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Aerobic exercise upregulates the BDNF-Serotonin systems and improves the cognitive function in rats

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ABSTRACT

Aerobic exercise (AE) benefits brain health and behavior. Serotonin (5-HT) and brain-derived neurotrophic factor (BDNF) are known to mediate and shape cognitive processes. Both systems share some actions: BDNF is involved in the maturation and function of 5-HT neurons. In turn, 5-HT is involved in neuroplasticity phenomena mediated by BDNF and stimulated by exercise. The aim of this work was to study the long-term effects of AE on BDNF- 5-HT systems and cognitive function in rats at different ages. A lifelong moderate-intensity aerobic training program was designed, in which aerobically exercised (E) and sedentary control (C) rats were studied at middle (8 months) and old age (18 months) by means of biochemical, immunohistochemical and behavioral assays. The levels and expression of BDNF, 5-HT, serotonin transporter (SERT) and 5-HT_{1A} receptor were determined in selected brain areas involved in memory and learning. Immunopositive cells to neuronal nuclear protein (NeuN) in the hippocampus CA1 area were also quantified. The cognitive function was evaluated by the object recognition test (ORT). Results indicate that AE enhanced spatial and non-spatial memory systems, modulated by age. This outcome temporarily correlated with a significant upregulation of cortical, hippocampal and striatal BDNF levels in parallel with an increase in the number of hippocampal CA1-mature neurons. AE also increased brain and raphe 5-HT levels, as well as the expression of SERT and 5-HT_{1A} receptor in the cortex and hippocampus. Old AE rats showed a highly conserved response, indicating a remarkable protective effect of exercise on both systems. In summary, lifelong AE positively affects BDNF-5-HT systems, improves cognitive function and protects the brain against the deleterious effects of sedentary life and aging.

1. Introduction

The current hypotheses that attempt to model and explain the effects of exercise on the central nervous system (CNS) and behavior have focused mainly on the study of changes in neurotrophins, neurotransmitter systems and vasculature (Van Praag, 2009; Vivar, Potter, & Van Praag, 2013).

BDNF is a neurotrophin mostly expressed in the CNS which has a critical role in hippocampal neurogenesis, experience-dependent neuroplasticity, neuronal shaping and survival (Deinhardt and Chao, 2014). The highest synthesis rate of BDNF occurs in hippocampal and cortical neurons, in parallel with the distribution of its tyrosine-kinase B (TrkB) receptor (Phillips, Baktir, Srivatsan, & Salehi, 2014).

Serotonin (5-HT) is a neurotransmitter with a modulatory role in almost all functions and biological processes: cardiovascular function, energy metabolism, sleep, stress response, cognitive function, sensory-motor and emotional regulation, etc (Ögren et al., 2008). The 5-HT neurons of the raphe nuclei are the only source of 5-HT in the brain, and their projections innervate most brain areas including cortical, limbic, midbrain, hindbrain and brainstem regions (Banerjee and Poddar, 2016). Although 5-HT acts through 14 different types of receptors, subtype 5-HT_{1A} is considered one of the main mediators of its action in the serotonergic neurons of the raphe nuclei as well as the non-serotonergic neurons of the cortex and hippocampus (Bert, Fink, Rothe, Walstab, & Bonisch, 2008; Meneses, 2013; Perez-García and Meneses, 2008). The 5-HT_{1A} receptor is involved in cognition and is thus considered a therapeutic target and a neural marker of memory deficits (Glickmann-Johnston et al., 2015; Meneses and Perez-García, 2007). The 5-HT transporter (SERT) performs the reuptake of 5-HT within the pre-synaptic terminals and inactivates 5-HT (Berumen, Rodríguez,

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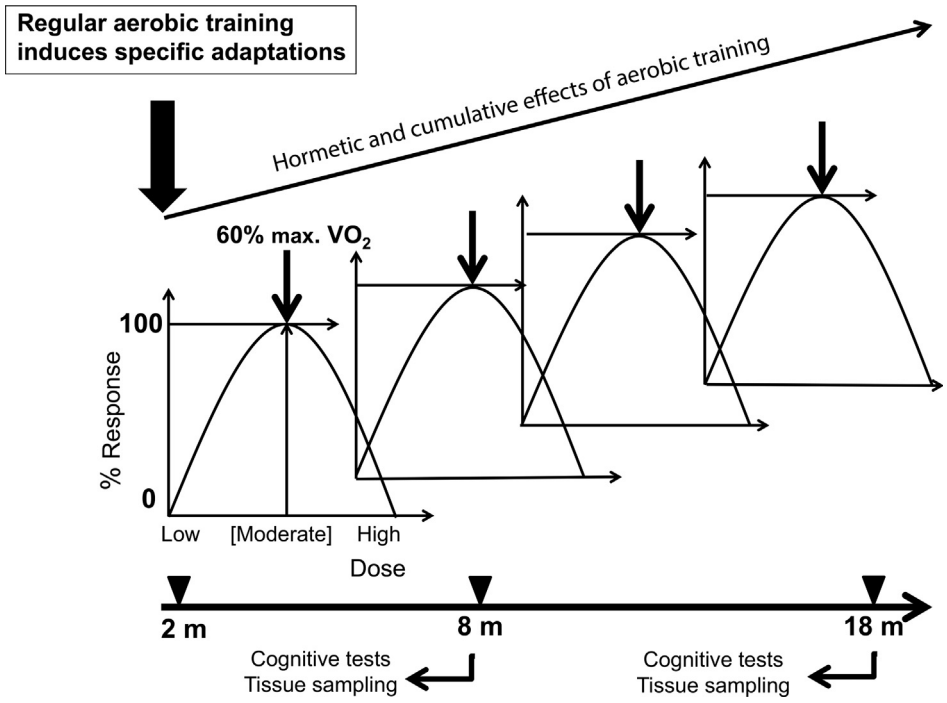

Fig. 1. Experimental design. Animals were exercised regularly from adolescence to old age. Two cut-off points (8–18 months) were established to study the cognitive function and obtain brain samples. The moderate intensity (60% max.VO_2) training was designed to increase aerobic power. Profiles represent successive states (cumulative effects) of supercompensation aimed to enhance the brain energy system and homeostatic capacity.

Miledi, & García-Alcocer, 2012). SERT is the main target of anti-depressant drugs and is used as an index of integrity of the axonal terminals of the cerebral 5-HT neurons. Alterations in their expression are associated with memory deficits and amnesic states (Meneses, 2013), and malfunction of the 5-HT system may contribute to the deterioration of memory during aging (Mitchell, McDevitt, & Neumaier, 2009).

Experimental evidence from rodent models, either pharmacologically treated or genetically modified, has demonstrated functional reciprocity between BDNF and 5-HT (Deltheil et al., 2007; Deltheil et., 2008; Homberg, Molteni, Calabrese, & Riva, 2014). This bidirectional system works through an auto/paracrine feedback loop whereby 5-HT up-regulates BDNF mRNA and, in turn, BDNF–TrkB regulates the serotonergic phenotype (Martinowich and Lu, 2008). BDNF and 5-HT converge and interact (Mattson, Maudsley, & Martin, 2004) within regions that control memory and learning processes: the medial prefrontal cortex, closely related to executive functions and working memory (Brockett, La Marca, Gould, 2015); the perirhinal cortex, associated to object (Hopkins and Bucci, 2010), place and context recognition memory (Morici et al., 2015; Warburton and Brown, 2015); the striatum, connected to high order cortical circuits and to the procedural memory (Fisher et al., 2017); the hippocampus, a critical brain structure for navigation, context-dependent learning and episodic memory (Geyller, Royer, & Choi, 2017; Inoue et al., 2015); and the medial dorsal thalamus (Ameen-Ali, Easton, & Eacott, 2015).

It is well known that physical activity generates plastic effects on cognitive functions and behavior mediated by BDNF, whose magnitude and quality of action depend on the model of exercise (2018; Berchtold, Castello, & Cotman, 2010; Gradari, Pallé, & Fontán-Lozano, 2016; Kamijo et al., 2009; Pietrelli et al., 2011; Pietrelli et., 2012). Klempin et al. (2013) have shown that exercise in young and old rodents requires the regulatory action of 5-HT and the integrity of its signaling pathways to induce BDNF-dependent adult neurogenesis in the hippocampus. However, it is not yet known to what extent regular exercise could alter this interaction, and whether this possible effect may have a long-term impact on the memory systems regulated by BDNF-5-HT. In this context, the present work aimed to study the long-term effects of lifelong aerobic exercise (AE) on the cognitive function and the BDNF-5-HT systems, in areas related with memory and learning, i.e. medial prefrontal and perirhinal cortex, striatum, hippocampus and raphe nuclei in rats at different ages. For this purpose, a chronic AE program was designed in order to activate brain metabolism and its homeostatic capacity.

On the other hand, it is a well-known fact that adult hippocampal neurogenesis comprises several developmental stages such as proliferation, differentiation, migration, targeting and synaptic integration, with various characteristic neuronal markers expressed at each stage. Therefore, to determine the possible effects of AE on neuronal maturation, the neuronal nuclear antigen (NeuN) as a specific marker of mature neurons was studied, as it is not expressed in non-neuronal or immature neuronal cells (Guselnikova and Korzhevskiy, 2015; Kim et al., 2013). In addition, (1) biochemical measurements to quantify the levels/expression of BDNF, 5-HT, 5-HT_{1A} receptor and SERT in the above-mentioned areas; (2) immunohistochemical studies of 5-HT and NeuN; and (3) behavioral assays to test the cognitive performance and the age-dependent decline that may occur in hippocampal, striatal and cortical functions were performed.

2. Materials and methods

2.1. Animals and experimental design

Animals: Sixty weaning male Wistar rats, obtained from the animal facilities of the Universidad Nacional de La Plata (UNLP), Facultad de Ciencias Veterinarias, weighing 70–100 g were housed in groups of two in standard laboratory cages, and randomized into two groups: sedentary control (C, n = 30) or exercised (E, n = 30). Animals were maintained at a temperature of 22 ± 2 °C, relative humidity 55–65%, and a daily photoperiod of 12:12 h light–dark (lights off at 6:00 am). Rats were subjected to microbiological monitoring every 6 months, and clinically evaluated every week. Food and water were supplied *ad libitum*. Weekly records of body weight, food, and drink were also obtained. All experiments were performed during the dark phase.

Experimental design (Fig. 1): Adult rats were physically trained from 2 to 18 months of age. Two cut-off points, representative of the physiological status, i.e. 8 months as middle-aged adult and 18 months as older adult, were established. Behavioral tests were performed 24 h post-training, after which animals were sacrificed to obtain brain samples. All efforts were made to minimize pain or discomfort and the

number of animals used, in accordance with international standards on animal welfare. This work was approved by the Institutional Ethics Committee for the Care and Use of Laboratory Animals of the School of Health Sciences (UCES) and complies with national regulations.

