



Research article

Treadmill exercise induces selective changes in hippocampal histone acetylation during the aging process in rats



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HIGHLIGHTS

- Exercise improved aversive memory retention.
- H4K12 acetylation levels increased in hippocampi of all exercised animals.
- Exercise increased H3K9 acetylation levels only in hippocampi of aged rats.

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ABSTRACT

Physical exercise and the aging process have been shown to induce opposite effects on epigenetic marks, such as histone acetylation. The impact of exercise on hippocampal histone acetylation on specific lysine residues, especially during the aging process, is rarely studied. The aim of this study was to investigate the effect of treadmill exercise (20 min/day during 2 weeks) on H3K9, H4K5 and H4K12 acetylation levels in hippocampi of young adult and aged rats. Male Wistar rats aged 3 or 20–21 months were assigned to sedentary and exercise groups. Single-trial step-down inhibitory avoidance conditioning was employed as an aversive memory paradigm. Hippocampal H3K9, H4K5 and H4K12 acetylation was determined by Western blotting. The daily moderate exercise protocol improved the aversive memory performance and increased hippocampal H4K12 acetylation levels in both tested ages. Exercise was also able to increase H3K9 acetylation levels in aged rats. An age-related decline in memory performance was observed, without any effect of the aging process on histone acetylation state. Our data suggest that treadmill exercise can impact hippocampal the histone acetylation profile in an age- and lysine-dependent manner. In addition, higher hippocampal H4K12 acetylation levels at both ages may be related to improvement of aversive memory performance.

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1. Introduction

A growing body of evidence suggests that moderate exercise is able to impact brain functions such as cognition, learning and memory performance, and cognitive decline in aging [4,25,26]. Epigenetic modifications, specifically global histone acetylation and

histone-modifying enzymes, have been linked to exercise effects on memory performance in rodent models. The histone acetylation corresponds to acetyl groups attached to lysine (K) residues on the amino-terminal tails of histones (H) and has been widely associated with enhanced transcriptional activity [2,17]. A single session of exercise (20 min) increased histone acetylase enzyme (HAT) (the enzyme that adds acetyl groups to histones) and decreased histone deacetylase enzyme (HDAC) (the enzyme that removes acetyl groups) activity immediately and one hour after exercise in the rat hippocampus [14]. Additionally, voluntary exercise protocols have induced changes in HDAC activity [1,16]. A previous study indicated that a moderate daily exercise protocol (20 min/day during 2 weeks) improved transiently aversive memory performance, (eval-

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uated by an inhibitory avoidance test) and concomitantly reversed age-induced decreases of hippocampal global H4 [22]. Despite these findings, exercise effects on specific lysine modifications in rodent hippocampi are rarely investigated [12,15].

Another remarkable point is the fact that aversive learning context exposure in different fear-motivated learning tasks can alter epigenetic marks, suggesting that the influence of the learning paradigm on acetylation levels should be considered. Interestingly, a training session of fear conditioning was able to alter hippocampal global acetylation of H2B, H3 and H4 [6,20] as well as specific lysine, such as H4K12, H3K9, H3K14, H4K5 and H4K8 in young adult rodents, while H4K12 acetylation state was unchanged in aged hippocampus [9,24]. The authors suggested that the lack of this specific acetylation during the acquisition stage can be related to age-dependent memory impairment [24]. In addition, it has been observed that exposure to a memory paradigm, including the test session, is able to alter H3K14 acetylation in the hippocampus of young adult rats [12]. However, little is known regarding its contribution to aversive memory consolidation in the aging process.

Despite the growing body of evidence describing the effects of exercise, the aging process and exposure to the aversive memory paradigm on epigenetic parameters [10,19], there are few studies evaluating the interaction among these factors, particularly histone acetylation on specific lysine residues, in brain areas. Thus, the aim of this study was to investigate the effect of a treadmill exercise protocol (20 min/day during 2 weeks) on histone acetylation levels on specific lysine residues, such as H3K9, H4K5 and H4K12, in hippocampi of 3 or 20–21-month-old Wistar rats. We asked whether exercise and aging effects are differentially modified by exposure to an inhibitory avoidance task.

2. Material and methods

2.1. Animals

Male Wistar rats aged 3 and 20–21 months old were used and maintained under standard conditions (12-h light/dark, 22 °C ± 2 °C) with food and water *ad libitum*. Animals were provided by Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS) and housed five per cage (Plexiglass cages, dimensions: 40 × 33.3 × 17 cm). The NIH “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 80-23, revised 1996) was followed in all experiments and the Local Ethics Committee approved all handling and experimental conditions (nr. 23997).

