats (18 mon). Note that middle-aged AT rats were the best performers, and older AT rats had better cognitive performance than SC. (g) Observe that the animals showed no anxiety-related behavior and that neither age nor exercise altered this response. Points represent the weekly mean±SEM. Asterisks represent the exercise effects, and show significant differences (*) when $P<0.05$, and highly significant (***) at $P<0.001$, which were detected by three-way RM ANOVA followed by Bonferroni's test. SC=sedentary control; $n=20$; AT=aerobically trained, $n=20$.

Table 1. Eight-arm radial maze. Summary of the three-way RM ANOVA results after 6 wk of testing, at middle age (8 mon) and old age (18 mon)

	Time/block			Age			Exercise			Interaction			
	Df	F	P	Df	F	P	Df	F	P	Df	F	P	
Correct entries (%)	5; 985	44.18	0.0001	1; 197	15.84	0.0001	1; 197	10.24	0.002	1; 197	8232.50	0.0001	Age×ex
										5; 985	4.65	0.0001	Block×ex
Number of arms	5; 985	44.26	0.0001	1; 197	2.20	0.139	1; 197	19.84	0.0001	1; 197	9748.23	0.001	Age×ex
										5; 985	5.09	0.002	Block×ex
Total time (s)	5; 985	74.64	0.0001	1; 197	33.90	0.0001	1; 197	13.73	0.0001	1; 197	2515.49	0.0001	Age×ex
Working memory errors	5; 985	27.42	0.0001	1; 197	12.53	0.0001	1; 197	0.03	0.864	1; 197	369.31	0.0001	Age×ex
										5; 985	3.83	0.003	Block×ex
Spatial memory errors	5; 985	37.21	0.0001	1; 197	2.33	0.128	1; 197	18.32	0.0001	1; 197	1307.97	0.0001	Age×ex
										5; 985	5.64	0.001	Block×ex
Success	1; 85	19.08	0.0001	1; 17	15.73	0.001	1; 17	24.73	0.0001	1; 197	249.71	0.0001	Age×ex
Grooming	5; 985	19.04	0.0001	1; 197	0.16	0.689	1; 197	0.77	0.379	1; 197	27.17	0.0001	Age×ex

Groups: SC (no-ex), $n=10$; AT (ex), $n=10$. $N_t=40$. Differences were considered highly significant when $P<0.001$, and significant when $P<0.05$.

Fig. 3b: The distance or squares crossed (horizontal activity) was significantly affected by exercise [$F_{(1,33)}=7.96$, $P=0.008$] and age [$F_{(1,33)}=79.35$, $P<0.0001$]. Interaction was significant [$F_{(1,33)}=11.53$, $P<0.001$], showing that aerobic training did not have the same effect at 8 and 18 months of age. When comparing middle-aged vs. old animals, regardless of exercise, we found that the former crossed more squares than the latter ($P<0.0001$). Middle-aged animals were more active and had a greater preference for exploring than the old ones.

Fig. 3c: Time spent in the central square (horizontal activity) was significantly affected by age [$F_{(1,33)}=56.41$, $P=0.0001$], but not by exercise. Interaction was not detected. Middle-aged rats, regardless of exercise, reentered to the central area more times than older rats.

Fig. 3d: Time spent in the peripheral squares (horizontal activity) was significantly affected by age [$F_{(1,33)}=41.14$, $P<0.0001$]. The old rats showed greater preference to remain in the peripheral areas related to middle-aged rats. This behavior was also significantly affected by exercise [$F_{(1,33)}=9.13$, $P<0.004$]. Post hoc analysis showed that old AT rats spent less time in the peripheral squares ($P<0.001$) than the age-matched controls. No interaction was detected.

Fig. 3e: The rearing and the thigmotaxis (vertical activity) gave a measure of interest in exploring the new environment. We observed a highly significant effect of age showing that middle-aged rats were more interested in exploring the open field than older animals. The exercise did not modify this response and we found no interaction.

Fig. 3f: The grooming behavior was significantly affected by exercise [$F_{(1,33)}=29.20$, $P<0.0001$] and age ($F_{(1,33)}=19.93$, $P<0.0001$). Interaction was also detected, indicating that physical activity did not have the same effect at 8 months and 18 months [$F_{(1,33)}=9.32$, $P=0.004$]. Results revealed that old AT rats groomed significantly less than SC of same age ($P<0.0001$). Exercise did not affect this response in middle age: SC rats had very few records, and AT, none.

Fig. 3g: The number of fecal pellets was significantly lower in AT than in SC rats [$F_{(1,33)}=7.30$, $P=0.01$]. Age did not affect this response. No interaction was detected.

Fig. 3h: The number of times that rats urinated was affected by exercise. We observed a significant reduction in it in AT rats compared with age-matched SC [$F_{(1,33)}=5.15$, $P=0.029$]. Data showed that this difference was very significant in AT old animals. Age did not affect this behavior, and no interaction was detected.

The overall results of the two-way ANOVA are widely reported in Table 2. Table 3 summarizes the mean±SEM of the variables (a–h) studied in the open field (OF).