2.2. Aerobic training protocol

The exercise model was based on two key concepts: hormesis and supercompensation. The hormesis model was proposed by Calabrese (2004) and claims that the dose–response curve is fundamentally U-shaped. That is, low doses of an exogenous or intrinsic factor (for example, exercise) may enhance response, while high factor doses may actually produce inhibitory or adverse effects (Calabrese, 2004; Mattson, 2008). In turn, supercompensation is a phenomenon known as Weigert's law, consisting in a metabolic rebound produced by the appropriate relationship between work and regeneration, which leads to superior physical, metabolic and neuropsychological adaptation. Training has immediate, delayed and cumulative effects (Bompa and Haff, 2009; Wilmore and Costill, 2007). Every time that supercompensation occurs, the subject establishes a new, increased homeostatic level with positive benefits for performance. On the basis of these notions, the main objective of this work was to progressively increase aerobic power through supercompensation to maximize brain homeostatic adaptation (Fig. 1).

Rats were trained in a motorized treadmill whose design was adapted from other commercial equipment (http://www.colinst.com/products/animal-treadmill-exer). It consisted in: 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 apparatus was connected to a computer and the following values were displayed on the screen in a real time while the rats run: 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). Since this is a forced exercise model, changes in the treadmill structure were performed to avoid the possible chronic stress effects including depression in the long term (Pietrelli et al., 2018). The main modification was to eliminate the electric device placed at the end of the running lane which through a light electric shock induces the animal to continue running. The other modification was to include the oxygen sensor inside the cage to measure the oxygen consumption (not the O_2/CO_2 ratio). Animals received no shockers to motivate them to run. At the end of each training session, C and E rats received a "frootloop" (a ring of fruity cereal) as a reward. Exclusion criteria: records were judged invalid when the rats touched the wall or remained inactive for > 15 sec. Before starting the training protocol at 2 months of age, both groups were habituated for 2 weeks.

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The training protocol was conducted as previously described (Pietrelli et al., 2011, 2012 and 2018). Briefly: E animals were regularly trained three times a week (Fig. 2a) at low-moderate intensity [60–70% of maximum oxygen consumption (max.VO_2)]. Controls were subjected to the same experimental conditions but within a motionless treadmill. Parameters such as duration, speed and slope (workload) (Fig. 2b, c, d) were set from monthly tests of max.VO_2. Test of max.VO_2 consisted in: six randomly selected rats (C, n = 6; E, n = 6) were individually tested. Each rat was subjected to a 10-min warm-up period at 6 m/min speed and 0° slope. Then, speed was increased 1 m/min every 3 min, and max.VO_2 was considered the point when oxygen consumption reached a plateau in spite of the increase in speed. The new workload selected were based on average VO_2 max measurements of the 6 trained rats that were tested and not based on individually determined VO_2 max values. Each monthly average of max.VO_2 was normalized by weight and expressed in terms of the specific VO_2 (ppm/g.min^{-1}). At 2 months, the initial workload was 15 min duration, 4–6 m/min speed, and 0° slope. The maximum overload was reached at 8 months (60 min duration, 12 m/min speed, and 5° slope) and the minimum was at 18 months-old (30 min duration, 4–6 m/min speed, and 0° slope). Middle-aged and old E rats performed 24 and 64 weeks of training, respectively.

2.3. Behavioral testing

At 8 and 18 months old (C, n = 15 × 2; E, n = 15 × 2) the object recognition test (ORT) was performed. Three experiments (Exp.) based on modified protocols of Winters, Saksida, and Bussey (2008) and Akkerman, et al. (2012), Akkerman, Prickaerts, Steinbusch, Blokland (2012) were designed with the open field (OF). The recommendations of Albasser et al. (2012) and Antunes and Biala (2012) were also followed. The OF (1 × 1 × 0.40 m high) was placed in a soundproof room and lit with 25 W red light, with no objects or signals. The arena was divided into 9 numbered squares, 16 × 16 cm each. Animals were habituated to the OF before testing (Fig. 3, Panel A). The ORT general procedure (Fig. 3, Panel B) consisted in: (A) *Sample phase:* (coding and memory acquisition) rat exposed to two identical objects for 5 min; (B) *Delay phase*: (consolidation) rat back to the cage and retained for 24 h; (C) *Choice/Test phase:* (memory retrieval in the long term) rat re-exposed to three different scenarios for 5 min: (1) two objects, one familiar, the other new; (2) two familiar objects, one positioned in another relative position; (3) two familiar objects, one positioned in another relative position in a changed physical context. Studies focused on the subject's ability to construct representations from perceptual, spatial and contextual information, and retrieve and use that information appropriately. The following criteria were established: (1) 24 h between experiments; (2) no animal performed multiple tests on the same day; (3) free access to food and drink; (4) each set of objects (by triplicate) had different color, shape, texture and size (plastic modules, porcelain cups and rubber balls filled with sand); (5) the relative distances between the objects (sample vs. choice phases) were kept constant; (6) the objects were sufficiently heavy to avoid displacement during the test; (7) no animal was rewarded. The procedure was doubly recorded by a manual score and filmed for further analysis. The arena and objects were thoroughly cleaned with 70% ethanol between trials.

2.3.1. Habituation protocol

The objective of the habituation to OF was to avoid interference of anxiety-related behavior with the acquisition and consolidation of memory processes. For five consecutive days, each rat was placed in the center of the OF and allowed to explore freely, without objects, for 5 min. We determined: (a) cumulative distance, defined as the sum of the squares crossed (animal entry with its 4 paws); (b) fecal pellets (number of fecal boli); (c) rearing postures (animal standing on two paws). Parameters: (a) was indicator of locomotion and exploratory activity; (b) was indicative of anxiety and emotionality, and (c) indicated anxiety.

2.3.2. Exp.1: Novel object recognition (NOR)

Sample Phase: the rat was placed on the opposite side and with its back to two identical objects, A1 and A2 (cubes of yellow plastic) (Fig. 3, Panel B, 1a). *Delay Phase:* 24 h. *Choice Phase:* the rat was re-exposed to two objects: one familiar (A3 or F, yellow cube, triplicated) and, in the same position, a new object (B, white cup) (Fig. 3, Panel B, 1b). It was evaluated**:** *Sample Phase:* (a) Exploration time [(A1 + A2), sec], defined as the sum of exploration time of each individual object; (b) Exploration time [(A1 vs A2), sec], identified as the exploration time of A1 + A2. This measure was performed to detect a possible preference to explore an object due to some hidden signal, undetectable to the operator that could induce a conduct pattern. *Choice Phase:* (c) Latency to B (sec), defined as the reaction time to the first contact with B (approaching the nose 1 cm or less); (d) Exploration time (sec) of the

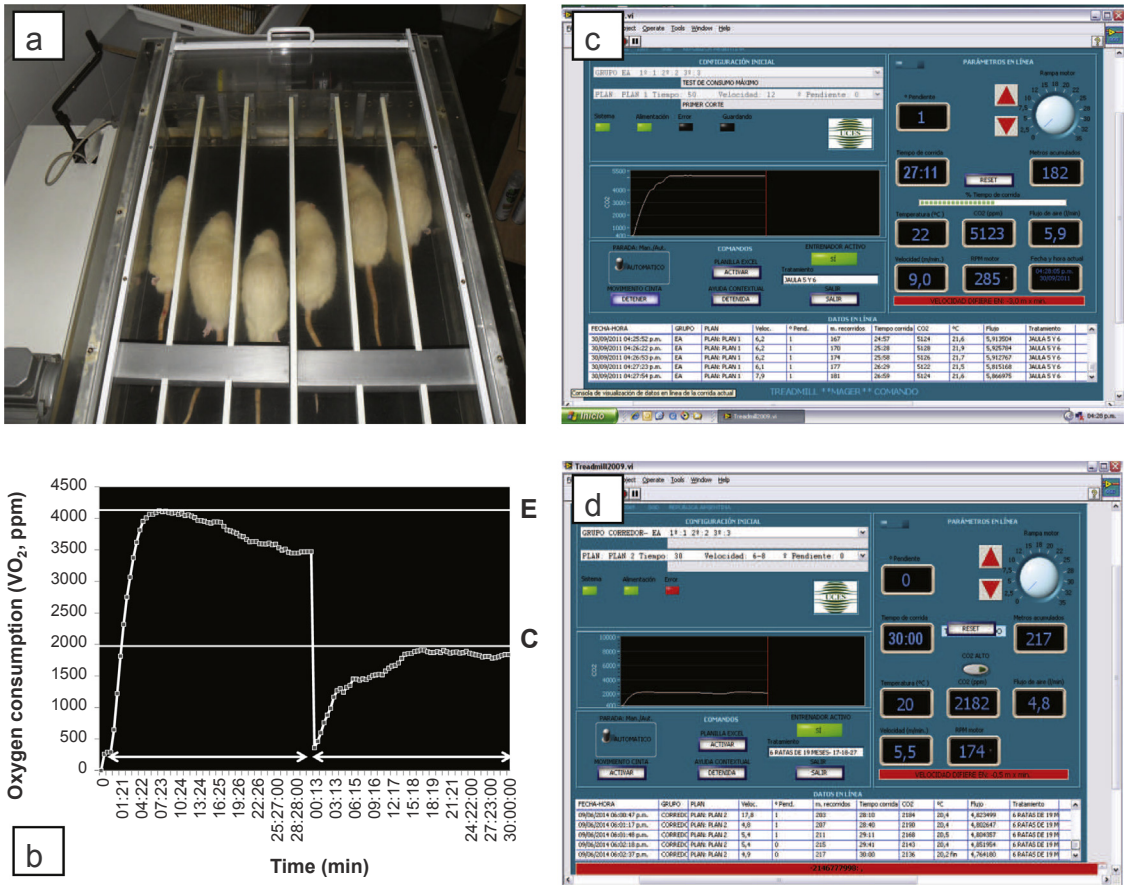

Fig. 2. Equipment overview and the main parameters measured. (a) Treadmill-running. (b) Oxygen consumption records (ppm) obtained from tests of max.VO_2, performed with middle-aged C-E rats. (c-d) Speed, slope, accumulated distance, oxygen consumption and air flow obtained in real-time and max.VO_2 records (ppm) belonging to E (top) and C (bottom) rats.

new object (B); (e) Exploration time of B (%), defined as the percentage of exploration time of the new object (B) relative to total exploration time (B/A3 + B × 100).

2.3.3. Exp.2: Displaced object recognition (DOR)

Sample Phase: the rat was placed on the opposite side and with its back to two identical objects, C1 and C2 (yellow/green modules) (Fig. 3, Panel B, 2a); *Delay Phase:* 24 h. *Choice Phase:* the rat was re-exposed to two identical objects, C1 and C2, both familiar (F/C3), but one of them displaced (D, triplicated) to change the relative positions they occupied in the *Sample Phase* (Fig. 3, Panel B, 2b). The following was evaluated**:** *Sample Phase:* (a) Exploration time [(C1 + C2), sec], the sum of exploration time; (b) Exploration time [(C1 vs C2), sec], exploration time of C1 and C2. The difference indicates the object preference. *Choice Phase:* (c) Latency to D (sec), is the reaction time to the first contact with the familiar displaced object (D) (approaching the nose 1 cm or less); (d) Exploration time (sec) of the displaced object (D); (e) Exploration time of D (%), measured the percentage of exploration time of the displaced object (D) relative to total exploration time (D/C3 + D × 100).