2.2. Exercise protocol

Rats were randomly divided into a sedentary (SED) or exercised (EXE) groups (n = 10–12; Fig. 1). The SED group animals was handled exactly as the EXE group and left on the treadmill while it was not operating for 5 min. The exercise training was performed on a motorized rodent treadmill (AVS Projetos, São Paulo, Brazil) with individual Plexiglas lanes. The peak oxygen uptake (VO₂) was indirectly measured in all animals prior to training.

Each rat ran on a treadmill at a low initial speed, and the speed was increased at a rate of 5 m/min every 3 min until the point of exhaustion (g, failure of the rat to continue running). The time to fatigue (in min) and workload (in m/min) were obtained as indices of exercise capacity, which, in turn, were taken as VO₂max [3,8]. The animals initially refusing to run were encouraged by gently tapping on their backs.

The exercise training consisted of running sessions at 60% of VO₂max. The rats were subjected to one 20-min running session daily for 2 weeks. Treadmill velocity progression is represented in

Table 1. In the first two sessions, rats were adapted to the treadmill. Neither electric shock nor physical prodding were used in this study. All of the procedures took place between 02:00 P.M. and 05:00 P.M.

2.3. Inhibitory avoidance

The animals were exposed or not exposed to an inhibitory avoidance task. We used single-trial step-down inhibitory avoidance conditioning as a model of fear-motivated memory. At step-down inhibitory avoidance training, the animals learned to associate a location in the training apparatus (a grid floor) with an aversive stimulus (footshock). In the training trial, rats were placed on a platform and immediately after stepping down on the grid received a 0.6 mA, 3.0 s footshock prior to removal from the apparatus. The test trial took place 24 h after the training trial, the rats were exposed to the platform and no footshock was delivered; latencies to step down were recorded and used as a measure of memory retention. Retention test latencies measurements were cut off at 180 s. The exercised and exposed to learning context groups, were subjected to inhibitory avoidance test session 30 min after the last exercise session and 30 min before euthanasia. The general procedures for inhibitory avoidance behavioral training and the retention test were described in a previous report [21,22].

2.4. Preparation of the samples

Rats were sacrificed by decapitation 1 h after the last training session of exercise. It has been demonstrated that our exercise protocol improved aversive memory performance as well as hippocampal global H4 at 1 h after the last training session of exercise, and the behavioral test was performed 30 min before euthanasia [22]. Hippocampi were quickly dissected, immediately snap-frozen in liquid nitrogen, and stored at –80 °C until the histone extraction. The hippocampi were homogenized (n = 5), placed in EDTA-free (Sigma-Aldrich, São Paulo, Brazil) solution 1 × containing a protease inhibitor cocktail tablet, and stored at –80 °C for subsequent analysis. Histones were extracted as previously described [11]. Briefly, PBS buffer (Phosphate-Buffered-Saline) containing 250 μL Triton and 10 mg NaN₃ was added to the homogenated samples to a 50 mL final volume. After 10 min on ice, samples were centrifuged at 6500g for 10 min at 4 °C. The supernatant was removed, and the pellet was collected. An equal volume of 0.2N HCl was added, and acid extraction of histones was carried out over night at 4 °C. The next day, samples were centrifuged at 6500g for 10 min at 4 °C, and this histone extract was stored at –80 °C. The protein concentration of each sample was measured using the Coomassie Blue method, and bovine serum albumin was used as a standard [7].

2.5. Western blot analysis

Twenty-five μg of total protein was separated on a 12% SDS-polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane. Membranes were blocked with 5% non fat dry milk in TBS containing 0.05% Tween-20 and were incubated overnight with the following antibodies: anti-β-actin (ab8227, Abcam) at 1:1500, anti-acetyl histone H3 (Lys-9, ab10812, Abcam) at 1:500, anti-acetyl histone H4 (Lys-5, K5, ab51997, Abcam) at 1:700, and anti-acetyl histone H4 (Lys-12, K12, ab61238, Abcam) at 1:700. Goat anti-rabbit (ab6721, HRP) horseradish peroxidase-conjugated secondary antibodies were used and detected using the ECL Western Blotting Substrate Kit (ab65628, Abcam, EUA). Pre-stained molecular weight protein markers (Benchmark marker, Invitrogen) were used to determine the molecular weight of the detected bands and confirm antibodies target specificity. Quantification was performed using a Kodak Gel Logic 2200 Imaging

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