Elevated plus maze

Fig. 4a: The latency was significantly affected by exercise and age, although no interaction was detected. AT rats needed less time to leave the central platform. When analyzing the effect of age, we found that aged rats presented longer latency, especially when comparing SC and AT.
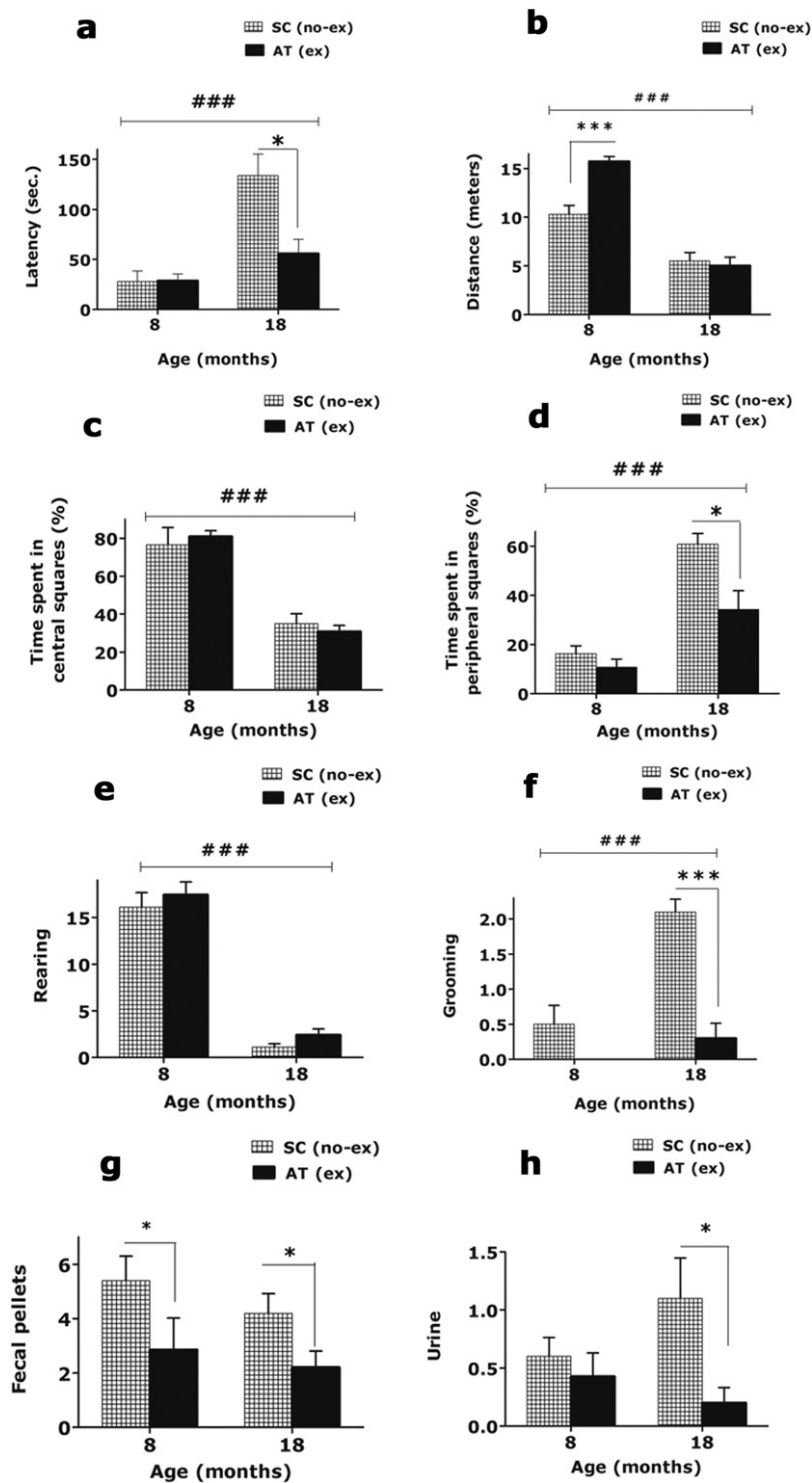
Fig. 3. Effects of aerobic exercise and age on anxiety-related behaviors in the open field. Rats subjected to aerobic routine were trained on the treadmill running for 24 and 64 wk. After the training period, SC and AT rats were assessed in middle age (8 mon) and old age (18 mon) in the open field. (a-h) note that aging had an anxiogenic effect. The exercise had an anxiolitic effect, particularly important in old age. Bars represent the mean±SEM. The effects of age were characterized by numerals ($^{\#}$), and the effects of exercise were indicated by asterisks (*). Differences were considered statistically significant [* or $^{\#}$] when $P<0.05$, and very significant [*** or $^{\#\#\#}$] when $P<0.001$. Statistical analysis was performed with two-way ANOVA followed by Bonferroni's test. SC=no-ex (no-exercise), $n=24$; AT=ex (exercise), $n=24$.

Fig. 4b: The number of entries into the open arms showed no significant differences between AT and SC rats ($P>0.05$). No interaction was detected; however, the response was affected significantly by age [$F(1,46)=36.33$,

Table 2. Open field. Summary of two-way ANOVA results, at middle age (8 mon) and old age (18 mon)

	Exercise			Age			Interaction		
	Df	F	P	Df	F	P	Df	F	P
Latency (s)	1; 33	6.70	0.014	1; 33	20	0.0001	1; 33	7	0.012
Distance (m)	1; 33	7.96	0.008	1; 33	79.35	0.0001	1; 33	11.53	0.001
Time spent in central square (%)	1; 33	0.001	0.98	1; 33	56.41	0.0001	1; 33	0.47	0.49
Time spent in peripheral squares (%)	1; 33	9.13	0.004	1; 33	41.14	0.0001	1; 33	3.88	0.05
Rearing thigmotaxis	1; 33	1.46	0.23	1; 33	190.90	0.0001	1; 33	0.0001	0.98
Grooming	1; 33	29.20	0.0001	1; 33	19.93	0.0001	1; 33	9.32	0.004
Fecal pellets	1; 33	7.30	0.01	1; 33	1.22	0.27	1; 33	0.10	0.74
Urine	1; 33	5.14	0.02	1; 33	0.33	0.56	1; 33	2.38	0.13

Groups: SC (no-ex), $n=12$; AT (ex), $n=12$; $N_t=48$. Differences were considered highly significant when $P<0.001$, and significant when $P<0.05$.

$P=0.0001$], indicating that middle-aged rats entered more times than the old ones.

Fig. 4c: The number of entries to closed arms was significantly affected by exercise [$F_{(1,46)}=10.77$, $P=0.002$] and age [$F_{(1,46)}=8.01$, $P=0.006$], although no interaction was detected. Subsequent comparisons showed that only the old AT rats had fewer entries to the enclosed arms than the SC rats of the same age ($P<0.01$), and that middle-aged animals entered more times than the old, regardless of group ($P<0.001$).

Fig. 4d: Time spent in open arms was significantly affected by exercise [$F_{(1,46)}=16.23$, $P=0.0002$], but not by age. No interaction was detected. When comparing group×age, significant differences were detected between AT and SC at 18 months of age ($P<0.001$). Trained rats spent more time exposed in the open arms than their sedentary counterparts.

Fig. 4e: Consistent with the results reported in (c), we observed that exercise significantly affected [$F_{(1,46)}=16.32$, $P=0.0002$] the time spent in closed arms. Old rats spent less time ($P<0.001$) than age-matched controls. There were no significant differences by age or interaction.

Fig. 4f: Rearing and thigmotaxis showed significant differences [$F_{(1,46)}=5.92$, $P=0.018$] by the effect of exercise. Age did not affect the response and no interaction was detected. When comparing group×age, it was observed that AT rats, regardless of age, had a greater number of rearing than SC, indicating a strong interest in exploring the new environment ($P<0.05$).

Fig. 4g: The grooming was not affected either by exercise or by age, no interaction was detected.