2.3.4. Exp.3: Displaced object recognition (DOR) in an inconsistent context

Sample Phase: the rat was exposed, in the same context, to two identical objects, C3 and C4 (rubber violet balls) (Fig. 3, Panel B, 3a). *Delay Phase:* after 24 h, 1) the floor of the OF was changed from black to white, and 2) the OF was re-located in a new experimental room, roughly similar to the previous one. *Choice Phase*: the rat was re-exposed in the new context (incongruent) to two identical objects both familiar (F/C5), but one of them displaced (D, triplicated) (Fig. 3, panel B, 3b). It was analyzed: *Sample Phase:* (a) Exploration time [(C3 + C4), sec], was the sum of exploration time; (b) Exploration time [(C3 vs C4), sec], comparison between the exploration time of C3 and C4 evaluates the possible preference among different objects. *Choice Phase:* (c) Latency to D (sec), is the reaction time to the first contact with the familiar displaced object (D) (approaching the nose 1 cm or less); (d) Exploration time (sec) of the displaced object (D); (e) Exploration time of D (%), is the percentage of exploration time of the displaced object (D) relative to total exploration time (D/C5 + D × 100).

2.4. Biochemical analysis

Brain tissue collection: Twenty-four hours after ORT, middle-aged and aged rats (C, n = 12 × 2 and E, n = 12 × 2) were sacrificed by decapitation (Harvard-Apparatus, South Natick, MA). Brains were rapidly dissected out, weighed and homogenized at a ratio of 1 g brain tissue/10 ml in a medium consisting of 0.32 M sucrose, 20 mM Tris-HCl, pH = 7,4, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 1mM sodium orthovanadate, 0,1 mM ammonium molybdate, 1 mM PMSF and protease inhibitor cocktail (CN P8340, Sigma Chemical Co., St. Louis, MO). Specific brain areas were identified following the guidance of The Rat Brain Atlas (Franklin and Paxinos, 2007). Homogenates were centrifuged at 10000*g* for 20 min to discard pellets. Then, supernatants were either analyzed immediately or kept at −80 ∘C before analysis. Protein concentration was measured by Bradford's method (Bradford, 1976) using bovine serum albumin (BSA) as standard.

2.4.1. ELISA assays

Samples (C, n = 6 × 2 and E, n = 6 × 2) of prefrontal and perirhinal

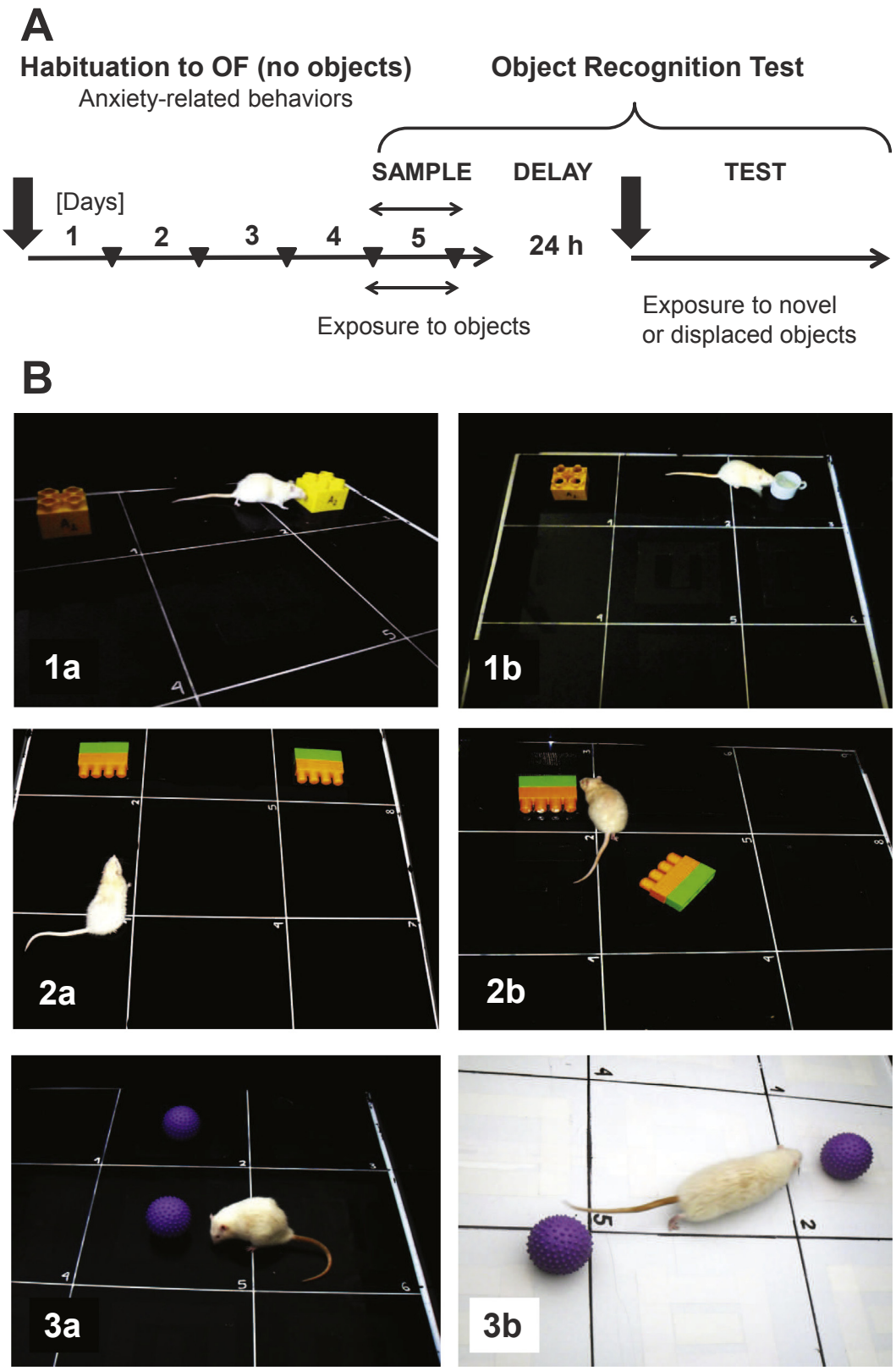

Fig. 3. ORT. Panel A. Scheme of the experimental design showing habituation (d1-d5), exposure to objects, delay and choice/test phase. Panel B. Phases of each experiment separated by a 24 h-delay. (a) Sample phase. (b) Choice/test phase. (1a-1b) Images correspond to: NOR. (2a-2b); to DOR-consistent context, and DOR-inconsistent context (3a-3b). Note that three sets of triplicate objects that differed in shape, color, size and texture were used.

cortex, hippocampus, striatum, and raphe were assayed to quantify the levels of BDNF and 5-HT with a commercial Enzyme Linked Immuno-Sorbent Assay (ELISA). Kit protocols followed were Rat BDNF CN BEK-2000-2P, Biosensis, Thebarton, South Australia 5031, and Rat 5-HT CN MBS725497, My Biosource, San Diego, CA. The total concentration of 5-HT in the brain was obtained from fresh samples of cerebral

hemispheres (including the raphe) without the olfactory bulb (The Rat Brain Atlas, Figs. 1–3, Bregma points: 4.28 mm to 3.56 mm), cerebellum (Fig. 75, Bregma point: −5.34 mm) and brainstem. Samples from C and E groups were assayed together in duplicate. The sensitivity of the BDNF ELISA Kit was < 2 pg/mL, intra and inter-assay CV (%) was < 10. The sensitivity of the 5-HT ELISA Kit was < 1.0 ng/mL, intra and inter-assay CV (%) was < 10. Both ELISA assays were highly specific. No significant cross-reactivity or interference between BDNF or 5-HT and analogues were observed.

2.4.2. Western-blotting (WB)

Samples (C, n = 6 × 2 and E, n = 6 × 2) of cortex and hippocampus containing 30 µg of protein were separated in 10 or 12% SDS-PAGE gel using a mini-cell system (Bio-Rad, Hercules, CA) and transferred onto a PVDF membrane (Bio-Rad). To quantify the expression of SERT and 5-HT_{1A} receptor markers in the cerebral cortex, a pool (total cortex) was obtained from samples of the medial prefrontal, perirhinal, and temporo-parietal (parahippocampal) cortex. Samples from C and E groups were assayed together in duplicate. Gels were stained with Coomassie blue as a transfer control, and membranes with Ponceau Red as protein loading control. Subsequently, blots were blocked in blotto blocking buffer in TBS (Thermo Fisher Scientific Inc., Waltham, MA) for 2 h at room temperature (RT). After three washes in TBST, blots were incubated with primary antibodies [rabbit anti-5HT_{1A} (1:3000); rabbit anti-SERT (1:4000)] overnight with gentle agitation at 4°C. Twenty-four hours later, the membranes were again washed in TBST and incubated with secondary antibodies [horseradish peroxidase-conjugated goat anti-rabbit IgG (1:500 or 1:1000, as appropriate), Sigma Chemical Co.] for 2 h at RT. After washing the blots with TBST, immunopositive bands were detected and visualized with Super ECL Plus Detection Reagent (Thermo Scientific P1010, Thermo Fisher Scientific, Waltham, MA). The blots were re-exposed to β-Actin [mAbcam 8226 (1:5000), Abcam, Cambridge, UK)] as the reference protein.

2.5. Immunohistochemistry (IHC)

Rats (C, n = 3 × 2 and E, n = 3 × 2) were anaesthetized by injection of pentobarbital sodium (IP,1 mg/kg) and perfused transcardially with saline containing 50 IU of heparin, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (all reagents from Sigma Chemical Co.). Brains were post fixed in the same fixative solution during 2 h followed by overnight immersion in a solution containing 5% sucrose in 0.1 M phosphate buffer, and then cut into coronal sections 40-µm thick. Tissue sections were used to detect: 5-HT in the raphe nuclei (Figs. 64–72, Bregma points: −4.04 mm to −4.96 mm) and NeuN in the hippocampus (CA1 area) (Figs. 43–50, Bregma points: −1.46 mm to −2.30 mm) (Franklin and Paxinos, 2007) by immunoperoxidase (IP) and immunofluorescence (IF), respectively. Briefly, free floating sections were washed three times in PBS, and incubated in a solution containing 3% BSA and 0.5% Triton X-100 in PBS for 1 h at RT. Then, sections were incubated with: rabbit anti-5-HT [Sigma Chemical Co., (1:2000)], and mouse anti-NeuN [Sigma Chemical Co., (1:1000)], for 48 h at 4 °C. Subsequently, sections were incubated with goat anti-mouse secondary antibody [Vector Labs., USA, (1:500)] for 2 h at RT, and goat anti-mouse IgG secondary antibody AlexaFluor 568 conjugate for IF [Thermo Fisher Scientific (1:500)]. Negative controls were obtained by omitting primary antibodies. Positive controls (DNA) were obtained by Hoechst staining (Sigma Chemical Co.). All sections were processed simultaneously using the same solution batch, mounted on gelatin-coated glass slides and coverslipped with mounting medium.

2.6. Imaging analysis

Immunolabeled sections were subsequently observed with an Axiophot-Zeiss light/epifluorescence microscope and digitized with an Olympus Q5 color digital camera connected to QCapture Pro software (minimum 2.5× and maximum 20× magnifications). Images were analyzed using the NIH Image software (Bethesda, MD, http://rsb.info.nih.gov/nih-image/). The quantification of NeuN-immunopositive mature neurons in the CA1 pyramidal layer was performed by manual cell counts within a standardized area (perimeter, 173 mm width × 130 mm height; scale, 300 pixels/inch). Only cell bodies clearly identified by NeuN immunofluorescence were included in the analysis, regardless of their label intensity, size and number and length of neuronal processes. All images (30 per group) were measured simultaneously by the same blind operator. To determine the percentage of NeuN reactive neurons, 200 immunopositive cells per group were quantified and calculated as: [(number of NeuN-immunopositive neurons belonging to middle-aged or aged rats/400) × 100].

2.7. Statistical analysis

Data were expressed as the mean ± SEM. Significance was established at $p < 0.05$. All data were tested for outliers, normality by Shapiro-Wilk's test and homoscedasticity by Levene's test, and shown to conform to the requirements for parametric statistics before ANOVAs were carried out. Two-way ANOVA (age × exercise) was used to analyze data from behavioral tests, biochemical determinations (ELISA and WB), and IHC. Specific differences were tested by *post hoc* comparisons with Bonferroni or Tukey's tests when appropriate. The correlation between SERT expression and 5-HT concentration in the raphe was analyzed using the Pearson coefficient (r). The habituation procedure to OF and the VO_2 data were analyzed by two-way ANOVA with repeated measures (RM ANOVA) on time factor (days/months) followed by Dunnett's or Bonferroni's test for multiple comparisons. All the variables were analyzed with SPSS v. 23.0 IBM (Armonk, NY) software 2015 and plotted with Graph Pad Prism v. 5.03 2010 (La Jolla, CA).

3. Results

3.1. Aerobic training protocol

Animals showed good adaptation to prolonged exertion and repetitive exercise, with increasing mechanical efficiency, motor control, locomotion and aerobic power. No rats needed to be removed from training because of pain, fatigue, diseases, osteo-articular disabilities, or for simply refusing to run. On the contrary, even old animals were well-willing to train. From the twelfth week of training we began to detect significant differences in the max. VO_2 between C and E animals, which showed the need for an initial period of cardiovascular adaptation. Regular AE produced specific responses of the oxidative energy system, as demonstrated by the average values obtained in the max.VO_2 (ppm/100 g·min^{-1}). The means obtained were: at 2 months (training start), C, 0.32 ± 0.04 and E, 0.34 ± 0.017 ($p > 0.05$, ns); at 8 months, C, 0.39 ± 0.02 vs E, 0.69 ± 0.01 (***$p < 0.001$); and at 18 months, C, 0.22 ± 0.007 vs E, 0.32 ± 0.005 (**$p < 0.01$). AE amplified aerobic power by approximately 77% in the middle-aged E and 45% in the aged E (Fig. 4).

3.2. Behavioral testing

3.2.1. Anxiety-related behavior

AE significantly [$F(3,40) = 25$, $p < 0.0001$] affected locomotor activity, which decreased until stability was achieved in all groups (Fig. 5a). However, in middle-aged E rats, locomotion stabilized at significantly higher values than the rest of the animals, which may be attributed to their greater motor capacity and not to the effects of anxiety. Defecation followed the same trend as locomotion [$F(3,37) = 8.3$, $p = 0.0002$]. *Post hoc* analysis showed that only the old C group had higher initial levels of emotionality. Finally, the number of fecal pellets decreased in all groups, being irrelevant on the day 4 (Fig. 5b). The

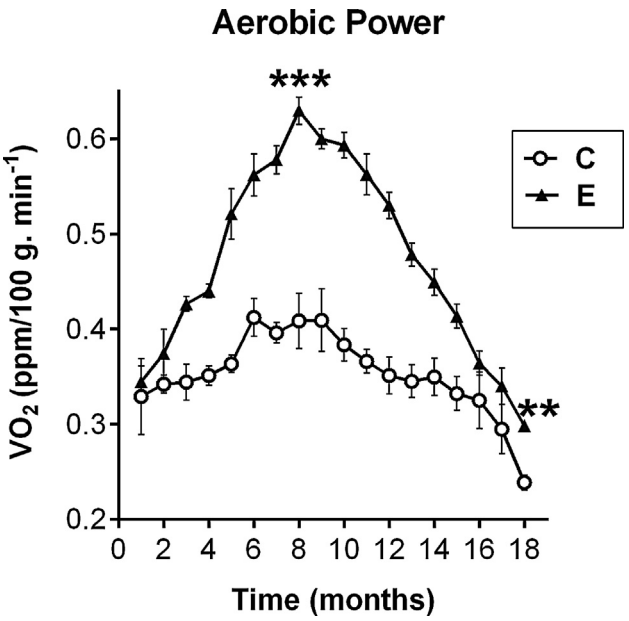

Fig. 4. Effects of E on aerobic power. Evolution of VO_2 of C and E rats throughout the experiment. Points represent the means ± SEM. The absolute values in ppm were normalized per 100 g of weight and per minute (ppm/100 g·min^{-1}). Significant differences: ***p < 0.001, 8C vs. 8E and **p < 0.01, 18C vs. 18E. Data from n = 30 rats/group, RM ANOVA, Bonferroni's *post hoc* test.

rearing postures were significantly affected by age [F (3,36) = 36.3, p < 0.0001]. Old animals showed physical limitations to stand on their hind legs. Middle-aged rats progressively decreased this behavior throughout the days until it was not significant (Fig. 5c).

3.2.2. Exp.1: NOR

Sample Phase: AE [F (1,37) = 4.60, p = 0.038] and age [F (1,37) = 5.40, p = 0.025] significantly affected the exploration time of objects A1 and A2. *Post hoc* analysis showed that the middle-aged E rats were the most active compared with the other groups (Fig. 6a). In terms of object preference (Exp.1), no significant differences (p > 0.05) were found in the exploration time when comparing A1 vs. A2 objects for any group (Fig. 6b). *Test/Choice Phase:* AE significantly affected [F (1,37) = 5.043, p = 0.0308] the latency time. *Post hoc* analysis showed that middle-aged E rats had the shortest reaction time, indicating higher speed of information processing (Fig. 6c). AE [F (1,37) = 21.29, p < 0.0001] and age [F (1,37) = 23.90, p < 0.0001] produced significant main effects on the ability to discriminate new objects. E rats demonstrated a higher cognitive response compared with their controls (Fig. 6d). When time of exploration was related to total time, significant effects of AE [F (1,37) = 47.49, p < 0.0001] and age [F (1,37) = 17.49, p < 0.0001] (Fig. 6e) were detected confirming the tendency observed in Fig. 6d

3.2.3. Exp.2: DOR-consistent context

Sample Phase: AE significantly affected [F (1,36) = 18.30, p = 0.0001] exploratory activity and age [F (1,37) = 13.90, p < 0.001] (Fig. 7a), in which the middle-aged E rats were the most active, showing the same trend observed in Fig. 6a. No significant differences (p > 0.05) were found in the exploration time when comparing C1 vs. C2 objects for any group (Fig. 7b). *Test/Choice Phase:* AE significantly affected [F (1,37) = 51.26, p = 0.029] the latency time to D. Significant main effects of age [F (1,37) = 13.84, p < 0.001] were also detected. *Post hoc* analysis showed that the largest differences were found between aged C and the remaining animals. (Fig. 7c). The exploratory activity was significantly affected by the main effects of AE [F (1,37) = 17.87, p < 0.0001] and age [F (1,37) = 12.81, p < 0.001] (Fig. 7d), showing the same trend that we observed in Fig. 6d. When time of exploration was related to total time, significant effects of AE [F (1,37) = 18.82, p < 0.0001] and age [F (1,37) = 16.87, p = 0.0002] (Fig. 7e) were observed confirming the tendency noticed in Fig. 7d.

3.2.4. Exp.3: DOR-inconsistent context

Sample Phase: Significant differences were induced by AE [F (1,37) = 10.59, p = 0.0025], while *post hoc* tests revealed the largest differences to be among old animals. Age significantly affected the exploration time [F (1,37) = 6.10, p < 0.05] (Fig. 8a). No significant differences (p > 0.05) were found in the exploration time when comparing C3 vs. C4 objects for any group (Fig. 8b). *Test/Choice Phase:* Reaction speed until the first contact with D was significantly affected by AE [F (1,37) = 6.42, p = 0.015] and age [F (1,37) = 8.68, p = 0.0056], with the aged C rats showing the longest latency to detect D as compared to the other animals (Fig. 8c). AE, significantly influenced [F (1,37) = 20.96, p < 0.0001] exploration time of D. Main effects due to age altered the exploration time [F (1,37) = 18.9, p < 0.0001] (Fig. 8d). When time of exploration was related to total time, significant effects of AE [F (1,37) = 67.33, p < 0.0001] and age [F (1,37) = 12.83, p = 0.001] (Fig. 8e) were determined confirming the tendency observed in Fig. 8d. In addition, the modified context increased exploratory activity and latency when comparing Fig. 7c–e to Fig. 8c–e.

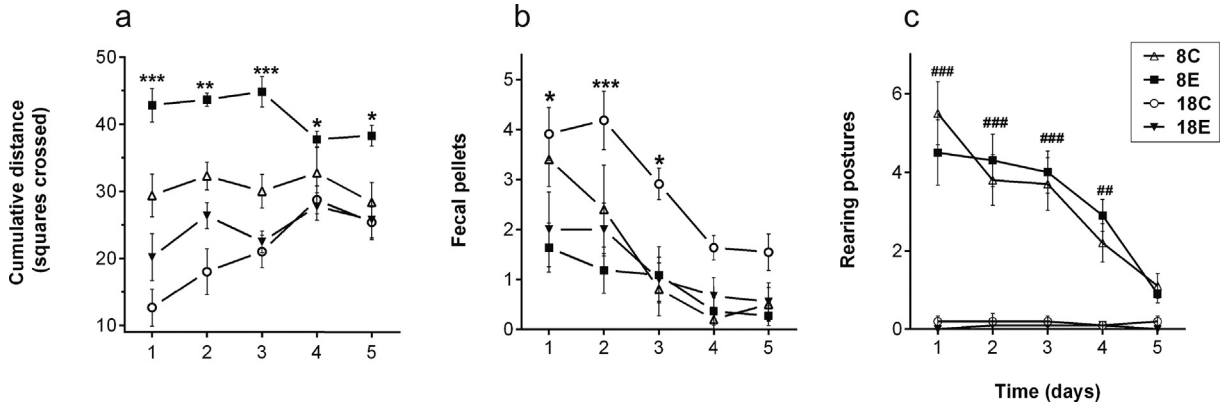

Fig. 5. Anxiety-related behavior. Five-days OF test to determine baseline levels of anxiety-related behavior prior to the ORT. Points represent the means ± SEM. (a) Cumulative distance. Significant differences due to E (days 1–3, ***p < 0.001; day 2, **p < 0.01; day 4–5, *p < 0.05, 8C rats vs. the other groups). (b) Fecal boli. Significant differences due to E (days 1–3, *p < 0.05; day 2, ***p < 0.001, 18C rats vs. the other groups). (c) Rearing postures. Significant differences due to age (days 1–3, ###p < 0.001, day 4, ##p < 0.01, middle-aged rats vs. old rats. Data from n = 15 rats/group. RMANOVA, Dunnett's *post hoc* test.