Fig. 4h: The number of fecal pellets was significantly lower in AT rats vs. SC [$F_{(1,46)}=5.01$, $P=0.03$], but there were no significant differences caused by age or interaction.

Fig. 4i: The number of times the rats urinated showed the same trend that we observed in (g).

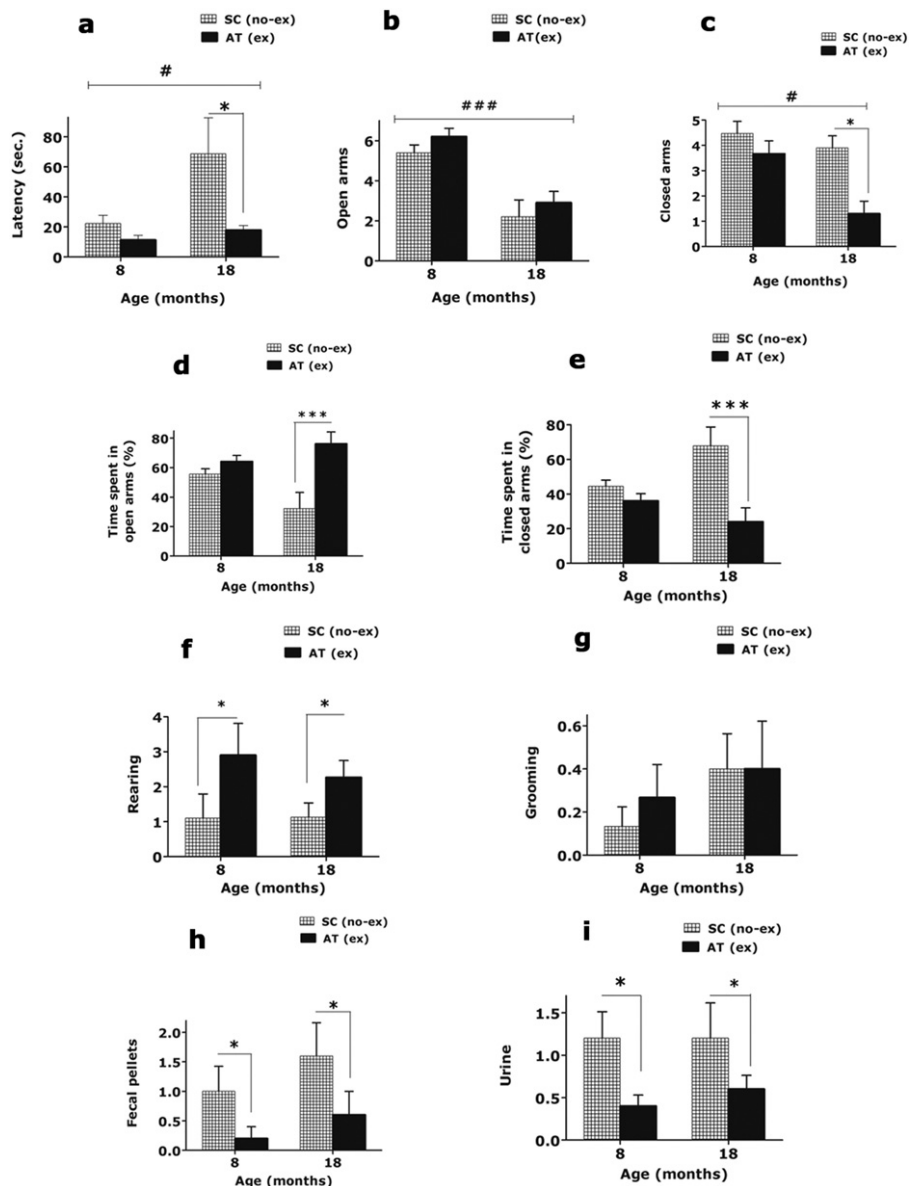AT rats urinated significantly [$F_{(1,46)}=6.44$, $P=0.014$] less than SC, both at 8 months and 18 months. Age did not alter this response and no interaction was detected.

The overall results of the two-way ANOVA are widely reported in Table 4. Table 5 summarizes the mean±SEM of the variables (a–i) studied in the elevated plus maze (EPM).

DISCUSSION

This study investigated the impact of chronic and moderate aerobic exercise and aging on cognitive parameters and anxiety-related behaviors. Based on the obtained results, the treadmill running was methodologically appropriate to study plastic and cumulative effects of aerobic exercise on the brain and behavior, and modeled realistically the human activity, practiced regularly from youth to old age. The AT animals showed good adaptation to the effort, greater mechanical efficiency, and a progressive increase in the physical performance.

Taking into account that forced and voluntary exercise differentially affects brain and behavior (Leasure and Jones, 2008) we discarded the model of voluntary exercise because it does not allow the specific manipulation of the stimulus (time and dose). Previous studies in humans and

Table 3. Open field. Summary of mean±SEM, $N_t=48$

	8 mon ($n=24$)		18 mon ($n=24$)	
	SC (no-ex, $n=12$)	AT (ex, $n=12$)	SC (no-ex, $n=12$)	AT (ex, $n=12$)
Latency (s)	27.77±10.69	28.66±6.85	133.80±21.48	56±14.19
Distance (m)	10.30±0.91	15.71±0.52	5.50±0.88	5.00±0.86
Time spent in central square (%)	76.60±9.01	80.85±3.08	35.00±5.24	30.90±3.14
Time spent in peripheral squares (%)	10.70±3.38	16.28±3.13	60.80±4.41	34.30±7.60
Rearing thigmotaxis	16.10±1.55	17.42±1.39	1.10±0.37	2.40±0.65
Grooming	0.50±0.26	0.00	2.10±0.17	0.30±0.21
Fecal pellets	5.40±0.89	2.85±1.16	4.20±0.72	2.20±0.61
Urine	0.60±0.16	0.42±0.20	1.10±0.34	0.20±0.13

Fig. 4. Effects of aerobic exercise and age on anxiety-related behaviors in the elevated plus maze. Rats subjected to aerobic routine were trained on the treadmill running for 24 and 64 wk. After the training period, SC and AT rats were assessed in middle age (8 mon) and old age (18 mon) in the elevated plus maze. (a-i) note that aging had an anxiogenic effect. Notice that the exercise had an anxiolitic effect, particularly important in old age, showing a trend similar to that observed in the OF. Bars represent the mean±SEM. The effects of age were characterized by numerals (#), and the effects of exercise were indicated by asterisks (*). Differences were considered statistically significant [* or #] when $P < 0.05$, and very significant [*** or ###] when $P < 0.001$. The analysis was performed with two-way ANOVA followed by Bonferroni's test. SC=no-ex (no-exercise), $n = 24$; AT=ex (exercise), $n = 24$.

animals (Kim et al., 2003; Berchtold et al., 2010) showed that the adaptations produced by exercise are time and dose-dependent, so it is reasonable to assume that the quantitative and qualitative physiological response varies depending on the type of exercise and the duration of their application.