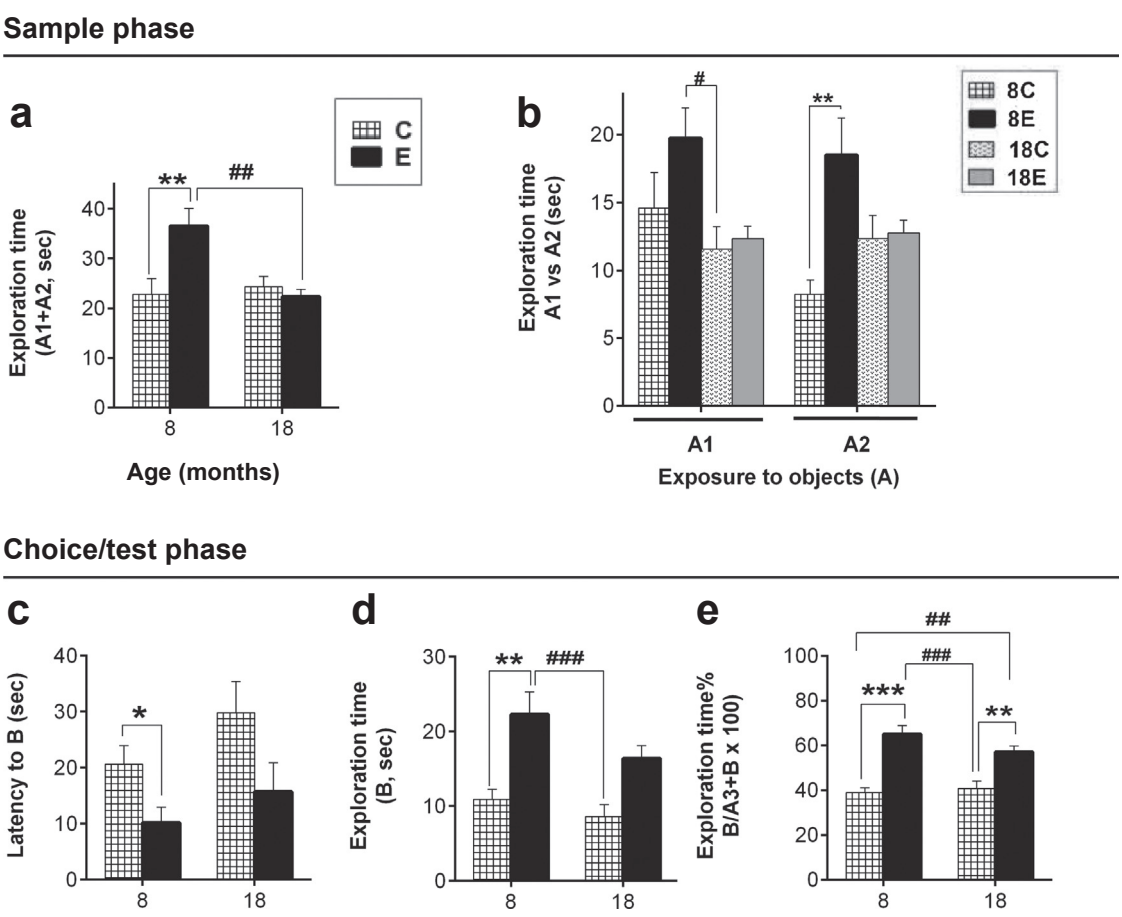

Fig. 6. Effects of E and age on recognition memory of new objects. Bars represent the mean ± SEM. *Sample phase.* (a) A1 + A2 exploration time. Significant differences due to E (**p < 0.01, 8C vs. 8E) and age (##p < 0.01, 8E rats vs.18E rats). (b) No significant differences (p > 0.05) were detected in the preference of A1 vs A2 objects. *Choice phase.* (c) Latency to B (new object). Significant differences due to E (*p < 0.05, 8C vs. 8E). (d) Exploration time to B. Significant differences due to E (**p < 0.001, 8C vs. 8E) and age (###p < 0.001, 8E vs. 18C). (e) Exploration time relative to total A3 + B exploration.time. Significant differences due to E (***p < 0.001, 8C vs. 8E; **p < 0.01 18C vs. 18E) and age (###p < 0.001, 8E vs. 18C; ##p < 0.01 8C vs. 18E). Data from n = 15 rats/group, two-way ANOVA, Bonferroni's *post hoc* test.

3.3. Biochemical analysis and immunohistochemistry

3.3.1. BDNF

The most significant effects of AE [F (1,48) = 14.91, p = 0.0003] and age [F (1,48) = 16, p = 0.0002] were detected on the levels of BDNF in the prefrontal cortical region, while *post hoc* tests determined that the middle-aged E rats had the greatest concentrations (Fig. 9a). BDNF levels in the perirhinal cortex were also affected by AE [F (1,46) = 4.808, p = 0.0334] and age [F (1,46) = 4.920, p = 0.0315], with multiple comparisons showing the lowest concentrations in old C rats. Interestingly, BDNF levels in this area were conserved in old E rats (Fig. 9b). Hippocampal BDNF content was significantly influenced by AE [F (1,33) = 10.01, p = 0.0033] and age [F (1,33) = 9.055, p = 0.0050], with AE increasing BDNF levels particularly in the middle-aged E rats as compared to old rats (Fig. 9c). Striatal BDNF also revealed significant effects of AE [F (1,27) = 4.259, p = 0.0488] and age [F (1,27) = 12.62, p = 0.0014], the lowest concentrations being observed in old C rats. Worth highlighting, the E rats showed conserved levels of BDNF in the striatum (Fig. 9d).

3.3.2. IHC (IF), Neu-N

Immunolabeled brain sections containing the CA1 area of the stratum pyramidale were observed under an epifluorescence microscope, and neurons positive for NeuN were subsequently quantified (Fig. 10, Panel A). Two-way ANOVA detected significant main effects of AE [F (1,45) = 121, p < 0.0001] and age [F (1,45) = 38.7, p < 0.0001]. AE significantly increased the number of mature neurons expressing NeuN (Fig. 10, Panel B). Regarding age, middle-aged rats showed 39% and 61% immunopositive NeuN cells in C and E, respectively, while old rats showed 33% and 67% in C and E, respectively (Fig. 10, Panel C).

3.3.3. 5-HT

Brain 5-HT levels were significantly altered by main effects of AE [F (1,32) = 16.18, p = 0.0003], while *post hoc* analysis determined the greatest differences between older animals (p < 0.001). Age significantly changed the 5-HT concentration [F (1,32) = 12.10, p < 0.01] (Fig. 11a, Panel A). Results revealed that raphe 5-HT levels were also significantly influenced by AE [F (1,20) = 85.28, p < 0.0001] and age [F (1,20) = 92.02, p < 0.0001], with the largest differences found among middle-aged E rats (Fig. 11b, Panel A). In this sense, Fig. 11a shows that 5-HT concentration was preserved by long-term AE in old runners. Optical microscope observation confirmed this trend and revealed qualitative differences in the distribution of 5-HT immunostaining in the raphe subregions, i.e. dorsal and ventral raphe nucleus and magnus raphe, induced by AE (Panel B, Fig. 11, a-c and b-d, C vs. E, respectively).

3.3.4. SERT and 5-HT$_{1A}$ (WB)

SERT expression was significantly altered by main effects of AE [F (1,20) = 75.89, p < 0.0001] and age [F (1,20) = 29.47, p < 0.0001] in the total cortex (Panel A, Fig. 12a) and the hippocampus (Panel A,

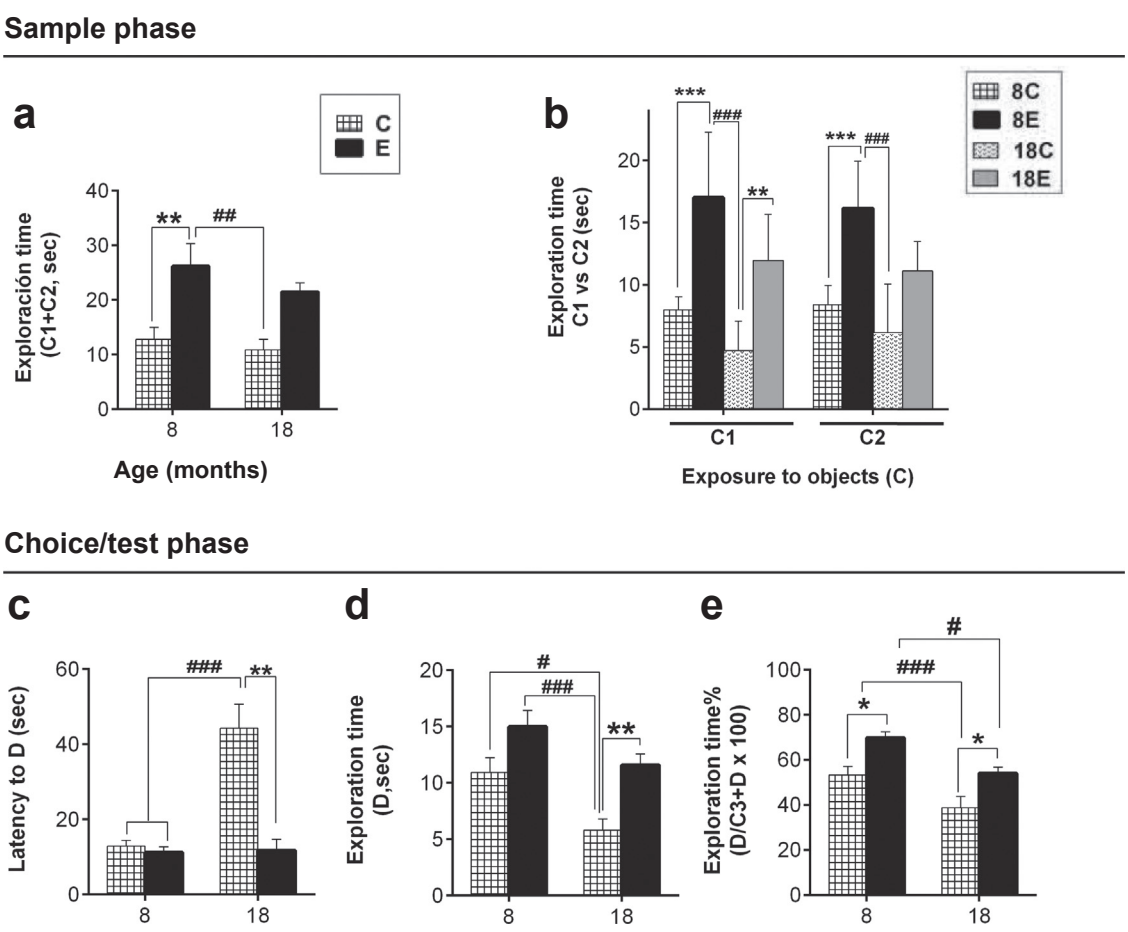

Fig. 7. Effects of E and age on recognition memory of displaced objects. Bars represent the mean ± SEM. *Sample phase.* (a) C1 + C2 exploration time. Significant differences due to E (**p < 0.01, 8C vs. 8E) and age (##p < 0.01, 8E vs. 18C). (b) No significant differences (p > 0.05) were detected in the preference of C1 vs C2 objects. *Choice phase.* (c) Latency to D (displaced familiar object). Significant differences due to E (**p < 0.01, 18C vs 18E) and age (###p < 0.001 8C and E vs. 18C). (d) D exploration time. Significant differences due to E (**p < 0.01, 18C vs. 18E) and age (###p < 0.001 8E vs. 18C; #p < 0.05, 8C vs. 18C). (e) Exploration time relative to total C3 + D exploration.time. Significant differences due to E (*p < 0.05, 8C vs. 8E and 18C vs. 18E) and age (###p < 0.001, 8C and E vs. 18C; #p < 0.05 8E vs. 18E). Data from n = 15 rats/group, two-way ANOVA, Bonferroni's *post hoc* test.

Fig. 12b) [F (1,44) = 26.04, p < 0.0001 and F (1,44) = 6.732, p = 0.0128, respectively]. AE upregulated SERT expression in both areas, which correlated with the raphe 5-HT concentration (Panel A, Fig. 12b). The Pearson coefficient (r) showed a significant positive linear correlation between the two variables in both the cortex and the hippocampus when controls versus exercised animals were compared. The control group showed a moderate association in the cortex, (r = 0.5, p = 0.025) and hippocampus (r = 0.6, p = 0.005). The exercised group showed a greater correlation in the cortex (r = 0.8, p < 0.0001) than in the hippocampus (r = 0.47, p = 0.030). 5-HT_{1A} receptor expression was significantly altered by main effects of AE [F (1,18) = 8.8, p = 0.0084] and aging [F (1,18) = 33, p < 0.0001] in the total cortex (Panel B, Fig. 12a) and hippocampus [F (1,20) = 14.69, p = 0.0010 and F (1,20) = 5.92, p = 0.0245, respectively] (Panel B, Fig. 12b). *Post hoc* tests revealed the greatest differences between middle-aged E rats and old animals.

4. Discussion

The main goal of this research was to study the possible alterations induced by AE on the brain levels and expression of two molecules: BNDF and 5-HT, both playing a part in memory acquisition, consolidation and recovery, as well as learning processes. Moreover, these molecules have shown functional convergence in cognition-related areas. It is also well-known that aging has deleterious effects on the signaling pathways of the BDNF-5-HT systems, affecting the functional and structural integrity of these brain areas (Luellen, Bianco, Schneider, & Andrews, 2007; Mattson et al., 2004; Ogren et al., 2008; Saleem, Tabassum, Ahmed, Perveen, & Haider, 2014).

In this study, it was demonstrated that an early and regular lifelong practice of low/moderate intensity AE significantly increased both the levels and expression of central components of the BDNF-5-HT system and reduced its age-induced decline, also preserving the integrity and functionality of their signaling pathways. This response temporarily correlated with more efficient cognitive behavior, which indicates a positive impact of AE on different memory systems associated with regions where 5HT and BDNF interact. Simultaneously, an increased response of neuronal maturation in the CA1 area, stratum pyramidale, was observed which could result in the amplification of the cellular substrate and connectivity.

In terms of behavioral findings, Exp.1 (NOR) demonstrated that AE improved animal ability to quickly detect objects with new attributes, indicating higher speed of information processing and discrimination capacity. Activation of the medial prefrontal cortical function by exercise could impact positively on executing control, involving the working memory, mental flexibility, and inhibitory control (Brockett et al., 2015; Kamijo et al., 2009). The amplification of perirhinal cortical function has been proven critical to memory achievement, encoding, consolidating and retrieval in tests of novelty discrimination capacity (Kinnavane, Albasser, & Aggleton, 2015; Morici et al., 2015;

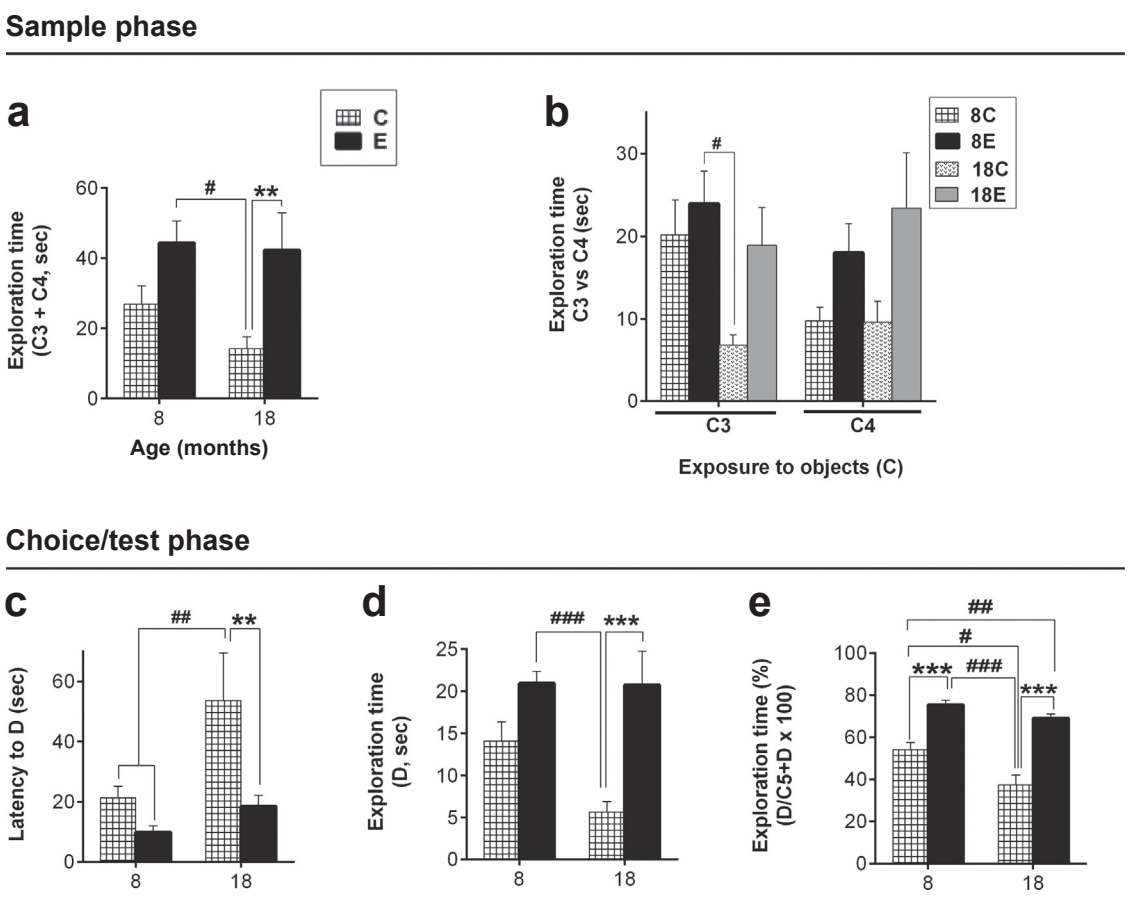

Fig. 8. Effects of E and age on the contextual memory. Bars represent the mean ± SEM. *Sample phase.* (a) C3 + C4 exploration time. Significant differences due to E (**p < 0.01,18C vs. 18E) and age (#p < 0.05, 8E vs.18C). (b) No significant differences (p > 0.05) were detected in the preference of C3 vs C4 objects. *Choice phase.* (c) Latency to D (displaced familiar). Significant differences due to E (**p < 0.01, 18C vs.18E) and age (##p < 0.01, 8C and E vs.18C). (d) D exploration time (***p < 0.001, 18C vs. 18E; ###p < 0.001 8E vs. 18C). (e) Exploration time relative to total C5 + D exploration.time. Significant differences due to E (***p < 0.001, 8C vs. 8E and 18C vs. 18E) and age (##p < 0.01, 8C vs. 18E; #p < 0.05 8C vs. 18C).Data from n = 15 rats/group, two-way ANOVA, Bonferroni's *post hoc* test.

Winters et al., 2008). AE also attenuated the loss of sensitivity to novelty by aging. According to Burke, Wallace, Nematollahi, Uprety, and Barnes (2010), it would not be a consequence of forgetting but of perirhinal cortex dysfunction.

In turn, Exp.2 (DOR-consistent context) showed that AE amplified the hippocampal function, which resulted in improved ability to discriminate spatial patterns and higher efficiency at detecting relative position changes during an event or within two ambiguous contexts (Tronel et al., 2012). The hippocampus is composed by the dentate gyrus (DG) and three subdivisions of the Cornu Ammonis (CA) area. The pyramidal cells in CA1 do not visually segregate in two distinct layers *per se*, but are distributed uniformly with a gradient of distinct morphological, molecular and physiological features along the radial axis. Both are different in the number and size of their cells. Moreover, there is a functional segregation between them that make their spatial representations to gradually change along the proximal–distal axis (Geyller et al., 2017). In agreement with findings reported by Mattson (2008), exercise significantly increased the number of mature NeuN-immunopositive neurons from the CA1 area, nonetheless, it cannot be concluded that exercise had a preferential effect on some of the CA1 layers (superficial and/or deep CA1). The spatial memory was deteriorated by aging and preserved in the long-term by exercise. In line with our results, Siette et al. (2013) have shown that hippocampal dysfunction observed in senescent rats during place-recognition memory tests strongly correlates with the loss of hippocampal synapses. In addition, the authors demonstrated that 12 weeks of running at the age of 20 months partially removes this deficit.

Furthermore, Exp. 3 (DOR-inconsistent context) indicates that AE increases contextual memory. A modified context requires the coordination of different memory systems (Robertson, Eacott, & Easton, 2015), greater executive control to avoid interference effects and speed of information processing (Burke et al., 2010; Kamijo et al., 2009). When comparing the consistent and inconsistent contexts, E rats maintained a greater capacity of discrimination in the two conditions. AE had a neuroprotective effect on contextual memory, as demonstrated by differences between old E rats and their controls. The incongruent context increased exploration time and produced a great cognitive demand due to the need to process multimodal information. This response could explain the difficulty of the old C rats to construct spatial representations of the environment and to recover the information in a new temporo-spatial framework that allows them to detect changes.