Our work demonstrated that aerobic exercise in rats affected chronically most of the cognitive variables in the radial maze, and potentiated the effects of learning along 30 days, both in middle and old age. Experimental rats, regardless of age, performed better than controls because

they learned faster and made fewer mistakes. This response indicated that the trained animals reached higher levels of acquisition, consolidation, and maintenance of the spatial, motor, and procedural memory than controls, in agreement with what was previously reported by Anderson et al. (2000) and Albeck et al. (2006). This prolonged consolidation occurs in the declarative memory system, a hippocampus-dependent system, and could involve interactions between the medial temporal region, the cerebral cortex, and also a pathway through the cortex and neostriatum that plays an important role in the acquisition of

Table 4. Elevated plus maze. Summary of two-way ANOVA results, at middle age (8 mon) and old age (18 mon)

	Exercise			Age			Interaction		
	Fd	*F*	*P*-value	Fd	*F*	*P*-value	Fd	*F*	*P*-value
Latency (s)	1; 46	6.60	0.014	1; 46	4.8	0.034	1; 46	2.80	0.105
Open arms	1; 46	1.93	0.17	1; 46	36.33	0.0001	1; 46	0.008	0.92
Closed arms	1; 46	10.77	0.002	1; 46	8019	0.006	1; 46	3.02	0.08
Time spent in open arms (%)	1; 46	16.23	0.0002	1; 46	0.77	0.38	1; 46	7.41	0.009
Time spent in closed arms (%)	1; 46	16.32	0.0002	1; 46	0.75	0.38	1; 46	7.42	0.009
Rearing	1; 46	5.92	0.01	1; 46	0.24	0.62	1; 46	0.30	0.58
Grooming	1; 46	0.20	0.66	1; 46	1.64	0.20	1; 46	0.18	0.67
Fecal pellets	1; 46	5.01	0.03	1; 46	1.54	0.21	1; 46	0.06	0.80
Urine	1; 46	6.44	0.01	1; 46	0.13	0.71	1; 46	0.13	0.71

Groups: SC (no-ex), $n=12$; AT (ex), $n=12$; $N_t=48$. Differences were considered highly significant when $P<0.001$, and significant when $P<0.05$.

habits, learned sequences, and sensorimotor adaptations (Seger, 2006). However, the exercise did not improve the working memory, a hippocampus-independent system (Dudchenko, 2004), more related to the prefrontal cortex, which is severely affected by aging (Bimonte et al., 2003). As expected, the analysis of age and exercise interactions revealed that the effects of exercise were modulated by age. In this sense, AT middle-aged rats were the most successful animals because they reached the criterion more times than the remaining rats. The AT old rats showed an amplified cognitive performance relative to age-matched controls, and, in the first week, were even more efficient than SC middle-aged rats, as demonstrated by subsequent analysis of correct entries, number of arms visited, time to find the right arms, and spatial memory errors. The success variable summarized the cognitive performance level, acquisition, and consolidation of memory achieved by animals, as it took into account the main indicators of learning and memory (see in section Eight-arm radial maze). The results indicated that AT older rats, again, outperformed the middle-aged SC rats in the last weeks of the trial. This interesting finding may suggest that the effects of regular training acted cumulatively over the long term, reversing the negative effects of physical inactivity, and partially restoring the cognitive function in old age.

In the case of grooming, all animals, regardless of age, showed some degree of anxiety only in the first week that quickly disappeared as a result of learning along the sessions. This initial response was probably because of a novelty, difficulty of the task, and/or social isolation; therefore, neither age nor exercise affected this behavior.

Based on the literature (Bimonte-Nelson et al., 2008; Van Praag, 2009; Rhyu et al., 2010) it is reasonable to consider that this adaptive response to aerobic exercise involved structural and functional changes in the brain through mechanisms that mediate neural plasticity, neurogenesis and neuronal survival, connectivity patterns, upregulation of neurotrophins and neurotransmitters, and stimulation of the vasculature, allowing us to assume that some of these phenomena have occurred in our rats, and may partially explain the results, particularly in AT aged rats.

A recent work from our laboratory (Pietrelli et al., 2011) showed a significant increase in the size and dendritic arborization of nitrergic cortical neurons, and upregulation of the nitrergic system in neurons belonging to the cerebral cortex, hippocampus, and striatum in 18-month old aerobically trained rats. Nitric oxide is essential in the acquisition phase of memory, but not in the consolidation or maintenance phases. This could be involved in the protective effect of chronic exercise on the function and survival of hippocampal, striatal, and cortical neurons, and thus on the prevention of cognitive age-dependent decline.

Several authors (Ennaceur et al., 2006, 2010; Stanford, 2007; Ramos et al., 2008) discuss that there is not an

Table 5. Elevated plus maze. Summary of mean±SEM. $N_t=48$

	8 mon ($n=24$)		18 mon ($n=24$)	
	SC (no-ex, $n=12$)	AT (ex, $n=12$)	SC (no-ex, $n=12$)	AT (ex, $n=12$)
Latency (s)	22.33±5.44	11.36±3.12	68.60±24.08	17.83±3.06
Open arms	5.40±0.38	6.20±0.41	2.20±0.84	2.90±0.56
Closed arms	4.46±0.48	3.66±0.51	3.90±0.48	1.30±0.49
Time spent in open arms (%)	55.66±3.60	64.13±4.24	32.30±10.93	76.10±8.13
Time spent in closed arms (%)	44.46±3.58	35.93±4.27	67.80±10.93	23.90±8.13
Rearing	1.10±0.69	2.90±0.91	1.13±0.40	2.26±0.48
Grooming	0.13±0.09	0.26±0.15	0.40±0.16	0.40±0.22
Fecal pellets	1±0.42	0.20±0.02	1.60±0.56	0.60±0.40
Urine	1.20±0.31	0.40±0.13	1.20±0.41	0.60±0.16

ideal model to measure anxiety and to discriminate its multidimensional components. From this perspective, each assay only provides a partial view of the subject, so it would be more appropriate to use multiple tests involving a wide range of stressful stimuli, such as novelty, contrast, brightness/darkness, open/closed spaces, elevated spaces, social isolation, and so forth.