AE increased the BDNF concentration in most of the brain regions studied: prefrontal cortex, perirhinal, striatum and hippocampus, nonetheless this response was modulated by age. The regional differences observed in this work are consistent with those described in previous reports (Baj et al., 2012; Hopkins and Bucci, 2010; Morgan et al., 2015), which correlated with the increase in the number of pyramidal mature neurons in CA1 that has been previously reported. This could explain the amplification observed in spatial memory which had already been described by other authors (Adlard, Perreau, & Cotman, 2005; Hopkins and Bucci, 2010; Sheikhzadeh, Asieh, Sahar, Naser, & Peyman, 2015; Soya et al., 2007). Interestingly, a comparable trend observed in the striatum could indicate the need of greater

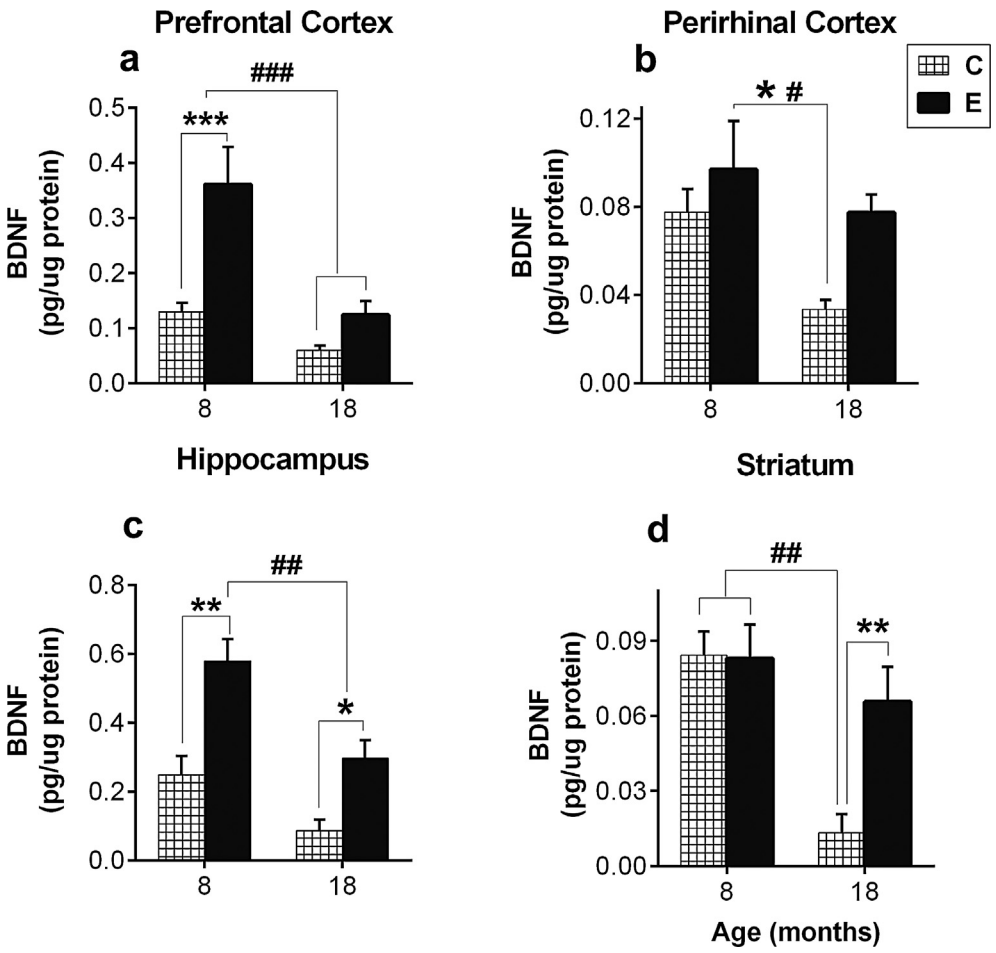

Fig. 9. Effects of E and age on BDNF levels in cognition related-areas. Bars represent the mean ± SEM. (a) Prefrontal cortex: Significant differences due to E (***p < 0.001, 8C vs. 8E) and age (###p < 0.001, 8C and E vs.18C and E). (b) Perirhinal cortex: Significant differences due to E and age (*p < 0.05, #p < 0.05, 8E vs.18C). (c) Hippocampus: Statistically significant differences due to E (**p < 0.01, 8C vs. 8E; *p < 0.05, 18C vs.18E) and age (##p < 0.01, 8E vs.18C and E). (d) Striatum: Significant differences due to E only among the old rats (*p < 0.05, C vs. E) and age (##p < 0.01, 8C and E vs. 18C). Data from n = 6 rats/group; Two-way ANOVA, Tukey's *post hoc* test.

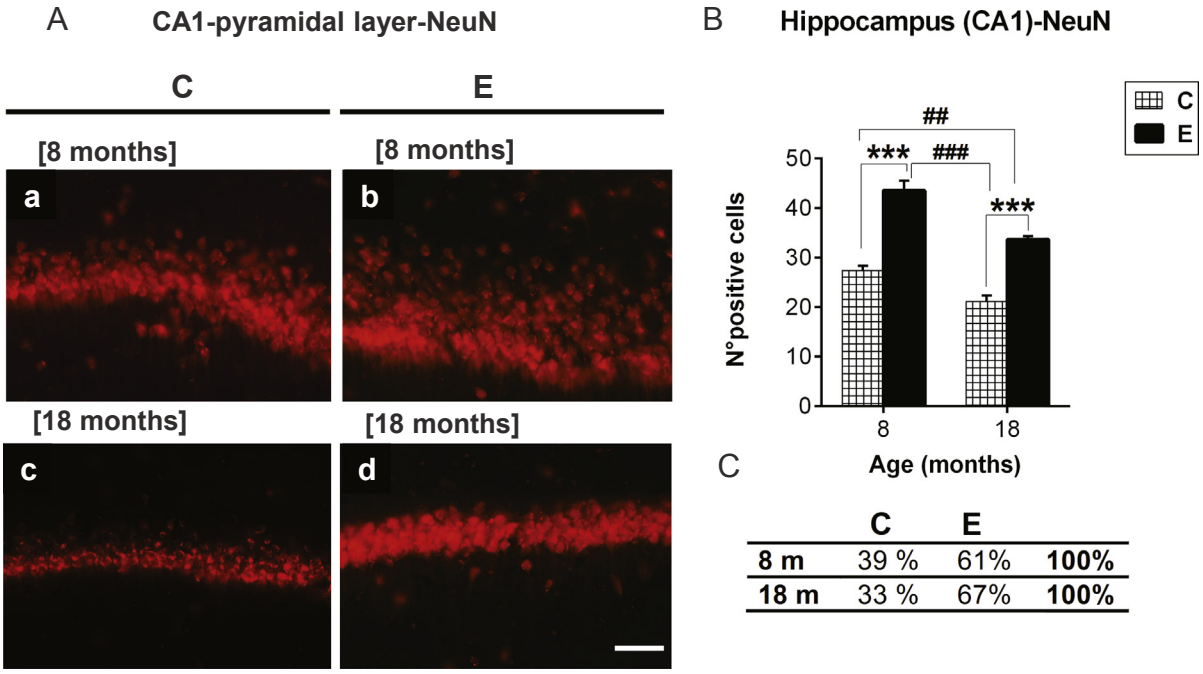

Fig. 10. Effects of E and age on the number of mature hippocampal CA1 neurons of the pyramidal layer. Panel A: (a-d) Photographs of brain sections showing the regional location and distribution of NeuN immunostaining in the hippocampus. Mature NeuN-immunopositive neurons corresponding to middle-aged rats, C vs. E (top) and old rats, C vs. E (bottom). Scale bar: 100 um. Magnification: (20 X). Panel B: Quantification of Neun-immunopositive neurons corresponding to panel A. Statistically significant differences due to E (***p < 0.001, 8C vs 8E; 18C vs. 18E), and age (### p < 0.001, 8E vs. 18C; ## p < 0.01, 8C vs. 18C and E). Bars represent the mean ± SEM. Data from n = 30 images/group. Two-way ANOVA, Bonferroni's *post hoc* test. Panel C: Data from panel B expressed as relative percentages of NeuN-immunopositive neurons within each age group (middle-aged rats 39% and 61%, C vs. E; old rats, 33% and 67%, C vs. E).

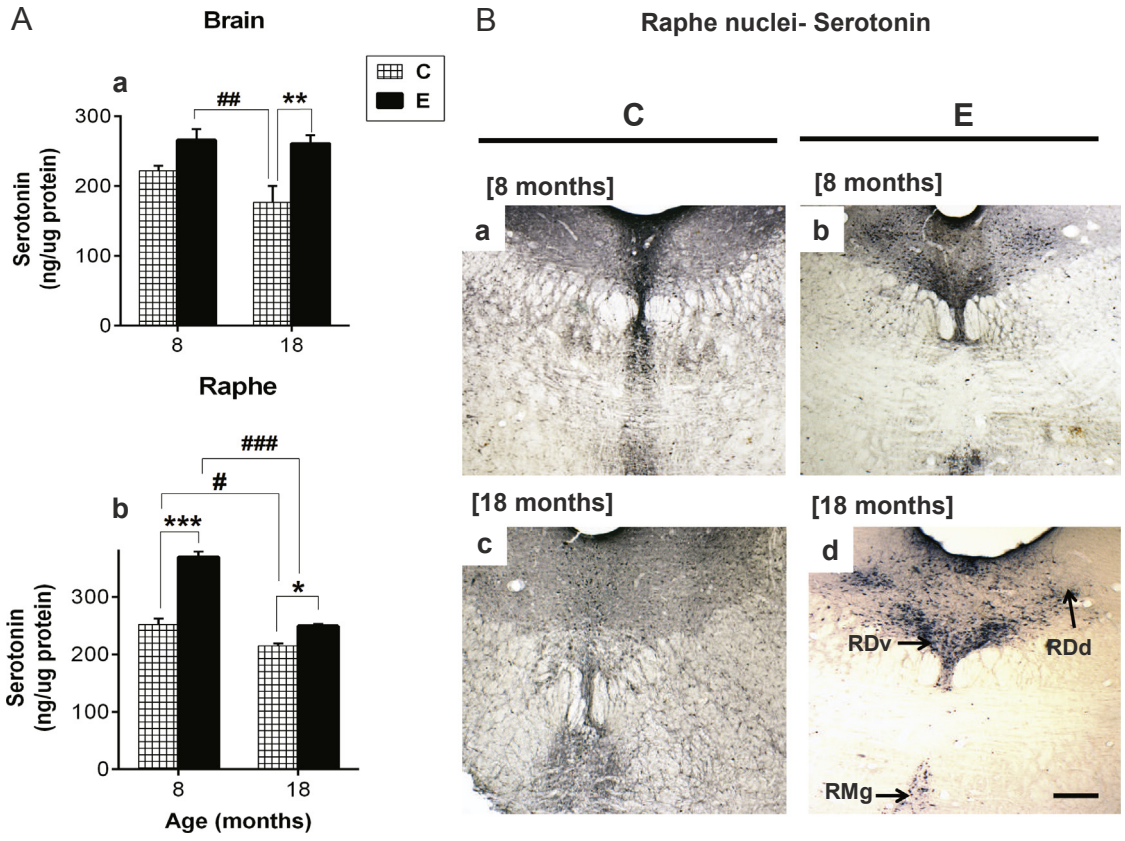

Fig. 11. Effects of E and age on 5-HT in the brain and raphe nuclei. Panel A: ELISA quantification. (a) Brain samples excluding the olfactory bulb. Significant differences due to E (**p < 0.01, 18C vs. 18E) and age (##p < 0.01, 8E vs. 18C). (b) Raphe nuclei. Significant differences due to E (***p < 0.001, 8C vs. 8E; *p < 0.05, 18C vs. 18E) and age (###p < 0.001, 8E vs. 18C and E; #p < 0.05, 8C vs.18C). Bars represent the mean + SEM. Data from n = 6 rats/group, two-way ANOVA, Tukey's *post hoc* test. Panel B. Raphe nuclei: Photographs (n = 30 images/group) of brain sections showing the regional location and distribution of immunostaining in the raphe subregions. Immunopositive-neurons corresponding to middle-aged rats, C vs. E (top) and old rats, C vs. E (bottom). *Arrows:* Dv: dorsal–ventral raphe; Dd: dorsal-dorsal raphe; Mg: magnus raphe. Scale bar: 100 um. Magnification: (10 X).

metabolic support for subcortical motor control and memory processes. According to Fisher et al. (2017), plasticity at synapses between the cortex and striatum is considered critical for learning novel actions.