Consistent with this view, we specifically investigated the effects of chronic aerobic exercise and aging on anxiety-related behaviors of naive rats, with two tests that are widely used for screening, the OF and the EPM. Both models are based on the study of patterns of spontaneous behavior, evoking a state of anxiety caused by the conflict approach/avoidance that produces a new (heterotypic stressor) and potentially threatening environment, and can be used as alternative indexes of the same emotional construct (Ramos, 2008).

Very few studies analyzed the relationship between exercise and anxiety in humans and animal models. The results were controversial. Some studies reported anxiolytic effect, other works reported anxiogenic effects or mixed effects depending on the task or variable considered. For example, a previous study (Burghardt et al., 2004) showed that the wheel running produced anxiogenic effects in the OF and EPM, whereas the treadmill running had no significant effect in either trial. Interestingly, it was noted that the greatest anxiogenic effect in the EPM was obtained after 8 weeks of exercise, whereas 4 weeks of training showed changes only in the OF. Another paper (García-Capdevila et al., 2009) investigated the effects of exercise on anxiety-related behavior in young rats (2 months) and showed that, after 1 month of training with voluntary wheel running, some indicators of emotional reactivity in the OF and EPM increased, regardless of the level of exercise. These results suggest that the type of exercise and duration of its application also appear to be important factors when evaluating the data in the OF and EPM.

Given that anxiety is an emotional response that shows different dimensions, and that emotion is a response to action that has a motor, physiological, expressive, and cognitive correlate (Belzung and Philippot, 2007), we decided to measure the largest number of indicators, including ethological variables such as defecation, urination, rearing, thigmotaxis, or grooming that were used to enhance the overall performance of the test, and even as indexes of anxiety-related behaviors in the absence of changes in other more conventional indicators of test (Rodgers and Dalvi, 1997). Moreover, our approach was to evaluate outcomes using the general context of each test.

Currently there is a tendency to assume that aging produces a hyper-reactivity to stress associated with high levels of anxiety. In this study, we found that most of the anxiety-related indexes measured in the OF, that is, latency, time spent in the central and peripheral area, and grooming, as well as variables related to exploratory behavior such as total distance (number of squares crossed), rearing, and thigmotaxis were affected by aging. The old rats, regardless of exercise, showed a significant increase

of the latency, which suggests a great emotional reactivity to conflict approach/avoidance and a reduction in decision making. Consistent with this behavior, the time spent in the central platform (reentries) significantly decreased in relation to middle-aged rats. There was also an increased grooming, and a significant decline of rearing and thigmotaxis that correlated with a decrease of the exploratory activity in parallel with the increase of the time spent in the peripheral sectors. This response could be partly explained by the physiological deterioration of the musculoskeletal and locomotor system that occurs in aging.

When we analyzed the effect of exercise on these variables, we found a significant reduction, especially in AT older rats, in the following anxiety-related indexes: latency, time spent in peripheral squares, grooming, urination, and fecal pellets. As expected, AT middle-aged rats crossed greater distances than the remaining animals, showing a higher exploratory activity and locomotive efficiency. Consistent with this observation, the increase in the time spent in peripheral areas in SC older rats was accompanied by a decrease in the time spent in the central platform.

The decrease in urination in old AT animals can be explained by reduced emotional reactivity in relation to the controls of the same age. We believe that this was not because of differences in the amount of drinking water. As runner rats drink more than controls because they are more prone to dehydration, we would expect an increase in the diuresis; however, the opposite occurred, the old SC urinated more often, showing higher levels of anxiety. We also rule out any pathology that may have affected the renal function. The biochemical parameters of our rats, evaluated every 6 months, were always within normal values.

The results from the OF showed an increase in indicators of anxiety-related behaviors with age, in disagreement with a previous report (Torras-Garcia et al., 2005) that found a significant and progressive decrease of anxiety in middle-aged (17 months) and old (24 months) animals when compared with young animals (3 months). They also indicated that regular moderate exercise had an anxiolytic effect, particularly important in old age, demonstrating its benefit in the management of stress, by reduced emotional reactivity.

Regarding EPM, and although data showed some overlap in certain anxiety-related indexes measured in the OF, we detected differences in behavioral responses, which can be attributed to the design characteristics, related to the type and degree of stressors (Nosek et al., 2008).

Aging affected critical indicators of anxiety such as latency and open arm entries. Aged rats increased the latency in relation to middle-aged animals, regardless of exercise, showing a similar trend that reported in the OF, and the number of entries into the open arms significantly decreased. These responses again showed an increase in the levels of anxiety with age. The locomotive activity, evaluated by the number of entries to the closed arms, was decreasing with age. This behavior correlated with an in-

crease in the number of entries to open arms and the time spent on them, in relation to age-matched controls.

The exercise also affected most of the variables indicative of anxiety in the EPM: latency, number and time spent in open arms, time spent in closed arms, number of fecal pellets, and urination. AT rats, regardless of age, had rearing and thigmotaxis behaviors significantly higher than SC rats, indicating a greater interest in exploring. This response was also different from that observed in the OF. Possibly the differences may be caused by the physical environment of the EPM, with more choices than the open field (Roy et al., 2009).

The decrease in urination in AT animals, regardless of age, can be explained by reduced emotional reactivity in relation to the SC rats. We believe that this was not because of differences in the amount of drinking water.

The results in the EPM showed the same trend detected in the OF, aging caused an increase in anxiety-related behaviors, and the aerobic training had an anxiolytic effect that was particularly notorious in older runners. This response was consistent with reports of Fulk et al. (2004), who subjected young rats to chronic moderate exercise on the treadmill for 10 weeks, and subsequently tested in the OF and EPM.

Finally, we want to discuss some conceptual and methodological aspects of our forced exercise model and its relationship to stress response as we observed in our rats.

Running, like other stressors, poses a threat to homeostasis that triggers the activation of both the sympathetic nervous system, thus resulting in epinephrine production, and the hypothalamic-pituitary-adrenal axis (HPA axis) resulting in glucocorticoid production. This is because of the increased demand for energy in somatic tissues. Stress exerts complex effects on the brain, periphery, and behavior dependent on the temporal profile and intensity of the stressor. The runner gets adapted to running over time (homotypic stress), and at different points during adaptation to the same stressor, the response to a different stressor (heterotypic stress) might be amplified or attenuated (Stranahan et al., 2008).

Different authors (Naylor et al., 2005; Campbell et al., 2009) reported that running initially causes an activation of the HPA axis resulting in increases of the circulating corticosterone. These levels are gradually restored to normal values with the long-term running. In rodents, aging is associated with elevated basal glucocorticoid secretion and prolonged stress-induced glucocorticoid release. Impaired shut-off of the glucocorticoid stress response may be associated with loss of glucocorticoid feedback inhibition of the HPA axis (Segar et al., 2009). This explains, at least in part, the anxiolytic effect of chronic exercise when the animals were exposed to a new stressor (OF or EPM) as well as the amplification of the behavioral response, especially in older ones.

The chronic running (repetitive stressor) shows controversial effects on function, shape, and proliferative capacity of brain cells that impact certain cognitive and behavioral parameters. Several researchers agree that long periods of voluntary running become stressful events that decrease the hippocampal progenitor proliferation, possibly through a general increase in some stress parameters and the opioid system (Droste et al., 2003; Joëls et al., 2007). According to Naylor and colleagues (2005) the voluntary exercise had a biphasic effect on the hippocampal progenitor proliferation in the adult rat that seemed to be dependent on the level of running activity. For example, rats that ran 9 days showed a remarkable increase in the neuronal proliferation, whereas rats that chronically ran for 24 days showed suppression of neurogenesis by 50%. They also observed a parallel increase of corticosterone levels, particularly during the lights-off cycle. Our results in the radial maze contradict some of these observations: the negative effects of chronic stimulation of the HPA axis, or the possible suppression of neurogenesis, even though this phenomenon took place, cannot explain the remarkable memory and learning levels achieved by runners. However, other authors emphasize the positive effects of chronic running on hippocampal function. In the brain of runners, dendritic branching and the density of dendritic spines are increased. These morphological alterations are indicative of enhanced synaptic function, which could play a critical role in facilitating hippocampal negative feedback on the HPA axis. Also, the regulation of hippocampal glucocorticoid receptor (GR) in rats may be duration-dependent of stressor (Stranahan et al., 2008).

Exercise is a paradoxical stressor, whether it is voluntary or forced, because the adaptive response to physical training is dual, "hormetic." Findings from studies of animal models and human populations suggest that hormesis (beneficial effects of low levels of stress) is an effective protection against many different diseases. Such stress-resistance mechanisms can be bolstered by diverse environmental factors including exercise, dietary restriction, cognitive stimulation, and so forth. (Mattson, 2008).

The training phenomenon called supercompensation was first described by Folbrot in 1941, and later discussed by Hans Selye, who called it the general adaptation síndrome (GAS). The GAS theory is the basis of training, and explains the hormetic response to effort. The greater the degree of adaptation to the training process, the greater the potential for high levels of functional performance. Supercompensation that occurs after exercise, therefore, is a relationship between work and regeneration that leads to homeostatic adaptations to higher levels, as well as to metabolic and neuropsychological amplification (Bompa and Haff, 2009).

The voluntary wheel running, considered a controllable, predictable, and rewarding stressor by many researchers, does not allow modeling this type of stimulation. Therefore, it is a non-specific exercise. The understanding of critical issues such as bioenergetic properties, volume and quality of exercise, and the impact of the timing of the presentation of training stimuli on the timeline allow modulating the adaptive response to the chronic effort, even direct it in a specific way. These characteristics determine the nature of the response and the extent of the findings. Therefore, the outcomes in forced and voluntary models,

although they seem to share some features, are not comparable because they are not the same.

Most studies examining the effects of wheel-running house animals are carried on a "one per cage basis" to facilitate quantification of individual activity levels. However, the social isolation can activate per se the HPA axis, making the animals more prone to the deleterious effects of stress. Running-induced elevations in corticosterone are prolonged in socially isolated runners. This suggests that social isolation can influence the response of runners to both a homotypic and a heterotypic stressor (Stranahan et al., 2008).

Although the traditional view has primarily focused on the negative effects of stress on a variety of somatic systems, emerging data support the idea that certain forms of stress can enhance cellular function. One of the desirable effects of the training is the increase in the homeostatic capacity through supercompensation. It is reasonable to assume that in the nervous system also, for example, at hippocampal level, adaptation occurs by the phenomenon of supercompensation. This response could be one reason that partially explains why our forced runners showed higher cognitive performance, whereas voluntary runners showed an initial increase in the neurogenesis, and subsequently a temporary stabilization followed by a gradual decline or suppression because of lack of adequate stimulation (Lucassen et al., 2010).

Although running indeed activates a number of systems related to the stress response, other mechanisms exist to reduce and compensate this reactivity. For example, the increase of the corticosterone level or the generation of reactive oxygen species (ROS) are part of the hormetic effects of the exercise because, in parallel, it also increases the resistance to stress, and overall the antioxidant capacity at the systemic level (Goto and Radák, 2010), acting as a protective factor (or neuroprotective), and not as a risk factor.

A recent work of Bayod and colleagues (2011) confirms and reinforces our results. They chronically subjected rats to treadmill running (9 months), and then examined the effects of long-term moderate training on adult middle-aged male rat cortex and hippocampus in order to identify the cellular mechanisms behind the effects of exercise. Chronic running induced an increase in IGF-1, a potent survival factor for neurons and oligodendrocytes; the up-regulation of sirtuin 1, a protein activated by stress that can extend life and protect cells against energy deprivation and oxidative stress; the stimulation of mitocondrial biogenesis and the prevention of signs of neurodegeneration, mainly in the hippocampus of the exercised animals.

In brief, upon the basis of the overall results obtained in the RM, OF, and EPM, we conclude that the mild-moderate stress produced during aerobic exercise results in favorable homeostatic adaptations that protect organisms against more severe stress or anxiety-related disorders, and the age-dependent cognitive decline.

As this report is a longitudinal study over the life we cannot know at what stage of the life cycle the deleterious effects of aging are higher than the exercise effects. Per- haps, it would be appropriate to set more cutoffs. We also want to emphasize that this work is part of a larger project involving numerous measurements at the cellular, morphological, and physiological levels that will be presented and discussed in future publications.

CONCLUSION

In summary, the magnitude and quality of adaptive response to chronic physical activity depend on the type of exercise and the cumulative effect of its application. In the present study, we demonstrated that aerobic exercise practiced throughout life, acted on a time and dose-dependent manner, produced plastic effects on the brain and behavior, and that these effects were modulated by age. Considering the results of the three tests in a broader context, we conclude that aging increased anxiety-related behaviors, and moderate and regular exercise improved the cognitive performance, and positively affected emotional aspects of behavior, leading to a better management of stress. These beneficial effects were important in old age.