It has been hypothesized that progressive physical stimulation within the hormetic range (60–70% of max. VO_2) applied in the over-compensation period may induce successive increases in BDNF levels, even in aging/senescent rats. According to other researchers (Gradari et al.; 2016; Heijnen, Hommel, Kibele, & Colzato, 2016), the biphasic pattern of BDNF may be assumed to coincide with this hormetic range (Bayod et al., 2011; Marosi and Mattson, 2014; Mattson, 2008). In the same direction, Inoue et al. (2015) demonstrated that moderate intensity training (60%) may satisfactorily stimulate the aerobic system to amplify spatial memory, and that only 20 min should be necessary to activate the monoaminergic response (Rabelo et al., 2015).

Present results demonstrate that AE increased the activity of the 5-HT system in the cortex, hippocampus and raphe nuclei, particularly in the old E rats. The qualitative differences observed in the pattern of distribution of serotonergic neurons between raphe subregions could be attributed to the complex expression pattern of tryptophan hydroxylase-2, the rate limiting enzyme for 5-HT biosynthesis (MacGillivray, Lagrou, Reynolds, Rosebush, & Mazurek, 2010). Also, the concomitant increase detected in the expression of SERT in the cortex and hippocampus could be due to the need to maintain an adequate balance between the intra-cellular and synaptic concentrations of 5-HT (MacGillivray, Reynolds, Rosebush, & Mazurek, 2012; Meneses and Perez-García, 2011). AE also increased 5-HT_{1A} receptor expression in the cortex and hippocampus of the middle-aged rats, confirming previous results by Kim et al. (2015). However, 5-HT_{1A} receptor expression was significantly diminished by age, as observed by other authors (Mitchell et al., 2009; Tauscher et al., 2001). Assuming that the good cognitive performance of the E rats was due, at least in part, to the activation of the 5-HT_{1A} receptor, it cannot be ruled out that 5-HT may be involved in cognitive function by some of its other receptors, or depending on the preferential stimulation of pre-synaptic 5-HT_{1A} auto-receptors and/or post-synaptic 5-HT_{1A} hetero-receptors. 5-HT can probably also act as a meta-modulator of other neurotransmitter systems involved in the formation of memory (Klempin et al., 2013; Ögren et al., 2008).

The sports training theory (Bompa and Haff, 2009) argues that systematic and regular training stimuli during the overcompensation period (between 24 and 72 h post-exercise) has cumulative effects on energy metabolic status and motor performance. Therefore, it is reasonable to assume that these effects are not limited to the peripheral tissues, but should also impact on brain metabolism. Prolonged exercise produces an increase in brain monoamines 5-HT and dopamine, not only to regulate central fatigue but also to activate the mobilization of energy substrates (Goekint et al., 2012).

Astrocytes are the major source of brain glycogen (2006; Blomstrand, Perrett, Parry-Bilings, & Newsholme, 1989; Caperuto et al., 2009; Langfort et al., 2006) and the cells responsible for providing sufficient energy substrates to the neurons in activity, especially for memory formation and the prevention of hypoglycemia (2012; Matsui et al., 2011). According to the "selfish brain" hypothesis (Peters, , 2011), the cortex, hippocampus, brainstem and, to a lesser extent, the hypothalamus, are brain regions showing great overcompensation. This phenomenon occurs earlier in the brain than in the skeletal muscles

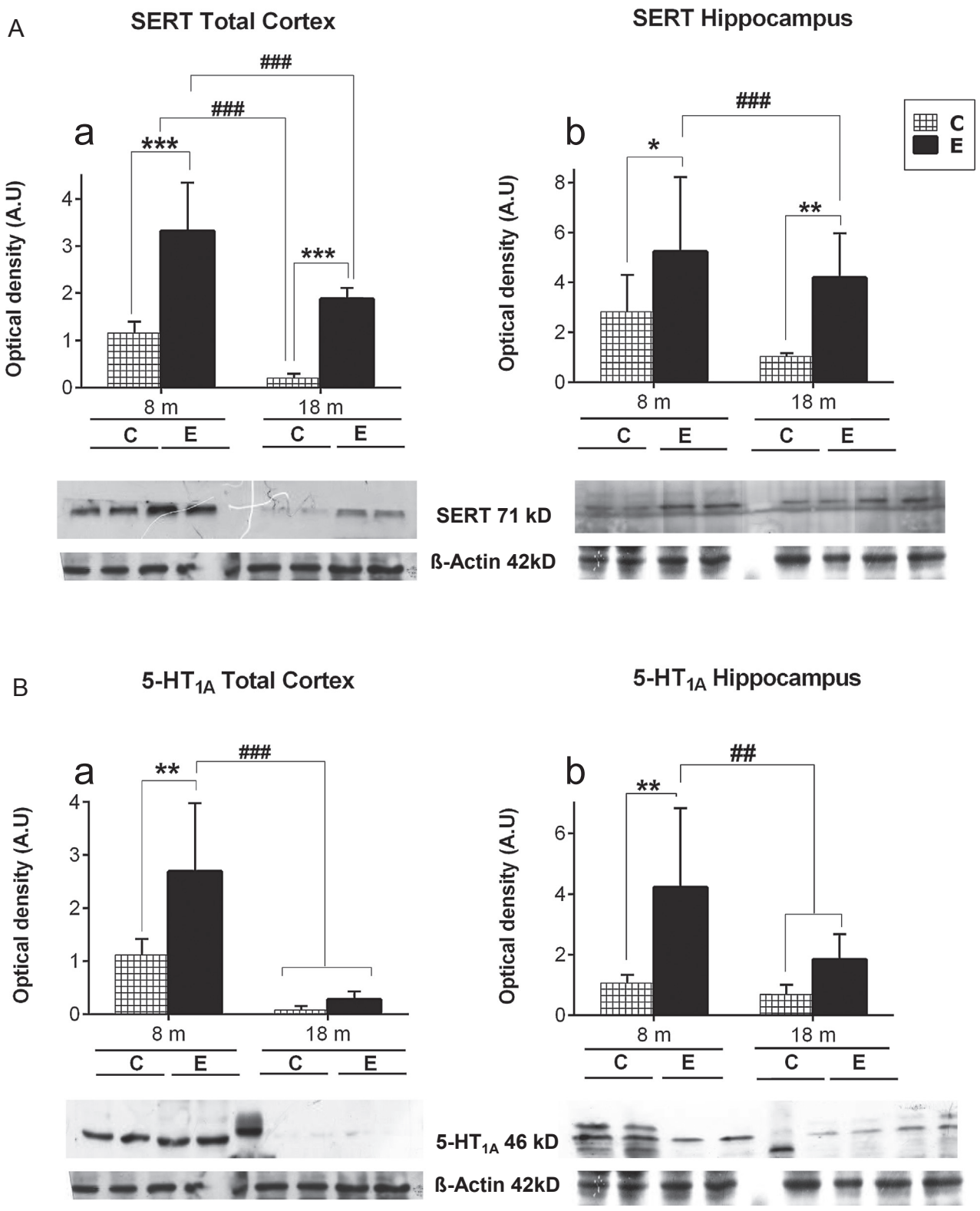

Fig. 12. Effects of E and age on SERT and 5-HT_{1A} expression in the cortex and hippocampus. Bars represent the mean ± SEM. Panel A: SERT. (a) Total cortex obtained from a pool containing prefrontal, perirhinal, and temporo-parietal regions. Statistically significant differences due to E (***$p < 0.001$, 8C vs. 8E and 18C vs. 18E) and age (### $p < 0.001$, 8C and 8E vs. 18C and 8E vs. 18E) (top), western blot corresponding to (bottom). (b) Hippocampus. Statistically significant differences were observed by E effect (*$p < 0.05$, 8C vs. 8E; **$p < 0.01$, 18C vs. 18E) and age (### $p < 0.001$, 8E vs. 18E) (top), western blot corresponding to b (bottom). PANEL B: 5-HT_{1A}. (a) Prefrontal, perirhinal, and temporo-parietal cortex. Significant differences due to E (**$p < 0.01$, 8C vs. 8E) and age (### $p < 0.001$, 8E vs.18C and E) (top), western blot corresponding to (bottom). (b) Hippocampus. Significant differences due to E (**$p < 0.01$, 8C vs 8E) and age (##$p < 0.01$, 8E vs. 18C and E) (top), western blot corresponding to b (bottom). Data from n = 6 rats/group; Two-way ANOVA, Tukey's *post hoc* test.

(24 h post-exercise) and may indicate a real competition for energy resources. Thus, it is possible to assume that regular AE stimulated overcompensation in the glycogen stores to satisfy the increased neuronal energy demand, proliferation and synaptic activity. Following these ideas and consistent with previous studies (Inoue et al., 2015; Klempin et al., 2013; Musumeci et al., 2015), the significant increase in 5-HT activity shown by E rats may be more closely related to the control of energy expenditure than to the cognitive or affective function themselves. On the other hand, the amplification of the memory systems with concomitant increases in BDNF expression could also be analyzed in the context of a lesser-known BDNF function as a master regulator of energy homeostasis (Gomez-Pinilla and Hillman, 2013; Marosi and Mattson, 2014). Perhaps, under certain circumstances, the BDNF-5-HT system could act as an interface between aerobic metabolism and the trophic and plastic effects produced by AE.

In brief: 1) the adaptive response of the brain to chronic AE is the result of cumulative hormetic effects that could result in compensatory and/or supercompensatory mechanisms favoring the cognitive function; 2) the coordinated action of 5-HT and BDNF could ensure the availability of energy resources in the cortex and hippocampus to meet the great cognitive and motor demand. This mechanism could be a prerequisite for the specific action of BDNF on memory processes, adult neurogenesis, neural remodeling and synaptic activity; 3) AE has a neuroprotective effect against cognitive impairment in older subjects and prevents the decline in serotonergic transmission and BDNF expression secondary to aging.

5. Conclusion

In conclusion, it can be suggested that regular training is a healthy habit to mitigate and/or prevent the physiological age-dependent decline in cognitive function. In addition, AE appears to be a complementary intervention to conventional pharmacological therapies, which are usually prescribed in certain neurodegenerative pathologies or neuropsychiatric disorders. Based on the present results, the practice of recreational aerobic activities of both low-moderate intensity and long duration such as walking, swimming, rowing, biking, jogging and dancing should be recommended.

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