Our work also provides new evidence of the neuroprotective and restorative properties of regular physical activity on the cognitive decline associated with aging, and its ability to reverse the potentially negative effects of inactivity. The mechanisms underlying these effects appear to operate throughout life when they are properly stimulated, but still do not know if they are the same through the years or stop acting when the exercise ends. Research in human exercise physiology and several observations in animal models suggest that it is unlikely that the adaptive mechanisms triggered by aerobic exercise do the same throughout life. Could there be similar responses in young and old subjects, but mediated by different mechanisms? Could there be a "priming effect" that functions as a cognitive reserve in the elderly? Which among these structural and functional changes are stable, partially or completely reversible beyond the period of exposure to the experience? There are still many questions to answer.

Finally, the knowledge of the physiological response to exercise in the long term will contribute to the development of non-pharmacological strategies aimed at the prevention and treatment of the deterioration of brain health, and the design of therapies for the physical and neurocognitive rehabilitation of older people.

REFERENCES

Albeck DS, Sano K, Prewitt GE, Dalton L (2006) Mild forced treadmill exercise enhances spatial learning in the aged rat. Behav Brain Res 168:345–348.

Anderson BJ, Rapp DN, Baek DH, McCloskey DP, Coburn-Litvak PS, Robinson JK (2000) Exercise influences spatial learning in the radial arm maze. Physiol Behav 70:425–429.

Baker LD, Frank LL, Foster-Schubert K, Green PS, Wilkinson Chw, McTiernan A, Plymate SR, Fishel MA, Watson GS, Cholerton BA, Duncan GE, Mehta PD, Craft S (2010) Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch Neurol 67:71–79.

Bayod S, Del Valle J, Canudas AM, Lalanza JF, Sanchez-Roigé S, Camins A, Escorihuela RM, Pallas M (2011) Long-term treadmill exercise induces neuroprotective molecular changes in rat brain. J Appl Physiol 111:1380–1390.

Belzung C, Philippot P (2007) Anxiety from a phylogenetic perspective: is there a qualitative difference between human and animal anxiety? Neural Plast, doi:10.1155/2007/59676.

Berchtold NC, Castello N, Cotman CW (2010) Exercise and time-dependent benefits to learning and memory. Neuroscience 167:588–597.

Bimonte HA, Nelson ME, Granholm AC (2003) Age-related deficits as working memory load increase: relationships with growth factors. Neurobiol Aging 24:37–48.

Bimonte-Nelson HA, Granholm AC, Nelson ME, Moore AB (2008) Patterns of neurotrophin protein levels in male and female Fischer 344 rats from adulthood to senescence: how young is "young" and how old is "old?" Exp Aging Res 34:13–26.

Bompa TO, Haff G (2009) Periodization: theory and methodology of training, 5th ed, p 424. Champaign, IL: Human Kinetics.

Boveris A, Navarro A (2008) Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radic Biol Med 44:224–229.

Buccafusco JJ (2009) Methods of behavior analysis in neuroscience, 2nd ed. (Taylor and Francis, ed), p 351. Boca Raton, FL: CRC Press.

Burghardt PR, Fulk LJ, Hand GA, Wilson MA (2004) The effects of chronic treadmill and wheel running on behavior in rats. Brain Res 1019:84–96.

Campbell JE, Rakhshani N, Fediuc S, Bruni S, Riddell MC (2009) Voluntary wheel running initially increases adrenal sensitivity to adrenocorticotrophic hormone, which is attenuated with long-term training. J Appl Physiol 106:66–72.

Colcombe SJ, Kramer AF, Erickson KI, Sclaf P, McAuley E, Cohen NJ, Webb A, Jerome GJ, Marquez DX, Elavsky S (2004) Cardiovascular fitness, cortical plasticity, and aging. Proc Natl Acad Sci U S A 101:3316–3321.

Cotman CW, Berchtold NC (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 25:295–301.

Cotman CW, Engesser-Cesar C (2002) Exercise enhances and protects brain function. Exerc Sport Sci Rev 30:75–79.

Dik M, Deeg DJ, Visser M, Jonker C (2003) Early life physical activity and cognition at old age. J Clin Exp Neuropsychol 25:643–653.

Dishman RK, Berthoud HR, Booth FW, Cotman CW, Edgerton VR, Fleshner MR, Gandevia SC, Gomez-Pinilla F, Greenwood BN, Hillman CH, Kramer AF, Levin BE, Moran TH, Russo-Neustadt AA, Salamone JD, Van Hoomissen JD, Wade CE, York DA, Zigmond MJ (2006) Neurobiology of exercise. Obesity 14:345–356.

Droste SK, Gensing A, Ulbricht S, Müller MB, Linthorst AC, Reul JM (2003) Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. Endocrinology 144:3012–3023.

Dudchenko PA (2004) An overview of the tasks used to test working memory in rodents. Neurosci Biobehav Rev 28:699–709.

Ennaceur A, Michalikova S, Chazot PL (2006) Models of anxiety: responses of rats to novelty in an open space and an enclosed space. Behav Brain Res 171:26–29.

Ennaceur A, Michalikova S, Van Rensburg R, Chazot PL (2010) Distinguishing anxiolysis and hyperactivity in an open space behavioral test. Behav Brain Res 207:84–98.

Erickson KI, Kramer AF (2009) Aerobic exercise effects on cognitive and neural plasticity in older adults. Br J Sports Med 43:22–24.

Etnier JL, Nowell PM, Landers DM, Sibley BA (2006) A meta-regression to examine the relationship between aerobic fitness and cognitive performance. Brain Res Rev 52:119–130.

Fulk LJ, Stock HS, Lynn A, Marshall J, Wilson MA, Hand GA (2004) Chronic physical exercise reduces anxiety-like behavior in rats. Int J Sports Med 25:78–82.

García-Capdevila S, Portell-Cortés I, Torras-Garcia M, Coll-Andreu M, Costa-Miserachs D (2009) Effects of long-term voluntary exercise on learning and memory processes: dependency of the task and level of exercise. Behav Brain Res 202:162–170.

Goto S, Radák Z (2010) Hormetic effects of reactive oxigen species by exercise: a view from animal studies for successful aging in human. Dose Response 8:68–72.

Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A (2007) Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39:1423–1434.

Joëls M, Karst H, Krugers HJ, Lucassen PJ (2007) Chronic stress: implications for neuronal morphology, function and neurogenesis. Front Neuroendocrinol 28:72–96.

Kamijo K, Hayash Y, Sakai T, Yahiro T, Tanaka K, Nishihira Y (2009) Acute effects of aerobic exercise on cognitive function in older adults. J Gerontol B Psychol Sci Soc Sci 64B:356–363.

Kim YP, Kim HB, Jang MH, Lim BV, Kim YJ, Kim H, Kim SS, Kim EH, Kim CJ (2003) Magnitude- and time-dependence of the effect of the treadmill exercise on cell proliferation in the dentate gyrus of rats. Int J Sports Med 24:114–117.

Kramer AF, Erickson KI, Colcombe SJ (2006) Exercise, cognition, and the aging brain. J Appl Physiol 101:1237–1242.

Kramer AF, Erickson KI (2007) Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function. Trends Cogn Sci 11:342–348.

Kregel K, Allen D, Booth F, Fleshner M, Henriksen E, Musch T, Leary D, Parks C, Poole D, Ra'anan A, Sheriff D, Sturek M, Toth L (2006) Resource book for the design of animal exercise protocols, 1st ed. Bethesda, MD: American Physiological Society.

Leasure JL, Jones M (2008) Forced and voluntary exercise differentially affect brain and behavior. Neuroscience 156:456–465.

Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E, Oomen CA, Czéh B (2010) Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: implications for depression and antidepressant action. Eur Neuropsychopharmacol 20:1–17.

Lytle ME, Vanderbilt J, Pandav RS, Dodge HH, Ganguli M (2004) Exercise level and cognitive decline. Alzheimer Dis Assoc Disord 18:57–64.

Markham JA, Greenough WT (2004) Experience-driven brain plasticity: beyond the synapse. Neuron Glia Biol 1:351–363.

Mattson MP (2008) Hormesis and disease resistance: activation of cellular stress response pathways. Hum Exp Toxicol 27:155–162.

Moraska A, Deak T, Spencer R, Roth D, Fleshner M (2000) Treadmill running produces both positive and negative physiological adap-

tations in Sprague-Dawley rats. AJP-regulatory, integrative and comparative. Physiology (Bethesda) 279:1321–1329.

Naylor AS, Persson AI, Eriksson PS, Jonsdottir IH, Thorlin T (2005) Extended voluntary running inhibits exercise-induced adult hippocampal progenitor proliferation in the spontaneously hypertensive rat. J Neurophysiol 93:2406–2414.

Nelson ME, Rejeski J, Blair SN, Duncan PW, Judge JO, King AC, Macera CA, Castaneda-Sceppa C (2007) Physical activity and public health in older adults. Recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39:1435–1445.

NIH (2011) Guide for the care and use of laboratory animals, 8th ed. Washington, DC: The National Academies Press.

Nosek K, Dennis K, Andrus BM, Ahmadiyeh N, Baum AE, Solberg Woods LC, Redei E (2008) Context and strain-dependent behavioral response to stress. Behav Brain Funct 4:23.

Pietrelli A, López-Costa JJ, Goñi R, López EM, Brusco A, Basso N (2011) Effects of moderate and chronic exercise on the nitrergic system and behavioral parameters in rats. Brain Res 1389:71–82.

Ramos A (2008) Animal models of anxiety: do I need multiple tests? Trends Pharmacol Sci 29:493–498.

Ramos A, Pereira E, Martins GC, Wehrmeister TD, Izídio GS (2008) Integrating the open field, elevated plus maze and light/dark box to asses different types of emotional behaviors in one single trial. Behav Brain Res 193:277–288.

Rhyu IJ, Bytheway JA, Kohler SJ, Lange H, Lee KJ, Boklewski J, McCormick K, Williams NI, Stanton GB, Greenough WT, Cameron JL (2010) Effects of aerobic exercise training on cognitive function and cortical vascularity in monkeys. Neuroscience 167:1239–1248.

Rodgers RJ, Dalvi A (1997) Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev 21:801–810.

Roy V, Chapillon P, Jeljeli M, Caston J, Belzung C (2009) Free versus forced exposure to an elevated plus-maze: evidence for new behavioral interpretations during test and retest. Psychopharmacology 203:131–141.

Segar TM, Kasckow JW, Welge JA, Herman JP (2009) Heterogeneity of neuroendocrine stress responses in aging rat strains. Physiol Behav 96:6–11.

Seger AC (2006) The basal ganglia in human learning. Neuroscientist 4:285–290.

Stanford SC (2007) The open field test: reinventing the wheel. J Psychopharmacol 21:134–135.

Stranahan AM, Lee K, Mattson MP (2008) Central mechanisms of HPA axis regulation by voluntary exercise. Neuromol Med 10:118–127.

Studeski S, Carlson MC, Fillit H, Greenough WT, Kramer A, Rebok GW (2006) From bedside to bench: can mental and physical activity promote cognitive vitality in late life? Sci Aging Knowledge Environ 2006(10):pe21.

Szabo AN, McAuley E, Erickson KI, Voss M, Prakash RS, Mailey EL, Wójcicki TR, White SM, Gothe N, Olson EA, Kramer AF (2011) Cardiorespiratory fitness, hippocampal volume, and frequency of forgetting in older adults. Neuropsychology 25:545–553.

Torras-Garcia M, Costa-Miserachs D, Coll-Andreu M, Portell-Cortés I (2005) Decreased anxiety levels related to aging. Exp Brain Res 164:177–184.

Van Praag H, Schubert T, Zhao Ch, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680–8685.

Van Praag H (2009) Exercise and the brain: something to chew on. Trends Neurosci 32:283–290.

Van Sluyters R, Ballinger M, Bayne K, Cunningham C, Degryse AD, Dubner R, Evans H, Gdowski F, Knight R, Mench J, Nelson R, Parks C, Stein B, Toth L, Zola S (2003), Guidelines for care and use of mammals in neuroscience and behavioral research, 1st ed. Washington, DC: The National Academies Press.

Voss MW, Prakash RS, Erickson KI, Basak Ch, Chaddock L, Kim JS, Alves H, Heo S, Szabo AN, White SM, Wojcicki TR, Mailey EL, Gothe N, Olson EA, McAuley E, Kramer AF (2010) Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. Fron Aging Neuroscience 2:1–17.

Wilmore J, Costill D (2007) Fisiología del Esfuerzo y del Deporte, 6ta. ed, p 776. Barcelona, España: Paidotribo.

Wisløff U, Helgerud J, Kemi OJ, Ellingsen O (2001) Intensity-controlled treadmill-running in rats: VO$_2$ max and cardiac hypertrophy. Am J Physiol Heart Circ Physiol 280:1301–1310.